CHANGES IN MUSCLE ACTION POTENTIALS DURING ACTIVITY: THE ROLE OF THE NA^{+}-K^{+} PUMP

By

© AUDREY L. HICKS

A Thesis
Submitted to the School of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree
Doctor of Philosophy

McMaster University

(June, 1988)

CHANGES IN MUSCLE ACTION POTENTIALS DURING ACTIVITY

DOCTOR OF PHILOSOPHY (1988) McMASTER UNIVERSITY

(Medical Sciences) Hamilton, Ontario

TITLE: Changes in Muscle Action Potentials During Activity: The Role of the Na^+-K^+ Pump

AUTHOR: Audrey Lyn Hicks, B.P.E., M.Sc.

SUPERVISORS: Dr. Alan J. McComas / Dr. Norman L. Jones

NUMBER OF PAGES: xii, 187.

ABSTRACT

The studies that make up this thesis have been designed to describe and analyse the changes in muscle action potentials associated with muscular activity. The initial experiments were carried out in human muscle to examine changes in the muscle compound action potential (M-wave) during both stimulated and voluntary activity. It was found that the M-wave increased in amplitude either during or following either type of activity, and that this increase in M-wave amplitude could not be explained by either a movement artefact or a greater synchronization of action potential firing. It was hypothesized that the potentiation of the M-wave was due to a hyperpolarization of the muscle fibre membrane which was increasing the amplitude of the individual fibre action potentials. Experiments were then conducted on rat soleus muscle (in vivo) in order to measure changes in resting membrane and action potentials associated with contractile activity. After 5 min of intermittent tetanic stimulation at 20 Hz, the mean resting membrane potential increased from a baseline value of -79.5 ± 4.8 mV to a mean maximum of -92.6 ± 4.2 mV 3-6 min post-stimulation ($p<.01$). There was a corresponding increase in action potential amplitude; it rose from a resting value of 82.2 ± 10.8 mV to 98.1 ± 7.8 mV in the recovery period ($p<.01$). It was hypothesized that increased activity of the electrogenic Na^+-K^+ pump was

causing the hyperpolarization. A series of experiments utilizing inhibitors of the Na^+-K^+ pump were then conducted; it was found that the administration of ouabain (1.25×10^{-4}M), cooling the bathing medium (from 37°C to 19°C) or removal of extracellular K^+ prevented the hyperpolarization following repetitive stimulation ($p<.05$). The magnitude of the electrogenic contribution of the Na^+-K^+ pump was then estimated by exposing stimulated muscle fibres to a high K^+ (20 mM) medium. While in the control (unstimulated) condition this caused an immediate depolarization of the muscle fibre membrane to approximately -58 mV, stimulated fibres maintained membrane potentials of -79.5 mV (± 8.6 mV) for at least 3 min, which was approximately -30 mV greater than that predicted by the Goldman-Hodgkin-Katz (GHK) equation. It is concluded that the Na^+-K^+ pump plays an important role in maintaining muscle fibre excitability during muscular activity, which is additional to its role in the restoration and maintenance of ionic gradients for Na^+ and K^+. The temporary hyperpolarization of the muscle fibre membrane during increased Na^+-K^+ activity offers an explanation for the potentiation of muscle compound action potentials observed during voluntary and stimulated contractions.

The author gratefully acknowledges support from NSERC, MRC, and the Leman Bros. Muscular Dystrophy Foundation.

ACKNOWLEDGEMENTS

This thesis could not have been completed without the help and support of many people. First, I would like to thank my supervisors - Dr. Norman Jones, whose insight and support were always much appreciated, and Dr. Alan McComas, for all his time, criticisms, and unwaivering determination to mold me into a scientist. I must also thank Dr. McComas for giving me the opportunity to present this work at international meetings; your support was greatly appreciated. Many thanks are also extended to the other members of my supervisory committee, Dr. John Rowe, Dr. Ethel Cosmos, and Dr. Digby Sale. A special note of thanks goes to Digby for always making the time to offer his support and guidance.

A great deal of thanks go to my fellow cell-mates, Jayne, Anne, and Vicki, who were the best support group one could ask for and brought a lot of enjoyment to my life as a graduate student. I would also like to give a special thank you to Frank, for his friendship, technical assistance, and for always letting me choose the radio station. I would also like to acknowledge the technical assistance of Glenn Shine, and the secretarial assistance of Anne Murray.

Finally, I want to thank those most near and dear to me - my two beautiful boys, Jason and Kyle, and Craig, for all his love, support, and wonderful ability of always being able to put things into perspective for me.

Table of Contents

Chapter 1. Introduction

1.1. Scope of present investigation 1

1.2. The muscle compound action potential 2

1.2.1. Definition 2
1.2.2. Applications of M-wave recordings 2

1.3. Relevance of M-wave measurements to the study of fatigue mechanisms 3

1.3.1. Definition of muscle fatigue 3
1.3.2. Possible sites of fatigue 4
1.3.3. Theoretical significance of M-wave changes in fatigue 5

1.4. Changes in M-wave during muscle activity 8

1.4.1. Voluntary contractions 8
1.4.2. Stimulated contractions 9

1.5. Ionic basis of resting and action potentials 12

1.5.1. Historical development of concepts 12
1.5.2. Studies of the squid giant axon 16
1.5.3. Goldman-Hodgkin-Katz equation 18
1.5.4. Influence of ionic concentrations on resting potential of squid axon 19

1.6. Resting and action potential measurements in striated muscle 20

1.6.1. Early studies 20
1.6.2. Effect of external ionic concentrations 20
1.6.3. Influence of protein in bathing medium on resting potential 21
1.6.4. Influence of tip potentials on resting potential measurements 22
1.6.5. Effect of temperature 23

1.7. The Na^+-K^+ Pump 25

1.7.1. Background 25
1.7.2. Early concepts 26
1.7.3. Contribution to steady state E_m: the Mullins-Noda equation 29
1.7.4. Energy dependence 30
1.7.5. Coupling ratio 32

Table of Contents (cont.)

1.7.6. Contribution to non-steady state E_m ... 33

1.7.7. Distribution of Na^+-K^+-ATPase in different fibre types 35
1.7.8. Effect of hormones 36
1.7.9. Inhibition of the Na^+-K^+ pump 38

1.8. Changes in Na^+-K^+ pump activity with muscular exercise 41

1.9. Summary and Hypothesis for Thesis 43

Chapter 2. Methodology

2.1. Introduction 44

2.2. Human studies 44

2.2.1. Experiments with intermittent tetanic stimulation 44
2.2.2. Intermittent voluntary contractions ... 46
2.2.3. Statistical treatment 52

2.3. Animal studies 52

2.3.1. Rationale 52
2.3.2. Animal preparation 52
2.3.3. Stimulation and recording system 54
2.3.4. Fatigue sequence 56
2.3.5. Assessment of Na^+-K^+ pump activity 59
2.3.6. Statistical treatment 62

Chapter 3. Results

3.1. Human studies 63

3.1.1. Experiments with intermittent tetanic stimulation 63
3.1.2. Experiments with voluntary contractions 65

3.2. Animal studies 67

3.2.1. Effect of tetanic stimulation on muscle compound action potentials............ 67
3.2.2. Effect of tetanic stimulation on resting and action potentials 72

Table of Contents (cont.)

Chapter 4. Discussion and Conclusions

4.1. M-wave during muscle activity in man 92

4.2. M-wave during muscle activity in rat soleus 93

4.3. Microelectrode recordings in rat soleus muscle fibres 97

4.3.1. Comparison of resting potentials to other published values 97
4.3.2. Effect of activity on resting membrane potential 99
4.3.3. Comparison of action potentials to other published values 101
4.3.4. Effect of activity on the action potential 101

4.4. Electrogenic Na^{+}-K^{+} pump activity 103

4.4.1. Findings at rest 103
4.4.2. Pump activity after muscle stimulation 105

4.5. Significance of Na+-K^{+} pump activity during muscular exercise 111

4.6. Clinical implications 117

4.6.1. Cardiac patients 117
4.6.2. Hyperkalaemic familial periodic paralysis 118
4.6.3. Myotonic muscular dystrophy 119

4.7. Future directions 120

4.8. Summary of results 123

Chapter 5. References 126

Appendix 1 141
Appendix 2 143
Appendix 3 146
Appendix 4 156
Appendix 5 162
Appendix 6 167
Appendix 7 172
Appendix 8 177
Appendix 9 181

List of Figures

Figure 1. Components of the muscle compound action potential 7

Figure 2. Changes in M-wave associated with voluntary fatigue 10

Figure 3. Placement of electrodes for thenar and EDB voluntary contractions 50

Figure 4. Experimental protocol for intermittent voluntary contractions 52

Figure 5. Measurement of E_m, AP and M-wave 56

Figure 6. Rat soleus preparation 58

Figure 7. Experimental protocol for stimulation of rat soleus 59

Figure 8. Effect of intermittent tetanic stimulation (15 Hz) on M-wave and twitch 64

Figure 9. Effect of intermittent tetanic stimulation on M-wave and twitch of single subject 66

Figure 10. Changes in M-wave during intermittent voluntary contractions of human thenar muscle 69

Figure 11. Changes in M-wave during intermittent voluntary contractions of thenar muscle in one subject 70

Figure 12. Changes in M-wave during intermittent voluntary contractions of human EDB muscle 71

Figure 13. Effect of intermittent tetanic stimulation (20 Hz) on rat soleus M-wave 73

Figure 14. Effect of ouabain (4mg/kg) on M-wave behaviour of stimulated rat soleus 74

Figure 15. Frequency distribution of resting and potentials in rat soleus 76

Figure 16. Effect of 5 min stimulation on resting and action potential in rat soleus 77

List of Figures (cont.)

Figure 17. Effect of ouabain (4mg/kg) on resting and action potential of rat soleus 79

Figure 18. Effect of ouabain (1.25×10^{-4}M) on resting and action potential of stimulated rat soleus 81

Figure 19. Effect of cooling (19°C) on resting and action potentials of stimulated rat soleus 84

Figure 20. Effect of removal of $[K^+]_o$ on resting and action potentials of stimulated rat soleus 85

Figure 21. Effect of Na^+-K^+ pump inhibition on resting and action potentials of unstimulated rat soleus 87

Figure 22. Effect of 20 mM $[K^+]_o$ on resting and action potentials of rat soleus 89

Figure 23. Estimated contribution of Na^+-K^+ pump to resting membrane potential 112

List of Tables

Table 1. Description of subjects for intermittent voluntary contraction experiment 47

Table 2. Changes in M-wave during intermittent voluntary contractions of thenar and EDB muscles 68

Table 3. Summary of results of rat soleus experiments 91

Table 4. Published values of resting and action potentials in rat muscle 98

List of Abbreviations

AP	= action potential
ATP	= adenosine triphosphate
Cl^-	= chloride ion
E_{Cl}	= chloride equilibrium potential
EDB	= extensor digitorum brevis
EDL	= extensor digitorum longus
E_K	= potassium equilibrium potential
E_m	= resting membrane potential
EMG	= electromyogram
F	= Faraday constant
E_{Na}	= sodium equilibrium potential
Hz	= hertz (cycles per second)
K^+	= potassium ion
kg	= kilogram
ln	= natural logarithm
M	= molar
mM	= millimolar
M-wave	= muscle compound action potential
min	= minute
mg	= milligram
ms	= millisecond
mV	= millivolt
Na^+	= sodium ion
Na^+-K^+ pump	= sodium-potassium pump
OS	= overshoot of action potential
R	= universal gas constant
T	= absolute temperature
μs	= microsecond

Introduction

1.1 Scope of the present investigation

The starting point for this thesis was the observation, by Fitch and McComas (1985), that the maximum muscle compound action potential evoked in the human tibialis anterior muscle increased in amplitude during repetitive stimulation of the common peroneal nerve. This increase in amplitude was of interest because it was in the opposite direction to that anticipated in the course of muscle fatigue. The studies that make up the body of this thesis have been designed to, first, describe the behavior of the muscle action potentials during activity, and second, to analyse the mechanisms responsible. The investigation has been carried out at two levels. First, a series of descriptive experiments were conducted on human muscle to examine the maximum electrical response which can be evoked from an entire muscle by stimulation of its motor nerve during different types of contractile activity. Second, the propagated impulses of single muscle fibres were studied, using an in vivo preparation of rat soleus muscle.

In the sections of the Introduction which follow, the maximum responses evoked in muscle are considered in relation to fatigue and to other situations. Then follows an account of the ionic basis of the resting and action potentials of single muscle fibres and the changes in

potential observed as a result of muscle activity. In view of the role that the sodium-potassium pump (Na^+-K^+ pump) plays in the maintenance of ionic gradients across the muscle membrane, a detailed description of the characteristics of the Na^+-K^+ pump is also included. The mechanisms by which the pump might contribute to the membrane potential of a muscle fibre during different states of activity will also be reviewed.

1.2 The muscle compound action potential (M-wave)

1.2.1 Definition. The muscle compound action potential is the electrical response which can be recorded from part, or all, of a muscle following stimulation of its motor nerve. The compound action potential is the algebraic sum of the impulses initiated in many muscle fibres. Magladery *et al* (1951) appear to have been responsible for assigning the term 'M-wave' to the muscle compound action potential; because of its convenience, the term 'M-wave' has been employed widely in this thesis.

1.2.2. Applications of M-wave recordings. The size of the M-wave response can be used as an indication of the fraction of the motor nerve population which has been stimulated. Indeed, if juxtathreshold stimuli are delivered, it is possible to determine the approximate

number of motor units in a muscle by comparing the responses of individual units to the maximum M-wave (McComas et al, 1971). M-wave recordings have found an important place in clinical electromyography (EMG) since the amplitudes of these responses are proportional to the excitable muscle mass and, as such, are diminished in diseases of both nerve and muscle. By stimulating a motor nerve maximally at two sites and recording the corresponding latencies of the M-waves, it is relatively easy to measure the impulse conduction velocities of the motor nerve fibres. Such determinations are of particular value in the detection of partial demyelination in localized or diffuse neuropathies. Finally, the M-wave responses to repetitive nerve stimulation are routinely employed to detect disorders of neuromuscular transmission in patients with weakness suggestive of either myasthenia gravis or the Lambert-Eaton syndrome.

1.3. Relevance of M-wave measurements to the study of fatigue mechanisms.

1.3.1. Definition of muscle fatigue. Fatigue is often defined as the "failure to maintain the required or expected power output" (Edwards, 1983). However, Bigland-Ritchie et al (1986) argue that this definition has different implications for maximum versus submaximum

effort, and thus, they prefer to define fatigue as any reduction in the maximum force generating capacity.

1.3.2. Possible sites of fatigue

The subject of muscle fatigue has intrigued researchers for the better part of this century, but the precise mechanisms responsible for fatigue are still a matter of conjecture. Rather than regarding fatigue as a failure of physiological function, it has recently been proposed that fatigue should be thought of more as a protective mechanism for survival (Bigland-Ritchie, et al, 1986).

The possible mechanisms underlying fatigue can be found anywhere in the continuum between the psyche, through the central and peripheral nervous systems, to crossbridge function within the myofilaments. In a given situation the site of the weak link will depend on a wide variety of factors, such as motivation, type and duration of muscular activity, and the physiological characteristics of the particular muscles being used. Basically, the potential sites of fatigue can be divided into either a central or peripheral location.

The central factors which might become impaired during muscular activity are: a) the excitatory drive to the higher motor 'centres', b) the balance between excitatory and inhibitory pathways converging on the lower

motoneuron pool, and c) the excitability of the spinal motoneurons (Bigland-Ritchie, et al, 1986). The peripheral factors that might be affected during fatigue include: a) the integrity of electrical transmission from nerve to muscle, b) impulse propagation along the sarcolemma and t-tubular system, c) the efficacy of excitation-contraction coupling, d) availability of muscle energy supplies, and e) crossbridge function (Bigland-Ritchie, et al, 1986).

In order to analyse changes associated with a loss in muscle force, it is useful to be able to distinguish between electric and metabolic events. In this context, measurement of the muscle compound action potential, or M-wave, has become a useful, non-invasive, index of neuromuscular transmission and muscle membrane excitability during fatigue experiments.

1.3.3. Theoretical significance of M-wave changes in fatigue.

The changes in M-wave offer different information regarding the nature of neuromuscular events associated with muscle fatigue (See Figure 1). Thus, the amplitude of the M-wave is representative of muscle membrane excitability as it is dependent on the resting membrane potential of the muscle fibre, and on the extent of the overshoot of the action potential. The size of the M-wave

will also reflect the number of muscle fibres (and motor units) participating in the response. Simple measurements of M-wave amplitude do not, however, distinguish between presynaptic failure of neuromuscular transmission, due to events in the nerve terminal, and loss of muscle fibre excitability.

The duration of the M-wave depends, in turn, on that of the single muscle fibre action potential and, hence, on the rate of activation of the outward potassium current channels and on the rate of inactivation of the inward sodium current channels. These mechanisms will be discussed in a later section (1.5). The M-wave duration is also affected by the degree of synchronization of the muscle fibre action potentials, becoming greater as impulse conduction slows in some of the fibres. As the M-wave becomes dispersed, however, the amplitude of the wave declines. It follows that, in fatigue studies, an increase in M-wave amplitude could result from larger muscle fibre action potentials or from more synchronous discharges; in the latter case, the area (voltage X time) of the M-wave should remain unchanged while in the former instance it should increase.

A decrease in M-wave amplitude, with constant duration, would signify either fewer responding muscle fibres or reduced action potential overshoots. An increase in M-wave duration would denote longer muscle

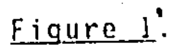
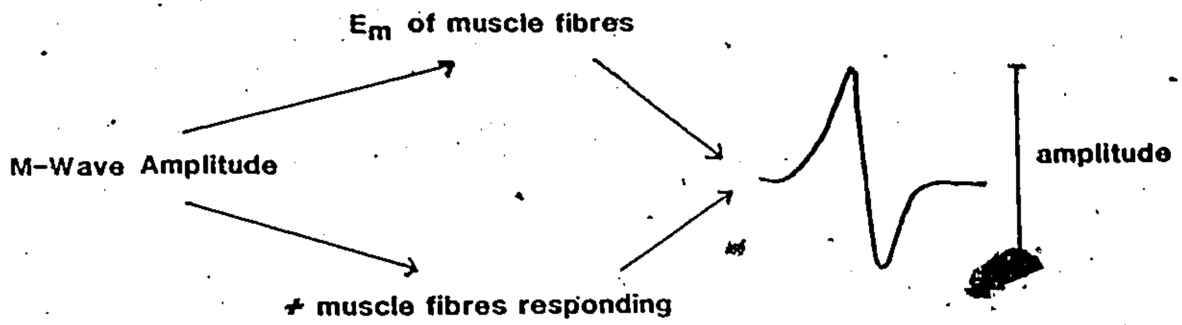
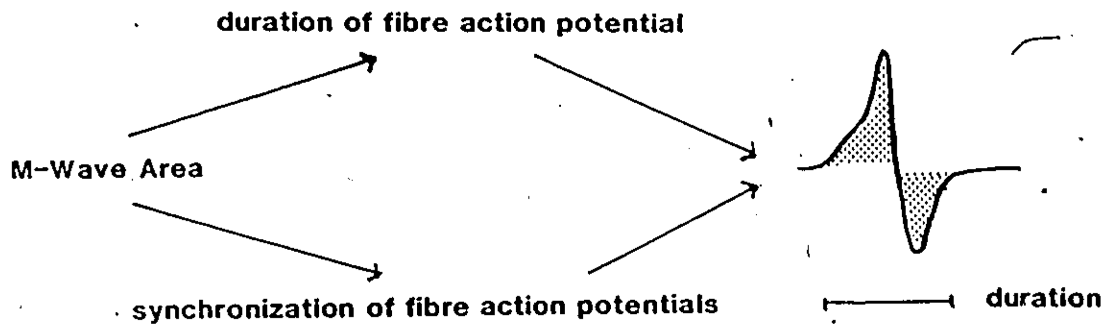


Figure 1.

Components of the muscle compound action potential.





Shaded area represents voltage/time integral, or M-wave area

fibre action potentials or greater dispersion of the muscle fibre volley; in the latter case the M-wave area should be unchanged while in the former it should increase. Finally, a decrease in M-wave duration would reflect either briefer muscle fibre action potentials or greater synchronicity of firing; there would be either a decrease or no change in the M-wave area respectively.

1.4. Changes in M-wave during muscle activity

1.4.1. Voluntary contractions. Reports on M-wave behavior during maximal voluntary contractions are conflicting. The pioneering study of Merton (1954), demonstrated that in the human adductor pollicis muscle, force decreased by more than 80% after 3 min of maintained maximum force, whereas there was no change in the size of the M-wave. Conversely, Stevens and Taylor (1972) reported a decline in M-wave amplitude within one minute of maximum voluntary contraction of the first dorsal interosseus muscle. Bigland-Ritchie *et al* (1982) attempted to resolve these discrepancies by investigating M-wave changes associated with voluntary fatigue of both the adductor pollicis and first dorsal interosseus muscles. The M-waves were shown to be well-maintained and it was concluded that no neuromuscular transmission failure occurs in either of these muscles. The

discrepancies in the literature were suggested to be due to either movement artifacts, inconsistent stimulus intensities, or inadequate methods of M-wave measurement.

The behavior of the M-wave during recovery from voluntary fatigue has received only limited attention. However, while not commented upon, M-wave potentiation is evident in the figures of Stevens and Taylor (1972) and of Milner-Brown and Miller (1986) in the recovery period following voluntary fatigue of the first dorsal interosseus muscle (See Figure 2).

1.4.2. Stimulated contractions

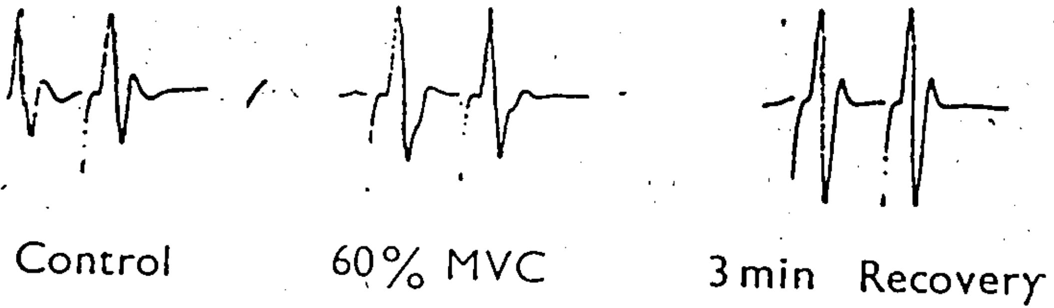
When a muscle is stimulated repetitively, the M-wave undergoes characteristic changes which are dependent on both the length and frequency of stimulation. Decline in the peak-to-peak amplitude of the M-wave is a commonly reported phenomenon during stimulated muscle fatigue experiments in humans (Hultman & Sjoholm, 1983; Fitch & McComas, 1985), and animals (Naess & Storm-Mathisen, 1955; Pagala, _et al_, 1984), but the amount of M-wave decline is dependent on the frequency of stimulation. Thus, the relative decline in M-wave amplitude is greater at frequencies above 30 Hz (Johns, _et al_, 1956; Bigland-Ritchie, _et al_, 1979) than at frequencies lower than 30 Hz (Johns, _et al_, 1956; Sandercock, _et al_, 1985). Associated with the decline in M-wave amplitude is an increase in its

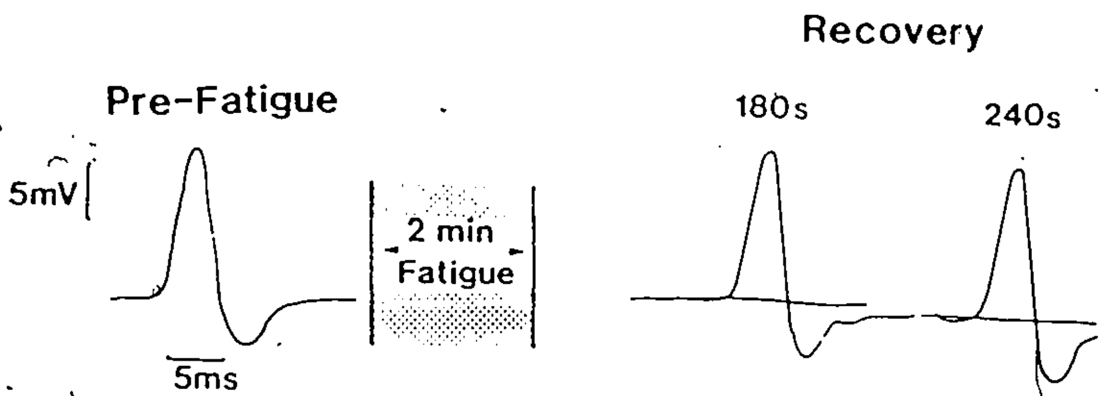
Figure 2A.

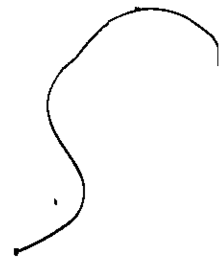
Changes in M-wave associated with voluntary contraction of human first dorsal interosseus muscle. Reproduced from Figure 7 (Stevens and Taylor, 1972). Note that the M-wave in the recovery period is greater than control.

Figure 2B.

Changes in M-wave during recovery from 2 min of maximum voluntary contraction of human first dorsal interosseus muscle. Reproduced from Figure 1, Milner-Brown and Miller (Muscle and Nerve, 1986). There appears to be an increased M-wave at 240s of recovery compared with pre-fatigue M-wave.


Control
60% MVC
3 min Recovery


Pre-Fatigue
5mV
5ms
2 min
Fatigue
Recovery
180s
240s



duration and/or area (Bigland-Ritchie, et al, 1979; Pagala, et al, 1984).

There is a fairly good relationship between the decline in force and decline in the M-wave at the higher frequencies of stimulation (Pagala, et al, 1984; Sandercock, et al, 1985). However, at lower frequencies, the M-wave behavior does not follow the decline in force (Sandercock, et al, 1985; Garner, Hicks, & McComas, 1986). Thus, high frequency fatigue has been attributed to neuromuscular transmission failure, with recovery being relatively quick (Bigland-Ritchie, et al, 1979; Jones, et al, 1979), whereas low frequency fatigue appears to be due to a failure in excitation-contraction coupling, with the recovery of tension being much slower (Edwards, et al, 1977).

While a great deal of attention has been given to the declining M-wave during muscle fatigue, there is often a brief period of M-wave potentiation before the eventual decline which is rarely commented upon. This enlargement was evident in the work of Fitch and McComas (1985); these authors suggested the supernormal period of excitability of the muscle fibres caused an increased synchronization of action potential firing. Alternatively, Duchateau and Hainaut (1984) proposed that a decreased temporal dispersion in transmitter release might increase the size of the M-wave. However, an alternative explanation for

the M-wave potentiation observed both during fatigue and in the recovery period is that the amplitudes of the individual fibre action potentials were increased, due to a hyperpolarization of the resting membrane potentials of the muscle fibres.

1.5. Ionic Basis of Resting and Action Potentials

1.5.1. Historical development of concepts

The combined work of Luigi Galvani and Allessandro Volta in the 18th century introduced the concept that electricity was involved in nerve transmission and muscle contraction. Galvani, in 1786, reported that amputated frog legs would contract convulsively if they were made part of an electric circuit (de Santillana, 1965). This led him to propose that muscles had an ability to produce electricity ("animal electricity"), which he claimed was independent of any metal in the circuit. This announcement was vigorously challenged by Volta, who at the same time was developing a theory that electrical transmission involved a difference of metals, and thus, a muscle could only contract if electricity was applied to it (de Santillana, 1965). Volta's subsequent invention of the battery which could stimulate nerves or muscles lent support to his theory. Later studies conducted by Galvani, in which he demonstrated that muscles could

contract without being in contact with any metal appeared to dismiss Volta's claim that two different metals were required for electric transmission from nerve to muscle (Needham, 1971). However, once adequate technology was developed to analyse the ionic currents in nerve and muscle, it became apparent that both Galvani and Volta were quite correct in their understanding of bio-electric currents.

Bernstein (1902) was the first to propose a satisfactory hypothesis for the origin of the resting membrane potential by applying the physiochemical ideas of Nernst (1888). His theory maintained that each cell had a membrane which was selectively permeable to potassium and impermeable to other cations and anions. This in itself was an important statement since at that time the idea of a membrane enveloping the cell was only an hypothesis. Bernstein proposed that there was a potential difference across the membrane at rest and that this was the result of the distribution of potassium ions on either side of the membrane: this potential could be predicted from the Nernst equation for the diffusion of potassium.

1) $$E_K = \frac{RT}{zF} \ln \frac{[K^{\pm}]_e}{[K^+]_i}$$

where E_K is the potassium equilibrium potential, R is the universal gas constant, T is the absolute temperature, z is the valency of the ion, and F is the faraday (Kuffler

and Nicholls, 1976).

Boyle and Conway (1941), disproved Bernstein's selective permeability theory, by demonstrating that the skeletal muscle fibre was permeable to both potassium and chloride. By soaking frog muscles in solutions containing various potassium chloride concentrations for 24 hrs, and then determining the intracellular potassium and chloride concentrations, they were able to show that the distribution of potassium and chloride across the membrane followed the rules of a Donnan equilibrium (Donnan, 1911). Boyle and Conway (1941) maintained that the cell membrane was impermeable to sodium and to large anions. Thus, the characteristic ion distribution inside the cell was a high concentration of K^+ and impermeable anions, with a low concentration of Na^+ and Cl^-. Outside the cell, the concentration of Na^+ and Cl^- was high, but K^+ was low. The [K][Cl] products on either side of the membrane were equal, following the Donnan rule.

The introduction of isotopic tracers in the late 1940's however, provided evidence that the membrane was also permeable to sodium. Thus, tracer flux studies by Levi and Ussing (1948) and Keynes (1954) showed that both Na^+ and K^+ were in a constant state of flux across the muscle membrane, with their flux rates being dependent on both the concentration and the electrical gradients.

In relation to the action potential, Bernstein

proposed that during excitation, the selective permeability of the membrane to K^+ was momentarily lost, such that other ions, namely sodium and chloride, could suddenly move across the membrane, resulting in temporary abolition of the membrane potential.

Bernstein's theory was not challenged until the advent of adequate technology to measure the potential differences across the membrane. In 1939, Hodgkin and Huxley, and in 1940, Curtis and Cole demonstrated that excitation led to a transient reversal, not a simple breakdown, of membrane potential in the nerve fibre, such that the action potential exceeded the resting potential. Later, Hodgkin and Katz (1949) showed that this overshoot of the action potential was dependent on the external sodium concentration. This latter finding had been anticipated by Overton (1902), who first recognized the importance of sodium to muscle excitation, when he showed that frog muscles became inexcitable when they were immersed in a medium with a low sodium concentration (Kepner, 1979). Overton prophesized a theory of membrane excitation which was remarkable in its accuracy: "The roles which sodium and lithium ions play in the spread of excitation and contraction of muscle are still unclear; perhaps there occurs, during these processes, a certain exchange between the potassium ions of the muscle fiber and the sodium ions in the surrounding solution, but there

are important problems with such an assumption" (Overton, 1902; in Junge, 1976).

1.5.2. Studies of the squid giant axon.

The suggestion by Young (1936), that the squid giant axon might be useful for the study of nerve fibre excitation, led to the first measurements of electrical potentials across excitable cell membranes. The axon was large enough in diameter (0.5 - 1.0 mm) to allow longitudinal insertion of fine wire electrodes without damaging or altering the electrical parameters of the membrane. Resting and action potentials in the squid axon were first recorded by Hodgkin and Huxley in 1939, and by Curtis and Cole in 1940. The resting potentials ranged from 50-70 mV (inside negative) and the action potentials between 80-120 mV.

The ionic mechanisms responsible for the action potential were not clarified until 1952, when Hodgkin and Huxley published 4 elegant papers describing results obtained by the new technique of voltage clamping.

While it was evident from Overton's work (1902; see above) that the action potential involved a sudden change in the membrane permeability to sodium, it was difficult to determine how this, and the resulting sodium current, changed over time since the action potential was so brief. The introduction of the voltage clamp technique

was a major breakthrough, because it allowed the membrane to be maintained at a set potential and, through a feedback amplifier, the necessary current required to keep the membrane potential at a particular voltage could be measured. Credit for this innovative technique goes to Cole and his collegues from Woods Hole, Mass. (Cole, 1949; Marmont, 1949). However, it was Hodgkin and Huxley who utilized the voltage clamp technique to conduct the necessary experiments to decipher exactly what happened to membrane currents and conductances during the action potential. Briefly, Hodgkin and Huxley were able to systematically reconstruct the action potential based on three important observations they made from voltage clamping the squid giant axon:

1) Identification of 2 ionic currents (Hodgkin, Huxley and Katz, 1952a).

When the membrane was depolarized to a value of approximately 0 mV, two separate currents were identified; an initial large inward current followed by a maintained outward current.

2) Separation of the Na^+ and K^+ currents (Hodgkin and Huxley, 1952b).

By substituting choline for Na^+ in the bathing fluid, the large inward current was eliminated. The resulting curve represented the K^+ current. By simple subtraction of the Na^+-free relationship from the normal

current-voltage relationship, the Na^+ current could then also be quantified.

3) Description of Na^+ and K^+ conductances (Hodgkin and Huxley, 1952c).

From Ohm's law (voltage = current x resistance) the conductance changes for Na^+ and K^+ over time were calculated at different depolarizations. These studies demonstrated that the change in conductance was more rapid for Na^+ than for K^+ and that maintained depolarizations inactivated the Na^+ conductance (gNa^+) but not the K^+ conductance (gK^+).

Finally, by knowing the membrane voltages associated with an action potential, and how gNa^+ and gK^+ changed with time, Hodgkin and Huxley (1952d) were able to account for and reconstruct the rising and falling phases of the action potential through the application of several equations describing the changes in Na and K conductance associated with changes in time and membrane potential.

1.5.3. Goldman-Hodgkin-Katz equation

In 1943, Goldman presented an equation based on Nernst's theory of electrodiffusion which would predict the potential difference across a membrane which was permeable to a single ion. Then, in 1949, Hodgkin and Katz modified Goldman's equation to incorporate a membrane that had different permeabilities for different ion

species (GHK equation). The equation was as follows:

$$2)\qquad E_m = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Na}[Na^+]_o + P_{Cl}[Cl^-]_i}{P_K[K^+]_i + P_{Na}[Na^+]_i + P_{Cl}[Cl^-]_o}$$

where, E_m is the membrane potential, R is the universal gas constant, T is absolute temperature, F is the Faraday constant, and P_K, P_{Na} and P_{Cl} are the permeability coefficients for K^+, Na^+ and Cl^- respectively.

Using this equation, Hodgkin and Katz (1949) were able to predict to within 1 mV the membrane potentials in squid axons in the resting state, with various ionic concentrations in the external fluid; they assumed the permeability coefficients for K^+, Na^+, and Cl^- to be 1:0.04:0.45, respectively.

1.5.4. Influence of ionic concentrations on resting potential of squid axon. One of the simplest tests of the GHK equation was to alter the external concentrations of potassium, sodium or chloride. In accordance with the equation, elevation of $[K^+]_o$ depressed the resting potential while lowering $[K^+]_o$ caused a hyperpolarization. However the extent of the hyperpolarization did not match the predicted value for E_m; this disparity was attributed to the effect of N_a permeability at low external K^+ concentrations. A further factor may have been inactivation of the Na^+-K^+ pump due to depletion of external K^+ (see below).

It was later found possible to extrude axoplasm from the squid giant fibre and the 'bag' of membrane could then be filled with solutions of known ionic composition. Thus Baker, Hodgkin and Shaw (1962) were able to reverse the polarity of the resting potential by making the K^+ concentration higher on the outside of the membrane versus the inside.

1.6. Resting and action potential measurements in striated muscle.

1.6.1. Early studies. Graham and Gerard (1946) were the first to use microelectrodes constructed from drawn-out glass capillaries filled with electrolyte solution. They were able to record membrane potentials in frog muscle fibres and reported a mean resting potential of -78.4 mV. This value increased to a mean of -97.6 mV after care was taken to maintain optimal temperature, external K^+, and to minimize the junction potential between the electrode tip and the myoplasm (Ling & Gerard, 1949).

1.6.2. Effect of external ionic concentrations In the case of striated muscle, altering $[Na^+]_0$ has little, if any, effect on the resting potential, and the ratio of $pNa^+:pK^+$ has been estimated as .013-.015 in frog muscle (Katz, 1966; Junge, 1976) and .03 in rat soleus muscle (Yonemura, 1967). In contrast, alterations of $[K^+]_0$ have

profound effects, though the correspondence between the observed resting potential and that derived from the GHK equation becomes less satisfactory at low values of $[K^+]_o$ (Adrian, 1956). The explanation given for this divergence was that the membrane was permeable to other ions than K^+, and that the effect of these ions became greater as $[K^+]_o$ decreased below physiological values. In contrast to the squid axon, mammalian muscle fibres appear to have a greater membrane permeability for Cl^-, and it is thought that Cl^- ions play a significant role in determining the membrane potential of mammalian muscle fibres, especially when $[K^+]_o$ increases (Dulhunty, 1978).

1.6.3. Influence of protein in bathing medium on resting potential. When skeletal muscle is immersed in a protein-free saline medium, there is a significant increase in the concentration of intracellular sodium (Creese, 1954; Carey and Conway, 1954; Creese and Northover, 1961). This change has been attributed to an increase in Na^+ permeability in the absence of protein on the outer surface of cell membranes (Creese and Northover, 1961). However, Na^+ permeability is not the only factor affected by protein since Macchia and co-workers (1984) have reported an increased anion permeability in toad semitendinosus muscles bathed in a normal Ringer solution versus a Ringer solution containing albumin. These

permeability changes will have obvious effects on membrane potentials in both the resting and active state. Thus, Kernan (1963) found the membrane potential of the rat extensor longus digitorum to be 12.4 mV greater in a bath containing rat plasma instead of Krebs-Ringer. Similarly, Shetty and co-workers (1985) found that the membrane potential of toad semitendinosus muscle in toad plasma was 9.9 mV greater than in Ringer solution. It has been suggested that serum proteins in the bathing medium modulate particular membrane receptors which affect ion conductances (Shetty, et al, 1985). The reliability of membrane potentials recorded in the absence of protein in the bathing fluid is therefore questionable.

1.6.4. Influence of tip potentials on resting potential measurements. The importance of minimizing the junction potential at the tip of the microelectrode was strongly stressed by Adrian, in 1956. Earlier, Nastuk and Hodgkin (1950) had reported higher membrane potential recordings in frog muscles fibres if the microelectrode was filled with 3M KCl rather than the isotonic KCl (118mM) which had traditionally been used, due to the lesser resistance the former had (eg.10-30 M vs. 50-150M). Indeed, the potentials recorded by Nastuk and Hodgkin were at least 10 mV greater than those reported by Ling and Gerard (1946). Additionally, Nastuk and Hodgkin claimed that using 3M KCl

in the microelectrode would reduce the junction potential between the myoplasm and the electrode fluid.

Adrian (1956) found that electrodes with high resistances were more likely to produce large junction potentials than those of low resistance. However, he also observed that even when filled with 3M KCl, microelectrodes can still have junction potentials, which are likely produced by some substance blocking the tip of the electrode and affecting the mobility of the ions. Only by accepting those electrodes with tip potentials of less than 5 mV could reliable membrane potential recordings be obtained.

1.6.5. Effect of temperature The GHK equation predicts that the membrane potential is proportional to absolute temperature. Ling and Woodbury (1949) noted that the resting membrane potentials of frog sartorius fibres increased almost linearly from 5 to 30°C, with a Q_{10} of 1.1. Nakanishi and Norris (1969) found a slightly greater effect of temperature on membrane potential in rat muscle (longissimus dorsi medialis), such that there was a 1.0-1.3 mV/^{o}C increase in membrane potential as the bath temperature was raised from 25 to 44°C; this corresponded to a Q_{10} value of approximately 1.15. In both of these studies the effect of temperature on membrane potential was greater than that predicted from the GHK equation.

Assuming that a normal resting potential at 37°C in rat muscle is -74.0 mV (Nakanishi and Norris, 1969), dropping the temperature to 20°C would cause the predicted potential to decline to only -70 mV. In the experiments of Nakanishi and Norris (1969) however, the potential decreased to -55.3 mV. Similarly, Senft (1967) found that the hyperpolarization of lobster giant axons in response to increased temperature was approximately twice that predicted from the GHK equation. Furthermore, ouabain would reduce the increase in E_m to the predicted levels. Thus, there appears to be a portion of the membrane potential that is dependent on metabolic energy, such as an electrogenic contribution by an active transport pump (see below).

The action potential is also affected by changes in temperature. In 1950, Nastuk and Hodgkin reported a larger overshoot (38 mV vs. 31 mV) of the action potential in frog muscles when the temperature was lowered from 18°C to 7°C. Additionally, because the conduction velocity decreases in proportion with a decrease in temperature, the action potential was considerably slower in the cooled condition. Alterations in temperature will affect the time courses of the conductance changes during an action potential. Briefly, Hodgkin and Huxley (1952d) predicted in their equations that the rate constants for the conductance changes increase threefold for every 10°C rise

in temperature. Thus, it could be expected that the overshoot of the action potential would enlarge with a declining temperature if there is a slower inactivation of Na^+ conductance and/or a slower activation of K^+ conductance during the course of an action potential.

1.7. The $Na^{\pm}$-$K^{\pm}$ pump

1.7.1. Background. The results of radioactive tracer studies on the frog sartorius revealed that, in the resting condition, there were matching inward and outward fluxes of Na^+ across the membrane due to the small, but nevertheless significant, permeability of the membrane to sodium (Levi and Ussing, 1948). The results from these studies were puzzling; how did the cytoplasm maintain such a low concentration of sodium and a high concentration of potassium if both ions were continually being exchanged across the membrane? Similarly, how did the cell maintain its internal negativity? The flux studies made it clear that there must be some active transport system moving sodium out of the cell against its electrochemical gradient; the discussion of this pump will now follow.

1.7.2. Early concepts. The suggestion of an active pumping mechanism within cells to get rid of sodium was first made by Dean (1941), in an effort to explain the results of Steinbach (1940). Steinbach had shown that

frog muscles which had gained sodium from being immersed in a K^+-free medium, could extrude Na^+ against a large electrochemical gradient if K^+ was readmitted to the bathing medium. Concomitant with the Na^+ extrusion, K^+ was reaccumulated in the muscle fibre against a concentration gradient of at least 10 to 1. Dean (1941) claimed that to maintain such an active concentration of ions would require work and proposed that "...there must be some sort of pump, possibly located in the fibre membrane, which can pump out the sodium or, what is equivalent, pump in the potassium."

This latter comment, regarding the pumping in of potassium, had been made 20 years earlier by Mitchell and Wilson (1921). They conducted experiments on toad muscles which showed that while potassium was lost when the muscle was stimulated, it was also taken back up. The evidence for this was that stimulated muscles would take up rubidium, but rested muscles would not. Mitchell and Wilson (1921) concluded that "... a condition favorable to the taking up of potassium probably occurs in a contracting muscle because rubidium and cesium, substances very similar to potassium in chemical and physiological behaviour, are absorbed in retainable form by a contracting muscle but not by a resting one."

Hodgkin (1951) first discussed the possibility of a coupled transport between sodium and potassium as an

explanation for the maintenance of intracellular ionic concentration gradients, and he was the first to raise the possibility that this sodium pump might be electrogenic. While discussing the role of metabolism in the maintenance of the resting membrane potential, Hodgkin (1951) proposed that the restoration of sodium pump activity after removal of a metabolic inhibitor would cause an immediate rise in resting potential. During the restoration of ionic gradients "...extrusion of sodium might temporarily raise the resting potential above its normal value."(Hodgkin, 1951). Although the concept of electrogenic Na^+-K^+ transport was periodically entertained in the ensuing years, the first experimental demonstration came in the late 1950's from studies on post-tetanic hyperpolarization (PTHP) in nerve fibres (Richie & Straub, 1957; Connelly, 1959). It was found that nerve fibres would hyperpolarize following a tetanic train of electrical stimulation, and it was concluded that this PTHP was due to increased activity of the Na^+ pump as a means of coping with the increased concentration of intracellular Na^+.

In the case of striated muscle, Conway and Hingerty (1948) were the first to suggest the presence of some sort of sodium pumping mechanism; they demonstrated that rat skeletal muscles which had gained sodium on a low-potassium diet would then extrude the sodium once a diet rich in potassium was reinstated. Desmedt (1953) provided

further evidence for a sodium pump in skeletal muscle, when he showed the the extrusion of Na by Na-loaded frog muscles was coupled with the restoration of normal K concentrations on either side of the membrane. Furthermore, during this time of sodium extrusion, both the resting membrane potential and the overshoot of the action potential increased. The electrogenicity of the sodium pump in skeletal muscle was clearly demonstrated by Kernan (1962) who showed that the active extrusion of Na^+ from Na^+-loaded frog muscles was accompanied by hyperpolarization of the muscle membrane potential, such that the latter exceeded the K equilibrium potential (E_K) by -11 mV. Later studies, also utilizing Na^+-enriched muscles, provided further support for the generation of electric current accompanying Na^+ extrusion; the amount of hyperpolarization could be as high as 40 mV (Adrian and Slayman, 1966; Frumento, 1965). Stimulation of the sodium pump by raising $[Na]_i$ has also been investigated quite extensively in mollusc neurons. For example, injection of Na^+ into snail neurons results in a consistent hyperpolarization, which can be inhibited by ouabain, K^+-free Ringer, and metabolic inhibitors (Moreton, 1969; Thomas, 1969; Kostyuk, 1970).

1.7.3. Contribution to steady state E_m: the Mullins-Noda equation. In determining the contribution of the Na^+-K^+

pump to the resting membrane potential of the cell, two approaches have been used; mathematically predicting the pump contribution with modification of the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949), and quantifying its contribution by measuring the difference in E_m with and without pump inhibition. Although the GHK equation has been successfully applied to the membrane potential of numerous cells, the equation does not take into account the effects of the electrogenic Na^+-K^+ pump, or any other transport mechanisms which might be generating an electric current. Thus, at times when Na extrusion rates are high, the GHK equation does not predict correct membrane potentials (eg. Kernan, 1962; Frumento, 1965). The GHK equation will never predict a membrane potential greater than E_K, but even under steady state conditions the predicted E_m usually underestimates the true resting membrane potential. Thus, Mullins and Noda (1963) modified the original GHK equation to make provision for the electrogenic Na-K pump, such that

3) $$E_m = \frac{RT}{F} \ln \frac{rP_K[K]_o + P_{Na}[Na]_o}{rP_K[K]_i + P_{Na}[Na]_i}$$

where r is the coupling ratio between Na^+ and K^+ transport with each cycle of the pump. If r=1 (ie. neutral pump) the equation is identical to the GHK equation. Thomas (1972) found that by giving r a value of 1.5 (3Na:2K), the predicted E_m of frog muscle fibres was extremely close to

the measured value. The electrogenic contribution of the pump with this coupling ratio was found to be approximately 10 mV. However, there is pharmacological evidence to suggest that the sodium pump makes an even greater contribution to the resting membrane potential of skeletal muscle. Locke and Solomon (1967) reported a drop in E_m of 15 mV in rat gastrocnemius muscle after ouabain exposure while Williams and co-workers (1971) observed a 25 mV decline in E_m in rat gracilis muscles. However, it is difficult to determine from these studies what portion of the drop in E_m was due to loss of electrogenic potential, and what portion was due to the gradual decline in ionic concentration gradients due to the absence of pump activity.

1.7.4. Energy dependence.

The active pumping of Na^+ and K^+ ions against their electrochemical gradients implies the expenditure of energy to drive the pump. The electrogenic Na^+-K^+ pump is a membrane-bound Na^+-K^+-ATPase that couples the extrusion of 3 Na^+ ions and uptake of 2 K^+ ions to the splitting of adenosine triphosphate (ATP).

Na-K-ATPase was first discovered by Skou, in 1957, in crab nerve, and by Bonting, Simon and Hawkins (1961) in striated muscle. Skou (1957) was able to demonstrate the presence of a specific Mg-activated ATPase which required

ATP, Na^+, and K^+ for activation, and was inhibited by Ca^{2+} and very high K^+. As the optimum Mg^{2+} concentration was found to be the same as the ATP concentration, it was concluded that the substrate for the enzyme was Mg-ATP. At this time, the actual location of the enzyme in the nerve could not be determined, since whole nerve homogenates were used for the enzymatic assay. A similar enzyme was then described by Post (1959) in the human erythrocyte membrane, which was responsible for the simultaneous active extrusion of sodium and accumulation of potassium across the cell membrane. The enzyme described by Post (1959) was also inhibited by Ca^{2+}, as well as by cardiac glycosides. Further evidence that the enzyme was located on the cell membrane was then provided by Hoffman (1960), when he showed that reconstituted human red cell ghosts would actively extrude sodium in the presence of ATP and that the sodium transport could be inhibited by either intracellular Ca^{2+} or extracellular cardiac glycoside. A broad study of the distribution of the Na^+-K^+-ATPase in various tissues of the cat was then conducted by Bonting, Simon and Hawkins (1961). The enzyme activity was determined in freeze-dried homogenates as the fraction of the total ATPase activity that was inhibited by ouabain and/or the omission of potassium. Na-K-ATPase was found to be present in nerve tissue, occular tissue, visceral organs, and muscular tissue.

In muscle, the major part of the enzyme activity appears to be associated with the sarcolemma, with only a very minor part being associated with the transverse tubules. Thus, Venosa and Horowicz (1981) found that when frog sartorius muscles were detubulated by glycerol, the total [^{3}H]ouabain binding capacity was only reduced by 20%.

Determination of the ATP:cation ratio has been done in a variety of tissues. There is general agreement that 1 ATP molecule is consumed in transporting 3 Na ions out of the cell, whether it be red blood cells (Garrahan and Glynn, 1967), nerve cells (Baker, 1965), or frog skeletal muscle (Harris, 1967). The metabolic cost of the Na-K pump is fairly significant, with about 16% of the resting oxygen uptake being ouabain-sensitive (Sjödin, 1982).

1.7.5. Coupling ratio. The electrogenic contribution of the Na^+-K^+ pump to the resting membrane potential is dependent on the actual coupling ratio of Na and K ions with each pump cycle. Various ratios have been reported for different cell types, which suggests that the coupling ratio is not fixed and might in fact depend on the physiological state of the cell. Only in the red blood cell has the evidence clearly suggested that the coupling ratio is 3Na out for every 2K in, thereby generating an outward current (Post, et al, 1967). However, in the

numerous nerve and muscle prepartions that have been investigated, the ratios cited have been 2Na:1K (Sjodin & Beauge, 1967 - squid axons), 3Na:1K (Caldwell, et al, 1960 - squid axons; Mullins & Noda, 1963 - frog striated muscle), 4Na:3K (Nakajima and Takahashi, 1966 - nerve cell bodies), and 3Na:2K (Mullins & Brinley, 1969 - squid axons). Mullins and Brinley (1969), using dialyzed squid axons, proposed that at low intracellular Na the coupling ratio would be close to 1:1, but that this ratio would increase as result of increasing $[Na]_i$. Similarly, Keynes and Rybova (1963) suggested that the sodium pump is a labile system and that the coupling between Na and K would vary under different ionic conditions. Thus, while the steady state coupling ratio in skeletal muscle might agree with Thomas' (1972) suggestion of 3Na:2K pump, there is no evidence to indicate that this ratio remains rigidly fixed under non-steady state conditions.

1.7.6. Contribution to non-steady state E_m. In view of the variability in experimentally determined coupling ratios, the contribution of the Na-K pump to the resting membrane potential should increase with increasing intracellular Na. While in a normal, steady state the membrane potential of most cells is just slightly less than E_K, if the Na-K pump is stimulated, E_m can increase to values much greater than E_K. Through modification of

Mullins and Noda's (1963) equation, Sjodin (1984) was able to predict membrane potentials more negative than E_K, as might occur under non-steady state conditions. He predicted that as $[Na]_i$ increased from 40 mM to 140 mM the contribution of the pump to E_m would increase from -10 mV to -50 mV. This is compatible with the observations of Kernan (1962) and Frumento (1963) who showed that the hyperpolarization induced by the pump increased with increasing rates of Na extrusion. In a later study by Kernan, in 1968, the actual contribution of the pump to E_m was found to be 22 mV during Na extrusion in rat muscle, as determined by the difference in the measured E_m and that predicted by the GHK equation. Furthermore, this electrogenic contribution of the sodium pump was completely abolished if the Na-loaded muscles were immersed in a cold bathing medium (8^oC). In a more recent study, Creese and co-workers (1987) have demonstrated that the sodium pump can contribute 33 mV to the resting membrane potential in the presence of depolarizing drugs.

1.7.7. Distribution of $Na^{\pm}$-$K^{\pm}$-ATPase in different fibre types.

The number of $[^3H]$ ouabain binding sites in skeletal muscle appears to differ according to fibre type

in some species. In the mouse, a slow-twitch muscle such as soleus, has been shown to contain significantly more binding sites than a fast-twitch muscle, such as EDL or gastrocnemius (Bray <u>et al</u>, 1977; Clausen and Hansen, 1982; Abdel-Aziz <u>et al</u>, 1985). Contradictory results have been found in rat muscle, where the fast-twitch muscles (EDL) have been shown to have both a greater density of $[^3H]$ ouabain binding sites (Clausen <u>et al</u>, 1982) and a greater Na^+-K^+-ATPase activity (Kjeldsen <u>et al</u>, 1984) than the slow-twitch soleus muscle. The distribution of $[^3H]$ ouabain binding sites between fibre types has been recently determined in human muscle, post mortem (Dorup <u>et al</u>, 1988). No significant differences were observed between muscles with a high ratio of type 1 to type 2 fibres (eg. soleus) versus those with low ratios of type 1 to type 2 fibres (eg. triceps brachii, rectus femoris). Thus, whether or not the density or distribution of Na^+-K^+ pumps is related to fibre type, and what the basis for this relationship might be, still remains to be determined.

It is known that the density of Na^+-K^+-ATPase changes with development. Kjeldsen <u>et al</u> (1984) have shown that the number of $[^3H]$ ouabain binding sites in skeletal muscle of both rats and mice first increase then decrease during development, reaching a constant value by 5 months of age. These results have been suggested as a

possible explanation for the low sensitivity to digitalis glycosides found in infants compared to either premature or adult individuals (Clausen et al, 1982; Kjeldsen et al, 1982).

1.7.8. Effect of hormones.

As early as 1941, Stickney demonstrated that epinephrine would reduce the net K^+ release induced by electrical stimulation by 40% in frog muscle, suggesting a direct effect of epinephrine on K^+ uptake in skeletal muscle. It was later found that this epinephrine-induced K^+ uptake was mediated by beta- rather than alpha-receptor activation (Powell and Skinner, 1966), and with the development of selective beta-adrenergic agonists came the discovery that the beta-2 receptors were specifically involved in the response (Lockwood and Lum, 1974). Coupled to an increased cellular K^+ uptake in the presence of catecholamines, a decline in intracellular Na^+ has also been observed, strongly suggesting that the Na^+-K^+-ATPase was mediating the response (Hays et al, 1974; Clausen and Flatman, 1977; Chinet and Clausen, 1984). Moreover, a catecholamine-induced hyperpolarization of the muscle membrane has been frequently reported; a response which is ouabain-inhibitable (Evans and Smith, 1973; Tashiro, 1973; Hays et al, 1974; Clausen and Flatman, 1977).

The exact mechanism by which beta-2-receptor

activation stimulates Na^+-K^+ transport is not really understood, but most evidence suggests a stimulation of cyclic AMP through adenylate cyclase, perhaps then leading to the activation of a protein kinase (Clausen & Flatman, 1977; McArdle & D'Alonzo, 1981; Clausen, 1986).

The effect of catecholamines on Na^+-K^+ transport has important implications during exercise, where plasma potassium can rise quite markedly (Hirche et al, 1980). The rise in catecholamines during exercise (Christensen & Galbo, 1980) acts as an important defense against exercise-induced hyperkalemia through beta-adrenergic stimulation of cellular potassium uptake. Thus, in the presence of beta-blockade, the exercise-induced rise in plasma potassium both during exercise and in the recovery period following, has been shown to be significantly enhanced (Williams, et al, 1985).

Insulin has been shown to increase the K content and decrease the sodium content in rat soleus muscle (Clausen & Kohn, 1977; Flatman & Clausen, 1979; Moore & Rabovsky, 1979), an effect which is blocked by ouabain, or reducing the temperature to below 15°C. Additionally, insulin has been found to increase E_m in rat soleus muscle (Flatman & Clausen, 1979) and other muscles in several species (Zierler, 1957, 1959; Wu & Zierler, 1985).

The mechanism by which insulin stimulates Na-K transport is not exactly known. Since propranolol has

been shown to have no effect on the hypokalemic action of insulin (Minaker & Rowe, 1982) it is believed that insulin acts at a site beyond cyclic AMP activation by adenyl cyclase. Thus, the effects of insulin and adrenalin on muscle membrane potential and muscle Na-K contents have been shown to be additive (Flatman and Clausen, 1979). Clausen and Kohn (1977) suggested that insulin might increase the relative affinity of the Na-K-ATPase to $[Na]_i$. This would result in an increased rate of Na-K transport, and E_m would increase due to the stoichiometry of the Na-K exchange. The resultant rise in $[K]_i$ could also contribute to the maintenance of the hyperpolarization (Clausen, 1986).

1.7.9. Inhibition of the $Na^{\pm}$-$K^{\pm}$ pump

(a) Cardiac glycosides. It has been known for some time that Na-loaded muscle fibres lose the ability to extrude Na ions and recover K if cardiac glycosides are present in the external medium (Matchett and Johnson, 1954; Johnson, 1956). Caldwell and Keynes (1959) found that injecting ouabain into the interior of squid axons did not inhibit the sodium pump, whereas adding it to the bathing medium did, strongly suggesting that the glycoside acts on the outside of the membrane, perhaps by competing with potassium for the active site on the enzyme. Ouabain (and other glycosides) has since been used as an important

index of Na-K pump activity, and the use of radio-actively labelled ouabain has provided insight into the number of active pump sites within a particular tissue.

(b) Metabolic inhibitors and cooling. The fact that the sodium pump must transport Na against its electrochemical gradient implies an active process; dependent on energy. Hodgkin and Keynes (1955) found that both metabolic inhibitors and cooling reduced the Na efflux and K uptake in squid axons. It is noteworthy that in that particular study, the authors found that the squid axon could maintain its original E_m for hours after pump inhibition. Similarly, Baker, Hodgkin and Shaw (1962) found that squid axons could still conduct thousands of impulses after pump blockade. However, three things can be said of the above two studies; firstly, the squid axon has a very low resistance compared to other cells, and thus, the potential generated by a pump current is much less than that from a cell with a greater membrane resistance. Second, the surface to volume ratio in the squid axon is much less than in other cells due to their large size, and thus for a given number of action potentials there would be a far lesser change in the concentration of internal ions due to the large intracellular volume. Finally, the squid axons studies were performed in vitro, where the extracellular volume was so large that even thousands of impulses would have very little effect on the

extracellular ion concentrations. Ritchie and Straub (1957) found that the PTHP in rabbit nerve fibres was markedly reduced if the fibre was soaked in 2,4-dinitrophenol (a metabolic inhibitor).

The rate of Na-K transport is very dependent on temperature. In vitro studies measuring the number of ions transported per [^{3}H]ouabain binding site per minute indicate that under in vitro conditions at 30-35°C, skeletal muscles only utilize 2-6% of their total capacity for Na-K transport at 37°C (Clausen & Hansen, 1974, 1982; Pflieger, et al, 1983; Clausen et al, 1987). Thus, while the predicted maximum transport capacity at 37°C is 16,000 K ions per [^{3}H]ouabain binding site per minute, the values obtained in studies just cited range from 300 to 700 K ions per binding site per minute. Clausen and associates (1987) have recently shown that with a combination of Na^+-loading and high $[K^+]_o$, the available Na^+-K^+ pumps (as determined by [^{3}H] ouabain binding in rat soleus muscle) can be activated to 90% of the theoretical maximum transport capacity at 30°C.

(c) Removal of Extracellular $K^{\pm}$. Perhaps the best evidence of a coupled transport of Na and K in excitable cells is the observation that Na extrusion cannot occur without K in the extracellular fluid. This observation was made very early (Steinbach, 1940), and since then the use of K+-free bathing media to Na-load cells has been an

an effective means of stimulating maximum Na^{+}-K^{+} pump activity upon replacement of K in the bathing medium.

1.8 Changes in $Na^{\pm}$-$K^{\pm}$ pump activity with muscular exercise.

There is evidence that both acute or chronic exercise can increase the Na^{+}-K^{+} pump activity, and thus enhance the clearance of potassium from the extracellular spaces. Brodal and co-workers (1975) examined the effect of 5 minutes of electrical stimulation of rat hindlimb muscles on the activity of Na-K-ATPase and observed a 28% increase in the activity of the enzyme. A 109% increase in ^{24}Na efflux from isolated frog muscle fibres was observed after 2.5 minutes of electrical stimulation (Hodgkin and Horowicz, 1959).

The Na^{+}-K^{+} pump activity appears to undergo considerable adaptation from chronic muscular exercise. Thus, 6 weeks of training led to a 2.6-fold rise in Na-K-ATPase activity in dog gracilis muscle (Knochel, et al, 1985), and an increase in the concentration of [^{3}H]ouabain binding sites in rat hindlimb muscles (Kjeldsen, et al, 1986). Additionally, the ouabain-suppressible component of E_m in dog intercostal fibres increased from 9mV to 33mV after training (Knochel, et al, 1985). In this same group of trained dogs, the E_m of the gracilis muscle was significantly greater than in untrained control animals.

Training has also been shown to increase the membrane potential in human subjects. Moss and co-workers (1983) reported significantly higher membrane potentials in the tibialis anterior muscle of trained (-98.8 mV) versus untrained (-91.5 mV) men.

The exercise-induced rise in plasma potassium is smaller after training in both humans (Tibes, et al, 1974; Kiens and Saltin, 1986; Kjeldsen et al, 1987) and animals (Knochel, et al, 1985), suggesting more efficient re-uptake of K^+. However, a recent study by Kjeldsen and co-workers (1987) has shown that while 10 weeks of training in humans resulted in a significant decrease in the rise in venous K^+ concentration at a constant exercise load, there was no change in [^{3}H] ouabain binding capacity in biopsies of the vastus lateralis muscle. Thus, while acute or chronic muscular activity leads to an improved capacity for clearing $[K]_e$, this could be due to either increased activity of the Na-K-ATPase due to increased sensitivity to $[K^+]_e$ and/or $[Na^+]_i$, or an increased density of the enzyme on the sarcolemmal membrane.

1.9. Summary and Hypothesis for Thesis.

It should be evident from the preceding sections of the Introduction that changes in the maximum response evoked from a muscle by stimulation of its motor nerve

reflect what is occurring to the membrane and action potential of the single fibre. Indeed, if an initial maximum M-wave becomes even larger as a result of contractile activity, this is strong evidence that at least some of the individual fibre action potentials have become larger. The purpose of this investigation was twofold. First, an analysis of M-wave behavior during stimulated and voluntary activity in humans was undertaken. An animal model (rat soleus) was then employed to see if similar changes in M-waves could be observed. The second purpose was to test the hypothesis that a hyperpolarization of the muscle fibre membrane, due to stimulation of the electrogenic Na^+-K^+ pump, was responsible for increasing the fibre action potentials and, hence the M-wave, during muscular activity.

2. Methodology

2.1. Introduction

Two separate studies were designed for this thesis, incorporating both a human and an animal model. This served to accomodate both purposes of the research; providing first a descriptive analysis of the M-wave during muscular activity and then an investigation of the mechanisms responsible.

2.2. Human studies

2.2.1. Experiments With Intermittent Tetanic Stimulation

Six subjects participated in this experiment (4 men, 2 women), all were volunteers and engaged in regular, moderate exercise; their ages ranged from 25 to 52 years. The experiment was performed on the right tibialis anterior muscle. The subject sat in a chair of adjustable height with the right leg flexed 90° at the knee and braced within a steel frame to maintain this angle; the foot was firmly strapped to an aluminum plate, below which strain gauges were attached. The ankle was plantarflexed by $10\text{-}20^{o}$ and the contractions were isometric.

(a) Stimulation and recording system. The stimulating electrodes were rectangular lead plates (4.5 cm by 2.5 cm) wrapped in moistened gauze impregnated with conducting cream; the cathodal electrode was placed over the common

peroneal nerve at the neck of the fibula while the anode lay slightly lower and more anterior. The distal placement of the anode appeared to allow a lower threshold of stimulus intensity. The stimuli were 50 μs pulses delivered from a high-voltage stimulator (Model 3072, Devices Ltd); the latter was triggered by a digital timing device (Digitimer Model 3290, Devices Ltd) through a gated pulse generator (Model 2521, Devices Ltd).

The recording electrodes were chlorided silver disc electrodes, 9 mm in diameter, filled with conducting cream and placed over the skin at the junction of the proximal and middle thirds of the anterior tibialis muscle; the initial negativity of the M-wave indicated that end-plates were situated beneath the electrode. A silver strip electrode (6 cm by 0.5 cm) was wrapped around the great toe as a reference while a second silver strip, mounted 8 cm below the knee, served as a ground. The gated pulse generator was set to deliver stimuli at 15 Hz; the tetani lasted 3 seconds and were repeated every 5 seconds for 3 minutes, or until complete mechanical fatigue had been induced. This frequency of stimulation was chosen based on previous observations in this laboratory that minimal neuromuscular junction failure occurs during intermittent repetitive stimulation at a 15 Hz frequency.

M-waves were evoked by delivering single stimuli

0.5 sec before each tetanus; this allowed for monitoring of the twitch torque as well. An arterial cuff was applied to the thigh to render the leg ischaemic. The recovery of the M-waves and twitch and tetanic torques was followed intermittently for approximately 50 minutes. The data were amplified and displayed on a variable persistance storage oscilloscope (Hewlett-Packard 141B), and were also stored on FM tape for detailed analysis after the experiment.

2.2.2. Intermittent Voluntary Contractions

(a) Rationale. Having observed the M-wave behavior during stimulated contractions (see above), it was important to determine if the M-wave would behave in a similar fashion under more physiological conditions. Thus, the next study incorporated a protocol of intermittent voluntary contraction of small muscles in the hand and foot.

(b) Subjects. Ten volunteers, 4 men and 6 women, volunteered for this study; they had no history of neurological or cardiovascular disease and most engaged in regular exercise; their details are summarized in Table 1. The study carried the approval of the university ethics committee.

(c) Stimulating and recording electrodes. In the hand, the muscles studied were those in the thenar

Table 1.

Description of subjects.

Table 1.

Subject	Sex	Age	Occupation
FS	M	30	Technician
JG	F	29	Grad. Student
LJ	M	26	Grad. Student
MJ	F	22	Med. Student
DH	M	28	Grad. Student
MT	F	27	Grad. Student
MM	M	28	Grad. Student
LS	F	29	Grad. Student
JW	F	26	Grad. Student
AH	F	34	Grad. Student

eminence supplied by the median nerve; these were the abductor pollicis brevis, opponens pollicis and flexor pollicis brevis. In the foot the muscle investigated was the extensor digitorum brevis. In order to minimize inadvertent movement of the electrodes, the wrist and hand were strapped inside a dorsal plastic cast; while the sole of the foot and the toes were pressed against the floor by the examiner.

To elicit M-waves, paired silver disc electrodes were used for stimulation. The discs were 9 mm in diameter and mounted in a Plexiglass holder so that their centres were 3 cm apart. The stimulating electrodes were held in position over the median nerve at the wrist by a Velcro strap; the cathode was distal. In the foot the same electrodes were positioned over the deep peroneal nerve at the ankle, again with the cathode distal.

The stigmatic recording electrode for the hand was a strip of silver foil, 6 mm wide and 3 cm long, placed over the main thenar end-plate zone. The latter ran perpendicular to the first metacarpal bone at the junction of its proximal and middle thirds (Fig 3A). The reference electrode was another strip of silver foil, 2.5 cm by 6 mm, wrapped around the little finger, while the ground electrode was another silver strip, placed over the back of the hand. The recording electrodes were lightly coated with conducting cream (Cardio-Cream, Ingram & Bell

Medical) as were the stimulating electrodes.

For the foot the stigmatic electrode was a strip of silver foil (3 cm by 6 mm) placed diagonally across the belly of the extensor digitorum brevis, as shown in Figure 3B. A reference strip was wrapped around the great toe, and a ground strip ran across the dorsum of the ankle. All recording electrodes were held in place by 1.25 cm wide adhesive plastic tape (Blenderm, No. 1525).

(d) Stimulating and recording equipment. The stimulator was of the high voltage type (Model 3072, Devices Ltd) and was set to deliver rectangular voltage pulses which were of super-maximal intensity (60 - 160 volts) and 50 usec duration; the stimulator was triggered by a digital timing device (Digitimer Model 3290, Devices Ltd). The signals from the recording electrodes were fed into amplifiers with bandwidths of 10 Hz - 1 kHz and were displayed on a 4 trace storage oscilloscope (Model 141B, Hewlett-Packard Ltd). The amplified data were recorded on magnetic tape (Model 3960 recorder, Hewlett-Packard Ltd) for subsequent analysis; response amplitudes and areas (voltage x time integral) were then calculated from the digitally acquired data with a custom in-house analysis program designed by Glenn Shine, using a Tecmar Labmaster Data Acquisition Module in a Texas Instrument Personal Computer (TI-PC).

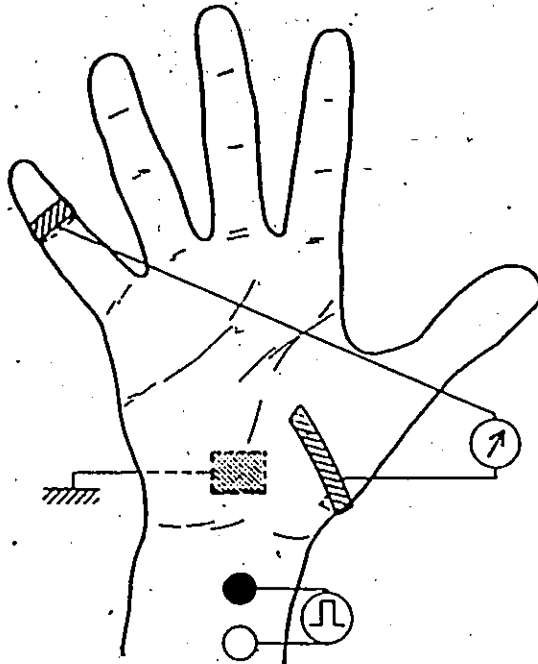
(e) Experimental protocol. Subjects were instructed to

Figure 3A.

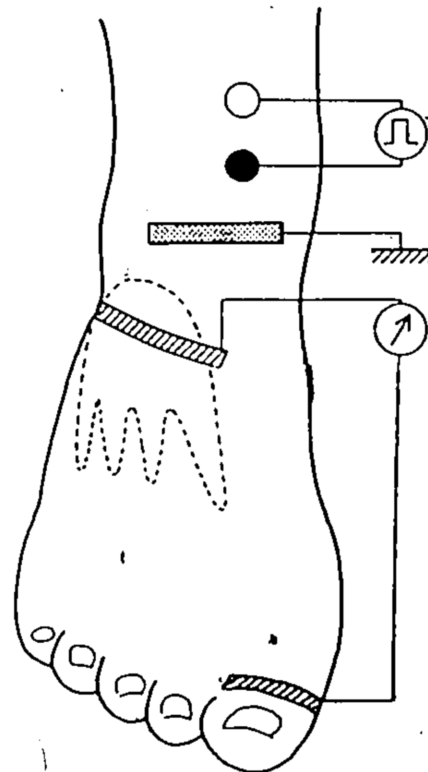
Placement of electrodes for thenar voluntary contractions.

Figure 3B.

Placement of electrodes for EDB voluntary contractions.



THENAR



EXT. DIG. BREVIS

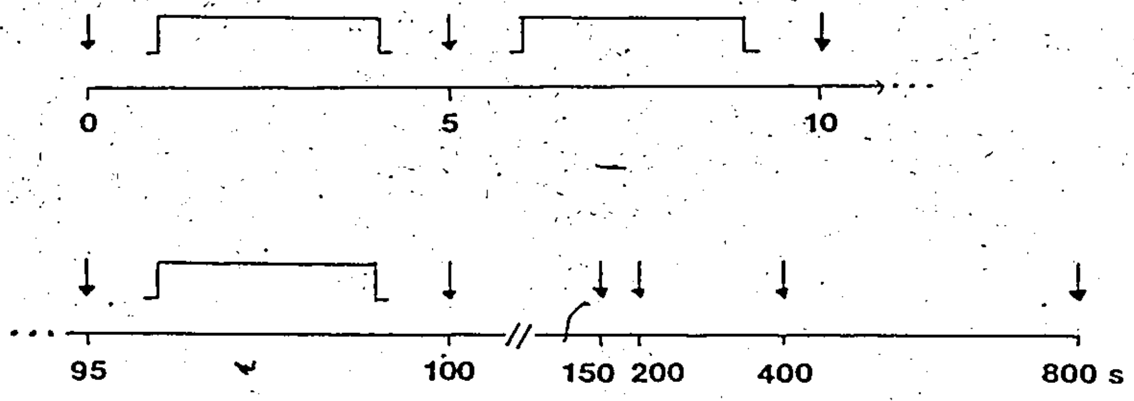
perform maximum voluntary contractions of the thenar muscle and the extensor digitorum brevis; the contractions were maintained isometric by the examiner supplying the opposing resistance. Each contraction lasted 3 seconds and was followed by a 2-second rest period; this sequence being continued for 5 minutes (See Figure 4). M-waves were evoked every 5 seconds for the 5 minutes of contractions, and were followed for 13 minutes of recovery. A control experiment was also done for each muscle in which the same number of M-waves were evoked, but with no voluntary contractions.

2.2.3. Statistical treatment. The data were analysed with a repeated measures analysis of variance design, in which M-wave changes at certain time periods were averaged and compared. Post-hoc Tukey A tests were then done to test selected differences between means.

2.3. Animal studies

2.3.1. Rationale. Having analysed the M-wave behavior in humans during both stimulated and voluntary contractions, it was now important to move the investigation to an animal model so that the changes in action potentials in single fibres, together with membrane potentials, could be measured.

Figure 4. Experimental protocol for intermittent voluntary contractions.


M-wave
Voluntary contraction
0
5
10
95
100
150
200
400
800 s

2.3.2. <u>Animal preparation</u>. Experiments were conducted on female Wistar rats weighing 220-280 grams. The animals were maintained in a humidity- and light-controlled environment (12 hours light, 12 hours dark) and were fed laboratory rat chow and water ad libidium. All animal handling and experimentation conformed to the University guidelines for animal care.

Under intraperitoneal sodium pentabarbitone anaesthesia (35 mg/kg body weight) the soleus muscle of the right leg was exposed, its nerve twig identified, and its tendon freed. The rat was placed prone on a warm brass plate and the limb was put in a Plexiglass chamber (volume = 175 ml) which contained Liley's solution (Liley, 1956; 140 mM NaCl, 4.5 mM KCl, 1 mM NaH_2PO_4, 1 mM $MgCl_2$, 11 mM glucose, 12 mM $NaHCO_3$, 2 mM $CaCl_2$; pH = 7.4) plus albumin (16 g/l). The bathing fluid was always maintained at 36-38^oC by circulating water and radiant heat (except in the cooling experiments). The limb was fixed to the base of the bath by Plexiglass clamps at the ankle and knee. The tendon of the soleus was attached by 5 cm of 3.0-gauge silk thread to a force transducer (Grass type FT036), the compliance of the entire system being 180 um/N; the muscle was set to its optimal length for twitch tension. At the end of each experiment, the animal was sacrificed by an overdose of anaesthetic.

2.3.3. Stimulation and recording system. Intracellular recordings were made from the first and occasionally the second layer of rat soleus muscle fibres with glass microelectrodes filled with 3M KCl solution. The positioning of the microelectrode, whether it be the first or second layer of muscle fibres, was guided by the deflections of the trace on the oscilloscope. The glass capillaries had outer diameters of 1.5 mm (WPI, Inc., #1B150F), and were pulled with a vertical puller (David Kopf Inst., Model 700C) to a tip diameter of approximately 1.0 um. Filling of the electrodes with the 3M KCl solution was accomplished with the use of fine stainless steel needle (28 gauge; Popper & Sons, Inc.); each microelectrode was checked under the microscope for the presence of any air bubbles and to ensure that the tip was not broken. The electrodes had d.c. tip resistances of 8-15 Mohms and tip potentials of 5 mV or less. All microelectrodes were used within 3 hours of filling. The reference electrode consisted of a length of polyethylene tubing (5 cm long; 3 mm diameter at the top and 1.5 mm diameter at the bottom) which contained a Ag:AgCl wire in agar-bathing solution; it was placed in the Plexiglass chamber.

The microelectrodes were positioned vertically above the soleus muscle with a Narishige (No. 1661) micromanipulator and fibre penetration was performed under

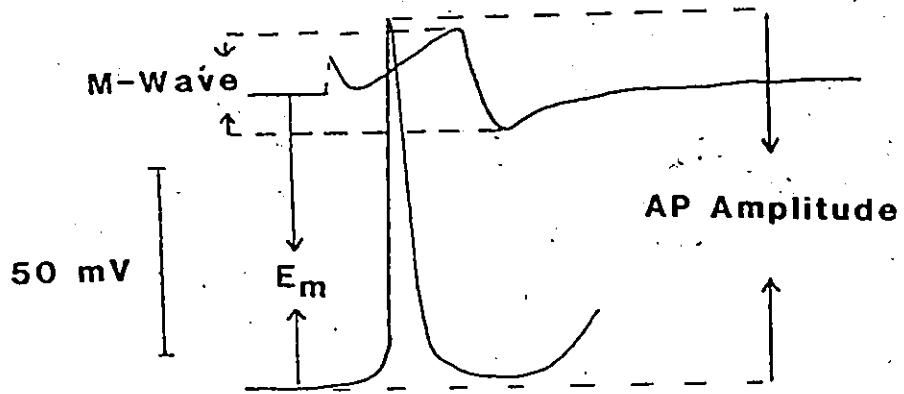
visual control. Resting membrane potentials were recorded with a high-input impedance amplifier (WPI Model M-707) and displayed on a 4 trace storage oscilloscope (Model 141B, Hewlett-Packard Ltd). Proper fibre penetration was assessed by the abruptness of the deflection on the screen; the potential across the membrane was then determined by means of a graticule or by "backing off" the voltage of the calibrator. Action potentials were evoked by supramaximal stimuli (20 us pulse width) applied to the soleus nerve twig through fine silver wire electrodes (0.5 mm diameter), manually positioned with a manipulator. The peak-to-peak amplitude of the action potentials were measured directly off the oscilloscope screen using a graticule. The overshoot (OS) of the action potential was measured as that potential difference, either positive or negative, from the zero potential baseline.

M-wave recordings were made using a silver wire electrode (0.1 mm diameter) twisted around the end of the muscle belly at its tendon of insertion; the reference electrode was an alligator clip on the foot. The peak-to-peak amplitude of the M-wave was also measured directly off the oscilloscope screen. A sample tracing showing the M-wave, membrane potential, and single fibre action potential is depicted in Figure 5.

In some instances, Polaroid photographs were taken of the oscilloscope tracings for subsequent comparisons

Figure 5.

Sample oscilloscope tracing showing how resting membrane potential, action potential, and M-wave were measured.


M-Wave
50 mV
E_m
AP Amplitude

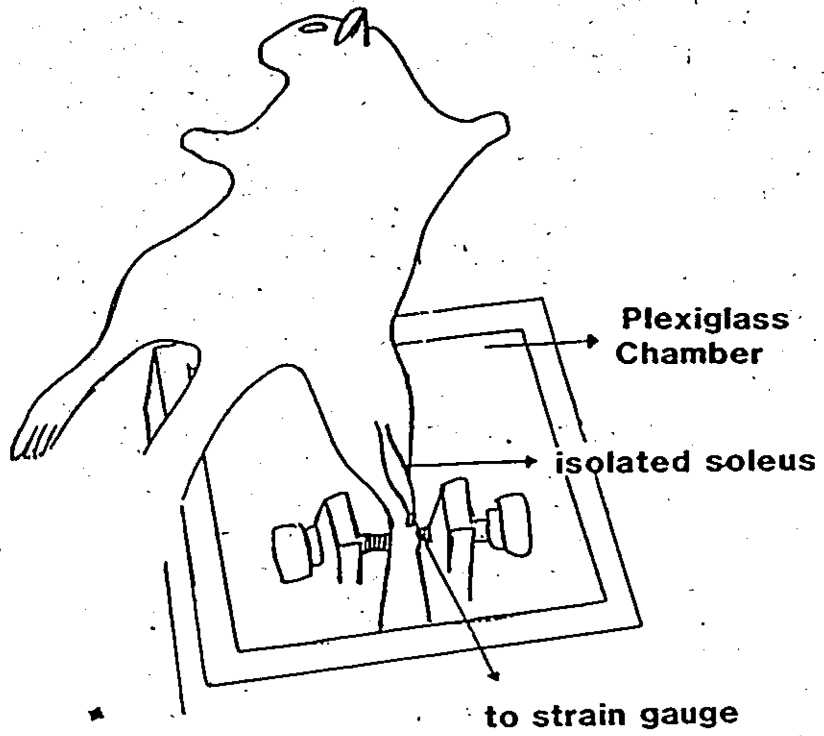
between experimental manipulations. An illustration of the placement of the hindlimb in the bathing chamber and the positioning of the electrodes is depicted in Figure 6.

2.3.4. <u>Fatigue sequence</u>. The fatigue protocol consisted of a series of 60 twitch-tetanus sequences, involving a twitch followed by a 4-second tetanus at 20 Hz, repeated every 5 seconds (See Figure 7). 20 Hz was chosed as the stimulation frequency because it produces an almost completely fused tetanus which can be maintained for long periods of time without any appreciable excitation failure at the neuromuscular junction (Kugelberg, 1973). The stimuli were 20 µsec in duration and approximately ten times maximal intensity, delivered from a Devices Ltd. stimulator (Model DS-2).

Baseline measurements of the twitch and maximal M-wave were made before the fatigue protocol began. As well, resting membrane and single fibre action potentials were recorded in 6-10 fibres to establish a baseline. Recovery of these parameters was then followed for 15 minutes after the stimulation; the total number of fibre impalements was approximately 30 for the entire recovery period (2 per minute). The first of these recovery measurements was made usually within 20 seconds of the end of the stimulation period.

Figure 6.

Placement of rat hindlimb in plexiglass chamber - the close-up shows the exposed soleus muscle with the various electrodes.


Plexiglass Chamber
isolated soleus
to strain gauge

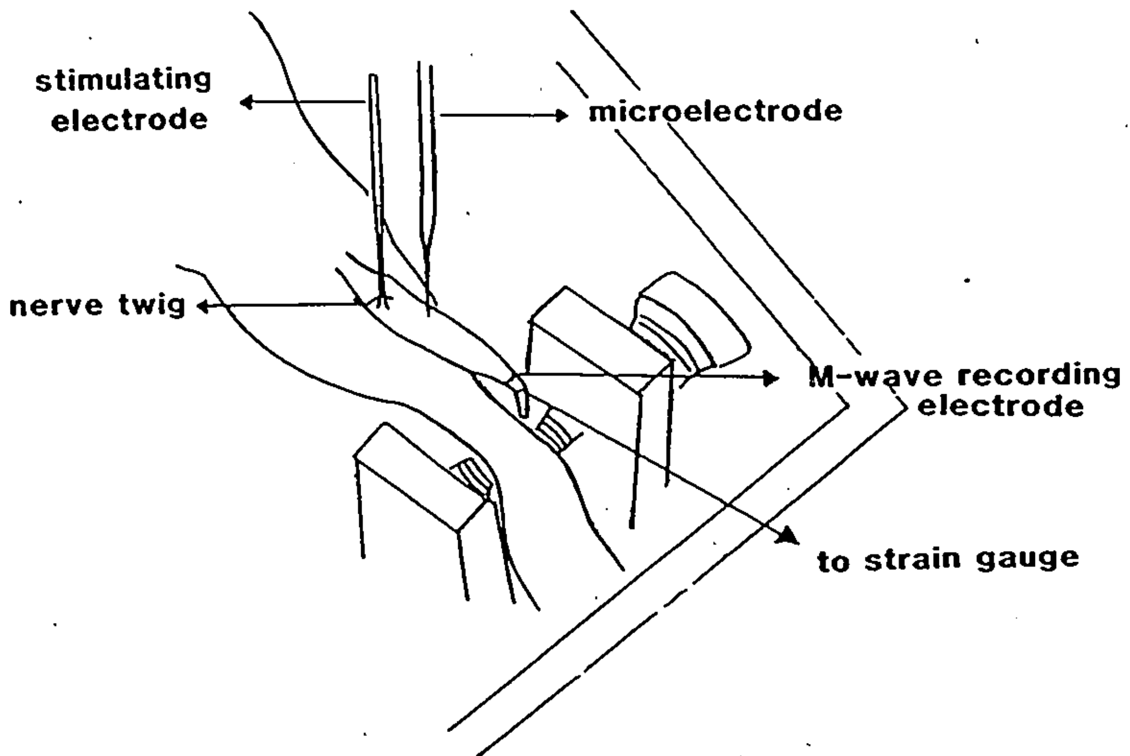
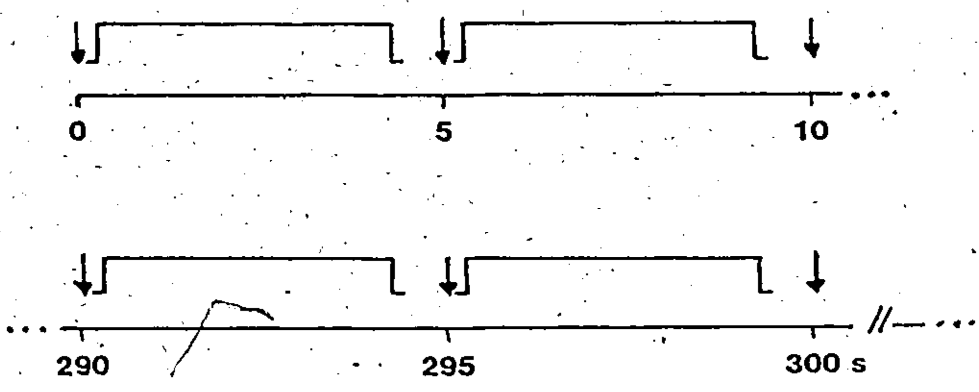

stimulating electrode
microelectrode
nerve twig
M-wave recording electrode
to strain gauge

Figure 7. Experimental protocol for stimulation of rat soleus.

- M-wave

- 20 Hz tetanus



Recovery ··· 1 3 5 7 9 11 13 15 min

random M-waves corresponding to fibre impalements
(approx. 2/min)

2.3.5. Assessment of $Na^{\pm}$-$K^{\pm}$ pump activity. The contribution of the Na^+-K^+ pump to the resting and action potential measurements was determined by inhibiting the pump activity by one of three methods:

1) Addition of ouabain. 5 ml of bathing fluid containing .128 mg ouabain was added to the bathing medium which was then stirred; the final concentration of ouabain being 1.25×10^{-4}M.

2) Lowering the temperature. The 37°C fluid in the chamber was replaced by an identical solution at 19°C.

3) Removal of $K^{\pm}$. The bathing medium was replaced by one which contained 0mM K^+ (the concentration of Na^+ was increased to 144.5 mM to maintain osmolarity).

Each of the above manipulations was conducted a few seconds before the termination of the fatiguing sequence in order to ensure that it was completed by the start of the recovery period. The presence of a drain at the bottom of the bath chamber allowed for rapid removal of the bathing medium in those studies in which the external medium was to be altered.

The magnitude of the electrogenic component of the Na^+-K^+ pump was estimated by exchanging the bathing medium for one containing 20mM K^+ (the Na^+ concentration was reduced to 124.5 mM to maintain osmolarity). This concentration of K^+ served to reduce the theoretical E_K to a level well below the control resting potential, so that

the size of the electrogenically-derived potential would be more readily apparent. The timing of the fluid exchange was as in the other manipulations described above.

The effect of all of the above bathing medium alterations on resting membrane and action potentials was also assessed in unstimulated soleus muscles for the same 15 minute period. This allowed for quantification of the contribution of the Na^+-K^+ pump to the normal steady state membrane potential of soleus muscle fibres.

2.3.6. Statistical treatment. The data were analyzed by a repeated measures analysis of variance to test the significance of differences in the variables as a result of both the fatigue sequence and the experimental intervention. Resting membrane and action potential measurements were averaged over 3 minute time periods during recovery; the means were then compared over time. Post-hoc Tukey A analysis was then done to test selected differences between means.

The baseline measurements of resting membrane and action potentials were very reproducible from experiment to experiment. There was no significant difference in resting values between the 68 animals studied, as determined by an analysis of variance. For this reason, the means of all the baseline measurements of resting and

action potentials were used as the standard to describe both the resting characteristics of the muscle membrane and to compare any changes as a result of experimental manipulations.

3. Results

3.1. Human Studies

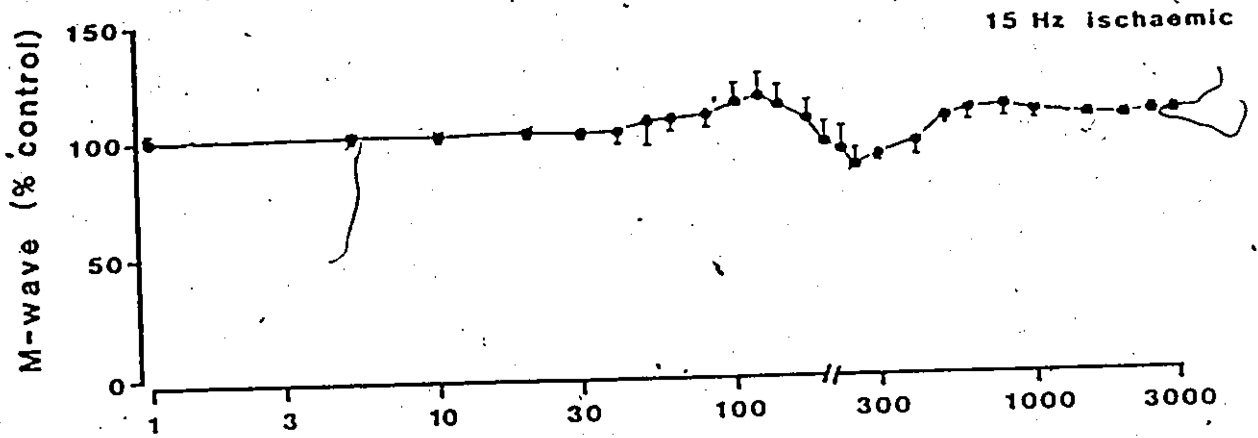
Studies were conducted on human muscle to follow the behavior of the M-wave during and after intermittent stimulation of the tibialis anterior muscle. These observations led to the investigation of M-wave behavior during maximum voluntary contractions, using the thenar and extensor digitorum brevis (EDB) muscles.

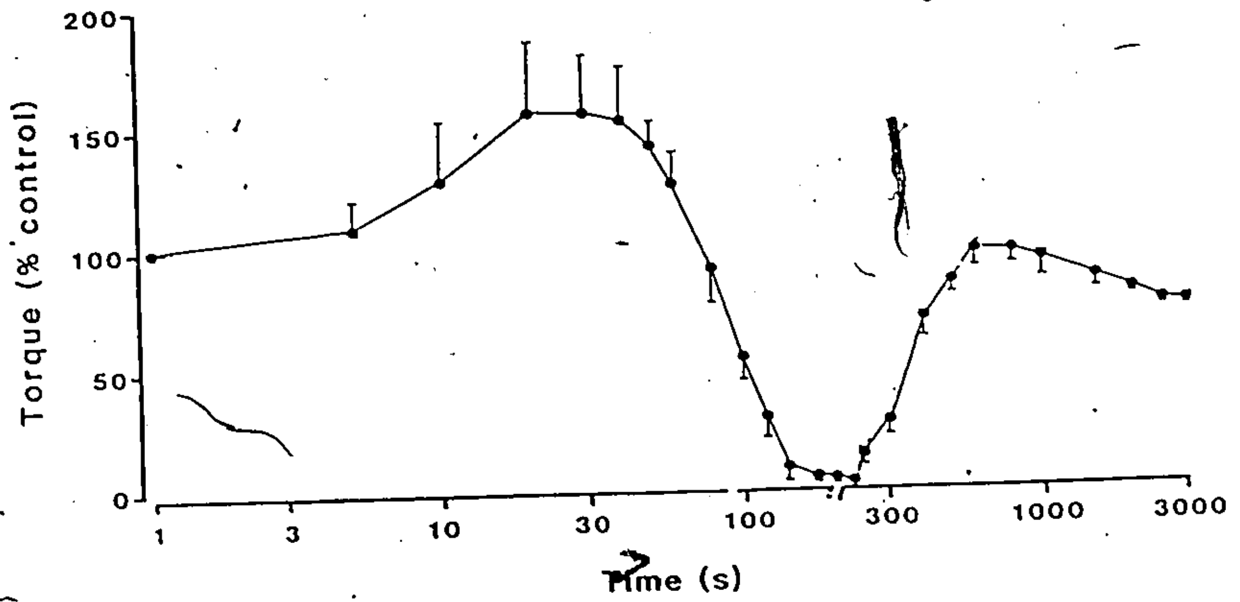
3.1.1. Experiments with intermittent tetanic stimulation

In the six subjects, intermittent electrical stimulation was applied to the peroneal nerve at a frequency of 15 Hz for 3 minutes. The leg was rendered ischaemic throughout the period of stimulation so as to hasten the onset of fatigue. M-wave responses were obtained by delivering single stimuli shortly before each tetanus. The mean changes in M-wave response and twitch torque during 15 Hz stimulation under ischaemic conditions are illustrated in Figure 8. The M-wave potentiated briefly during the intermittent stimulation, declined to a value of 85% of baseline at the end of the stimulation, and then recovered within 5 min of the recovery period. Twitch torque, on the other hand, showed an earlier potentiation than the M-wave during the stimulation and then

Figure 8.

Changes in M-wave amplitude (top) and twitch torque (bottom) during and after 15 Hz stimulation of human tibialis anterior muscle, under ischemic conditions. Intermittent stimulation was continued for 225 secs (break in abcissa). Values are mean ± standard deviation. Note logarithmic time scale..


15 Hz ischaemic
M-wave (% control)
150
100
50
0
1
3
10
30
100
300
1000
3000


Torque (% control)
200
150
100
50
0
1
3
10
30
100
300
1000
3000
Time (s)

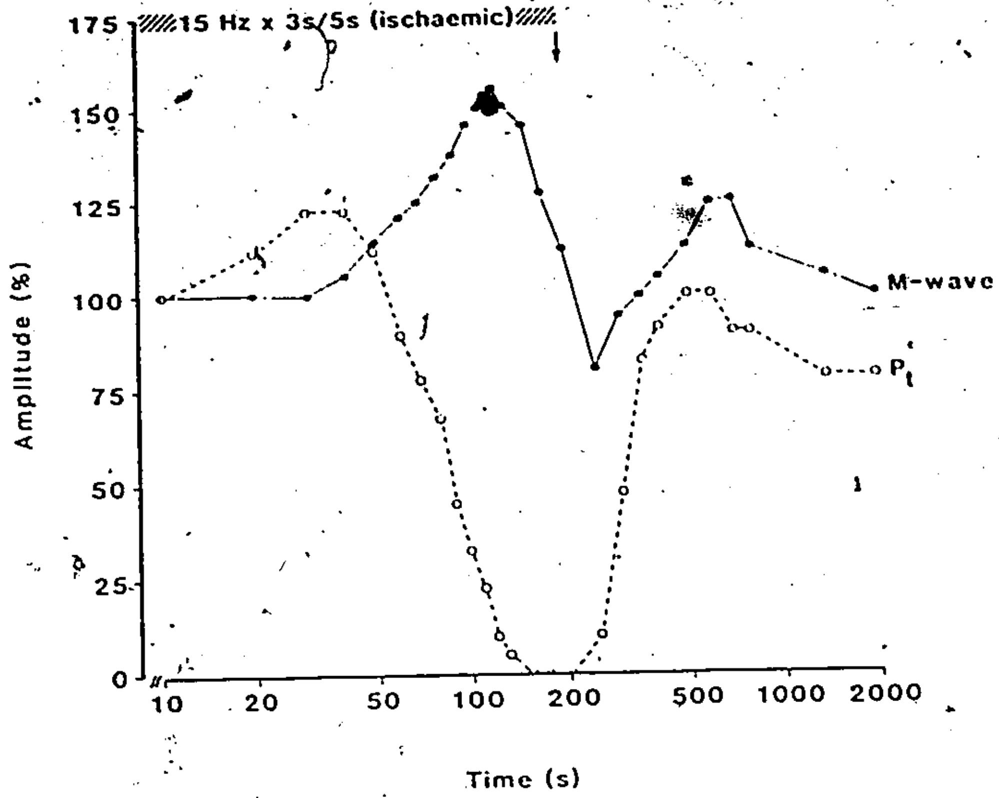
progressively declined to zero. Figure 9 is of interest because it shows the results of one particular subject (J.K.) in which a very pronounced potentiation of the M-wave occurred both during and following the repetitive stimulation. The M-wave increased to 150% of baseline during the stimulation before declining to approximately 80% at the end. In the recovery period, the M-wave potentiated again to approximately 125% of baseline. These results prompted a search for the physiological mechanisms underlying the M-wave enlargement. First, however, a more complete study of M-wave behaviour during muscular activity was undertaken. In the next experiments to be described, voluntary contractions replaced stimulated activity in an effort to simulate more physiological contractions for the muscle.

3.1.2. <u>Experiments with voluntary contractions</u>

In all 10 subjects who volunteered for this study, significant enlargement of the M-wave was observed either during or after 100 seconds of intermittent voluntary contractions of both the thenar and EDB muscles. These results are depicted in Figures 10 and 11, and the mean values are given in Table 2. In the thenar muscle, the M-wave increased in both amplitude (Figure 10A) and area (Figure 10B) during the course of the MVC's, both of these changes being significantly different from what occurred in

Figure 9.

Twitch torques (interrupted lines) and M-waves (solid lines) recorded between successive 3 sec tetani at 15 Hz in an ischemic tibialis anterior muscle of 31 yr old male.


15 Hz x 3s/5s (ischaemic)
175
150
125
100
75
50
25
0
Amplitude (%)
M-wave
P_t
10
20
50
100
200
500
1000
2000
Time (s)

the control condition ($p<.05$). The changes occurred quite rapidly, within 15-35 seconds of the start of the intermittent contractions. The fact that the M-wave area increased at least as much as M-wave amplitude dismisses the possibility that greater action potential synchronization was responsible for the increased amplitudes (See Discussion). Figure 11 shows the results from a single subject, where the potentiation of the M-wave during the 100 sec of intermittent maximal effort is quite marked.

In the case of the EDB muscle, the M-wave enlargement occurred somewhat later, and was most prominent in the recovery period following the intermittent contractions (Figure 12A and 12B). The M-wave area showed the greatest enlargement, rising to 126% of baseline by the end of the voluntary effort ($p<.05$). The M-wave amplitude changes were somewhat less and slower to occur, reaching 114% of baseline at approximately the 2 min mark of the recovery period ($p<.05$).

3.2. Animal Studies

3.2.1. Effect of tetanic stimulation on muscle compound action potentials

The effect of 5 minutes of electrical stimulation at 20 Hz on the soleus M-waves was studied in 14 animals;

Table 2.

Changes in M-wave amplitude and area associated with intermittent voluntary contractions of human thenar and EDB muscle (n = 10). Values are mean ± standard deviation.
* $p<.05$.

THENAR				
Time (sec)	M-wave Amplitude (%)		M-wave Area (%)	
	Control	Experimental	Control	Experimental
5	101.1 ± 1.5	114.8 ± 8.0	100.8 ± 1.5	106.3 ± 7.8
15	101.1 ± 1.2	119.8 ± 8.3*	100.3 ± 1.2	111.3 ± 12.7
25	100.7 ± 1.2	121.5 ± 9.5*	100.0 ± 1.0	115.1 ± 12.9
35	101.1 ± 1.9	122.2 ± 12.7*	99.8 ± 1.6	118.7 ± 12.5*
45	101.0 ± 1.1	123.6 ± 12.6*	99.4 ± 1.0	119.9 ± 14.8*
60	100.9 ± 1.9	121.8 ± 15.7*	99.8 ± 3.4	121.7 ± 14.9*
80	101.5 ± 2.9	122.9 ± 16.9*	99.6 ± 2.7	127.2 ± 12.0*
100	101.2 ± 2.6	121.8 ± 18.6*	99.3 ± 3.1	129.3 ± 14.1*
150	100.3 ± 1.8	101.7 ± 9.9	97.2 ± 4.1	116.3 ± 9.9*
200	99.8 ± 2.4	101.0 ± 9.5	98.4 ± 3.2	109.2 ± 8.6
400	99.7 ± 3.1	99.8 ± 10.2	99.8 ± 4.4	99.7 ± 9.8
800	101.1 ± 4.1	99.8 ± 8.7	100.3 ± 5.6	96.7 ± 9.4

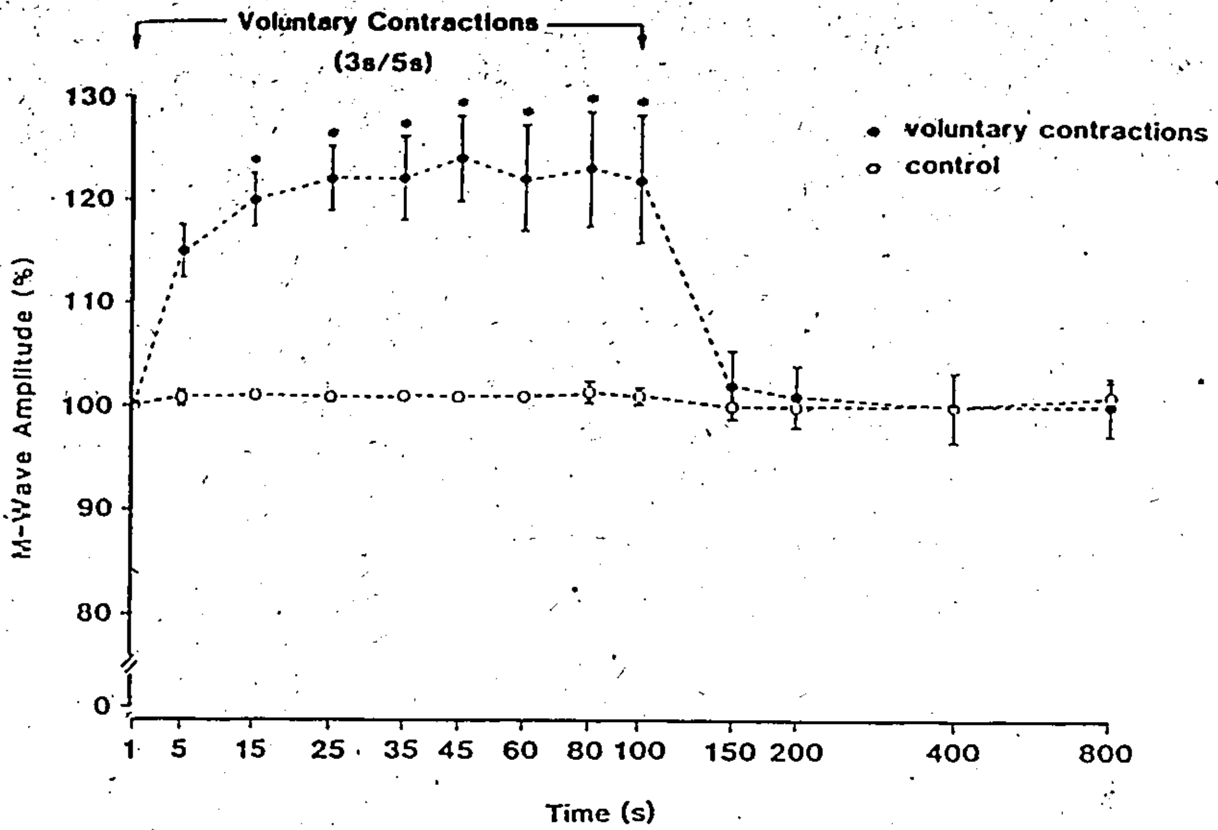
EDB				
5	100.0 ± 0.5	105.8 ± 4.9	99.6 ± 3.2	103.4 ± 6.0
15	101.1 ± 1.8	109.4 ± 6.9	100.3 ± 1.8	111.0 ± 9.9
25	101.1 ± 2.1	109.1 ± 7.7	100.5 ± 2.5	115.3 ± 11.5
35	100.8 ± 2.0	106.4 ± 8.4	99.8 ± 2.1	112.2 ± 11.8
45	101.2 ± 2.5	109.4 ± 7.7	100.1 ± 3.3	117.9 ± 11.2
60	100.9 ± 2.8	111.4 ± 9.6	100.5 ± 3.9	124.0 ± 17.3*
80	100.8 ± 3.0	110.7 ± 10.6	99.8 ± 3.7	121.8 ± 12.9*
100	100.4 ± 3.5	112.3 ± 9.6	99.8 ± 4.8	125.9 ± 15.2*
150	99.0 ± 3.4	112.7 ± 13.3*	97.8 ± 5.9	120.7 ± 15.3*
200	99.3 ± 3.7	114.2 ± 13.0*	97.0 ± 10.4	118.9 ± 15.4*
400	99.4 ± 4.1	111.8 ± 14.2	98.5 ± 10.6	112.7 ± 13.6
800	99.1 ± 3.9	109.7 ± 15.7	99.7 ± 11.5	111.4 ± 16.3

Figure 10A.

Changes in M-wave amplitude during intermittent voluntary contractions of human thenar muscle (n = 10). Values are mean ± standard error. Note logarithmic time scale.
* $p<.05$.

Figure 10B.

Changes in M-wave area during intermittent voluntary contractions of human thenar muscle (n = 10). Values are mean ± standard error. Note logarithmic time scale.
* $p<.05$.


Voluntary Contractions
(3s/5s)
voluntary contractions
control
M-Wave Amplitude (%)
130
120
110
100
90
80
0
1
5
15
25
35
45
60
80
100
150
200
400
800
Time (s)

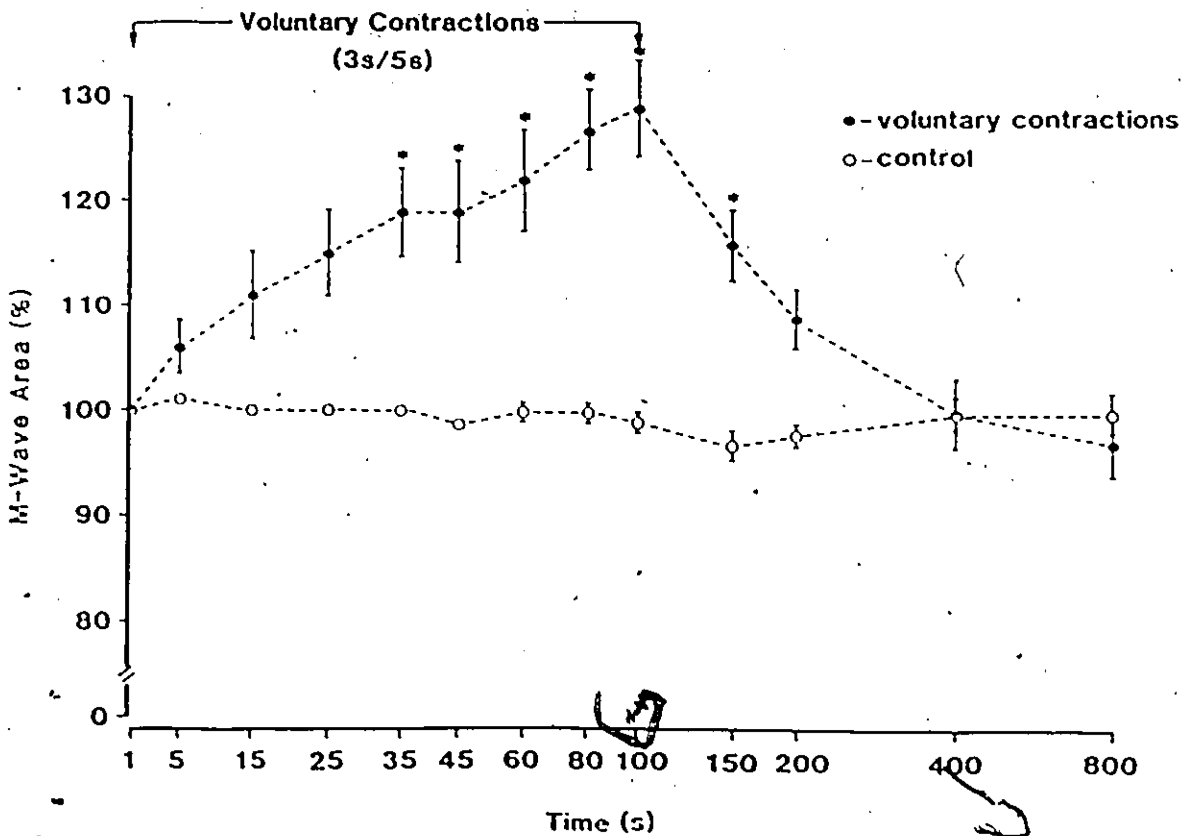

Voluntary Contractions
(3s/5s)
•-voluntary contractions
o-control
M-Wave Area (%)
130
120
110
100
90
80
0
1
5
15
25
35
45
60
80
100
150
200
400
800
Time (s)

Figure 11.

Data from one subject (29 yr old female) showing the changes in M-wave amplitude associated with intermittent voluntary fatigue of the left thenar muscle. Intermittent effort occurred for 100 seconds; the remaining time points are in the recovery period.

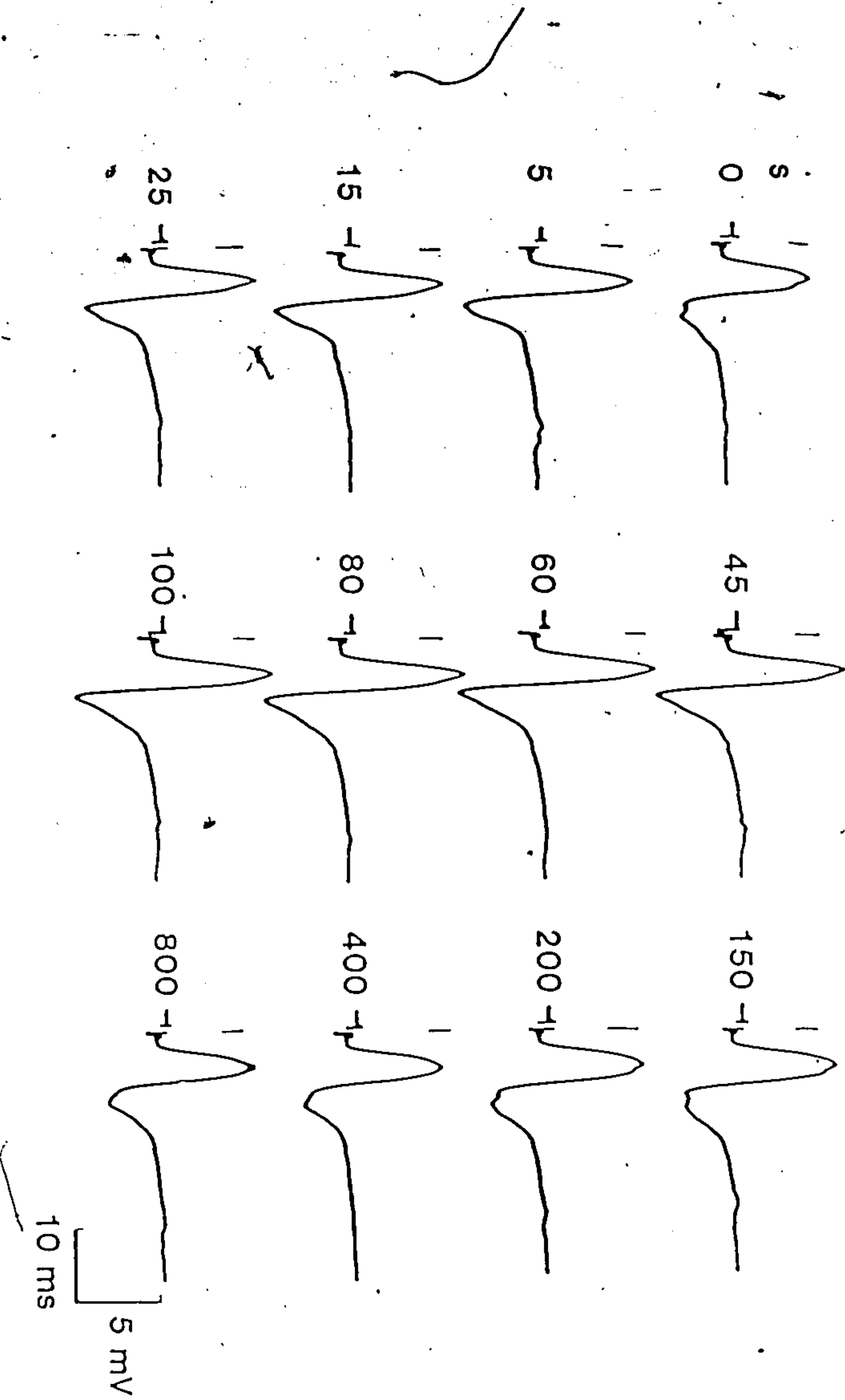
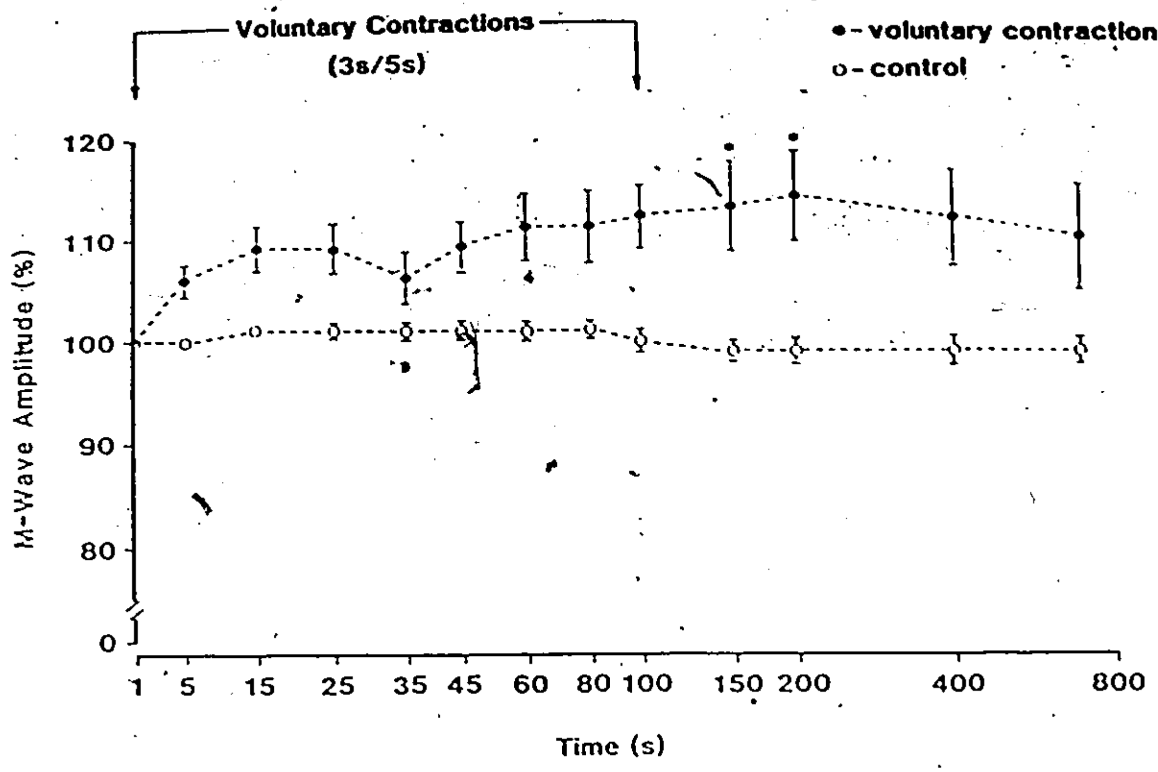

J.G., ♀, 29 yr.
(L) THENAR
s
0
5
15
25
45
60
80
100
150
200
400
800
10 ms
5 mV

Figure 12A.

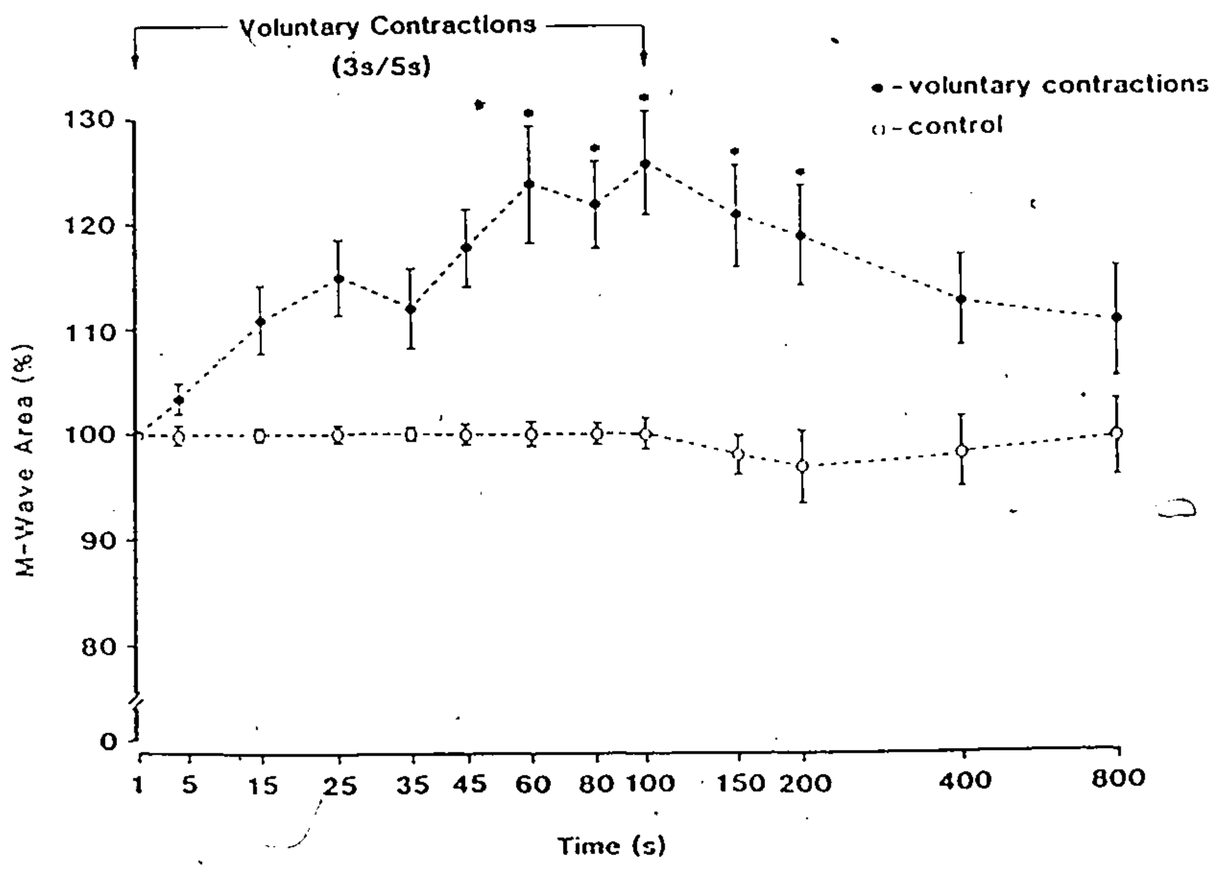
Changes in M-wave amplitude during intermittent voluntary contractions of human EDB muscle (n = 10). Values are mean ± standard error. Note logarithmic time scale.
* $p<.05$.

Figure 12B.

Changes in M-wave area during intermittent voluntary contractions of human EDB muscle (n = 10). Values are mean ± standard error. Note logarithmic time scale.
* $p<.05$.



Voluntary Contractions
(3s/5s)
• - voluntary contractions
o - control
M-Wave Amplitude (%)
120
110
100
90
80
0
1
5
15
25
35
45
60
80
100
150
200
400
800
Time (s)



Voluntary Contractions
(3s/5s)
• - voluntary contractions
o - control
M-Wave Area (%)
130
120
110
100
90
80
0
1
5
15
25
35
45
60
80
100
150
200
400
800
Time (s)

these results are depicted in Figure 13. At the end of the stimulation period, the mean M-wave had decreased to 75.7% (± 22%) of its original value. Twitch tension, at the end of the stimulation, had dropped to a mean level of 40.2% (± 17.9%) of its initial value. Recovery of the M-wave after stimulation was very rapid, with full recovery being evident within the first minute. There was then a consistent potentiation of the M-wave in the subsequent 10 minutes; the M-wave enlarged to a mean maximum of 149% (± 25%) of its starting value ($p<.01$). It can be seen that the peak potentiation occurred primarily between 5 and 10 minutes of the recovery period.

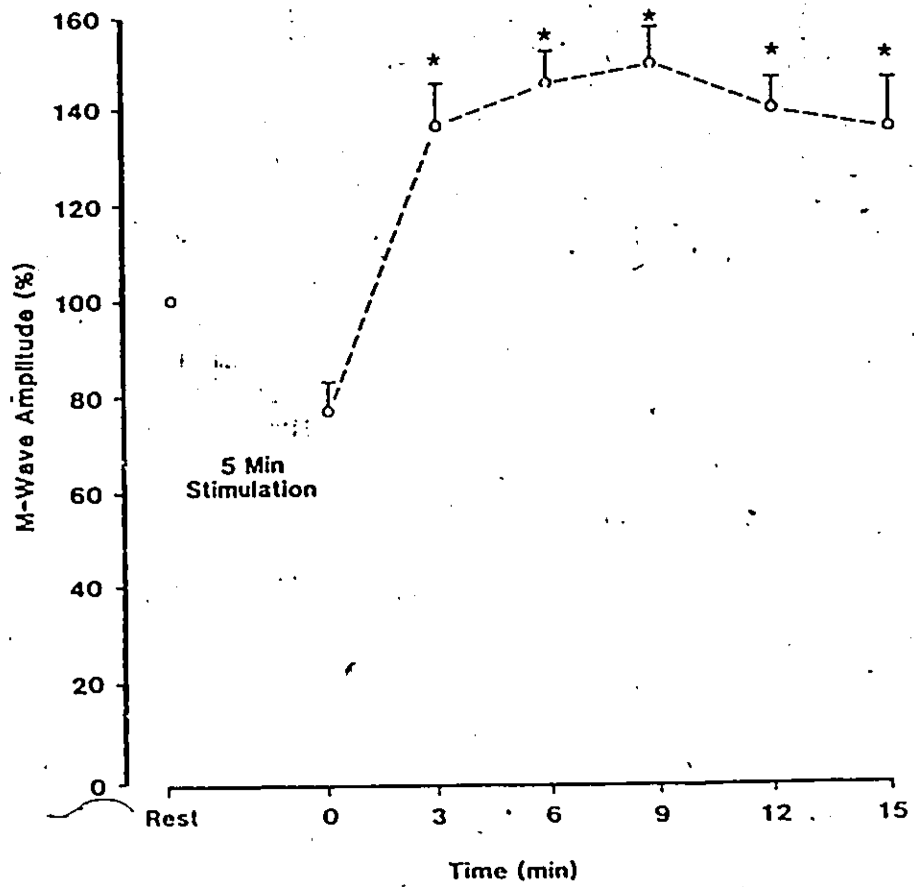
Figure 14 shows the effects of intraperitoneal injection of ouabain (4mg/kg) on the behavior of the M-wave during and after the 5 minutes of stimulation. The resting M-wave was significantly affected by the ouabain injection, dropping by more than 50% ($p<.01$) before the stimulation had begun. There was still, however, a potentiation of the M-wave after stimulation, which was significant at the 6 min mark of the recovery period ($p<.05$).

3.2.2. <u>Effect of tetanic stimulation on resting and action potentials</u>

Under control conditions the mean resting potential of 400 soleus muscle fibres, studied in 68 animals, was

Figure 13.

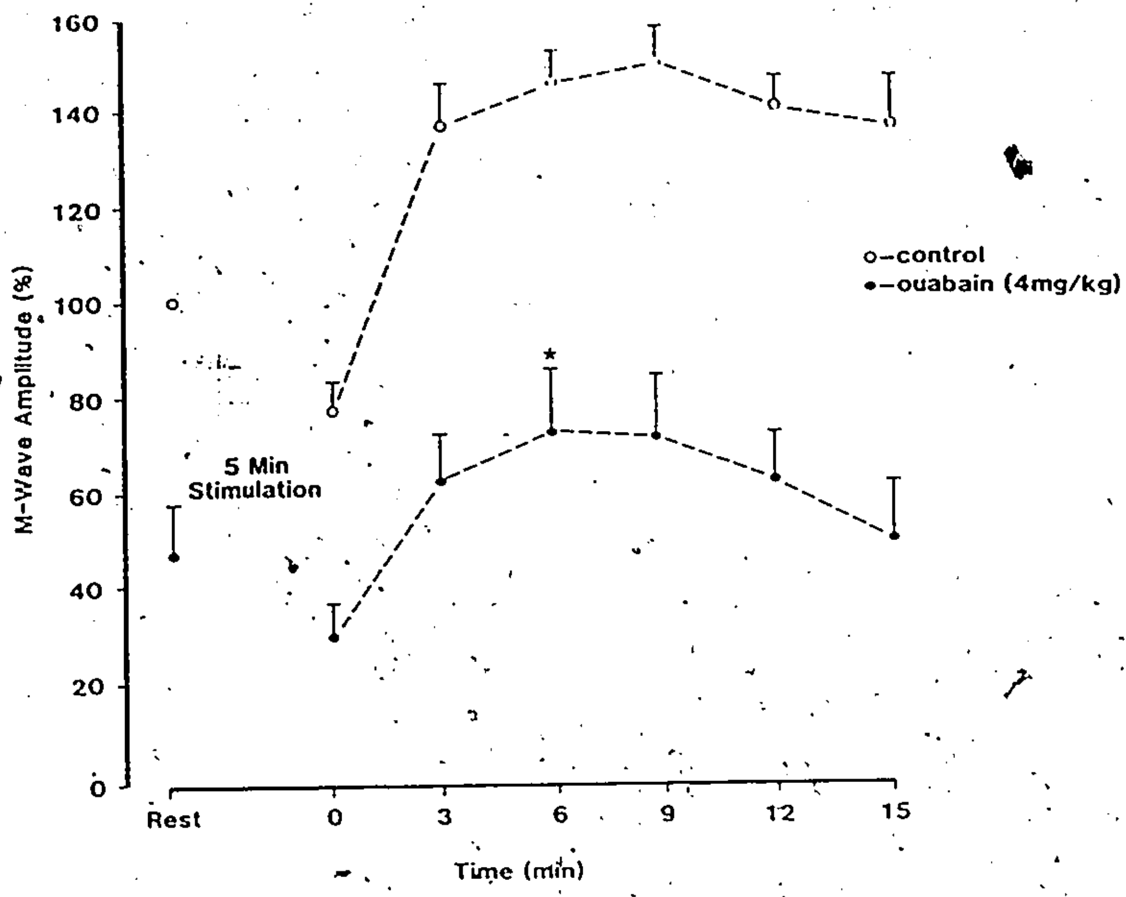
Changes in M-wave amplitude after intermittent stimulation of rat soleus muscle at 20 Hz. Values are mean ± standard deviation.
* Significant difference from rest ($p<.01$).



160
140
120
100
80
60
40
20
0
M-Wave Amplitude (%)
5 Min
Stimulation
Rest
0
3
6
9
12
15
Time (min)

Figure 14.

Effect of intraperitoneal injection of ouabain (4mg/kg) on the behavior of the M-wave after intermittent tetanic stimulation of the rat soleus muscle. Note the depressed M-wave after ouabain injection at rest (●) before the stimulation began. The control condition (○) represents the normal changes in M-wave after stimulation, as in Figure 13.
* Significant difference from rested (post-injection) condition ($p<.05$).


160
140
120
100
80
60
40
20
0
M-Wave Amplitude (%)
o–control
•–ouabain (4mg/kg)
5 Min
Stimulation
*
Rest
0
3
6
9
12
15
Time (min)

-79.5 ± 4.8 mV, and the mean action potential amplitude was 82.2 ± 10.8 mV. There was no significant difference in resting and action potentials between animals as determined by an ANOVA test. After the muscles had been stimulated tetanically, further determinations of resting and action potential amplitude were made during a 15 min recovery period; the distributions of the measurements are shown in Figure 15. It can be seen that the resting potentials were increased as a result of the stimulation, the new mean value being -91.6 ± 4.4 mV. Although the individual muscle fibres exhibited considerable variability, there was a corresponding rise in the mean action potential amplitude, the post-stimulation value being 97.8 ± 8.9 mV. The changes in mean resting potential and action potential amplitudes were both statistically significant ($p < 0.01$). The top right corner in Figure 15 shows typical recordings of resting and action potentials in two different fibres during the control and recovery periods respectively; the significant enlargement of the M-wave can also be seen in this figure.

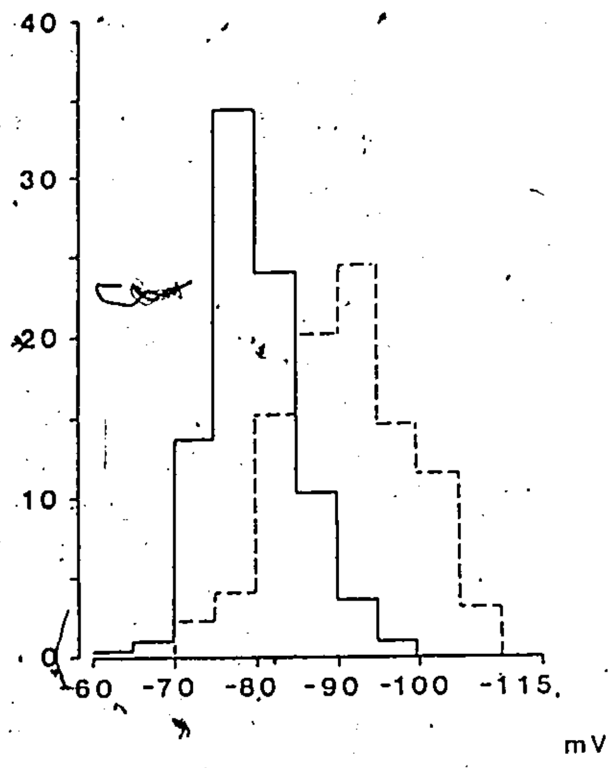
In Figure 16A, the time-course of the change in resting potential has been plotted (n = 13 animals). It was found that even the first fibre impalements, performed at the end of the tetanus, showed elevations of resting potential; the mean value in the first 3 minutes of recovery was -11 mV greater than that at rest (-79.5 mV ->

Figure 15.

Frequency distribution of resting and action potentials in rat soleus muscle. The solid lines represent the resting state and the dotted lines represent post-stimulation. The inset illustrates the changes in the M-wave (top) and single fibre action potential (bottom) as seen on the oscilloscope in a typical experiment. The hyperpolarization of the membrane after the stimulation is quite evident (right hand side of the inset).

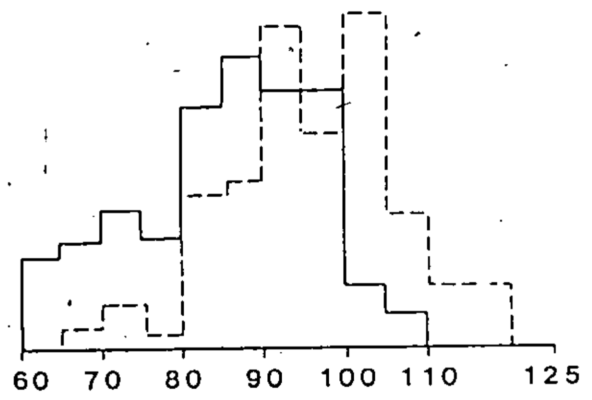
Resting Potentials ($\bar{x} \pm SD$)

-79.5 ± 4.8 mV ⟶ -91.6 ± 4.4 mV



Action Potentials ($\bar{x} \pm SD$)

82.2 ± 10.8 mV ⟶ 97.8 ± 8.9 mV



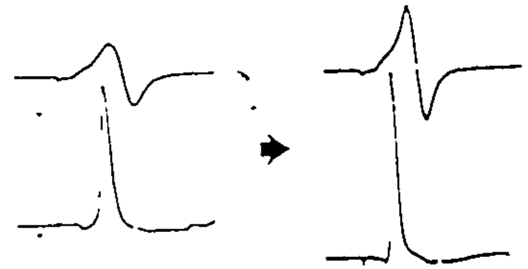
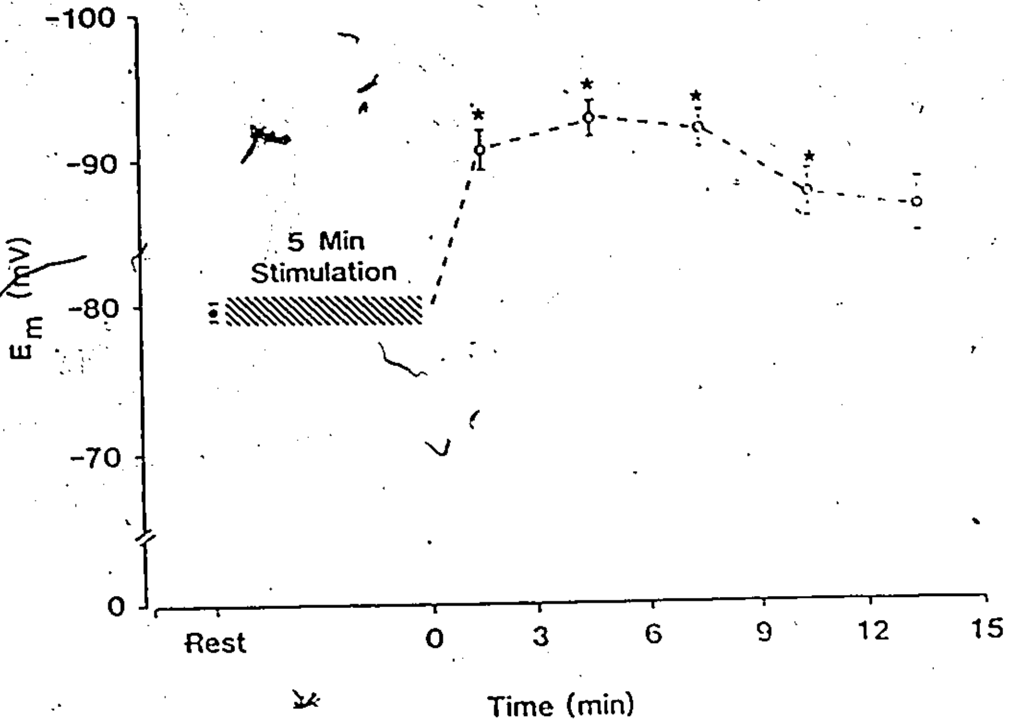


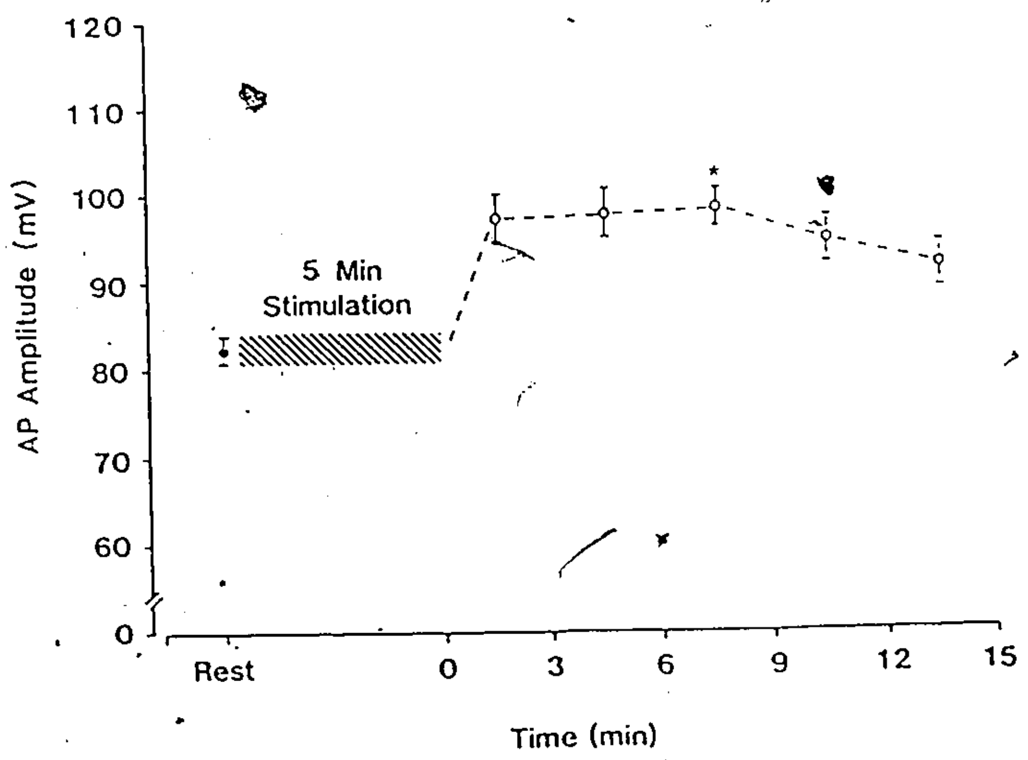
Figure 16A.

Effect of 5 min of intermittent tetanic stimulation (20 Hz) on the resting membrane potential of rat soleus muscle fibres (n = 13). Values are mean ± standard error.
* Significant difference from resting value ($p<.05$).

Figure 16B.

Effect of 5 min of intermittent tetanic stimulation (20 Hz) on the single fibre action potential of rat soleus muscle fibres (n = 13). Values are mean ± standard error.
* Significant difference from resting value ($p<.05$).


-100
-90
-80
-70
0
E_m (mV)
5 Min
Stimulation
Rest
0
3
6
9
12
15
Time (min)


120
110
100
90
80
70
60
0
AP Amplitude (mV)
5 Min
Stimulation
Rest
0
3
6
9
12
15
Time (min)

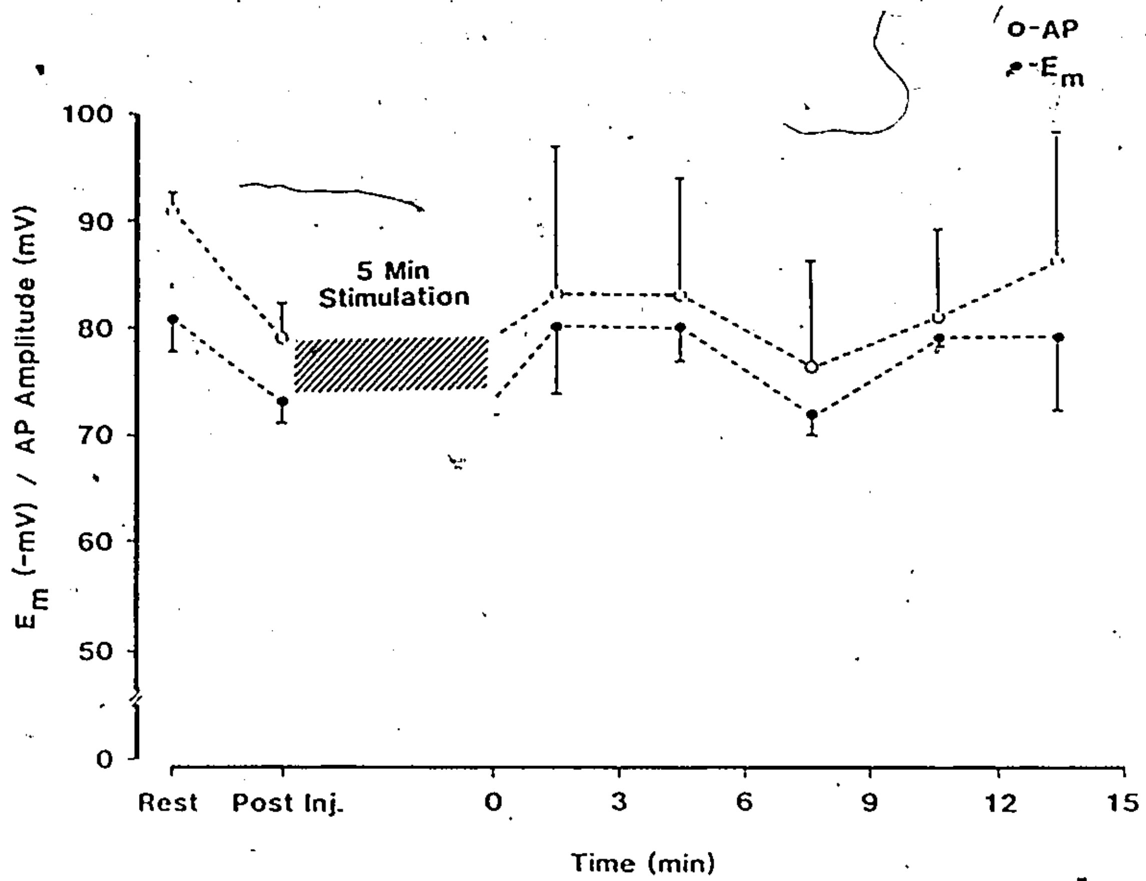
-90.9mV; $p<.05$). Figure 16A also shows that, although the hyperpolarization began to diminish 9 minutes after the end of the tetanic stimulation, it was still evident at 15 minutes.

The amplitudes of the single fibre action potentials followed a similar time-course to that of the resting potentials (Figure 16B). In the first 3 minutes after stimulation, the mean amplitude had risen to 97.0 ± 9.4 mV, and reached a maximum value of 98.1 ± 7.8 mV 6-9 minutes after stimulation ($p<.05$). The amplitudes of the action potentials then began to decrease during the remainder of the recovery period.

(a) Effects of ouabain. To test the hypothesis that the post-tetanic hyperpolarization was due to the electrogenic effect of the Na^{+}-K^{+} pump, an attempt was made to inhibit pump activity by the administration of ouabain. In the initial experiments, ouabain was injected intraperitoneally (4 mg/kg) approximately 30 minutes prior to the start of the stimulation. Results from 5 experiments are presented in Figure 17. One can see that although the stimulation-induced hyperpolarization was not nearly as marked in the ouabain experiments compared with Figure 16, the membrane potentials during recovery were still larger than those recorded prior to the tetanic stimulation. The fact that the injection of ouabain

Figure 17.

Effect of intraperitoneal injection of ouabain (4 mg/kg) on the resting and action potential of rat soleus muscle fibres before and after 5 min of intermittent tetanic stimulation (20 Hz). Values are mean ± standard error (n=5).


o-AP
•-E_m
100
90
80
70
60
50
0
5 Min
Stimulation
E_m (-mV) / AP Amplitude (mV)
Rest
Post Inj.
0
3
6
9
12
15
Time (min)

caused a progressive depolarization of the muscle fibres makes it difficult to compare these experimental results with those in Figure 16 since the muscle did not begin the stimulation in the same state of excitability in each condition. Thus, under ouabain-induced depolarization, repetitive stimulation would have had uncertain effects on the muscle since an increasing proportion of the fibres were becoming unresponsive.

The alternative and preferred approach was to stimulate the muscles repetitively first, assuring consistent impulse-mediated ionic changes in the intra- and extracellular fluids, and then to add the ouabain to the bathing medium a few seconds before the end of the stimulation period. Any immediate effect of the drug should have been evident in the surface fibres used for micro-electrode impalement, even if deeper fibres were affected more slowly.

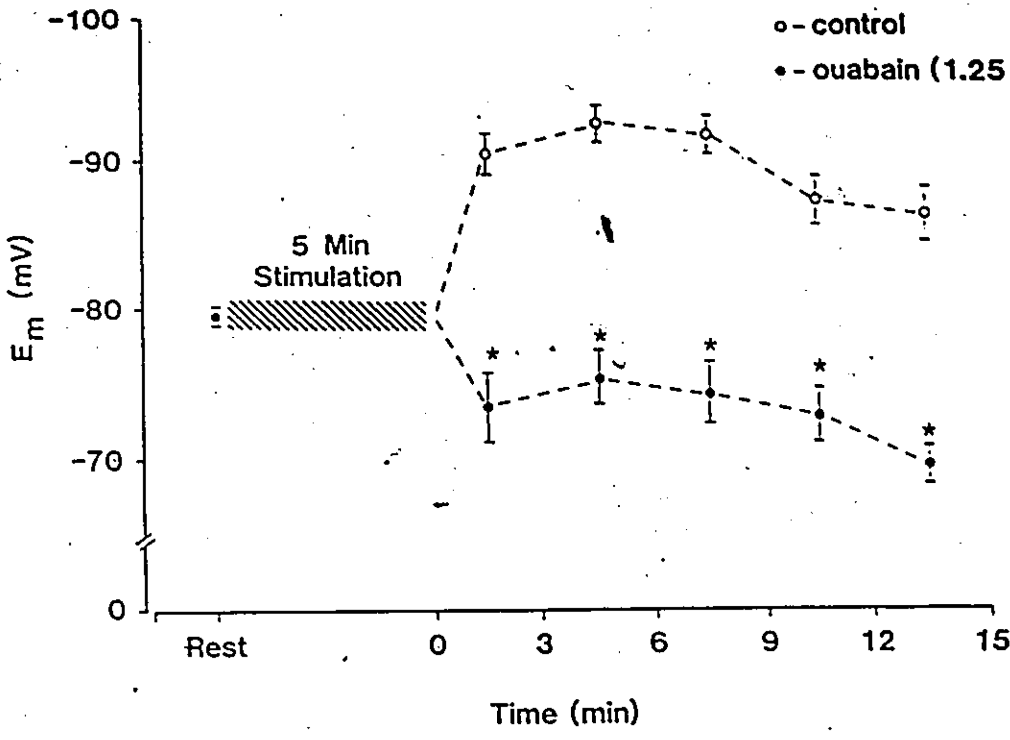
When ouabain, in a concentration of 1.25×10^{-4}M, was added to the bathing medium at the end of the stimulation period, the surface fibre membranes no longer exhibited a hyperpolarization in the recovery period, nor were the action potentials enlarged. Figure 18 illustrates that, following tetanic stimulation and in the presence of ouabain, the mean resting potential ranged from -74.1 ± 4.6 mV, in the first 3 minutes, to -70.1 ± 2.5 mV at 12-15 minutes (n = 5). During this time, the

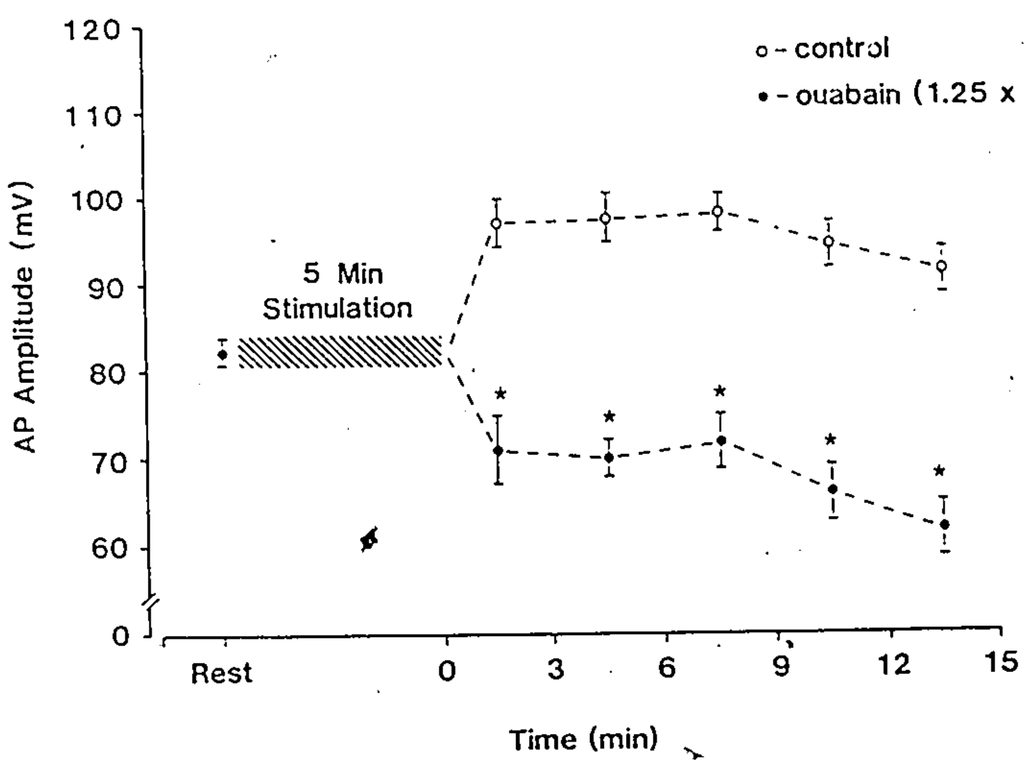
Figure 18A.

Effect of ouabain (1.25×10^{-4}M) administered at the end of 5 min intermittent tetanic stimulation (20 Hz) on the resting potential of rat soleus muscle fibres (n=5). Values are mean ± standard error.
* Significant difference between control and ouabain conditions ($p<.01$).

Figure 18B.

Effect of ouabain (1.25×10^{-4}M) administered at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the single fibre action potential of rat soleus muscle fibres (n = 5). Values are mean ± standard error.
* Significant difference between control and ouabain conditions ($p<.01$).


o - control
• - ouabain (1.25 x 10-4 M)
5 Min
Stimulation
-100
-90
-80
-70
0
Em (mV)
Rest
0
3
6
9
12
15
Time (min)


o - control
• - ouabain (1.25 x 10-4 M)
5 Min
Stimulation
120
110
100
90
80
70
60
0
AP Amplitude (mV)
Rest
0
3
6
9
12
15
Time (min)

mean amplitude of the action potentials decreased in amplitude from the resting value of 82.2 ± 10.8 mV to 61.8 ± 6.7 mV. The resting membrane and action potential measurements during recovery in stimulated fibres in the presence of ouabain were significantly different from the control conditon without ouabain ($p<.01$).

(b) <u>Effects of cooling</u>. The second technique used to investigate the activity of the sodium pump in stimulated muscle was to rapidly cool the muscle fibres, thereby diminishing active transport. As in the experiments with ouabain, the cooling was delayed until the end of the 5 min tetanic stimulation, so as not to diminish the ionic changes resulting from impulse propagation in the muscle fibres. The temperature of the bathing fluid was reduced from 37°C to 19°C by replacing one solution with another, this procedure taking approximately 15 seconds (See Methods, pg 61).

At 19°C the mean resting potential of stimulated fibres remained between -77.0 ± 5.3 mV and -79.9 ± 4.7 mV over the 15 minute recovery period, neither value being significantly different from the resting mean of -79.5 ± 4.8 mV ($n = 7$). The fibre action potentials responded differently, in that their amplitudes began to rise after 3 minutes of recovery, reaching a mean maximum value of 99.4 ± 7.6 mV at 9-12 minutes. These results are

depicted in Figure 19. The mean overshoot of the action potential at 9-12 minutes was 21.0 ± 6.6 mV and was significantly larger ($p < .05$) than those in rested and stimulated muscles at 35-37°C (4.0 ± 12.9 mV and 8.4 ± 12.2 mV respectively; see Table 3).

(c) Effects of K^+-free bathing medium. The final experiment designed to inhibit Na^+-K^+ pump activity was to replace the bathing solution with one which was K^+-free; as in the cooling and ouabain experiments, this intervention was carried out at the end of the tetanic stimulation (n = 5).

In the immediate recovery period (0-3 min), the mean resting potential of surface fibres bathed in the K^+-free medium was significantly lower than in the normal bathing medium ($p < .05$). However, by 3-6 min both the resting and action potentials started increasing; at 6-9 min the mean resting potential was -91.7 ± 4.6 mV and was significantly different from the pre-stimulation value of -79.5 ± 4.8 mV ($p < .01$). These elevated membrane potentials persisted for the remainder of the 15 minute recovery period. These results are illustrated in Figure 20A.

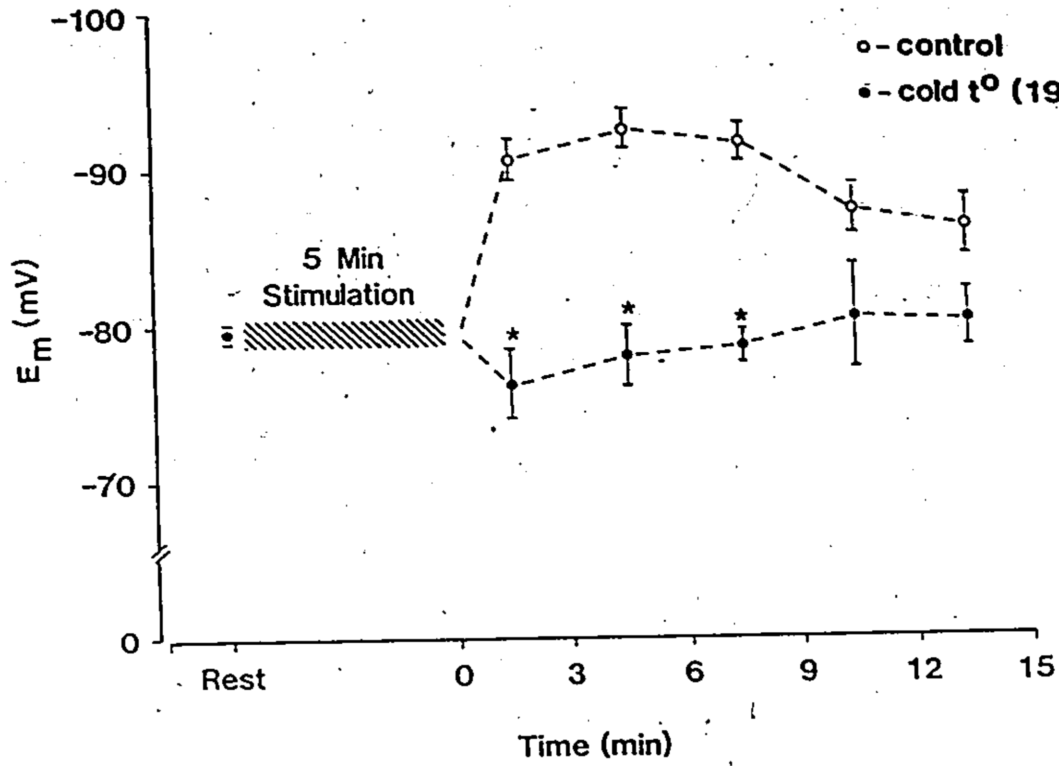
The action potential amplitudes also rose significantly above the control level after 3 minutes of recovery. This delayed increase in resting and action

Figure 19A.

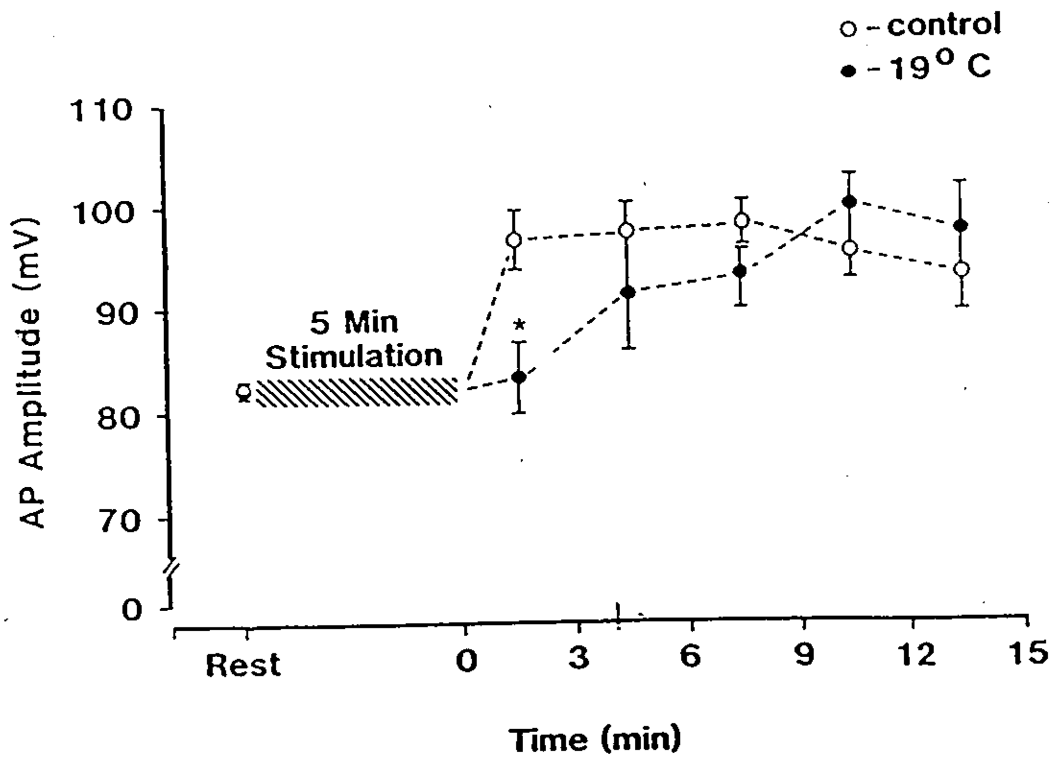
Effect of cooling the bathing medium to 19°C at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the resting potential of rat soleus muscle fibres (n = 7). Values are mean ± standard error.
* Significant differnce between control and cold conditions ($p<.01$).

Figure 19B.

Effect of cooling the bathing medium to 19°C at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the single fibre action potential of rat soleus muscle fibres (n = 7). Values are mean ± standard error.
* Significant difference between control and cold conditions ($p<.05$)



o - control
• - cold t° (19°C)
5 Min
Stimulation
E_m (mV)
-100
-90
-80
-70
0
*
*
*
Rest
0
3
6
9
12
15
Time (min)



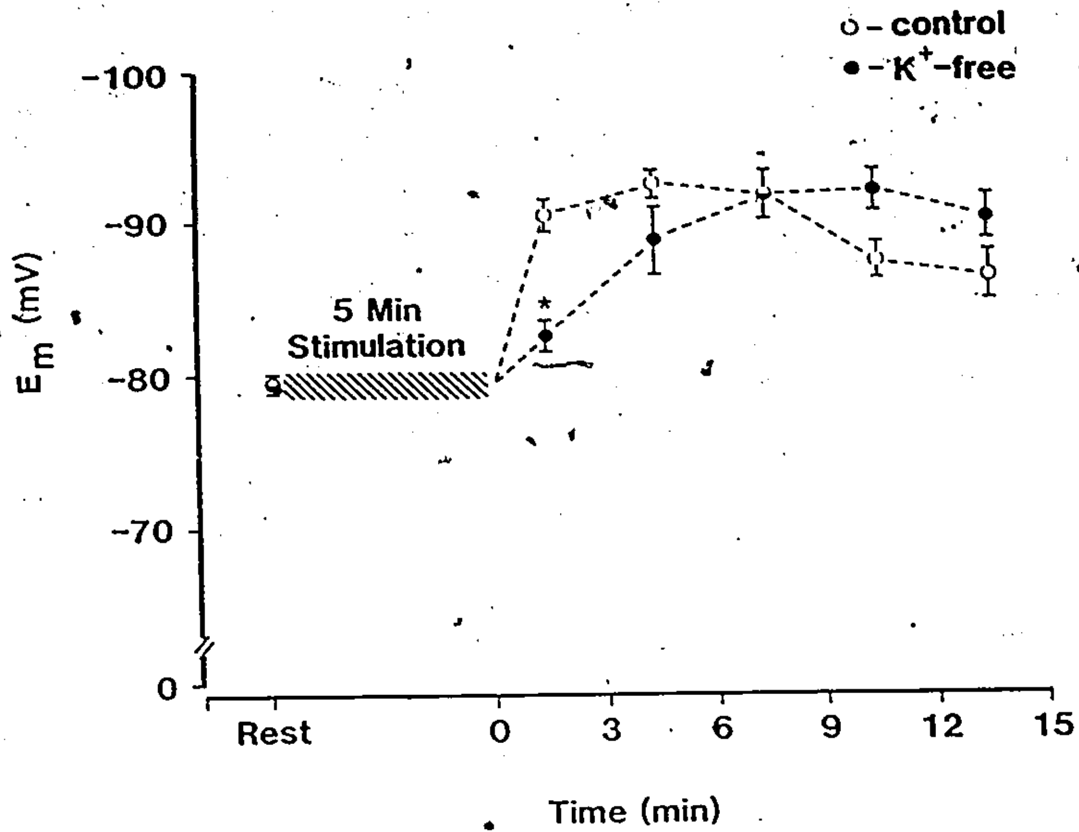
o - control
• - 19° C
5 Min
Stimulation
AP Amplitude (mV)
110
100
90
80
70
0
*
Rest
0
3
6
9
12
15
Time (min)

Figure 20A.

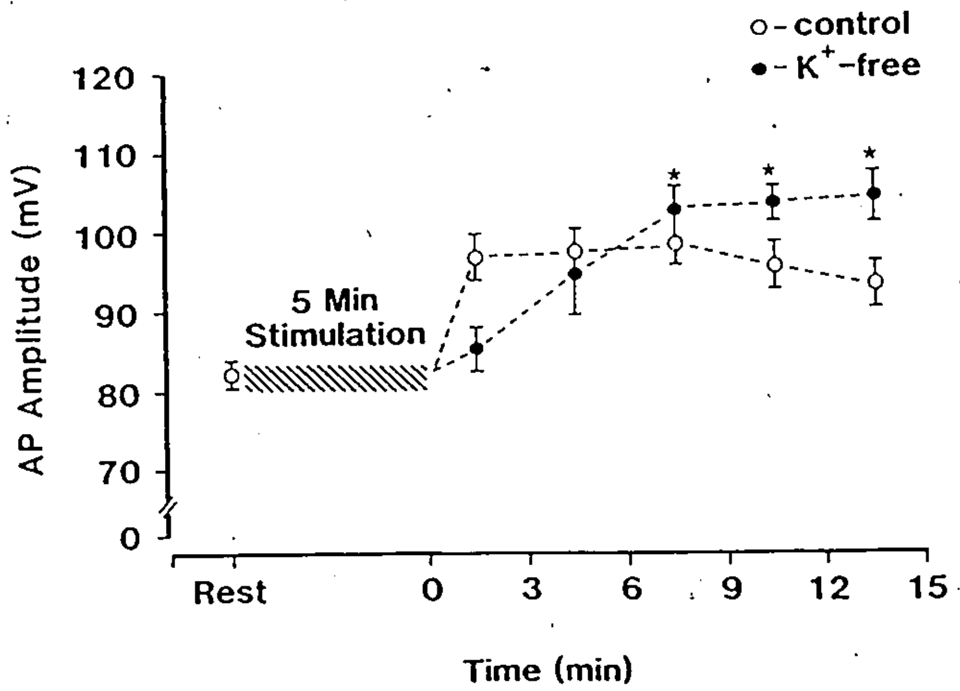
Effect of removal of $[K^+]_e$ at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the resting potential of rat soleus muscle fibres (n = 5). Values are mean ± standard error.
* Significant difference between control and K^+-free conditions (p<.05).

Figure 20B.

Effect of removal of $[K^+]_e$ at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the single fibre action potential of rat soleus muscle fibres (n = 5). Values are mean ± standard error.
* Significant difference in AP amplitude from rest (p<.05).



o - control
• - K+-free
-100
-90
-80
-70
0
Em (mV)
5 Min
Stimulation
*
Rest
0
3
6
9
12
15
Time (min)



o - control
• - K+-free
120
110
100
90
80
70
0
AP Amplitude (mV)
5 Min
Stimulation
*
*
*
Rest
0
3
6
9
12
15
Time (min)

potentials was probably due to renewed activity of the sodium pump, following diffusion of K^+ from deeper parts of the muscle; discussion of this possibility follows in the next chapter (pg 109).

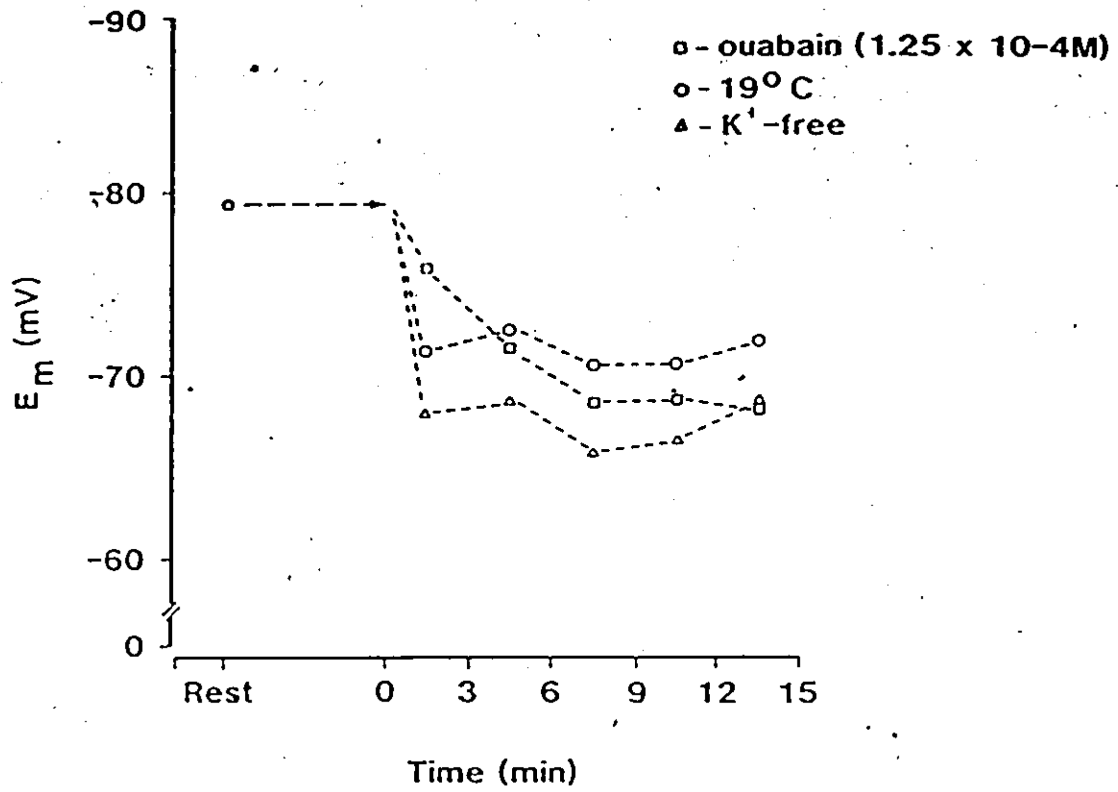
(d) Effect of ouabain, cooling, and removal of $[K^+]$ on unstimulated muscle. Estimation of the contribution of the Na^+-K^+ pump to the normal steady state membrane potential was done by studying the effects of the various pump inhibitors on unstimulated muscle fibres. These results are shown in Figure 21A. There was a significant decrease in the resting membrane potential from baseline with all three pump inhibitors; the mean decrease being 10.1 ± 2.4 mV ($p<.05$). There was no significant difference between the various pump inhibitors in the magnitude of the depolarization they induced. The depolarization resulting from pump inhibition was evident within 3 minutes when cold or removal of K^+ was utilized; the effect of ouabain on membrane potential took slightly longer, with significant depolarization being evident within 3-6 minutes. Figure 21B illustrates the effect of pump inhibition on the action potential amplitude; a significant decrease was observed with all three methods ($p<.05$).

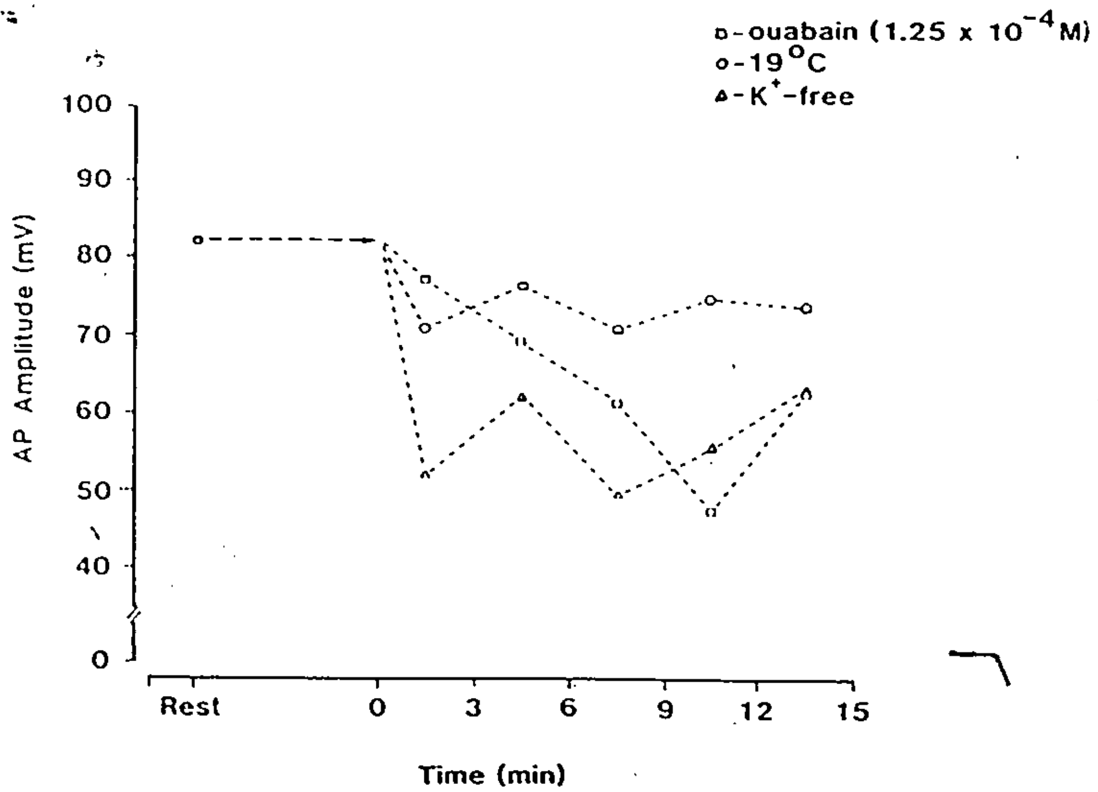
Figure 21A.

Effect of ouabain (1.25 x 10^{-4}M; n = 3), cooling (19°C; n = 3) or removal of extracellular [K^{+}] (n = 5) on the resting potential of unstimulated rat soleus muscle fibres.

Figure 21B.

Effect of ouabain (1.25 x 10^{-4}M; n = 3), cooling (19°C; n = 3), or removal of extracellular [K^{+}] (n = 5) on the single fibre action potential of unstimulated rat soleus muscle.


-90
-80
-70
-60
0
E_m (mV)
o - ouabain (1.25 x 10-4M)
o - 19° C
▵ - K⁺-free
Rest
0
3
6
9
12
15
Time (min)


o - ouabain (1.25 x 10⁻⁴M)
o - 19°C
▵ - K⁺-free
100
90
80
70
60
50
40
0
AP Amplitude (mV)
Rest
0
3
6
9
12
15
Time (min)

(e) Effect of 20mM $K^{\pm}$. In the final part of the study, an attempt was made to estimate the magnitude of the electrogenic component of the Na^{+}-K^{+} pump. To do this, soleus muscles were tetanized for 5 minutes; following which the bathing fluid was rapidly exchanged for one containing 20mM K^{+}. This concentration was chosen to reduce the theoretical E_K to a level well below the control resting potential, so that the size of the electrogenically-derived potential would be more readily apparent.

It was found that, in the first 3 minutes of the recovery period, the mean resting potential of fibres bathed in 20 mM K^{+} was -79.5 ± 8.6 mV, which was identical to the pre-stimulation value (n = 5). However, this value was remarkably different from that measured in unstimulated muscle fibres exposed to the same high K^{+} medium. Fibres in unstimulated muscle underwent an immediate depolarization in the high K^{+} medium, the mean resting potential falling to 59.9 ± 3.5 mV in the first 3 min (Figure 22A).

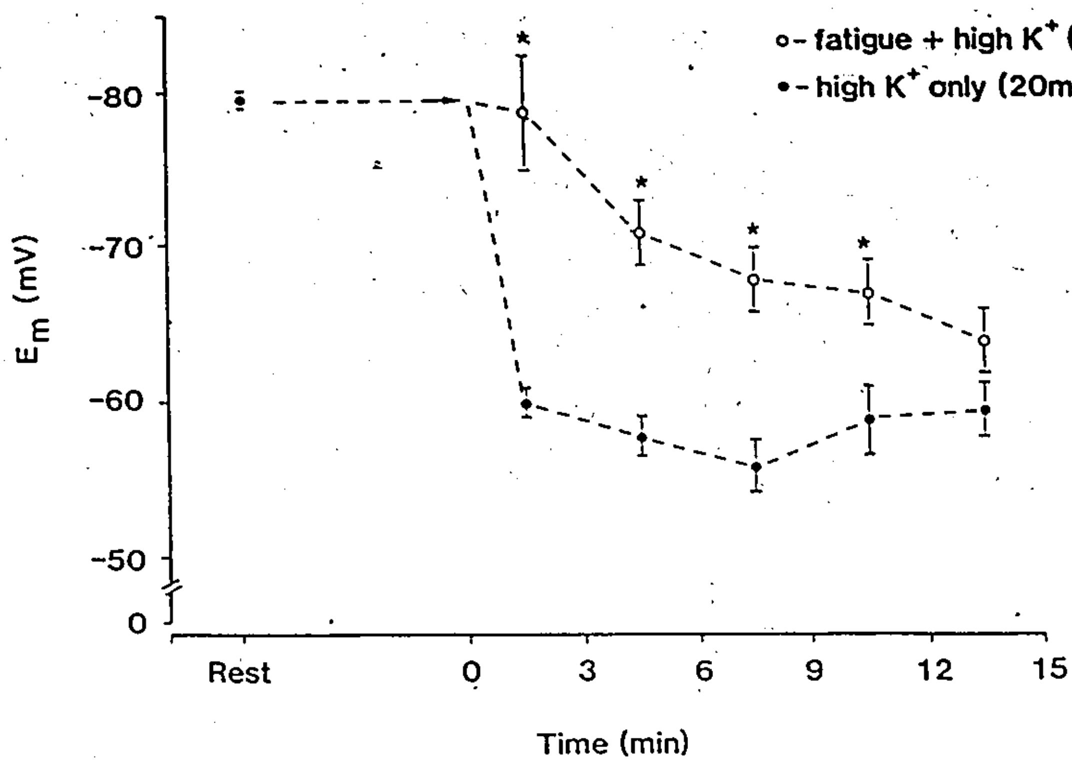
The increase in $[K^{+}]_{o}$ had a significant effect on the action potentials of stimulated muscle fibres; the mean value declined from 82.2 ± 10.8 mV to 59.7 ± 8.3 mV (Figure 22B). By 6 minutes after the end of tetanic stimulation no action potentials could be evoked by indirect stimulation and by this time the mean resting

Figure 22A.

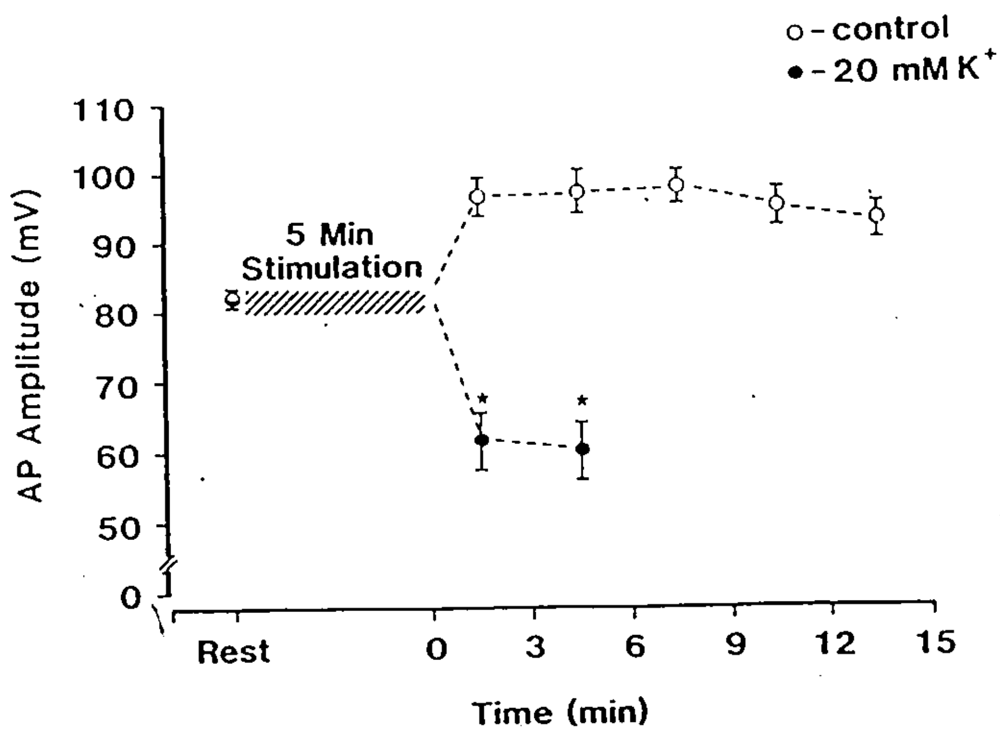
Effect of 20mM $[K^+]_e$ on the resting membrane potential of stimulated (○) versus unstimulated (●) rat soleus muscle fibres (n = 5). Values are mean ± standard error.
* Significant difference between fatigue plus 20mM K^+ and 20mM K^+ only ($p<.01$).

Figure 22B.

Effect of 20mM $[K^+]_e$ added at the end of 5 min of intermittent tetanic stimulation (20 Hz) on the single fibre action potential of rat soleus muscle fibres (n = 5). Values are mean ± standard error.
* Significant difference between control and 20mM K^+ conditions ($p<.05$).



o- fatigue + high K+ (20mM)
•- high K+ only (20mM)
Em (mV)
-80
-70
-60
-50
0
Rest
0
3
6
9
12
15
Time (min)



o- control
•- 20 mM K+
AP Amplitude (mV)
110
100
90
80
70
60
50
0
5 Min
Stimulation
Rest
0
3
6
9
12
15
Time (min)

potential had fallen to 68.1 ± 5 mV. In unstimulated muscle fibres, the high K^+ bathing medium depolarized the surface fibre membranes to such an extent that they were inexcitable, and action potentials could not be evoked.

The results from all the studies on the resting and action potentials in the rat soleus muscle are presented in Table 3.

Table 3.

Summary of results of resting and action potential measurements in stimulated rat soleus muscles under different conditions. Values are means ± standard deviations.

Time = minutes post stimulation.
E_m = resting membrane potential
AP = action potential amplitude
OS = action potential overshoot

Table 3.

Experimental Condition	Time (min)	E_m (mV)	AP (mV)	OS (mV)
Rest		-79.5 ± 4.8	82.2 ± 10.8	4.0 ± 12.9
Stimulation	1.5	-90.9 ± 4.7	97.0 ± 9.4	6.2 ± 8.8
	4.5	-92.6 ± 4.2	97.4 ± 10.1	4.8 ± 13.3
	7.5	-91.6 ± 4.3	98.1 ± 7.8	7.7 ± 6.4
	10.5	-88.2 ± 6.1	94.5 ± 9.4	8.4 ± 12.2
	13.5	-88.2 ± 6.1	91.6 ± 9.4	5.8 ± 7.8
Stim. + ouab. (1.25×10^{-4}M)	1.5	-74.1 ± 4.6	71.0 ± 8.7	-3.1 ± 12.7
	4.5	-76.0 ± 3.5	69.9 ± 4.4	-6.0 ± 8.4
	7.5	-74.2 ± 3.9	71.8 ± 6.9	-2.5 ± 4.2
	10.5	-73.4 ± 3.7	66.0 ± 7.3	-7.4 ± 11.6
	13.5	-70.1 ± 2.5	61.8 ± 6.7	-8.2 ± 8.9
Stim. + 19°C	1.5	-77.0 ± 5.3	83.8 ± 8.1	8.0 ± 9.3
	4.5	-77.2 ± 4.7	90.0 ± 13.3	13.0 ± 0.8
	7.5	-78.7 ± 2.6	93.6 ± 7.9	15.2 ± 8.5
	10.5	-79.9 ± 8.6	99.4 ± 7.6	21.0 ± 6.6
	13.5	-79.9 ± 4.7	95.8 ± 11.2	18.3 ± 5.9
Stim. + 0mM K^+	1.5	-82.5 ± 4.5	85.0 ± 5.2	2.4 ± 2.0
	4.5	-91.1 ± 6.4	98.0 ± 10.1	6.9 ± 5.3
	7.5	-91.7 ± 4.6	102.9 ± 6.5	11.2 ± 5.2
	10.5	-92.5 ± 4.0	103.4 ± 4.6	10.8 ± 3.8
	13.5	-90.1 ± 5.6	103.9 ± 6.5	13.9 ± 2.8
Stim. + 20mM K^+	1.5	-79.5 ± 8.6	59.7 ± 8.3	-19.8 ± 14.5
	4.5	-71.5 ± 4.9	63.1 ± 8.7	-10.5 ± 6.7
	7.5	-68.1 ± 5.0		
	10.5	-67.2 ± 4.5		
	13.5	-63.7 ± 4.7		

4. Discussion and Conclusions

This thesis was designed to study the potentiation of muscle compound action potentials (M-waves) during stimulated and voluntary activity. After ruling out several mechanisms that could be involved, it was hypothesized that a hyperpolarization of the muscle fibre membrane due to increased activity of the electrogenic Na^+-K^+ pump was responsible for increasing the individual fibre action potentials, and hence, the M-wave, both during and following muscular activity. After initial observations of the behaviour of the muscle compound action potential (M-wave) in both humans and animals during stimulated and voluntary fatigue, an in vivo preparation of the rat soleus muscle was employed to compare the M-wave changes with single fibre measurements of the resting membrane and action potentials. The role of the Na^+-K^+ pump in mediating these changes was then assessed by blocking the pump with several pump inhibitors. The results suggest that the rise in $[Na^+]_i$ and $[K^+]_o$ associated with impulse activity stimulates the membrane-bound Na^+-K^+-ATPase, causing a temporary hyperpolarization of the muscle fibre membrane. Moreover, the electrogenic contribution of the Na^+-K^+ pump to the resting membrane potential becomes substantially larger, thereby helping to maintain muscle membrane excitability during muscular activity.

4.1. M-wave during muscle activity in man

In the early part of this research, observations were made on the maximum muscle compound action potential, or M-wave, during indirect stimulation of the human tibialis anterior muscle. These results confirmed the finding of Fitch and McComas (1985) that repetitive stimulation of the ischemic muscle induces a transient enlargement of the M-wave. Little attention has been given to this phenomenon in previous studies; this omission is rather surprising since later changes in the M-wave during repetitive electrical simulation, such as the decline in amplitude and prolongation, have been investigated in detail (Bigland-Ritchie et al, 1979; 1982). Passing references to action potential enlargement have been made in studies employing repetitive stimulation of single motor units (Sandercock et al, 1985) and of whole muscles (Uramoto, et al, 1983; Fitch and McComas, 1985), while in EMG textbooks the M-wave enlargement is termed 'pseudofacilitation' (Kimura, 1983). There do not appear to be any previous reports of M-wave enlargement during recovery from fatigue although it is evident in the study of Stevens and Taylor (1972), and in the more recent one by Milner-Brown and Miller (1986; see Figure 2, Section 1.4.1). The discrepancy between the sizes of the M-waves during recovery and in the control period in the Stevens and Taylor (1972) study have been interpreted as

indicating that the control stimuli were not supramaximal (Bigland-Ritchie et al, 1982), but the results from this thesis offer an alternative explanation, namely that the fibre action potentials had increased in amplitude.

It is clear that the potentiation of the M-wave is not a mechanical artefact due to shortening of the muscle belly under the stigmatic recording electrode, since increased M-waves were seen in response to single stimuli, at a time when the twitch response had barely started, in all of the present experiments. Fitch and McComas (1985) had also dismissed the possibility that M-wave potentiation was due to movement artefact since they noted that during continuous stimulation, the enlargement of the M-wave began some seconds after the muscle had shortened to its tetanic length.

The experiments on the human thenar and EDB muscles demonstrated that M-wave enlargement also occurred under physiological circumstances, in which muscles underwent repeated voluntary contractions without the use of an arterial occlusion cuff. These experiments were technically more satisfactory than the earlier ones utilizing repetitive stimulation of tibialis anterior in that the small muscles studied, the extensor digitorum brevis and the median-innervated thenar group, had focal innervation zones and therefore yielded simpler, biphasic, M-waves for analysis. Not only were the amplitudes of the

M-waves measured in these last experiments but also the voltage-time integrals ('areas'). Both of these variables increased significantly as a result of voluntary effort in the thenar and EDB muscles. The time course of enlargment of the M-waves, however, was different in the two muscles. Whereas in the thenar muscle the M-wave had reached its maximum potentiation during the voluntary effort, in the EDB muscle the greatest enlargment of the M-wave was observed in the recovery period following the voluntary effort. The significance of these differences is difficult to explain, but it may be related to the activity/patterns of the two muscles or perhaps the fibre type distributions, both of which may affect the number or activity of the Na^{+}-K^{+} pumps.

In the intrinsic muscles of both the hand and the foot the increase in M-wave area was at least as great as the change in peak-to-peak amplitude. This finding was important when the possibility was considered that the increase in M-wave amplitude was due to better synchronization, and hence summation, of the single muscle fibre action potentials. Such an explanation had been put forward by Duchateau and Hainaut (1984) who postulated that neuromuscular transmission had been facilitated by activity. However, an explanation of this kind is unlikely on theoretical grounds since the interval between the arrival of the impulse in the motor nerve terminals

onset of the end-plate potential is normally 0.2 ms (Hubbard and Schmidt, 1963) and the release of transmitter is spread over 0.3 ms (Eccles and Liley, 1959); both times being short in comparison with the duration of the M-wave (10-15 msec).

Based on these arguments, it was then necessary to investigate the one remaining possibility, namely, that the increase in M-wave amplitude was the result of larger single fibre action potentials. To pursue this possibility, an animal model of M-wave potentiation was sought.

4.2. M-wave during muscle activity in rat soleus

Before embarking on the experiments employing microelectrode measurements of resting and action potentials in the rat soleus, it was important to establish whether or not the compound action potential of this muscle would behave in a similar way to human M-waves. The results of the initial experiments on the rat soleus showed very clearly that this was the case, the M-wave potentiating by a mean of 49%. This led to the next series of experiments involving intracellular measurements of resting and action potentials in stimulated rat soleus muscle, to determine if the increased M-wave was due to a hyperpolarization of the muscle fibre membrane.

4.3. Microelectrode recordings in rat soleus muscle fibres

4.3.1. Comparison of resting potentials to other published values.

The mean value of the resting membrane potential in the present study was -79.5 ± 4.8 mV which falls in the range of other reported values for rat muscle in Table 4. From this Table it can be seen that the published values vary considerably, ranging from -62 to -83 mV.

Part of the variability may be due to the lower temperatures employed in some of the in vitro investigations; thus a lower temperature would affect the membrane potential through the rate of diffusion, reflected in the GHK equation, and through reduced activity of the Na^+-K^+ pump (see below). A more important source of variability is likely to have been the presence or absence of protein in the bathing fluid. Creese and Northover (1961) observed that the sodium content of isolated rat diaphragm was markedly increased if plasma proteins were absent from the bathing fluid, and Kernan (1963) found that the mean membrane potential of rat EDL muscle fibres was -12.4 mV larger when the muscle was immersed in plasma rather than in Krebs-Ringer solution.

Table 4.

Published values of resting membrane and action potentials in rat muscle. Values are mean ± standard deviation.

EDL = extensor digitorum longus
gastroc. = gastrocnemius
N/A = not given

Table 4.

Ref.	Muscle	Preparation	t°	Protein?	E_{ms} (mV)	AP (mV)
4	Rat soleus	in vitro	25°	no	-69.0 ± 5.5	91 ± 12.9
4	Rat EDL	in vitro	25°	no	-72.0 ± 3.4	89 ± 14.6
174	Rat soleus	in vitro	26°	no	-66.0 ± 3.7	65.4 ± 8.0
174	Rat EDL	in vitro	26°	no	-78.2 ± 6.6	87.6 ± 4.0
65	Rat soleus	in vitro	37°	no	-73.7 ± 2.8	93.8 ± 6.2
65	Rat EDL	in vitro	37°	no	-77.1 ± 3.9	102.9 ± 6.8
108	Rat soleus	in vivo	N/A	no	-68.8	
108	Rat gastroc.	in vivo	N/A	no	-83.5	
56	Rat soleus	in vivo	31°	no	-73.4 ± 3.2	
56	Rat gastroc.	in vivo	31°	no	-76.7 ± 2.9	
120	Rat diaphragm	in vitro	25°	no	-62.2 ± 2.2	84.3 ± 2.5

There is also more recent evidence that the membrane conductances of sodium, potassium, and chloride may all be affected by the presence or absence of a plasma protein fraction (Page, et al, 1980; Macchia, et al, 1984; Shetty, et al, 1985).

The differences in E_m between fast and slow muscles of the rat have generally been attributed to higher $[Na^+]_i$ and lower $[K^+]_i$ in the soleus versus the fast extensors (Sreter & Woo, 1963; Yonemura, 1967).

4.3.2. Effect of activity on resting membrane potential

In all the experiments employing intermittent repetitive stimulation of the rat soleus, there was an increase in resting potential and there was strong evidence to suggest that this was due to the electrogenic Na^+-K^+ pump (see below). The hyperpolarization was striking; the mean increase in membrane potential was approximately -12 mV, a value similar to that reported recently following repetitive stimulation in mouse soleus muscle (Fong, Atwood and Charlton, 1986). Except for a brief mention of an increased membrane potential in rat soleus muscle fibres after repetitive stimulation (Hanson, 1974), there are no other reports of such a phenomenon; in fact, activity-induced depolarization of the muscle membrane has traditionally been found in both whole mammalian muscles (Hanson, 1974; Juel, 1986; Metzger and Fitts, 1986) and

amphibian single fibres (Grabowski, et al, 1972; Westerblad and Lannergren, 1986). None of these studies, however, were conducted under conditions in which the Na^{+}-K^{+} pump would be optimally activated, either because the temperature was less than 37°C or, in the case of the single fibre studies, the preparation itself prevented any appreciable change in external ion concentrations. Furthermore, protein was not added to the bathing media.

Stimulus-induced depolarization has also been reported in vivo (Locke and Solomon, 1967). It is likely, however, that in those experiments the impulse-mediated K^{+} efflux caused a fall in E_K which could not be compensated for by the electrogenic sodium pump. Thus, the stimulus frequency of 300 Hz employed by these authors was far higher than the physiological range (Kugelberg, 1973) and higher also than the frequency used in the present study (20 Hz).

The time course of the muscle membrane hyperpolarization in the present study was of interest in that the hyperpolarization was already well established at the end of the stimulation period and persisted for more than 15 minutes. The effects of shorter periods of stimulation were not investigated but future studies might determine what amount of activity is necessary to bring about a detectable rise in resting membrane potential.

4.3.3. Comparison of action potentials to other published values.

Table 4 gives published values for the mean amplitudes of action potentials in rat muscle fibres. These values may be compared with the mean value of 82.2 ± 10.8 mV found in the present study. As with the resting potentials , the reported values vary widely and probably for the same reasons (Section 4.3.1).

4.3.4. Effect of activity on the action potential

After intermittent tetanic stimulation the mean action potential amplitude was found to have increased by 14.9 mV from its control (resting) value. This increase, like the hyperpolarization, was evident in the earliest recordings and persisted for at least 15 minutes. The time-course of the change in single fibre action potentials differed from that of the M-wave in some muscles, since the latter was slower and the peak amplitude was not achieved for at least 5 minutes. It is probable that the discrepancy was due to the different fibre populations involved in the two measurements. Whereas the M-wave reflected the impulse activity of the entire muscle, the microelectrode recordings were made from only the most superficial fibres. In the latter situation most of the K^+ released by impulse activity would be expected to diffuse into the bathing medium and thus tend to cause only limited

depolarization. For deeper fibres, however, K^+ from impulse activity might be temporarily confined to the interstitial spaces and would tend to cause a depolarization, the magnitude of which could be predicted by the GHK equation. The time required for this K^+ to either diffuse to the surface fibres or be pumped back into the fibre could explain the delayed rise in the M-wave in the recovery period.

Only one other investigation has reported an increased amplitude of single fibre action potentials after continuous stimulation of rat muscle. Metzger and Fitts (1986), employing continuous 5 Hz (1.5 min) or 75 Hz (1 min) stimulation of rat diaphragm muscle, noted a significant increase in action potential amplitude during recovery. In addition, Stalberg (1966) showed a significant increase in extracellularly recorded single muscle fibre action potentials during human voluntary contractions.

4.4. Electrogenic Na^+-K^+ pump activity

4.4.1. Findings at rest.

In an earlier section (1.7) the evidence was presented for the existence of a Na^+-K^+-ATPase as part of the surface membranes of mammalian striated muscle fibres. It was shown that under specific experimental conditions, such as sodium loading (Kernan,

1968) or the administration of muscle depolarizing drugs (Creese et al, 1987), the Na^+-K^+ pump was capable of generating a substantial electrogenic potential across the sarcolemma. In resting muscle fibres, however, the contribution of the pump is much smaller and may be predicted from the Mullins-Noda (1963) equation, in which:

$$3) \qquad E_m = \frac{RT}{F} \ln \frac{rK^{+}[K^{+}]_o + bNa^{+}[Na^{+}]_o}{rK^{+}[K^{+}]_i + bNa^{+}[Na^{+}]_i}$$

where E_m is the resting potential, R is the universal gas constant, T is the absolute temperature, F is the Faraday, b is the ratio of Na^+ to K^+ permeability, and r is the coupling ratio of Na^+ to K^+ during sodium pumping.

In the surface fibres examined in the present experiments, E_m was -79.5 mV while the values for $[K^+]_o$ and $[Na^+]_o$ were those of the bathing fluid and were 4.5 mM and 140 mM respectively. Assuming the coupling ratio, r, to be 1.5 (Thomas, 1972) and taking Sreter's (1963) values of 163 mM and 13 mM for $[K^+]_i$ and $[Na^+]_i$ respectively in rat soleus, then b must be .04, which is similar to Yonemura's (1967) estimate of .03.

$$-79.5 = \frac{RT}{F} \ln \frac{1.5(4.5) + b(140)}{1.5(163) + b(13)}$$

$$b = .04$$

When this value for b is used in the GHK equation, the calculated value for E_m becomes -73.9 mV, suggesting that

the electrogenic contribution of the sodium pump is -(79.5 - 73.9) = -5.6 mV. This value is rather smaller than the depolarizations observed after inhibition of the sodium pump by ouabain. For example, Locke and Solomon (1967) reported a drop of -15 mV in rat gastrocnemius after ouabain while Williams *et al* (1971) observed a -25 mV depolarization in rat gracilis fibres. In the present study inhibition of the pump in rested muscle fibres produced an average -10 mV depolarization. The larger values reported by Locke and Solomon (1967) and by Williams *et al* (1971) may have been due partly to more complete inhibition of the pump, with greater loss of the electrogenic component of the resting membrane potential. Another factor may have been the gradual running-down in the $[K^+]_i/[K^+]_o$ gradient, with consequent depolarization, due to pump inhibition. This factor may be especially pertinent in the study of Locke and Solomon (1967) in which the stimulus frequency of 300 Hz was far higher than the physiological range (Kugelberg, 1973).

4.4.2. Pump activity after muscle stimulation

(a) Experimental design considerations. Experiments were undertaken to explore the possibility that the membrane hyperpolarization and muscle fibre action potential enlargement, observed after repetitive stimulation, were due to enhanced electrogenic Na^+-K^+ pump

activity. The studies in which the M-wave was recorded in the rat soleus after intraperitoneal injection of ouabain were inconclusive (Section 3.2); enlargement of the M-wave could still be seen after tetanic stimulation even though the response was depressed before the stimulation began. It is possible that this paradoxical behaviour was due to inhomogeneities among the muscle fibre population in the presence of ouabain. Thus, some fibres would undergo depolarization block, due to inhibition of the pump, and would be responsible for the declining M-wave; other fibres, not yet affected by ouabain, might be able to develop larger electrogenic potentials due to stimulation of the pump. These possibilities could only be resolved by recording from single fibres rather than entire populations.

A related problem was to ensure that the methods used to inhibit the pump did not interfere with the ionic perturbations associated with the tetanic stimulation i.e. K^+ efflux and Na^+ influx. For this reason, the inhibitory challenge could only be given at the end of the period of tetanic stimulation. This consideration led to the use of bath-applied ouabain; also, by developing a means of rapidly changing the bathing fluid, it was possible to inhibit the pump by cooling and by withdrawing extracellular K^+ at the end of the stimulation period. Even though the deeper fibres of the muscle would not be

immediately affected by either ouabain or a change in the bathing medium, only the surface fibres were used for microelectrode impalement; thus any effect of the intervention should have been immediately obvious. This strategy proved very effective, combining the advantages of in vivo and in vitro techniques. A further reason for applying the inhibitory challenge at the end of the tetanic stimulation was that any latent pump sites might then have become unmasked (Grinstein & Erlij, 1974), and could have become more readily accessible to the particular pump inhibitor.

(b) Resting potential measurements. Regardless of the method employed to inhibit the pump (ouabain, cooling, or K^+ withdrawal), care was taken to ensure that the pump was not totally inactivated; had it been, the fibres would have become inexcitable and the action potential could not have been studied. Thus, under resting steady state conditions, inhibition of the Na^+-K^+ pump with either ouabain, cooling, or removal of $[K]_e$ caused a rapid depolarization of the surface fibres, ranging from -7.8 mV (cooling) to -11 mV (ouabain, K^+-free solution; Section 4.4.1.). However, if the same pump inhibitor was applied to stimulated muscle, the resting potentials were maintained fairly close to the initial baseline value of-79.5 mV, especially when cooling or removal of K^+ were used to inhibit the pump (see Figs 14-16). These results

provided strong evidence that the sodium pump had been responsible for hyperpolarizing the muscle fibres following repetitive stimulation.

The delayed rise in membrane potential in the K^+-free experiments deserves comment. Whereas the removal of K^+ would be expected to inhibit the Na^+-K^+-ATPase on the superficial fibres in contact with the bathing medium, there would presumably still be some active pump sites on the underside of those same fibres in contact with interstitial [K^+], owing to the cylindrical shape of the muscle fibre. Furthermore, in the case of the K^+-free bathing medium, it is unlikely that the pump activity was inhibited for more than a few minutes in the stimulated muscles, since diffusion of K^+ from deeper parts of the muscle into the vicinity of the surface fibres would renew activity of the pump. Renewed pump activity, coupled with the increased E_K, explains why the muscle fibres gradually became hyperpolarized in the K^+-free experiments.

(c) Action potential measurements. The application of ouabain, cooling or a K^+-free bathing solution caused an immediate reduction in the overshoot of the action potential following a period of repetitive stimulation (See Table 3). These results showed that the enlargement of the muscle fibre action potential, normally observed after tetanic stimulation, depended on Na^+-K^+ pump activity. However, in the cooling experiments, the action potential

began to enlarge as the recovery period continued. An increased overshoot following cooling was also found by Nastuk and Hodgkin (1950) in single amphibian muscle fibres. It is probable that the reduced temperature altered the Na^+ and K^+ channel kinetics such that the Na^+ channels were slower to inactivate and/or the K^+ channels were slower to open. According to the original Hodgkin-Huxley equations describing the action potential (1952d), the rate constants for the Na^+ and K^+ conductance changes increase threefold for every 10°C rise in temperature. Thus, the cold bathing medium in the present study may have altered the time course of the electrical changes constituting the action potential such that the peak of the action potential came closer to E_{Na}. Immediately after the stimulation period, E_{Na} was likely reduced due to the impulse-mediated rise in $[Na^+]_i$ and fall in $[Na^+]_o$. However, the gradual increase in the overshoot of the action potential, seen in the recovery period, suggested that enough sodium pumping was taking place to gradually restore the Na^+ concentration gradient, thereby increasing E_{Na} and the action potential overshoot. A delayed rise in action potential amplitude was also seen in the K^+-free experiments after tetanic stimulation. As with the delayed hyperpolarization in these conditions, the increase in action potential amplitude was probably due to renewed activity of the Na^+-K^+ pump following diffusion of K^+ from

deeper parts of the muscle into the vicinity of the surface fibres.

(d) <u>Experiments with 20 mM $[K^{\pm}]_o$</u>. The experiments employing a high K^+ bathing medium provided convincing evidence that the electrogenic contribution of the pump can increase substantially during heightened pump activity. After the addition of the high K^+ (20mM) medium to stimulated fibres the membrane potential predicted from the GHK equation (assuming $[Na^+]_i$ = 19mM, $[Na^+]_o$ = 120.0mM, $[K^+]_i$ = 152mM, from Sreter, 1963, and $[K^+]_o$ = 20mM) was -47.9mV. The measured membrane potential however, was -79.5mV, suggesting a -30mV electrogenic contribution. This value was strikingly different from the membrane potential of -58 mV recorded in unstimulated fibres exposed to the same high K^+ medium; this observation further emphasized how the electrogenic contribution of the Na^+-K^+ pump increases in response to contractile activity. A -30 mV electrogenic contribution may appear large but is commensurate with those found in other circumstances. Thus, Creese, Head and Jenkinson (1987) have recently shown that the sodium pump can contribute at least -33mV to the membrane potential in the presence of depolarizing drugs. Also, Kernan (1968) found that rat muscle fibres, which had been sodium loaded in a K^+-free medium, developed electrogenic potentials of -22mV on re-exposure to external K^+. The observation that the membrane potential gradually

decreased in the present experiments with high K^+ suggests that sodium pump activity was gradually decreasing as $[Na]_i$ was restored to normal values.

It is also possible to predict the size of the electrogenic component of the resting potential in the deeper fibres of the muscle, using ion concentrations found in other studies. Following the impulse-mediated rise in $[K]_o$, K^+ would be expected to diffuse relatively poorly away from the deeper fibres; hence higher values for $[K]_o$ would be anticipated in deeper parts of the muscle than close to the surface. Studies with ion-sensitive microelectrodes have yielded values of 7-10mM for interstitial K^+ following repetitive stimulation (Hnik et al, 1976; Hirche et al, 1980; Juel, 1986), though higher concentrations up to 14mM have occurred during voluntary contraction of human forearm muscles (Vyskocil, et al, 1983). Indeed, Saltin and co-workers (1988) have recently estimated that interstitial K^+ can be 15mM or higher at the end of sustained isometric contractions of human knee extensors. If, in the present study, the deeper fibres had similar resting potentials to the surface fibres after stimulation, with the same ionic concentrations as above, only $[K]_o$ = 9mM, then the electrogenic contribution may also have been as high as -30mV. Sjodin (1971) has demonstrated that the activity of the Na^+-K^+ pump is heightened with increasing interstitial $[K^+]$ in the range

of 0-10 mM.

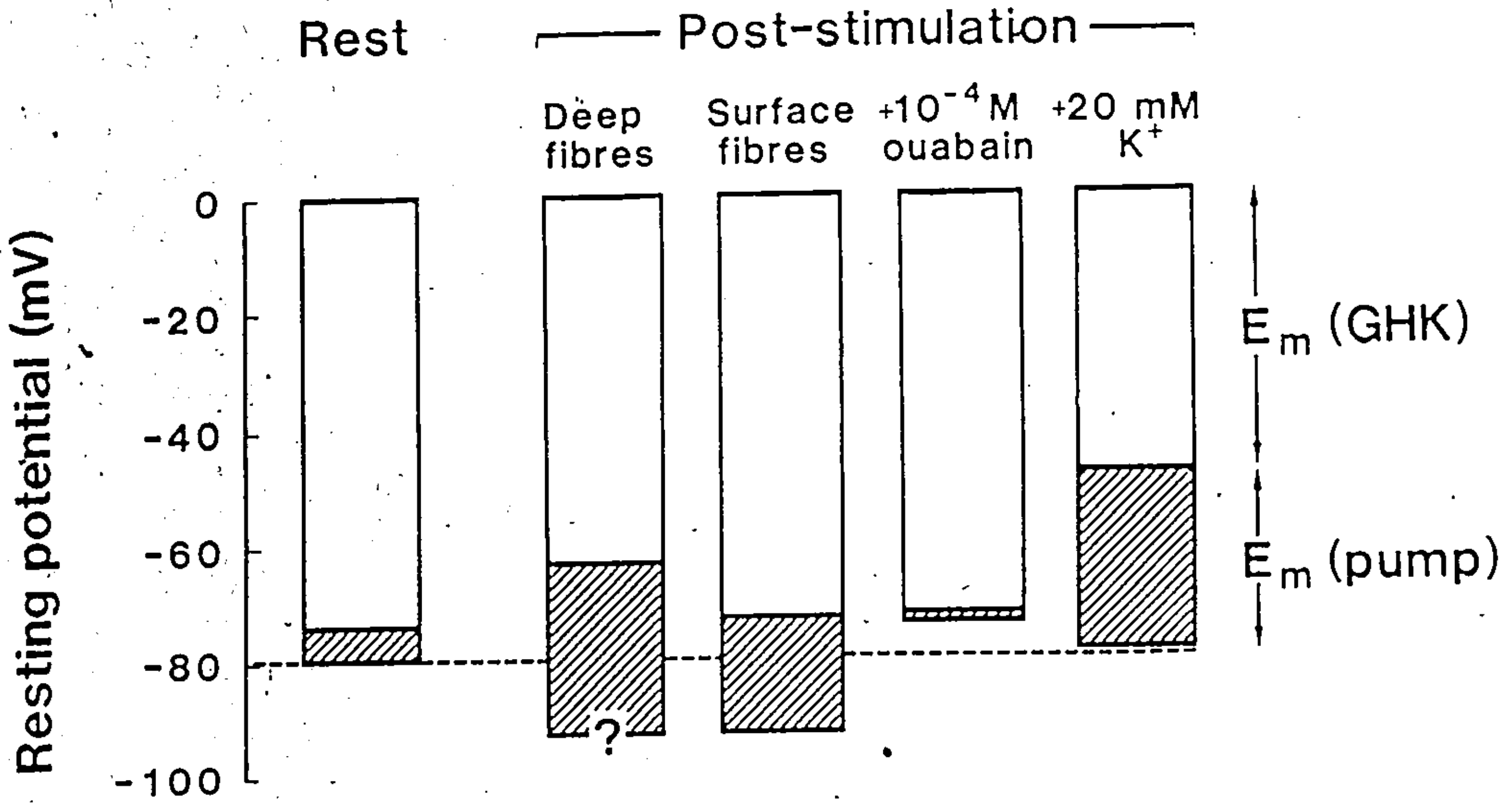
Figure 23 summarizes many of the preceding comments by showing the predicted contribution of the electrogenic Na^+-K^+ pump under various conditions.

4.5. Significance of $Na^{\pm}$-$K^{\pm}$ pump activity during muscular exercise.

The role of the Na^+-K^+ pump has traditionally been thought to be in the restoration of the ionic gradients for Na^+ and K^+. However, when faced with the challenge of continuing muscular activity, the pump also appears to play a major role in contributing to the membrane potential of the muscle fibre and thereby maintaining excitability. A more complete understanding of this function of the sodium pump can be had if one considers the potassium dynamics during muscular exercise. It has long been recognized that contractile activity causes a net efflux of K^+ from muscle fibres, resulting in an elevation of plasma K^+ (Fenn and Cobb, 1935; Fenn, 1938). With every impulse, K^+ is released by the muscle fibre into the interstitial fluid; at the same time there is an approximately equal entry of Na^+. Studies using bicycle exercise or running in humans have shown arterial K^+ concentration may reach 6-7 mM; values as high as 8 mM have been reported in individual cases (Sjogaard and Saltin, 1982; Medbo and Sejersted, 1985). However, if all the K^+ released by the muscle were

Figure 23.

Estimated contribution of the Na^+-K^+ pump to the resting membrane potential of surface and deep muscle fibres under different conditions. The value of the membrane potential predicted from the Goldman-Hodgkin-Katz (GHK) equation is also indicated.



Rest
Post-stimulation
Deep fibres
Surface fibres
+10⁻⁴M ouabain
+20 mM K⁺
Resting potential (mV)
0
-20
-40
-60
-80
-100
?
Em (GHK)
Em (pump)

to gain access to the venous blood, there is a risk that the concentration of K^+ would rise to a cardiotoxic level. Clinical and experimental observations have indicated that a rapid rise in plasma K^+ concentration to 8mM or more results in depressed excitation of atrial and Purkinje fibres followed by ventricular fibrillation or asystole (Pick, 1966; Ettinger, et al, 1974). Mohrman and Sparks (1974) measured changes in $[K^+]$ in the venous effluent of isolated dog hindlimb muscles following a brief tetanus, and predicted that less than 10% of the impulse-mediated efflux of K^+ was actually lost to the blood; the majority was maintained in the interstitium until it was taken back up by the muscle in recovery. However, this poses another problem; if the K^+ efflux is retained within the muscle belly, a K+-induced fall in muscle fibre membrane potential would be predicted from the GHK equation.

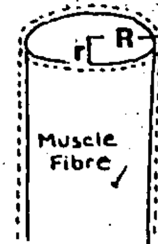
Potassium efflux during muscle activity has been determined with the aid of radioactive tracers. A value of 9.6 pmole/cm^2/impulse was given for single frog semitendinosus fibres (Hodgkin and Horowicz, 1959) and 10.7 pmole/cm^2/impulse for whole rat diaphragm muscles (Creese, et al, 1958). If the width of the interfibre cleft is taken as 1 um (Kugelberg, 1973; Carpenter & Karpati, 1984) and the equivalent circular diameter of a hexogonal fibre as 50 um, the ratio of intracellular to extracellular space would be 25:1. The corresponding increase in interfibre

[K] per impulse would be .2mM, based on the following calculations:

K^+efflux/fibre/impulse = $2\pi rl \times 10 \times 10^{-12}$ moles

Extracellular space = $\pi R^2 l - \pi r^2 l$

if $r = 25 \times 10^{-4}$ cm, then $R = 25.5 \times 10^{-4}$ cm



then,

Change in $[K]_e$ per impulse:

$$\frac{2\pi rl \times 10^{-11}\text{moles}}{\pi R^2 l - \pi r^2 l} = \frac{2r \times 10^{-11}}{R^2 - r^2} = 2 \times 10^{-7}\text{moles/cm}^3$$

$$= 0.2 \text{ mM}$$

Mohrman and Sparks (1974) predicted that interstitial $[K^+]$ in dog muscle increased by only .04-.075 mM/impulse during their 1 second tetanus at 32 Hz. They assumed, however, that 15% of the muscle tissue volume was interstitial (compared to the 4% prediction in the previous example). Using these values, they calculated that 10.8 - 19 pmoles of K^+ crosses each square centimeter of surface membrane per impulse, which was in the range of the direct tracer-flux studies cited above (Creese et al, 1958; Hodgkin and Horowicz, 1959).

Based on intracellular recordings made in situ in normal human muscle, the mean critical membrane potential was found to be -71 mV (McComas, et al, 1968). Thus, it would be reasonable to suppose that all fibres would

undergo depolarization block if the resting potential was held below this value, say at -63 mV. Whether one uses the predicted rise in $[K]_e$ of :2mM/impulse or .04mM/impulse just mentioned above, significant rises in $[K^+]_e$ could occur rapidly during a single maximum voluntary contraction, since motor units can discharge transiently at rates as high as 100 Hz (Marsden et al, 1973). A rise in $[K^+]_e$ to 9 mM would be expected to depolarize the muscle fibre membrane below the critical threshold. While it is evident that the Na^+-K^+ pump activity is increased during muscular exercise to cope with the rise in $[Na^+]_i$ and $[K^+]_e$, the uptake of K^+ between action potentials does not appear to equal the impulse-mediated efflux of K^+. This explains the net efflux of K^+ into the interstitium and blood. The electrogenic nature of the pump, however, serves to protect the muscle fibre membrane from the impending impulse-mediated depolarization block; by raising the membrane potential it renders the Na channels available for excitation while at the same time ensuring that the size of the action potential will be sufficient for onward propagation. This function of the sodium pump during stimulated or voluntary muscle contraction is additional to its role in restoring the intracellular concentrations of Na^+ and K^+ during the recovery period.

There is supporting evidence in the literature of enhanced sodium pump activity during muscular exercise.

Brodal and co-workers (1975) observed a 28% increase in the activity of Na^+-K^+-ATPase following indirect stimulation of rat hindlimb muscle. In the present study, the fact that membrane potentials were still elevated 15 min post-stimulation suggests that the Na^+-K^+-ATPase maintains its accelerated activity well into the recovery period. Hirche and co-workers (1980) observed that, after 10 minutes of recovery, dog gastrocnemius muscle had only regained approximately one third of the K^+ it had lost during 12 minutes of intermittent tetanic stimulation. This is commensurate with the maintained decline in plasma [K^+] during recovery from exercise reported in humans (Mebo and Sejersted, 1985; McKelvie, et al, 1987).

There are several reports of the effects of chronic muscular exercise on Na^+-K^+ pump activity; a 2.6 fold rise in Na^+-K^+-ATPase was observed in dog gracilis muscle after 6 weeks of training (Knochel, et al, 1985). Similarly, the concentration of [^{3}H] ouabain binding sites in rat hindlimb muscles has been shown to increase following 6 weeks of training (Kjeldsen, et al, 1986). Presumably, an increased sodium pump activity should manifest itself by either an increased resting membrane potential and/or a lowered $[K^+]_e$. Both of these observations have been cited; Moss and associates (1983) reported higher membrane potentials in trained versus untrained human anterior tibialis muscle. Similarly, in a longitudinal study, Knochel and co-workers

(1985) reported significantly higher membrane potentials in trained versus untrained dog gracilis muscles. The latter study also found that the ouabain-suppressible component of the resting membrane potential in the intercostal muscles increased from -9 mV to -33 mV after training, suggesting an enhanced electrogenic effect of the Na^+-K^+ pump in response to training. Also, the exercise-induced rise in plasma potassium is smaller after training in both humans (Tibes, et al, 1974; Kiens and Saltin, 1986; Kjeldsen et al, 1987) and animals (Knochel, et al, 1985), suggesting more efficient re-uptake of K^+ from the extracellular spaces.

4.6. Clinical Implications

4.6.1. Cardiac patients. It is known that circulating catecholamine levels rise during exercise (Christensen and Galbo, 1983), and the resulting B-adrenergic stimulation lowers plasma potassium levels by mediating cellular potassium uptake (Silva, et al, 1977). As mentioned in Chapter 1 (Section 1.7.8), the B_2-receptors are specifically involved in this response, and studies have shown that the exercise-induced rise in plasma potassium is exaggerated in the presence of B-blockade (Rosa, et al, 1980; Linton, et al, 1984; Williams, et al, 1985) due to impaired Na^+-K^+ transport. Clinically, this exaggerated rise in plasma potassium in propranolol-treated cardiac

patients might be a contributing factor to the increased muscle fatigue often reported in conjunction with B-blockade therapy (Carlsson, et al, 1978; Breckenridge, 1982).

4.6.2: Hyperkalaemic familial periodic paralysis. The absence of effective Na^+-K^+ transport during muscular exercise has also been connected to the rare clinical syndrome, hyperkalemic familial periodic paralysis. This disorder is manifested by periodic attacks of paralysis, most commonly occurring after exercise, although attacks may also be elicited by ingestion of K^+-rich nutrients or cooling (Streeten, et al, 1971; Clausen, et al, 1980). It has been suggested that these hyperkalemic attacks could be the result of deficient Na^+-K^+ transport (Brooks, 1969). Clausen and co-workers (1980), however, were unable to find any difference in the total number of ouabain binding sites or in the ouabain-sensitive component of ^{42}K-influx in cells from patients compared with controls. Nevertheless, the fact that the episodes of paralysis and hyperkalemia can be prevented by the administration of catecholamines (Wang and Clausen, 1976) or continued muscular exercise, strongly suggests that impaired Na^+-K^+ transport is at least one of the fundamental features of the disease.

4.6.3. Myotonic muscular dystrophy. Myotonic dystrophy is

another inherited disorder in which deficiencies in Na^+-K^+ transport could be implicated. This is exemplified by the work of Hull and Roses (1976) who found that although no differences appeared to exist between myotonic dystrophy patients and controls in mean ouabain-sensitive K^+-influx, there was a significant difference in the stoichiometric ratio of ouabain-sensitive transport of Na^+ and K^+. Thus, while the normal exchange ratio in erythrocytes was $3Na^+:2K^+$, in myotonic dystrophy patients the ratio was determined to be $2Na^+:2K^+$. This suggests that the pump in these patients is not electrogenic, and one would therefore predict that the muscle membranes would not hyperpolarize in the presence of heightened pump activity. Recent work conducted in this laboratory has shown that the time course of M-wave potentiation during voluntary fatigue is considerably slower in myotonic patients than in healthy normals. These last findings would be consistent with a decreased coupling ratio in these patients, or at the very least, a slower activation of the sodium pump. Hull and Roses (1976) have suggested that the abnormality in myotonic dystrophy patients might be the absence of, or low affinity for, one of the three Na binding sites on the Na^+-K^+-ATPase. Further work in this area is warranted to determine the exact nature of the muscle membrane disorder in myotonic dystrophy.

4.7. Future directions

The results from this thesis provide several directions for future research. Much of the Discussion revolved around the potassium dynamics of muscular exercise, and how it might affect muscle fibre excitability. That the Na^+-K^+ pump plays a major role in maintaining fibre excitability during activity has been quite well demonstrated, but in order to completely understand the extent to which the muscle can balance K^+ efflux with K^+ uptake, precise measurements of intra- and extracellular K^+ concentrations are needed.

These measurements could best be made with double-barrelled ion-sensitive microelectrodes, which would provide both measures of the membrane potential and of the concentration of K^+. In addition, it would be worthwhile to determine what proportion of $[K^+]_o$ lies in the interfibre and T-tubular compartments, since there is some evidence that the T-tubule system may act as a holding compartment for K^+ (Hodgkin & Horowicz, 1960; Grabowski, et al, 1972; Gonzalez-Serratos, et al, 1978). A temporary resevoir of this kind could serve to prevent too dangerous a rise in the concentration of K^+ in the circulation.

The presence of plasma protein in the bathing medium has been shown to be vital in the maintenance of the normal ion conductances across the muscle fibre membrane. That this fact has been neglected in most previous

electrophysiological studies of muscle fibre membranes is rather surprising. It would be interesting to know whether the protein effect is exerted entirely on the ion channels or whether the Na^{+}-K^{+} pump is also affected. One approach would be to stimulate muscles and then see if hyperpolarization occurred in the absence of protein. The component conductances of the membrane could also be determined by conventional intracellular stimulation and recording techniques (eg. Hutter and Noble, 1960).

As also mentioned in the Discussion, the results from this study have significant implications with regard to several clinical syndromes. In view of the close relationship shown in this study between the behavior of the compound muscle and single fibre action potentials in fatigue, relatively simple, non-invasive studies could be done on clinical populations that would yield meaningful information regarding the effectiveness of the Na^{+}-K^{+} transport system. For example: the experiments on M-wave behaviour in voluntary fatigue of thenar and EDB muscles could be performed on the following patient groups;

eg. a) hyperkalemic familial periodic paralysis

b) myotonic dystrophy

c) cardiac patients on ß-blockade therapy

d) cardiac patients on digitalis therapy

If, as suggested, each of the above patient groups have some abnormality in Na^{+}-K^{+} transport, it should be evident

in the behaviour of the M-wave during and following fatigue, i.e. potentiation should be reduced, delayed, or absent.

It would also be interesting to see if the variability in M-wave potentiation between muscles observed in this study could be related either to activity patterns in different muscles or to the distribution of Na^{+}-K^{+}-ATPase in different muscle fibre types.

5.0. Summary of Results

1. A study has been made of the M-wave (muscle compound action potential) during muscle activity.

2. In human subjects, the peak-to-peak amplitude of the M-wave was found to enlarge following voluntary contraction. M-wave potentiation was also evident during tetanic stimulation at 15 Hz, under ischemic conditions. The amount of M-wave enlargement varied considerably from one subject to another. As the muscle activity was continued, the enlargement was followed by a reduction in amplitude. In some subjects there was a further enlargement of the M-wave during the recovery phase.

3. The enlargement of the M-wave was associated with an equal, if not greater, increase in the voltage-time integral. This observation, and other considerations, made it unlikely that the greater amplitude of the M-wave could be accounted for solely by better synchronization of evoked muscle fibre action potentials.

4. The possibility was then explored that the M-wave enhancement resulted from potentiation of the individual muscle fibre action potentials. It is this work which formed the greater part of the thesis research.

5. In the soleus muscles of rats anaesthetized with sodium pentobarbitone, it was possible to show that the M-wave enlarged following, and occasionally during, intermittent repetitive stimulation at 20 Hz. The enlargement was maximal at 6 min post-stimulation, but was still present at 15 min post-stimulation. Ouabain, administered intraperitoneally in doses of 4-5 mg/kg, diminished the resting M-wave but did not completely abolish the increase resulting from tetanic stimulation.

6. Intracellular recordings were made from rat soleus muscle fibres _in vivo_, using glass microelectrodes filled with 3M KCl solution. In untreated muscles, the mean resting potential was -79.5 mV and the mean action potential amplitude was 82.2 mV. Following 5 min of intermittent tetanic stimulation (4 sec at 20 Hz, repeated every 5 s), the mean resting potential had increased to -92.6 mV and the mean action potential amplitude to 98.1 mV. Both elevations were maintained for approximately 5 min and then became smaller, but were still present at 15 min.

7. The immediate rises in resting and action potential following tetanic stimulation were abolished by any of the following 3 modifications to the mammalian bathing fluid:

(i) addition of ouabain at a concentration of 1.25 x

10^{-4}M, (ii) cooling from 37°C to 19°C, and (iii) removing K^+. These results were interpreted as evidence that the elevations in resting and action potential were dependent on the electrogenic effect of the Na^+-K^+ pump.

8. The electrogenic contribution of the Na^+-K^+ pump to the resting membrane potential was then assessed by replacing the bathing medium of stimulated muscle fibres with one containing a high concentration of K^+ (20mM). In spite of the high [K^+], the stimulated fibres were able to maintain membrane potentials approximately -30 mV greater than that predicted from the Goldman-Hodgkin-Katz (GHK) equation for at least 3 min. In unstimulated fibres the addition of a high K^+ medium caused an immediate depolarization to approximately -58 mV.

9. Enhanced activity of the Na^+-K^+ pump is envisaged as preventing depolarization block of muscle fibres during impulse-induced K^+ efflux, in addition to restoring the intracellular and extracellular concentrations of Na^+ and K^+ following muscular activity.

References

1. Abdel-Aziz, M., W. Manning, KM Ward and AC Wareham. (1985) ^{3}H-ouabain binding in normal and dystrophic mouse skeletal muscles and the effect of age. J. neurol. Sci. 70: 47-53.

2. Adrian, RH. (1956) The effect of internal and external potassium concentration on the membrane potential of frog muscle. J. Physiol. 133: 631-658.

3. Adrian, RH and CL Slayman. (1966) Membrane potential and conductance during transport of sodium, potassium, and rubidium in frog muscle. J. Physiol. 184: 970-1014.

4. Albuquerque, EX and S. Thesleff (1966) A comparative study of membrane properties of innervated and chronically denervated fast and slow skeletal muscles of the rat. Acta. Physiol. Scand. 73: 471-480.

5. Baker, PF, AL Hodgkin and TI Shaw. (1962b) The effects of changes of internal ionic concentrations on the electrical properties of perfused giant axons. J. Physiol. 164: 355-374.

6. Baker, PF, AL Hodgkin and TI Shaw. (1962a) Replacement of the axoplasm of giant nerve fibres with artificial solutions. J. Physiol. 164: 330-354.

7. Baker, PF. (1965) Phosphorus metabolism of intact crab nerve and its relation to the active transport of ions. J. Physiol. 180: 383-423.

8. Bernstein, J. (1902) Investigations on the thermodynamics of bioelectric currents. In: Cell Membrane Permeability and Transport, GR Kepner (ed) Dowden, Hutchison & Ross, Inc.: Stroudsberg, Pa., 1979.

9. Bigland-Ritchie, B, F. Bellemare and JJ Woods. (1986) Excitation frequencies and sites of fatigue. In: Human Muscle Power NL Jones, N. McCartney, AJ McComas (eds) Human Kinetics Publishers, Inc.: Champaign, Ill.

10. Bigland-Ritchie, B, DA Jones and JJ Woods. (1979) Excitation frequency and muscle fatigue: electrical responses during human voluntary and stimulated contractions. Exp. Neurol. 64: 414-427.

11. Bigland-Ritchie, B., CG Kukulka, OCJ Lippold and JJ Woods. (1982) The absence of neuromuscular transmission failure is sustained maximal voluntary contractions. J. Physiol. 330: 265-278.

12. Bonting, SL, KA Simon and NM Hawkins. (1961) Studies on sodium-potassium-activated adenosine triphosphatase. I. Quantitative distribution in several tissues of the cat. Arch. Biochem. Biohpys. 95: 416-423.

13. Boyle, PJ and EJ Conway. (1941) Potassium accumulation in muscle and associated changes. J. Physiol. 100: 1-63.

14. Bray, GM, W. Wilcox and AJ Aguayo. (1977) Ouabain binding sites in skeletal muscle from normal and dystrophic mice. J. Neurol. Sci. 34: 149-156.

15. Breckenridge, A. (1982) Jogger's blockade. Br. Med. J. 284: 532-533.

16. Brodal, BP, NL Eeg-Larsen, OE Iversen, E. Jebens and A. Roed. (1975) Enhanced Na,K-activated ATPase activity after indirect electric stimulation of rat skeletal muscle in vivo. Life Sci. 17: 329-332.

17. Brooks, JE. (1969) Hyperkalemic periodic paralysis. Intracellular electromyographic studies. Arch. Neurol. 20: 13-?.

18. Caldwell, PC, AL Hodgkin, RD Keynes and TI Shaw. (1960) The effects of injecting 'energy-rich' phosphate compounds on the active transport of ions in the giant axons of loligo. J. Physiol. 152: 561-590.

19. Caldwell, PC and RD Keynes (1959) The effect of ouabain on the efflux of sodium from a squid giant axon. J. Physiol. 148: 8-10P.

20. Carey, MJ and EJ Conway. (1954) Comparison of various media for immersing frog sartorii at room temperature, and evidence for the regional distribution of fibre Na^+. J. Physiol. 125: 232-250.

21. Carlsson, E., E. Fledenius, P. Lundborg and L. Svensson. (1978) Beta-adrenoceptor blockers, plasma potassium, and exercise. Lancet 2: 424-425.

22. Carpenter, S. and G. Karpati. (1984) Pathology of Skeletal Muscle. Churchill Livingstone: New York.

23. Chinet, A. and T. Clausen. (1984) Energetics of active sodium-potassium transport following stimulation with insulin, adrenalin or salbutamol in rat soleus muscle. Pfleugers Arch. 401: 160-166.

24. Christensen, J. and H. Galbo. (1983) Sympathetic nervous activity during exercise. Ann. Rev. Physiol. 45: 139-153.

25. Clausen, T. (1986) Regulation of active Na^+-K^+ transport in skeletal muscle. Phys. Rev. 66: 542-580.

26. Clausen, T., ME Everts and K. Kjeldsen. (1987) Quantification of the maximum capacity for active sodium-potassium transport in rat skeletal muscle. J. Physiol. 388: 163-181.

27. Clausen, T. and JA Flatman. (1977) The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. J. Physiol. 270: 383-414.

28. Clausen, T. and O. Hansen. (1974) Ouabain binding and Na^+-K^+ transport in rat muscle cells and adipocytes. Biochim. Biophys. Acta. 345: 387-404.

29. Clausen, T. and O. Hansen. (1982) The Na^+-K^+ pump, energy metabolism, and obesity. Biochem. Biophys. Res. Commun. 104: 357-362.

30. Clausen, T., O. Hansen, K. Kjeldsen and A. Norgaard. (1982) Effect of age, potassium depletion and denervation on specific displaceable ^{3}H-ouabain binding in rat skeletal muscle in vivo. J. Physiol. 333: 367-381.

31. Clausen, T. and PG Kohn. (1977) The effect of insulin on the transport of sodium and potassium in rat soleus muscle. J. Physiol. 265: 19-42.

32. Clausen, T., P. Wang, H. Orskov and O. Kristensen. (1980) Hyperkalemic periodic paralysis. Relationships between changes in plasma water, electrolytes, insulin and catecholamines during attacks. Scand. J. Clin. Lab. Invest. 40: 211-220.

33. Cole, KS. (1949) Some physical aspects of bioelectric phenomena. Proc. Nat. Acad. Sci: 35: 558-566.

34. Connelly, GM. (1959) Recovery processes and metabolism of nerve. Rev. Mod. Phys. 31: 474-484.

35. Conway, EJ and D. Hingerty. (1948) Relations betwen potassium and sodium levels in mammalian muscle and blood plasma. Biochem. J. 42: 372-376.

36. Creese, R. (1954) Measurement of cation fluxes in rat diaphragm. Proc. Roy. Soc. Biol. 142: 497-513.

37. Creese, R., JL D'Silva and J. Northover. (1958) Effect of insulin on sodium in muscle. Nature 181: 1278.

38. Creese, R., SD Head and DF Jenkinson. (1987) The role of the sodium pump during prolonged end-plate currents in guinea-pig diaphragm. J. Physiol. 384: 377-403.

39. Creese, R. and J. Northover. (1961) Maintenance of isolated diaphragm with normal sodium content. J. Physiol. 155: 343-357.

40. Curtis, HJ and KS Cole. (1940) Membrane action potentials from the squid giant axon. J. Cell. Comp. Physiol. 15: 147-157.

41. Dean, RB. (1941) Theories of electrolyte equilibrium in muscle. In: Cell Membrane Permeability and Transport. GR Kepner (ed) Dowden, Hutchinson & Ross, Inc.: Stroudsberg, Pa., 1979.

42. Desmedt, JE. (1953) Electrical activity and intracellular sodium concentration in frog muscle. J. Physiol. 121: 191-205.

43. Donnan. (1911) Theory of membrane equilibrium and membrane potential in the presence of nondialysing electrolytes. A contribution to physical-chemical physiology. In: Cell Membrane Permeability and Transport. GR Kepner (ed) Dowden, Hutchison & Ross, Inc.: Stroudsberg, Pa., 1979.

44. Dorup, I, K. Skajaa and T. Clausen. (1988) A simple and rapid method for the determination of the concentrations of magnesium, sodium, potassium and sodium, potassium pumps in human skeletal muscle. Clinical Sci. 74: 241-248.

45. Duchateau, J. and K. Hainaut. (1984) Isometric or dynamic training: differential effects on mechanical properties of a human muscle. J. Appl. Physiol. 56: 296-301.

46. Dulhunty, AF. (1978) The dependence of membrane potential on extracellular chloride concentration in mammalian skeletal muscle fibres. J. Physiol. 276: 67-82.

47. Eccles, and Liley, (1959). Factors controlling the liberation of acetylcholine at the neuromuscular junction. Am. J. Phys. Med. 38: 96-103.

48. Edwards, RHT. (1983) Biochemical bases of fatigue in exercise performance: catastrophe theory of muscle fatigue. Int. Ser. Sprts Sci. 13: 3-28.

49. Edwards, RHT, DK Hill,k DA Jones and PA Merton. (1977) Fatigue of long duration in human skeletal muscle after exercise. J. Physiol. 272: 769-778.

50. Ettinger, PO, TJ Regan and HA Oldewurtel. (1974) Hyperkalemica, cardiac conduction, and the electrocardiogram: a review. Am. Heart J. 88: 360-371.

51. Evans, RH and JW Smith. (1973) Mode of action of catecholamines on skeletal muscle. J. Physiol. 232: 81P.

52. Fenn, WO and DM Cobb. (1936) Electrolyte changes in muscle during activity. Am. J. Physiol. 115: 345-356.

53. Fenn, WO. (1938) Factors affecting the loss of potassium from stimulated muscles. Am. J. Physiol. 124: 213-229.

54. Fitch, S. and AJ McComas. (1985) Influence of human muscle length on fatigue. J. Physiol. 362: 205-213.

55. Flatman, JA and T. Clausen. (1979) Combined effects of adrenalin and insulin on active electrogenic Na^+-K^+ transport in rat soleus muscle. Nature. 281: 580-581.

56. Fong, CN, HL Atwood, KN Jeejeebhoy and MP Charlton. (1987) Nutrition and muscle potassium: differential effect in rat slow and fast muscles. Can. J. Physiol. Pharmacol. 65: 2188-2190.

57. Fong, CN, HL Atwood and M. Charlton. (1986) Intracellular sodium-activity at rest and after tetanic stimulation in muscles of normal and dystrophic (dy^{2J}/dy^{2J})C57BL/6J mice. Exp. Neurol. 93: 359-368.

58. Frumento, AS. (1965) Sodium pump: its electrical effects in skeletal muscle. Science. 147: 1442-1443.

59. Garrahan, PJ and IM Glynn. (1967) The stoichiometry of the sodium pump. J. Physiol. 192: 217-235.

60. Garner, S, A. Hicks and AJ McComas. (1986) M-wave potentiation during fatigue and recovery in man and rat. J. Physiol. 377: 108P.

61. Goldman, DE. (1943) Potential, impedence and rectification in membrane. J. Gen. Physiol. 27: 37-60.

62. Grabowski, W., A. Lobsiger and H. Luttgau. (1972) The effect of repetitive stimulation at low frequencies upon the electrical and mechanical activity of single muscle fibres. Pfleugers Arch. 334: 222-239.

63. Graham, J. and RW Gerard. (1946) Membrane potentials and excitation of impaled single muscle fibres. J. Cell. Comp. Physiol. 28: 99-117.

64. Grinstein, S. and D. Erlij. (1974) Insulin unmasks latent sodium pump sites in frog muscle. Nature 251: 57-58.

65. Hanson, J. (1974) The effect of repetitive stimulation on the action potential and the twitch of rat muscle. Acta. Physiol. Scand. 90: 387-400.

66. Harris, EJ. (1967) The stoichiometry of sodium ion movement from frog muscle. J. Physiol. 193: 455-458.

67. Harris, JB and AR Luff. (1970) The resting membrane potential of fast and slow muscle fibres in the developing mouse. Comp. Biochem. Physiol. 33: 923-931.

68. Hays, ET, TM Dwyer, P. Horowicz and JG Swift. (1974) Epinephrine action on sodium fluxes in frog striated muscle. Am. J. Physiol. 227: 1340-1347.

69. Hirche, HE, E. Schumacher and H. Hagemann. (1980) Extracellular K^+ concentration and K^+ balance of the gastrocnemius muscle of the dog during exercise. Pfleugers Arch. 387: 231-237.

70. Hnik, PH, I. Krekule, N. Kritz, J. Mejsnar, V. Smiesko, E. Vjec and F. Vyskocil. (1976) Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. Pfleugers Arch. 362: 85-94.

71. Hodgkin, AL. (1951) The ionic basis of electrical activity in nerve and muscle. Biol. Rev. 26: 338-409.

72. Hodgkin, AL and B. Katz. (1949) The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. 108: 37-77.

73. Hodgkin, AL and P. Horowicz. (1959) Movements of Na and K in single muscle fibres. J. Physiol. 145: 405-432.

74. Hodgkin, AL and AF Huxley. (1939) Action potentials recorded from inside a nerve fibre. Nature. 144: 710.

75. Hodgkin, AL, AF Huxley and B. Katz. (1952a) Measurement of current-voltage relations in the membrane of the giant axon of loligo. J. Physiol. 116: 424-448.

76. Hodgkin, AL and AF Huxley. (1952b) Currents carried by sodium and potassium ions through the membrane of the giant axon of loligo. J. Physiol. 116: 449-472.

77. Hodgkin, AL and AF Huxley. (1952c) The components of membrane conductance in the giant axon of loligo. J. Physiol. 116: 474-496.

78. Hodgkin, AL and AF Huxley. (1952d) A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. 117: 500-544.

79. Hodgkin, AL and RD Keynes. (1955) Active transport of cations in giant axons from sepia and loligo. J. Physiol, 128: 28-60.

80. Hoffman, JF (1960) The link between metabolism and the active transport of Na in human red cell ghosts. Fed. Proc. 19: 127.

81. Hubbard JI and RF Schmidt. (1963) An electrophysiological investigation of motor nerve terminals. J. Physiol. 166: 145-167.

82. Hull, KL and AD Roses. (1976) Stoichiometry of sodium and potassium transport in erythrocytes from patients with myotonic dystrophy. J. Physiol. 254: 169-181.

83. Hultman E. and H. Sjoholm. (1983) Electromyogram, force and relaxation time during and after continuous electrical stimulation of human skeletal muscle in situ. J. Physiol. 339: 33-40.

84. Hutter, OF and D. Noble. (1960) The chloride conductance of frog skeletal muscle. J. Physiol. 151: 89-102.

85. Johns, R, D. Grob and AM Harvey. (1956) Studies in neuromuscular function. II Effects of nerve stimulation in normal subjects and in patients with myasthenia gravis. Bulletin of the Johns Hopkins Hospital 99: 125-135.

86. Johnson, JA. (1956) Influence of ouabain, strophanthidin, and dihypdrostrophanthidin on sodium and potassium transport in frog sartorii. Am. J. Physiol. 217: 525-531.

87. Jones, DA, B. Bigland-Ritchie and RHT Edwards. (1979) Excitation frequency and muscle fatigue - mechanical responses during voluntary and stimulated contractions. Exp. Neurol. 64: 401-413.

88. Juel, C. (1986) Potassium and sodium shifts during in vitro isometric muscle contraction, and the time course of the ion-gradient recovery. Pfleugers Arch. 406: 458-463.

89. Junge, D. (1976) Nerve and Muscle Excitation. Sunderland, Mass.: Sinauer Associates, Inc.

90. Kernan, RP. (1962) Membrane potential changes during sodium transport in frog sartorius muscle. Nature. 193: 986-987.

91. Kernan, RP. (1963) Resting potential of isolated rat muscles measured in plasma. Nature. 200: 474-475.

92. Kernan, RP. (1968) Membrane potential and chemical transmitter in active transport of ions by rat skeletal muscle. J. Gen. Physiol. 51: 204S-210S.

93. Keynes, RD. (1954) The ionic fluxes in frog muscle. Proc. Roy. Soc. Biol. 142: 359-382.

94. Keynes, RD and R. Rybova. (1963) The coupling between sodium and potassium fluxes in frog sartorius muscle. J. Physiol. 168: 58P.

95. Kiens, B. and B. Saltin. (1986) Endurance training of man decreases muscle potassium loss during exercise. Acta Physiol. Scand. 126: 20A.

96. Kimura, J. (1983) Electrodiagnosis in Diseases in Nerve and Muscle: Principles and Practice. Davis: Philadelphia, Pa.

97. Kjeldsen, K., A. Norgaard and T. Clausen. (1984) The age-dependent changes in the number of ^{3}H-ouabain and Na-K-contents in mammalian skeletal muscle. Pflugers Arch. 402: 100-108.

98. Kjeldsen, K, A. Norgaard, C. Estrup, C. Hau and T. Clausen. (1987) Effects of training on exercise-induced hyperkalemia and the concentration of Na,K-pumps in human skeletal muscle. Acta Physiol. Scand. 129: 23A.

99. Kjeldsen, KA, EA Richter, H. Galbo, G. Lortie and T. Clausen. (1986) Training increases the concentration of [^{3}H]ouabain-binding sites in rat skeletal muscle. Biochim. Biophys. Acta. 860: 708-712.

100. Knochel, JP, JD Blachley, JH Johnson and NW Carter. (1985) Muscle cell electrical hyperpolarization and reduced exercise hyperkalemia in physically conditioned dogs. J. Clin. Invest. 75: 740-745.

101. Kostyuk, PG. (1970) Active transport of ions in a nerve cell and its connection with the electrical processes on surface membrane. Ukr. Biochem. J. 43: 9-16.

102. Kuffler, SW and JN Nicholls. (1976) From Neuron To Brain. Sinauer Associates, Inc.: Sunderland, Mass.

103. Kugelberg, E. (1973) Histochemical composition, contractile speed and fatigueability of rat soleus motor units. J. Neurol. Sci. 20: 177-198.

104. Levi, H. and HH Ussing. (1948) The exchange of sodium and chloride ions across the fibre membrane of the isolated frog sartorius. Acta Physiol. Scand. 16: 232-249.

105. Liley, AW. (1956) An investigation of spontaneous activity at the neuromuscular junction of the rat. J. Physiol. 132: 650-660.

106. Ling, G. and W. Woodbury. (1949) Effect of temperature on the membrane potential of frog muscle fibres. 1J. Cell. Comp. Physiol. 34: 407-412.

107. Linton, R. M. Lim, C. Wolfe, P. Wilmshurst and D. Band. (1984) Arterial plasma potassium measured continuously during exercise in man. Clin. Sci. 67: 427-431.

108. Locke, S. and HG Soloman. (1967) Relation of resting potential of rat gastrocnemius and soleus muscle sto innervation, activity and the Na-K pump. J. Exp. Zool. 166: 377-386.

109. Lockwood, RH and BKB Lum. (1974) Effects of adrenergic agonists and atagonists on potassium metabolism. J. Pharmacol. Exp. Ther. 189: 119-129.

110. Macchia, D., M. Didelot and W. Anderson. (1984) Anion permeability of toad skeletal muscle incubated in plasma and Ringer solution. Muscle Nerve. 7: 415-423.

111. Magladery, JW, WE Porter, AM Park and RD Teasdall. (1951). Electrophysiological studies of nerve and reflex activity in normal man. Bull. Johns Hop. Hosp. 88: 499-537.

112. Marmont, G. (1949) Studies on the axon membrane. I. A new method. J. Cell. Comp. Physiol. 34: 351-382.

113. Marsden, OD, JC Meadows and PA Merton. (1971) Isolated single motor units in human muscle and their rate of discharge during maximal voluntary effort. J. Physiol. 217: 12-13P.

114. Matchett, PA and JA Johnson. (1954) Inhibition of sodium and potassium transport in frog sartorii in the presence of ouabain. Fed. Proc. 13: 384.

115. McArdle, JJ and AJ D'Alonzo. (1981) Effects of terbutaline, a $beta_2$-adrenergic agonist, upon the membrane potentials of innervated and denervated fast- and slow-twitch muscles. Exp. Neurol. 71: 134-143.

116. McComas, AJ, PC Fawcett and MJ Campbell. (1971) Electrophysiological estimation of the number of motor units within a human muscle. J. Neurol. Neurosurg. Psych. 34: 121-131.

117. McComas, AL, K. Mrozek, D. Gardner-Medwin and WD Stanton. (1968) Electrical properties of muscle fibre membranes in man. J. Neurol. Neurosurg. Psych. 31: 434-440.

118. McKelvie, RS, MI Lindinger and GJF Heigenhauser. (1987) Ion changes in arterial red blood cells in maximal exercise. Med. Sci. Sports Exer. 19: S86.

119. Merton, PA. (1954) Voluntary strength and fatigue. J. Physiol. 123: 553-564.

120. Metzger, JM and RH Fitts. (1986) Fatigue from high- and low-frequency muscle stimulation: role of sarcolemma action potentials. Exp. Neurol. 93: 320-333.

121. Milner-Brown, HS. and RG Miller. (1986) Muscle membrane excitation and impulse propagation velocity are reduced during muscle fatigue. Muscle Nerve 9: 367-374.

122. Minaker, KL and JW Rowe. (1982) Potassium homeostasis during hyperinsulinemia: effect of insulin level, B-blockade, and age. Am. J. Physiol. 242: E373-E377.

123. Mitchell, PH and JW Wilson. (1921) The selective absorption of potassium by animal cells. J. Gen. Physiol. 4: 45-56.

124. Moore, RD and JL Robovsky. (1979) Mechanism of insulin action on resting membrane potential of frog skeletal muscle. Am. J. Physiol. 236: C249-C254.

125. Moreton, RB. (1969) An investigation of the electroginic sodium pump in snail neurones, using the constant field theory. J. Exp. Biol. 51: 181-201.

126. Moss, RF, PB Raven, JP Knochel, JR Peckham and JD Blachley. (1983) The effect of training on resting muscle membrane potentials. Int. Ser. Sports Sci. 13: 806-811.

127. Mullins, LJ and FJ Brinley. (1969) Potassium fluxes in dialysed squid axons. J. Gen. Physiol. 53: 704-740.

128. Mullins, LJ and K. Noda. (1963) The influence of sodium-free solutions on the membrane potential of frog muscle fibres. J. Gen. Physiol. 47: 117-132.

129. Naess, K and A. Storm-Mathisoen. (1955) Fatigue of sustained tetanic contractions. Acta Physiol. Scand. 34: 351-366.

130. Nakajima, S. and K. Takahashi. (1966) Post-tetanic hyperpolarization and electrogenic Na pump in stretch receptor neurone of crayfish. J. Physiol. 187: 105-127.

131. Nakanishi, T. and FH Norris. (1970) Effect of local temperature on the resting membrane potential in rat muscle. Electroenceph. Clin. Neurophysiol. 28: 633-636.

132. Nastuk, WL and AL Hodgkin. (1950) The electrical activity of single muscle fibres. J. Cell. Comp. Physiol. 35: 39-74.

133. Needham, D. (1971) Machina Carnis. University Press: Cambridge.

134. Nernst. (1888) On the kinetics of substances in solution. In: Cell Membrane Permeability and Transport GR Kepner (ed) Dowden, Hutchison & Ross, Inc.: Stroudsberg, Pa., 1979.

135. Overton, E. (1902) In: Cell Membrane Permeability and Tansport. GR Kepner (ed) Dowden, Hutchison & Ross, Inc.: Stroudsberg, Pa., 1979.

136. Pagala, M, T. Namba and D. Grob. (1984) Failure of neuromuscular transmission and contractility during fatigue. Muscle Nerve 7: 454-464.

137. Page, E., PI Polimeni and DD Macchia. (1980) Anion distribution and exchange in vertebrate striated muscle. Ann. NY Acad. Sci. 341: 524-533.

138. Pflieger, G., I. Szabo and T. Kovacs. (1983) The influence of catecholamines on Na,K transport in slow- and fast-twitch muscles of the rat. Pfleugers Arch. 398: 236-240.

139. Pick, A. (1966) Arrhythmias and potassium in man. Am. Heart J. 72: 295-306.

140. Post, RL. (1959) Relationship of an ATPase in human erythrocyte membranes to the active transport of sodium and potassium. Fed. Proc. 19: 127.

141. Post, RL, CD Albright and K. Dayani. (1967) Resolution of pump and leak components of sodium and potassium ion transport in human erythrocytes. J. Gen. Physiol. 50: 1201-1220.

142. Powell, WJ and NS Skinner. (1966) Effect of the catecholamines on ionic balance and vascular resistance in skeletal muscle. Am. J. Cardiol. 18: 73-82.

143. Ritchie, JM and RW Straub. (1957) The hyperpolarization which follows activity in mammalian non-medullated fibres. J. Physiol. 136: 80-97.

144. Rosa, RM, P. Silva, JB Young, L. Landsberg, RS Brown, JW Rowe and FH Epstein. (1980) Adrenergic modulation of extrarenal potassium disposal. N. Eng. J. Med. 302: 431-434.

145. Saltin, B., G. Sjogaard, S. Strange and C. Juel. (1987) Redistribution of K^+ in the human body during muscular exercise; its role to maintain whole body homeostasis. (Unpublished manuscript).

146. Sandercock, TG, JA Faulkner, J. Albers and P. Abbrecht. (1985) Single motor unit and fibre action potentials during fatigue. J. Appl. Physiol. 58: 1073-1079.

147. de Santillana. (1965) Alessandro Volta. Sci. Amer. 212: 82-91.

148. Senft, JP. (1967) Effects of some inhibitors on the temperature dependent component of resting potential in lobster axons. J. Gen. Physiol. 50: 1835-1848.

149. Shetty, R., D. Krishna and D. Macchia. (1985) Effects of albumin on resting membrane potential of toad semitendinosus muscles. Pflugers Arch. 404: 83-85.

150. Silva, P., RS Brown and FH Epstein. (1977) Adaptation to potassium. Kidney Int. 11: 466-475.

151. Sjodin, RA. (1971) The kinetics of sodium extrusion in striated msucle as functions of the external sodium and potassium ion concentrations. J. Gen. Physiol. 57: 164-187.

152. Sjodin, RA. (1982) The transport of electrolytes in muscle. J. Membr. Biol. 68: 161-178.

153. Sjodin, RA. (1984) Contributions of electrogenic pumps to resting membrane potentials: The theory of electrogenic potentials. In: Electrogenic transport. Fundamental principals and physiological implications. MP Blaustein and M. Lieberman (eds) Raven Press: New York, p105-127.

154. Sjodin, RA and LA Beauge. (1967) The ion selectivity and concentration dependence of cation coupled active sodium transport in squid giant axon. Curr. Mod. Biol. 1: 105-115.

155. Skou, JC. (1957) The influence of some cations on an adenosine triphosphatase from peripheral nerve. Biochim. Biophys. Acta. 23: 394-401.

156. Sreter, FA. (1963) Cell water, sodium, and potassium in stimulated red and white mammalian muscles. Am. J. Physiol. 205: 1295-1298.

157. Stalberg, E. (1966) Propagation velocity in human muscle fibers in situ. Acta. Physiol. Scand. 70 (Suppl. 287): 1-42.

158. Steinbach, HB. (1940) Sodium and potassium in frog muscle. J. Biol. Chem. 133: 695-701.

159. Stevens, JA. and A. Taylor. (1972) Fatigue of maintained voluntary muscle contraction in man. J. Physiol. 220: 1-18.

160. Stickney, JC. (1941) The effect of epinephrine on the potassium balance of the hind limbs of the frog. Am. J. Physiol. 132: 9-17.

161. Streeten, DH, TG Dalakos and H. Fellerman. (1971) Studies on hyperkalemic periodic paralysis. Evidence of changes in plasma Na and Cl and induction of paralysis by adrenal glucocorticoids. J. Clin. Invest. 50: 142-155.

162. Tashiro, N. (1973) Effects of isoprenaline on contractions of directly stimulated fast and slow muscles of the guinea-pig. Br. J. Pharmacol. 48: 121-131.

163. Thomas, RC. (1969) Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. J. Physiol. 201: 495-514.

164. Thomas, RC. (1972) Electrogenic sodium pump in nerve and muscle cells. Phys. Rev. 52: 563-594.

165. Tibes, U., B. Hemmer, U. Schweigart, D. Boning and D. Fotescu. (1974) Exercise acidosis as cause of electrolyte changes in femoral venous blood of trained and untrained man. Pfleugers Arch. 347: 145-158.

166. Uramoto, I., K. Watanbe and T. Totsuka. (1983) Contrast of time courses of changes in musclular potentials to prolonged stimulation at 5 Hz in rat medial gastrocnemius and soleus muscles. Exp. Neurol. 80: 479-483.

167. Venosa, RA and P. Horowicz. (1981) Density and apparent location of the sodium pump in frog sartorius muscle. J. Membr. Biol. 59: 225-232.

168. Vyskocil, F., P. Hnik, H. Rehfeldt, R. Vejsada and E. Vjec. (1983) The measurement of K_e concentration changes in human muscles during volitional contractions. Pfleugers Arch. 399: 235-237.

169. Wang, P. and T. Clausen. (1976) Treatment of attacks in hyperkalemic familial periodic paralysis by inhalation of salbutamol. Lancet 1: 221-223.

170. Westerblad, H. and J. Lannergren. (1986) Force and membrane potential during and after fatiguing intermittent tetanic stimulation of single Xenopus muscle fibres. Acta. Physiol. Scand. 128: 369-378.

171. Williams, ME, EV Gervino, RM Rosa, L. Landsberg, JB Young, P. Siva and FH Epstein. (1985) Catecholamine modulation of rapid potassium shifts during exercise. N. Engl. J. Med. 312: 823-827.

172. Williams, JA, CD Withrow and DM Woodbury (1971) Effects of ouabain and diphenylhydratoin on transmembrane potentials, intracellular electrolytes, and cell pH of rat muscle and liver in vitro. J. Physiol. 212: 101-115.

173. Wu, FS and KL Zierler. (1985) Insulin stimulation of an electrogenic pump at high extracellular potassium concentration. Am. J. Physiol. 249: E12-E16.

174. Yonemura, K. (1967) Resting and action potentials in red and white muscles of the rat. Jap. J. Physiol. 17: 708-719.

175. Young, JZ. (1936) The structure of nerve fibres in cephalopods and crustacea. Proc. Roy. Soc. Biol. 121: 367-386.

176. Zierler, KL. (1957) Increase in resting membrane potential of skeletal muscle produced by insulin. Science. 126: 1067-1068.

177. Zierler, KL. (1959) Hyperpolarization of muscle by insulin in a glucose-free environment. Am. J. Physiol. 197: 523-526.

Appendix 1

Raw Data from Intermittent Tetanic Stimulation at 20 Hz

Appendix 1.

Individual subject data of M-wave changes during intermittent tetanic stimulation of tibialis anterior at 20 Hz (under ischaemia). Repetitive stimulation (3 secs on, 2 secs off) stopped at 225 secs.

National Library of Canada

Bibliothèque nationale du Canada

Canadian Theses Service

Service des thèses canadiennes

NOTICE

AVIS

The quality of this microfiche is heavily dependent upon the quality of the thesis submitted for microfilming.

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage.

Please refer to the National Library of Canada target (sheet 1, frame 2) entitled:

Veuillez consulter la cible de la Bibliothèque nationale du Canada (microfiche 1, image 2) intitulée:

NOTICE

AVIS

M-WAVE AMPLITUDE DURING 15 HZ STIMULATION (ischaemic)

Subject	Time (s)												
	5	10	20	30	40	50	60	80	100	120	140	175	200
SG	100	100	104	112	108	108	112	117	112	108	96	83	83
NK	100	100	100	100	100	105	105	109	109	109	105	100	100
JK	106	106	100	100	106	119	125	125	144	156	150	131	113
GJ	100	100	100	100	100	100	100	100	105	105	105		
JO	100	100	95	95	95	90	90	95	95	100	100	110	
AM	100	100	106	100	100	100	100	96	100	106	106	94	81
Mean	101	101	100.8	101.2	100.5	103.7	105.3	106.7	110.8	114	110.3	103.6	94.3
SD	2.4	2.4	3.9	5.7	4.2	9.7	12	12.6	17.4	20.8	19.8	18.2	15.1

	Time (s)											
	225	250	300	400	500	600	800	1000	1500	2000	2500	3000
SG	83	94	104	108	112	112	117	112	104	102	100	
NK	95	68	82	86	91	91	91	95	95	100	100	100
JK	113	94	88	94	[illegible]	119	113	110	105	100	113	113
GJ		79	79	89	105	116	116	111	107	105	105	100
JO		98	95	100	100	100	100	100	100	100	100	100
AM	75	81	88	100	106	106	109	113	113	113	115	106
Mean	91.5	85.6	85.3	92.8	10[illegible].5	107.3	107.7	106.8	104	103.3	105.5	103.8
SD	16.5	12.7	9.1	9	[illegible].2	10.5	10.2	7.5	6.1	5.1	6.9	5.8

Appendix 2.

Raw Data from Intermittent Voluntary Contractions of Thenar and EDB Muscles

Appendix 2.

Individual subject data of M-wave changes during intermittent voluntary contractions of thenar and EDB muscles. Intermittent effort (3 sec/5 sec) continued for 100 secs; M-waves were evoked every 5 secs.

Thenar Muscle - M-Wave Amplitude (% Baseline)

Subject	Time (s)												
	0	5	15	25	35	45	60	80	100	150	200	400	800
Control													
AH	100	100	101	100	100	101	100	99	98	99	97	100	104
FS	100	101	100	100	101	100	100	100	100	98	96	94	93
JG	100	104	102	102	106	102	104	107	104	103	101	96	98
MJ	100	102	99	99	100	101	99	100	101	101	100	104	108
LJ	100	101	103	103	100	102	101	103	103	100	102	101	99
LS	100	103	102	101	101	100	101	100	100	98	97	97	100
JW	100	99	101	101	101	101	101	102	101	101	101	101	102
MT	100	100	102	101	101	101	101	104	103	101	102	103	102
DB	100	101	101	101	102	103	104	103	105	103	103	100	105
MM	100	100	100	99	99	99	98	97	97	99	99	101	100
Fatigue													
AB	100	109	111	111	116	119	104	116	112	97	99	97	100
FS	100	112	110	116	106	114	109	109	107	85	83	75	84
JG	100	128	130	134	140	147	149	155	154	116	115	107	114
KJ	100	107	117	108	105	104	101	96	90	92	93	98	96
LJ	100	121	126	133	132	128	125	128	126	111	111	110	108
LS	100	102	114	116	120	123	124	129	136	102	99	97	96
JW	100	118	124	124	121	127	126	121	117	93	97	96	94
KT	100	110	113	121	130	122	130	129	132	110	111	116	109
DH	100	122	134	134	139	139	140	138	136	109	101	94	96
MM	100	119	119	118	113	113	110	108	108	102	101	104	101

Thenar Muscle - M-Wave Area (% Baseline)

Subject	0	5	15	25	35	45	60	80	100	150	200	400	800
Control													
AB	100	99	101	100	100	100	98	99	96	100	98	103	108
FS	100	101	100	99	100	100	99	99	99	96	95	92	89
JG	100	101	101	101	102	99	107	106	106	102	103	102	100
MJ	100	100	98	98	98	98	98	97	98	97	97	103	105
LJ	100	100	101	101	100	100	100	100	99	88	93	96	94
LS	100	103	99	100	99	99	96	99	99	95	94	93	97
JW	100	100	100	99	97	98	97	97	96	96	96	101	101
MT	100	101	102	101	102	101	101	102	102	100	100	103	107
DB	100	100	100	101	101	100	104	100	101	102	104	101	101
MM	100	104	101	100	99	99	98	97	97	96	95	104	103
Fatigue													
AB	100	106	105	106	109	111	100	120	125	115	109	104	91
FS	100	108	108	120	111	121	117	129	133	107	97	80	80
JG	100	104	104	110	126	125	127	130	133	129	124	104	108
MJ	100	105	120	124	122	125	129	130	125	110	107	107	107
LJ	100	110	115	121	122	118	118	121	119	122	107	100	96
LS	100	102	107	104	110	113	116	122	127	109	108	94	92
JW	100	103	107	108	108	109	116	114	113	104	95	95	92
MT	100	95	96	101	113	103	117	123	126	119	113	115	107
DB	100	124	140	142	147	154	155	156	163	132	119	98	90

EDB Muscle - M-Wave Amplitude (% Baseline)

Subject	Time (s)												
	0	5	15	25	35	45	60	80	100	150	200	400	800
Control													
AE	100	100	101	100	100	101	101	100	101	100	101	102	102
FS	100	101	100	102	99	101	101	100	100	98	97	96	95
JG	100	100	100	100	101	101	101	101	100	100	100	100	100
MJ	100	100	100	100	100	99	100	100	99	99	99	100	101
LJ	100	100	100	100	100	100	100	100	100	99	100	97	100
LS	100	100	100	98	100	98	95	95	93	93	96	100	100
JW	100	100	106	106	106	107	106	107	107	106	107	104	104
MT	100	100	102	102	102	103	103	103	103	101	101	100	100
DB	100	100	101	101	99	100	100	100	99	98	99	99	99
MM	100	99	101	102	101	102	102	102	102	96	93	92	90
Fatigue													
AE	100	113	113	107	111	108	111	111	116	108	108	105	103
FS	100	100	101	97	99	100	99	93	95	97	102	106	109
JG	100	106	102	103	102	101	101	102	109	114	114	108	104
MJ	100	114	117	120	121	119	123	121	115	136	137	141	145
LJ	100	102	117	115	93	120	125	121	127	116	117	111	108
LS	100	107	114	111	110	109	106	102	106	110	113	115	115
JW	100	104	108	105	103	106	111	113	111	95	98	94	92
DB	100	102	100	105	105	104	106	108	108	109	107	100	94
MM	100	104	113	119	114	118	121	125	124	129	132	126	117

EDB Muscle - M-Wave Aréa (% Baseline)

Subject	0	5	15	25	35	45	60	80	100	150	200	400	800
Control													
AE	100	100	101	101	101	101	103	102	103	102	104	105	109
FS	100	101	101	100	99	100	100	100	99	97	97	96	98
JG	100	100	100	100	99	99	99	100	99	99	100	101	101
MJ	100	101	101	101	101	100	102	101	101	101	102	105	109
LJ	100	102	101	101	101	101	101	101	100	101	101	100	102
LS	100	101	97	95	97	93	91	90	88	92	93	100	100
JW	100	98	102	102	103	103	102	102	103	104	105	108	108
MT	100	102	102	103	102	102	102	103	103	101	101	101	101
DB	100	100	97	98	97	97	99	98	97	97	98	99	100
MM	100	91	101	104	98	105	106	101	105	84	69	70	69
Fatigue													
AE	100	106	112	108	111	110	110	111	112	109	106	107	106
FS	100	105	104	103	110	110	106	103	109	110	109	114	122
LG	100	98	105	115	111	117	120	123	140	127	122	104	97
MJ	100	114	128	130	133	129	134	133	134	141	140	136	143
LJ	100	95	118	119	98	134	158	131	139	135	134	121	116
LS	100	106	113	117	121	126	127	132	140	128	129	121	120
JW	100	97	95	95	95	99	101	103	104	95	98	96	96
DB	100	108	105	127	109	112	130	125	116	109	102	94	90
MM	100	102	119	124	122	124	130	135	139	132	130	121	113

Appendix 3.

Baseline Measurements of Resting and Action Potentials
in Rat Soleus Muscle Fibres.

Appendix 3.

Individual measurements of resting and action potentials from rat soleus muscle fibres (n = 68). Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (mV)
AP = action potential amplitude (mV)

Baseline Data

RMP	AP	RMP Mean	SD	AP Mean	SD
74					
80					
88	82	79.3	5.3	75.5	9.8
76	88				
84					
72					
84	68				
76	64				
84	68				
72	80	76.3	3.9	77.7	7.4
74	70				
78	76				
74	90				
76	82				
80	66				
86	62	85.2	3.0	71.6	10.5
88	70				
84	68				
88	92				
74	86				
77	88	77.7	3.4	86.8	1.6
84	88				
76	84				
80					
75	88				
76	80				
80	96	80.9	3.0	93.7	7.6
84	102				
80	100				
84	92				
78	86				
84	100				
72.0	92.0				
76	88	76.7	2.7	89.3	3.2
76	94				
80	90				
80	88				
76	84				
75	80				
80	92	78.5	3.6	84.7	8.2
80	70				
85	95				
75	85				
76	86				

80	100				
88	92	82.2	4.9	96.6	2.8
88	96				
76	96				
79	99				
78	86				
77		78.9	3.6	98.7	7.9
78	98				
87	103				
76	103				
80	110				
76	92				
76	72				
78	110	76.7	0.9	93.0	15.8
76	97				
86	70				
84	72	86.6	2.5	73.8	4.2
90	70				
89	81				
84	76				
80	60				
86	89	81.0	4.0	73.5	12.3
82	90				
74	74				
84					
77	60				
84	68				
84	88				
78	96	80.4	3.2	88.4	7.0
84	96				
80	84				
76	78				
86	86				
90	86	87.6	2.3	90.2	8.2
84	103				
90	80				
88	96				
76	96				
76	94	78.0	1.8	94.4	0.8
80	94				
80	94				
78	94				
96	74				
92	80	89.7	6.0	87.0	12.8
80	90				
86	90				
87	76				
97	112				

84	88	81.0	2.5	88.0	5.8
82	86				
80	84				
78	80				
78	98				
84	92				
88	70	80.8	3.7	84.8	9.2
78	92				
80	80				
78	86				
80	96				
76	92	84.4	4.8	82.8	6.9
84	83				
90	86				
88	71				
84	82				
88	70	82.0	3.1	82.4	8.3
80	76				
80	84				
82	90				
80	92				
84	90	83.0	6.3	87.3	7.2
76	80				
92	94				
76	88				
80	76				
90	96				
79	78	79.9	5.3	75.7	6.0
78	68				
80	82				
90	86				
80					
84	72				
78	72				
70	72				
84	92	80.3	6.4	87.7	5.7
92	76				
72	92				
78	86				
76	92				
80	88				
84	64	76.3	4.3	81.0	11.3
80	70				
75	91				
73	77				
72	92				
74	92				

76	82				
76	94	75.6	3.2	84.8	8.4
70	70				
80	90				
76	88				
74	94				
74	90	76.8	3.7	88.0	5.2
76	80				
76	84				
84	92				
70	60				
72	54	72.7	2.5	75.2	13.7
72	86				
76	77				
70	82				
76	92				
78	96				
72	72	77.7	5.8	87.3	11.1
80	102				
69	96				
80	78				
87	80				
80	90				
78	92	78.8	5.3	84.8	12.8
76	86				
88	96				
72	60				
80	76				
76	72	75.0	3.6	78.0	7.3
74	72				
68	72				
76	90				
76	86				
84	70				
74	74	77.6	2.0	72.4	2.0
76	74				
74	74				
80	70				
84	96				
80	82	83.6	2.0	92.8	7.2
84	92				
84	104				
86	90				
84	64				
76	82	76.8	3.9	74.0	6.4
72	72				
76	80				
76	72				

80	90				
78	88	79.0	0.7	90.0	3.1
79	95				
79	87				
88	86				
80	88	83.2	3.9	91.6	4.1
88	92				
80	96				
80	96				
80	74				
80	80	79.6	0.8	83.2	6.0
80	84				
78	86				
80	92				
80	70				
76	72	73.2	4.1	70.4	2.7
70	66				
70	70				
70	74				
88	82				
84	70	82.4	3.2	79.6	5.9
80	78				
80	80				
80	88				
86	76				
76	80	80.8	3.7	78.8	6.5
84	78				
80	70				
78	90				
76	82				
78	60	78.2	1.3	67.6	7.5
79	66				
78	64				
80	66				
80					
78		79.0	0.9	68.0	
79					
78	68				
80					
74	74				
76	56	78.2	4.8	75.3	9.6
88	74				
80	80				
75	82				
76	86				
94	90				

79	87	80.4	7.7	80.4	9.0
70	70				
79	69				
80	86				
80	80				
80	75	77.6	2.3	72.2	8.4
78	74				
74	76				
76	56				
80					
84	72	79.0	2.8	75.6	5.0
76	74				
76	84				
78	78				
80	70				
78	72				
90	68	81.7	4.8	74.7	5.0
80	70				
76	78				
86	82				
80	78				
86	106				
78	74	80.0	3.3	88.8	10.4
80	90				
80	90				
76	84				
78	82				
78	90	80.3	2.7	84.3	8.1
78	90				
84	84				
80	68				
84	92				
74	66				
76	74	75.2	2.0	78.4	8.6
72	76				
78	90				
76	86				
76	72				
74	78	79.7	3.9	83.0	6.6
86	82				
80	88				
80	92				
82	86				
74	64				
80	62	80.0	3.8	69.2	7.0
80	70				
80	68				
86	82				

80	88	80.4	4.1	89.2	3.5
88	88				
80	88				
76	86				
78	96				
76	70	81.6	4.6	78.4	7.8
78	70				
88	90				
80	84				
86	78				
80	76	79.0	3.0	64.7	9.3
82	64				
82	56				
80	50				
76	68				
74	74				
76	66	78.3	5.9	74.7	7.3
70	78				
76	76				
88	72				
76	68				
84	88				
86	94	80.0	3.8	95.2	5.2
80	100				
80	102				
74	92				
80	88				
82	90	78.8	1.6	90.4	2.3
78	94				
78	88				
78	92				
78	88				
76	82	77.3	2.2	79.7	7.6
76	80				
74	68				
80	86				
80	72				
78	90				
80	80	76.3	2.7	77.3	2.5
78	78				
74	78				
78	78				
72	72				
76	78				
80	88	80.8	4.1	81.6	3.9
80	76				
84	80				

74	82				
86	82				
80	78				
78	74	80.4	2.0	78.8	3.2
80	78				
84	80				
80	84				
74	58				
80	92	78.7	2.2	83.7	16.2
80	64				
80	96				
80	96				
78	96				
80	90				
76	84	77.6	2.9	85.6	2.7
82	86				
76	86				
74	82				
78	84				
78	88	77.5	3.8	82.5	4.1
79	79				
84	86				
74	82				
72	76				
78	78				
80	80	78.4	2.3	85.6	7.1
80	92				
74	82				
80	96				
76	70				
74	74	76.4	2.3	74.8	3.2
74	76				
80	80				
78	74				
80	76				
78	74	78.0	2.2	77.2	4.8
74	86				
80	72				
78	78				
74	78				
80	88	81.6	4.3	90.8	8.4
84	94				
86	90				
84	104				
Mean					
79.5	82.2				

SD	
4.8	10.8

Appendix 4.

Raw Data of Changes in Resting and Action Potentials
After 5 min Intermittent Tetanic Stimulation at 20 Hz.

Appendix 4.

Effect of 5 min of intermittent tetanic stimulation at 20 Hz on resting and action potentials in soleus muscle fibres (n = 13). Time intervals denote time after stimulation; individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)

Fatigue

Time Interval	RMP		AP		MWAVE
0 - 3:00	84	88.0	90	94.0	105.5
	92		98		116.7
	96	96.0	92	92.0	
	90	96.0		102.0	
	102		102		
	104	96.7	103	109.0	100
	90		110		111
	96		114		111
	88	93.0	100	104.5	111
	88		104		122
	94		102		155.5
	102		112		200
	96	92.0		94.0	
	90		98		
	90		90		110
	85	87.7	95	91.7	175
	90		100		183
	88		80		183
	92	95.5	88	85.0	110
	99		82		120
	96	94.0	116	116.0	
	92				
	92	84.0	90	89.0	
	76		88		
	84	87.3	102	106.0	
	90		108		
	88		108		
	80	82.0	88	82.7	
	78		80		
	88		80		
	88	89.0	88	95.5	75
	86		102		80
	86		94		85
	96		98		95
MEAN	90.5	90.9	96.8	97.0	123.6

SD	6.3	4.7	10.0	9.4	36.2
3:00 - 6:00	92	97.3	108	100	122.2
	100		92		127.8
	100				127.8
	88	91.3	92	92.7	
	92		92		
	94		94		
	104	98.0	108	98.0	
	92		88		
	94	90.7	106	108.7	122.2
	90		104		122.2
	88		116		133.3
	108	92.0	108	100.0	122
	84		104		122
	84		88		122
	88	88.5	90	102.0	130
	92		104		130
	88		108		140
	86		106		140
	83	91.0	93	94.3	192
	100		100		192
	90		90		192
	104	98.0	67	70.5	140
	92		74		150
	96	87.0		114.0	
	78		114		
	92	98.0	92	91.3	
	100		90		
	102		92		
	104	95.2	88	101.4	
	96		106		
	94		103		
	92		104		
	90		106		
	94	91.3	98	90.7	
	100		80		
	80		94		
	85	85.0	93	102.3	100
	86		106		110
	84		108		115

MEAN	92.5	92.6	97.5	97.4	135.8
SD	7.1	4.2	10.6	10.1	24.0
6:00 - 9:00	100	96.0			133.3
	92				127.8
	88	88.0	104	110.0	
	88		116		
	96	96.0	80	85.0	
	96		90		
	86	89.5	104	99.0	144.4
	104		90		144.4
	84		100		144.4
	84		102		144.4
	88	91.3	108	102.0	122
	98		98		144.4
	88		100		155.5
	85	86.5	109	109.5	140
	88		110		140
	100	94.5	96	97.0	200
	98		89		200
	90		100		208
	90		103		208
	100	96.0	108	100.3	
	88		112		
	100		81		
	100	96.0	82	93.0	
	92		104		
	99	90.3	99	97.7	
	88		100		
	84		94		
	76	83.0	76	87.0	115
	90		98		120
MEAN	91.7	91.6	98.3	98.1	152.4
SD	6.6	4.3	10.1	7.8	30.4
9:00 - 12:00	84	84.0	80	80.0	122.2

	84				122.2
	92	90.0	100	94.0	
	88		88		
	104	103.0		84.0	
	102		84		
	96	90.0	100	98.0	144.4
	90		98		144.4
	84		96		133.3
	84	84.8	104	101.8	144.4
	94		98		155.5
	81		101		122
	80		104		122
	84	85.0	112	107.0	
	86		102		
	100	89.3	96	94.0	
	84		92		
	84		94		
	78	80.0	86	84.5	
	84		84		
	78		84		
	80		84		
	88	88.0	108	107.0	120
	88		106		105
MEAN	87.4	88.2	95.5	94.5	130.5
SD	7.1	6.1	8.9	9.4	14.3
12:00 - 15:00	80	80			105.5
	100	100.0	84	84.0	
	92	85.0	92	92.0	133.3
	78		92		133.3
	90	90.0	94	94.0	122
	88	85.0	83	84.0	200
	82		85		200
	86	89.0	104	111.0	
	92		118		
	84	81.0	104	94.3	
	80		90		

	79		89		
	75	75.0	78	78.0	
	98	92.0	90	95.3	100
	88		106		95
	90		90		90
MEAN	86.4	86.3	93.3	91.6	131.0
SD	7.0		10.2		39.7

Appendix 5.

Effect of Ouabain on Resting and Action Potentials
of Stimulated Rat Soleus Muscle Fibres

Appendix 5.

Effect of ouabain (1.25 x 10^{-4} M) added at the end of 5 min of intermittent tetanic stimulation at 20 Hz on the resting and action potentials of soleus muscle fibres (n = 5). Time intervals denote time after stimulation. Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)

Ouabain (1.25×10^{-4} M) added at the end of fatigue

Time Interval	RMP		AP		MWAVE
0 - 3:00	80	80.0		56.0	36
	80		56		45
	76	78.0	72	78.0	71.4
	80		84		81
	76	68.0	76	68.0	77
	60		60		85
	72	70.0	82	80.8	55.5
	76		92		55.5
	68		80		61
	66		74		61
	68		76		61
	76	74.3	66	72.0	82
	78		74		82
	78		86		88
	68		64		88
	72		68		88
	74		74		82
MEAN	73.4		74.0		70.6
		74.1		71.0	
SD	5.6		9.4		15.8
		4.6		8.7	
3:00 - 6:00	84	79.6	56	70.0	45
	80		50		45
	80		72		45
	76		88		45
	78		84		45
	80	77.8	44	63.8	86
	76		72		86
	83		77		86
	72		62		81
	88	79.0	60	66.4	92
	81		54		92
	74		74		100
	72		74		100
	80		70		100
	72	71.0	80	74.7	61
	70		74		61
	72		80		61
	64		64		61

	79		79		61
	66		70		61
	74		76		61
	72	72.6	70	74.9	82
	68		76		82
	72		82		82
	68		72		82
	78		76		82
	76		76		76
	74		72		76
MEAN	75.3	76.0	70.9	69.9	72.8
SD	5.6	3.5	10.2	4.4	17.8
6:00 - 9:00	86	80.4	86	82.4	45
	86		86		45
	76		68		45
	74		86		45
	80		86		45
	82	77.0	82	74.5	81
	72		76		81
	74		80		76
	80		60		76
	71	72.3	72	65.3	100
	64		64		100
	82		60		92
	68	69.5	68	63.3	61
	68		52		55.5
	70		70		55.5
	72				50
	68	72.0	70	73.4	76
	78		82		76
	72		74		70.5
	64		62		76
	80		76		70.5
	78		82		70.5
	64		68		70.5
MEAN	74.3	74.2	73.2	71.8	68.0
SD	6.6	3.9	9.7	6.9	17.0
9:00 - 12:00	82	76.5	75	68.8	45
	86		82		45

	74		66		45
	64		52		45
	76	68.7	72	72.0	76
	70				76
	60				67
	78	78.0	60	52.0	77
	78		44		61.5
	82	74.0	72	66.0	44
	70		54		44
	76		72		44
	78		66		44
	68		64		39
	70		68		39
	70	69.7	68	71.0	64.7
	70		72		64.7
	70		78		53
	70		70		53
	70		70		53
	68		68		47
MEAN	72.9		67.0		53.7
		73.4		66.0	
SD	6.2		8.9		12.3
		3.7		7.3	
12:00 - 15:00	78	74.0	78	59.3	45
	74		40		36
	70		60		36
	66	68.7		72.0	57
	72		72		52
	68				52
	72	72.0	50	54.0	61.5
	64		58		54
	80				54
	72				46
	68	68.5		67.0	39
	72		80		33
	66		54		28
	68				22
	70	67.1	54	56.9	47
	68		56		35
	70		56		35
	64		56		29.4
	64		56		29.4
	64		56		23.5
	70		64		23.5

MEAN	69.5	70.1	59.3	61.8	39.9
SD	4.3	2.5	10.1	6.7	11.8

Appendix 6.

Effect of Cooling on the Resting and Action Potentials
of Stimulated Rat Soleus Muscle Fibres

Appendix 6.

Effect of cooling the bathing medium to 19°C at the end of 5 min of intermittent tetanic stimulation at 20 Hz on the resting and action potentials of soleus muscle fibres (n = 7). Time intervals denote time after stimulation. Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)
AP Overshoot = potential difference in AP amplitude from zero potenti

Cold Bath (19-20°C) added at end of fatigue

Time Interval	RMP		AP		AP Overshoot
0 - 3:00	76	82.0	94	82.0	18
	88		70		-18
	88	84.0	84	82.0	-4
	80		80		0
	76	78.0	104	102.0	28
	80		100		20
	70	73.0	78	74.0	8
	76		70		-6
	70	71.5		80.0	
	60		56		-4
	80		84		4
	76		100		24
	68	69.3	92	81.3	24
	80		100		20
	60		52		-8
	84	81.3	76	85.3	-8
	80		88		8
	80		92		12
MEAN	76.2		83.5		6.9
		77.0		83.8	
SD	7.8		14.8		13.4
		5.3		8.1	
3:00 - 6:00	84.0				
	88	82.4	94	92.5	6
	80		90		10
	80		96		16
	84				
	80		90		10
	76	82.0	78	94.0	2
	88		104		16
	86		86		0
	78		108		30
	68	69.3	68	62.0	0
	72		70		-2
	68		48		-20
	70	74.6	94	95.6	24
	74		95		21

	75		95		20
	78		102		24
	76		92		16
	64	75.0	75	90.8	11
	88		94		6
	68		92		24
	80		102		22
	76	80.0	104	105.0	28
	80		100		20
	80		104		24
	84		112		28
MEAN	77.9		91.4		14
		77.2		90.0	
SD	6.6		14.3		11.8
		4.7		13.3	
6:00 - 9:00	68.0	79.3			
	88				
	82				
	78	79.3	92	96.7	14
	80		98		18
	80		100		20
	81	83.0		98.0	
	76		96		20
	92		100		8
	72	78.8	102	104.3	30
	79		105		26
	80		104		24
	84		106		22
	80	79.0	84	78.5	4
	70		50		-20
	84		94		10
	82		86		4
	66	73.5	84	93.3	18
	70		96		26
	76		105		29
	82		88		6
	84	78.0	88	91.0	4
	72		94		22
MEAN	78.5		93.3		15
		78.7		93.6	
SD	6.3		12.4		11.8
		2.6		7.9	

9:00 - 12:00	80	81.0	90	96.0	10
	80		92		12
	80		100		20
	84		102		18
	76	76.0	96	96.0	20
	80	81.5	112	105.0	32
	80		102		22
	80		104		24
	86		102		16
	88	88.0	104	114.0	16
	88		124		36
	74	78.0	78	90.0	4
	82		102		20
	66	66.0	90	90.0	24
	68	73.0		102.0	
	78		102		24
MEAN	79.4		100.0		19.9
		77.6		99.0	
SD	6.0		10.1		7.8
		6.5		8.0	
12:00 - 15:00	78	75.0	80	74.0	2
	72		68		-4
	88	84.0	108	103.0	20
	80		98		18
	82	83.0	102	98.0	20
	84		94		10
	80	80.0	116	108.0	36
	80		106		26
	80		106		26
	80		104		24
	84	84.0	106	106.0	22
	70	70.0	82	82.0	12
	80	80.0	94	94.0	14
	88	83.0	102	101.0	14
	78		100		22

MEAN	80.3	79.9	97.7	95.8	17.5
SD	4.7		12.1		9.6

Appendix 7.

Effect of Removal of K^+ on the Resting and Action Potentials of Stimulated Rat Soleus Muscle Fibres

Appendix 7.

Effect of removal of K^+ at the end of 5 min of intermittent tetanic stimulation at 20 Hz on the resting and action potentials of rat soleus muscle fibres (n = 5). Time intervals denote time after stimulation. Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)

No K+ (0mM) added to bath after fatigue

Time Interval	RMP		AP	
0 - 3:00	80	76.0	88	78.0
	80		80	
	76			
	56			
	88		66	
	84	87.0	92	91.5
	88		92	
	76		88	
	100		94	
	74	88.0		90.4
	88		80	
	94		98	
	88		88	
	98		90	
	86		96	
	86	80.0		84.0
	80		84	
	74		80	
	80		88	
	80	81.7	62	81.0
	80		84	
	76		80	
	90		96	
	80		80	
	84		84	
MEAN	82.6		85.2	
		82.5		85.0
SD	8.7		8.9	
		4.5		5.2
3:00 - 6:00	56		44	
	100	99.5	96	108.0
	98		116	
	100		120	
	100		100	
	88	94.8	104	108.0
	100		110	
	106		124	
	80		92	
	100		110	
	84	83.6	94	88.8

	88		84	
	84		94	
	86		96	
	76		76	
	86	86.3	86	87.0
	74		70	
	84		92	
	88		86	
	86		94	
	100		94	
MEAN	88.8	91.1	94.4	98.0
SD	11.5	6.4	17.4	10.1
6:00 - 9:00	68	82.8	78	92.8
	80		92	
	80		92	
	90		98	
	96		104	
	86	93.0	104	111.5
	90		104	
	100		120	
	96		118	
	80	93.0	96	102.5
	100		108	
	86		100	
	106		106	
	98	95.6	106	100.0
	88		100	
	92		84	
	96		106	
	104		104	
	95	94.2	115	107.8
	94		114	
	94		104	
	96		112	
	92		94	
MEAN	91.6	91.7	102.6	102.9
SD	8.6	4.6	10.0	6.5
9:00 - 12:00	96	90.0	104	98.5
	88		88	

	80		98	
	96		104	
	100	100.0	116	108.0
	100		100	
	88	88.7	100	97.3
	80		76	
	98		116	
	100	93.0	108	105.0
	88		104	
	92		108	
	88		96	
	98		110	
	92		104	
	102	91.0	118	108.0
	92		98	
	80		102	
	90		114	
MEAN	92.0		103.4	
		92.5		103.4
SD	6.9		9.9	
		4.0		4.6
12:00 - 15:00	96	91.0	114	104.0
	88		100	
	78		96	
	102		106	
	100	91.7	116	109.7
	78		96	
	98		122	
	96		114	
	90		114	
	88		96	
	82	86.0	90	101.0
	78		102	
	98		108	
	86		104	
	98	99.0	110	111.7
	100		112	
	102		110	
	98		110	
	98		112	
	98		116	
	100	82.7	106	93.3
	80		100	
	86		106	

	80		92	
	74		74	
	76		82	
MEAN	90.3	90.1	104.2	103.9
SD	9.3	5.6	11.0	6.5

Appendix 8.

Effect of High $[K^+]_o$ on the Resting and Action Potentials of Stimulated Rat Soleus Muscle Fibres

Appendix 8.

Effect of high $[K^+]_o$ added at the end of 5 min of intermittent tetanic stimulation at 20 Hz on the resting and action potentials of rat soleus muscle fibres (n = 5). Time intervals denote time after stimulation. Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)

High K+ (20mM) added to bath after fatigue

Time Interval	RMP		AP	
0 - 3:00	100	93.3	50	49.3
	96		46	
	84		52	
	84	83.6	54	71.6
	88		78	
	80		84	
	82		74	
	84		68	
	80	77.6	64	55.6
	80		60	
	70		54	
	86		48	
	72		52	
	72	67.5	50	55.0
	78		60	
	60			
	60			
	78	75.3	82	67.0
	74		80	
	80		58	
	70		70	
	80		62	
	70		50	
MEAN	78.6		61.7	
		79.5		59.7
SD	9.4		11.8	
		8.6		8.3
3:00 - 6:00	78	74.0	64	64.0
	70			
	88	78.0	82	76.0
	74			
	80		70	
	70			
	70	72.8	56	61.0
	78		60	
	70		60	
	76		68	
	70			
	58	63.2		
	70			
	60			

	68			
	60		—	
	78	69.7	64	51.5
	70		50	
	70		44	
	70		48	
	74			
	62			
	64			
MEAN	70.8	71.5	60.5	63.1
SD	7.0	4.9	10.4	8.7
6:00 - 9:00	80	74.0		
	68			
	70	63.3		
	56			
	64			
	76	74.0		
	74			
	74			
	72			
	60	62.5		
	66			
	64			
	60			
	66	66.7		
	66			
	68			
MEAN	67.8	68.1		
SD	6.2	5.0		
9:00 - 12:00	74	72.0		
	72			
	70			
	68	69.3		
	76			
	64			
	60	60.5		
	68			

	60	
	54	
	52	63.3
	80	
	58	
	63	70.8
	66	
	78	
	76	
MEAN	67	67.2
SD	8.2	4.5
12:00 - 15:00	70	72.0
	78	
	68	
	72	64.0
	60	
	60	
	58	58.0
	60	
	56	
	64	61.0
	58	
	74	63.5
	60	
	60	
	60	
MEAN	63.9	63.7
SD	6.6	

Appendix 9.

Control Data on the Effects of Various Pump Inhibitors on the Resting and Action Potentials of Unstimulated Rat Soleus Muscle Fibres

Appendix 9.

Effects of ouabain (1.25×10^{-4} M), cooling ($19^{o}C$), removal of $[K^+]_o$, and raising $[K^+]_o$ to 20 mM on the resting and action potentials of rat soleus muscle fibres. Time intervals denote time after the addition of the specific inhibitor. Individual experiments are separated by a space in the RMP column.

RMP = resting membrane potential (-mV)
AP = action potential amplitude (mV)

Control Data (pump inhibition without stimulation)

Time Interval	Ouabain		Cold	
	RMP	AP	RMP	AP
0 - 3:00	96	98	74	58
	80	94	72	76
	70	68	76	60
	70	78	62	70
	74	82	62	68
	70	70	76	80
	78	78		
	70	70		
	64	64		
	79	71	80	84
	80	80	76	
	86	80	68	
	70	70	68	
	76	76		
MEAN	75.9	77.1	66.4	64.3
SD	7.9	9.3	16.1	18.2
3:00 - 6:00	76	60	77	78
	76	88	70	80
	72		78	80
			68	
	68	66	68	62
	68	76		
	70	62	70	62
			68	80
	76	76	74	86
	68	62	70	
	68	68		
	74	64	60	
			78	70
			64	54
			92	82
			82	92
			70	88
MEAN	71.6	69.1	72.6	76.2
SD	3.4	8.6	7.6	11.2
6:00 - 9:00	70	74	60	
	76	80	66	54

			64	24
	61	48	68	70
	66	58		
	62	56	74	82
	72	80	80	80
	68	68	62	66
			68	80
	70	60		
	70	36	70	90
	68	48	72	68
	72	62	84	74
			80	88
MEAN	68.6	60.9	70.7	70.5
SD	4.2	13.3	7.3	17.8
9:00 - 12:00	80	58	70	70
			74	82
	64	46	78	78
	60	48	62	62
	60	38		
			76	80
	68	44	74	
	68		64	
	74	54		
	76	40	76	88
			68	68
			80	82
			60	68
			68	66
MEAN	68.8	46.9	67.5	68.4
SD	6.9	6.7	16.3	18.8
12:00 - 15:00	76	68	68	74
	60		86	76
	68	78	68	76
	70	66	74	76
			80	62
	64	66	76	70
	72	56		
	56		74	50
	62		62	
	74	62	64	
			68	
	68		58	
	76	38	74	78
	74			
			88	96
			70	

MEAN	68.3	62.0		72.1	73.1
SD	6.3	11.6		8.3	11.7

Control Data (pump inhibition without stimulation)

Time interval	High K+		No K+	
	RMP	AP	RMP	AP
0 - 3:00	64		58	
	62		69	74
	50		64	
			64	38
	64	50		
	60	60	72	50
	62		66	50
	60		60	30
	60	40	66	58
			60	46
			68	60
	60			
	60		78	
			80	
			72	
			76	60
			66	64
	60		60	
	56		60	
	56			
	64		68	
	60		78	40
			70	
			68	
			84	52
			64	
			60	
			68	
			70	
			64	
			68	
MEAN	59.9	50.0	67.9	51.8
SD	3.5	8.2	6.5	11.8
3:00 - 6:00	60		66	70
	60		66	66
	64		78	60
	56		66	74
			72	72
	58			
	58		76	60
	56		74	80
			66	54
	58		60	54
	48			

		70	
	64	64	
	60	76	
	58	82	68
	48	62	54
	60	60	
		68	
		66	56
		72	82
		64	
		74	
		70	
		72	40
		64	
		64	
		66	38
MEAN	57.7	68.7	61.9
SD	4.6	5.6	12.6
6:00 - 9:00	60	56	28
	48	72	72
		66	72
	50	66	68
	68		
	60	72	62
	52	60	40
		66	64
	56	64	54
	52	64	50
	58		
	60	64	
	50	60	20
	56	60	30
		64	
		66	
		74	50
		64	
		68	30
		64	48
		64	
		76	46
		74	50
MEAN	55.8	65.9	49.0
SD	5.5	5.1	15.7
9:00 - 12:00	80	68	76

	74	64	58
		72	74
	56	70	64
	64		
	48	66	28
		68	56
	66	62	
	60	68	
	56		
	56	64	
	56	66	
		60	
	54	60	
	58		
	54	70	
	52	66	60
	50	66	
		64	36
		68	
		72	
		66	48
		72	52
MEAN	58.9	66.6	55.2
SD	8.5	3.5	14.4
12:00 - 15:00	70	78	78
	72	64	64
		68	66
	58	70	74
	62	68	88
	54		
	56	60	
	68	60	
	60	80	28
	60	76	100
	64	80	72
	50	62	
	54	72	52
	55	74	
	52		
	56	62	
		60	50
		68	50
		74	60
		70	66
		64	30
MEAN	59.4	68.9	62.7
SD	6.4	6.6	19.3