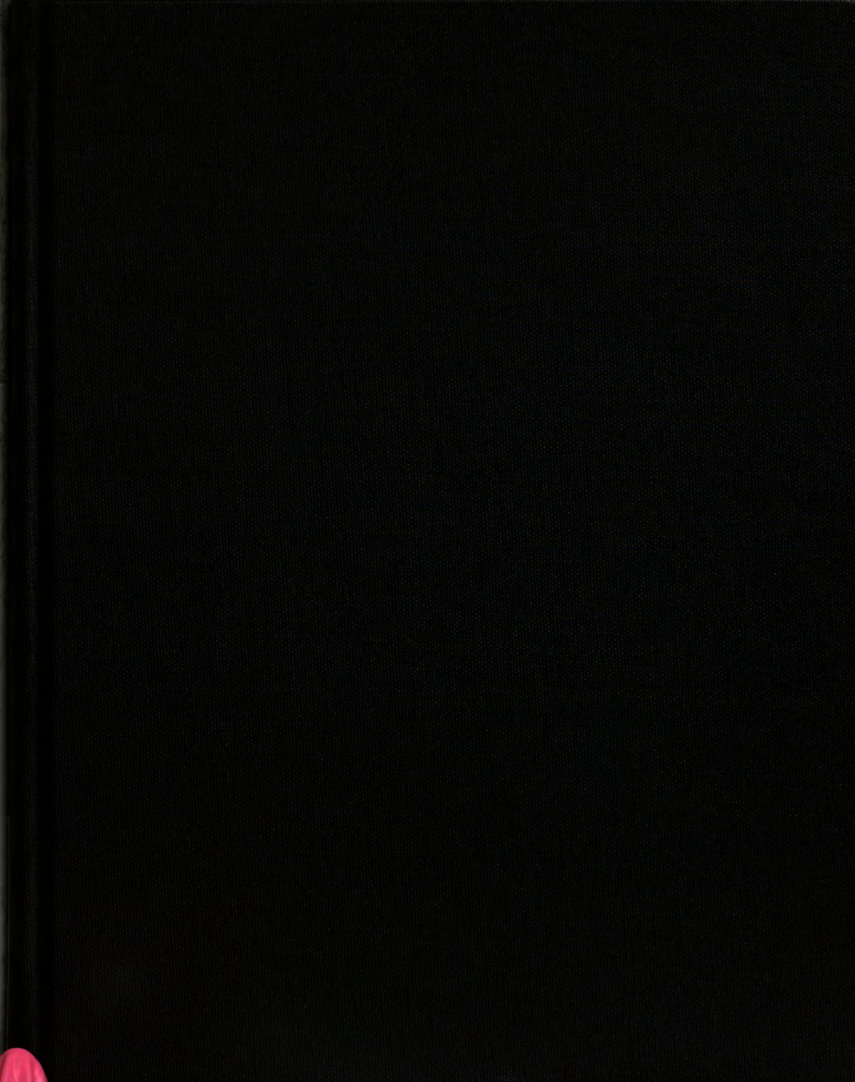






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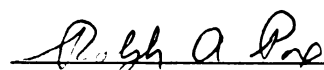
ELECTROPHYSIOLOGYICAL AND PHARMACOLOGICAL
COMPARISON OF TWO KINDS OF
SCHISTOSOMA MANSONI MUSCLE FIBERS

presented by

MING TIAN

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MASTERS degree in ZOOLOGY


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**ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL COMPARISON OF
TWO KINDS OF SCHISTOSOMA MANSONI MUSCLE FIBERS**

By

Ming Tian

A THESIS

**Submitted to
Michigan State University
In partial fulfillment of the requirements
For the Degree of**

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1997

ABSTRACT

Electrophysiological and Pharmacological Comparison of Two Kinds of *Schistosoma mansoni* Muscle Fiber

By

Ming Tian

Two morphologically dissimilar muscle fibers have been isolated from *Schistosoma mansoni*. Microperfusion and whole-cell patch clamp experiments have been performed on these two kinds of muscle fibers to determine differences in receptors and ion channels.

Microperfusion experiments revealed that there are different receptors existing on both kinds of muscle fibers.

Both kinds of muscle fibers contract when exposed to a series of neurotransmitters. Elevated extracellular K^+ also elicited contraction on both kinds of muscle fibers with different sensitivity.

The results revealed that there are two voltage-dependent K^+ currents of both kinds of muscle fibers. There are subtle differences between the K^+ channels on each of the muscle fibers in the kinetics of activation and inactivation and toxin sensitivity.

To my dear family

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my adviser, Dr. Ralph A. Pax for his kind, generous and patient support and guidance through my whole graduate study. Thank you!

Also thanks are due to Dr. Tim Day, who taught the patch-clamp technic to me. His help is very important and highly appreciated. Lots of thanks to Dr. Bennet and Dr. Band who have given me valuable guides.

Best wishes for all my lab mates: Cindy Miller, Eunjoon Kim, George Chen, Mary Thomas and Mary Lou Pax who have given me countless great help and supports.

TABLE OF CONTENTS

LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
INTRODUCTION.....	1
I. Introduction to <i>Schistosoma</i>	1
1. Schistosomiasis and its world economic effect..	1
2. <i>Schistosoma</i> as a primitive invertebrate.....	2
II. <i>Schistosoma</i> Muscle Fibers.....	3
1. Individual muscle fibers.....	4
2. General physiology and electrophysiology of frayed muscle fibers.....	11
3. Effect of neurotransmitters on <i>Schistosoma</i> and their isolated muscle fibers.....	13
a. Serotonin.....	13
b. Elevated extracellular K ⁺	14
c. FMRFamide and related peptides.....	15
d. Glutamate.....	15
OBJECTIVES.....	17
MATERIALS AND METHODS.....	19
I. Enzyme Treatment.....	19
II. Electrophysiological Recording.....	20
III. Microperfusion Setup.....	21
RESULTS.....	23
I. Electrophysiological Results from Whole-cell Clamp Recording.....	23
1. Two outward rectifying currents on the large fibers.....	23
2. Voltage-dependence of the two potassium currents.....	29
3. Kinetics of the two K ⁺ currents.....	34
4. Pharmacological characters of the channels conducting the two currents.....	41

II. Results of microperfusion of different ligands.....	44
1. K ⁺ solution.....	44
2. Glutamate.....	44
3. FMRFamide.....	44
DISCUSSION.....	52
I.Receptors Present in <i>Schistosoma</i> Muscle Fibers.	52
1. The two kinds of muscle fibers are differentially sensitive to elevated K ⁺	52
2. Glutamate receptors on Schistosoma muscle fibers.....	53
3. FMRFamide receptors exist on both types of muscle fibers.....	53
II.The Pharmacological and Electrophysiological Difference in the K ⁺ Channels on the Large and the Frayed Fibers... ..	54
1. Both the currents on large muscle fibers are mediated by K ⁺ channel.....	54
2. Comparison of the Schistosoma K ⁺ channels to K ⁺ channels in other animals.....	57
SUMMARY.....	59
REFERENCE.....	60

LIST OF TABLES

Table 1 - Properties of the two voltage-dependent K^+ currents of schistosome frayed muscle fibers...	12
Table 2 - Comparison of the two outward K^+ currents on the two different muscle fibers.....	56

LIST OF FIGURES

Figure 1 - Large muscle fibers appear less frequently than frayed muscle fibers in the preparation...	8
Figure 2 - The morphological comparison between the large muscle fibers and the frayed muscle fibers....	10
Figure 3 - Two examples of voltage-dependent currents in the large muscle fibers from <i>S. mansoni</i>	26
Figure 4 - Separation of the small fast current from the total current in the large muscle fiber of <i>S. mansoni</i>	28
Figure 5 - The current-voltage (I-V) relationship for the large slow current.....	31
Figure 6 - The current-voltage (I-V) relationship for the "A" current as derived by the subtraction method	33
Figure 7 - The relationship between the time constant of activation and amplitude of test pulse applied for the delayed current and the "A" current...	38
Figure 8 - The time constant of inactivation of the "A" current and the delayed current.....	40
Figure 9 - Effect of 10 mM 4-aminopyrimidine and 30 mM tetraethylammonium on the delayed outward current	43
Figure 10 - The percentage of fibers' contracting in response to exposure to elevated external K^+	47
Figure 11 - The percent fiber contracting in response to exposure to various concentrations of	

L-glutamate.....49

Figure 12 - The percent fiber contracting in response to
exposure to various concentrations of
FMRFamide 51

INTRODUCTION

I. INTRODUCTION TO SCHISTOSOMA

1. Schistosomiasis and its Socioeconomic Effect.

Schistosomes, or blood flukes are medically and economically important because they cause one of the major human diseases, schistosomiasis (Noble and Noble, 1976). Schistosomiasis results from a heavy infection with schistosome trematode worms, and in particular from the eggs laid in the human body by the female worms. This disease affects 74 countries, in which some 200 million are infected. Of these, 20 million suffer clinical morbidity or disability. The disease kills few people, but its sapping chronic effects, very high prevalence, and association with agricultural and economic water development projects, makes it a problem of great public health concern. According to the World Health Organization's Twelfth Program Report of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Medicine(1995), in terms of the consequent disability-adjusted life-years (DALYs) lost, it is second only to malaria.

Four of the 19 varieties of schistosome seriously

affect people (Mullers, 1975). They are transmitted through one of several species of water-associated snails which can act as hosts of the sporocyst intermediate stage of the parasite. Once in the human body, female worms produce eggs continuously and can live for up to 35 years, but normally they last no more than three to five years. Each female worm can produce between 40 to 3500 eggs a day depending on the species and other factors. About half of the eggs get trapped around the bladder or in the liver, where they are attacked and destroyed by the body's immune system. In this process granulomas are formed by destroying neighboring host cells. In heavy infections, over long periods of time, these granulomas surrounding the eggs may cause extensive fibrosis of the liver and obstructive kidney damage. Hepatic enlargement, portal hypertension, kidney failure, blood in the urine (hematuria), anaemia, and cancer of the bladder can all be associated with schistosomiasis (WHO report, 1995) .

2. *Schistosoma* as a Primitive Invertebrate

The four species of *Schistosoma* which can affect

humans are *S. mansoni*, *S. japonicum*, *S. haematobium* and *S. mekongi*. They all have a similar life cycle. Eggs are passed from the adult *Schistosoma* into human urine or feces and contain fully formed miracidia (Mullers, 1975). On immersion in fresh water, particularly under conditions of warmth and light, the miracidia hatch and attempt to penetrate fresh water snails which serve as intermediate hosts. After an asexual reproductive stage in the snail, cercariae emerge in 4-7 weeks. They can penetrate human skin and enter the peripheral lymphatic or venous vessels. For *S. mansoni*, they are carried to the lungs 4-7 days after penetration. The schistosomes then move from the lungs to the portal vessels. They grow into adults and mate in the portal vessels and remain in pairs.

In all species of schistosomes, the male worm is characteristically boat-shaped, with a gynaecophoric canal in which the female lives. *Schistosoma* is the only genus in the Class Trematoda which has separate sexes.

II. SCHISTOSOME MUSCLE FIBERS

Muscle fiber function is critical to schistosomes in both their parasitic and free-living stages. Migration between and within the host, attachment in the host and reproduction are all dependent on muscle activity. Several anti-schistosomal medicines, such as metrifonate and praziquantal, exert their effect by disturbing the schistosome muscle function (Pax et al., 1981; Fetterer, et al., 1980). It is very important to understand the properties of muscle fibers of this parasite.

1. Individual Muscle Fibers

The somatic muscle of the schistosome is grouped into circular muscle, longitudinal muscle and oblique muscle (Spence, 1971). Underneath the tegument is a thin layer of circular muscles. Under this layer of circular muscle is a thicker layer of longitudinal muscle fibers. The longitudinal muscle is the main component of the mass of the schistosome muscles. There are also a small number of oblique fibers which perhaps represent transverse somatic muscles.

To get a better understanding of the physiology of

individual muscle fibers in *Schistosoma*, an enzyme treatment protocol using a combination of papain digestion and mechanical dissociation has been developed to obtain a dispersion of individual muscle fibers on which electrophysiological and pharmacological research can be accomplished. (Blair, et al. 1991) (This method is described in detail in the Materials and Methods section).

The identified individual muscle fibers have been separated into three major groups according to their morphology: frayed fibers, crescent-shaped and spindle-shaped fibers. The frayed fiber is most prevalent in the dispersed fiber preparation (Figure. 1). Because of this, most previous electrophysiological and pharmacological studies have been focused on this category of muscle fibers.

Frayed fibers are characterized by their bifurcated or frayed endings and a length of about 20 μm . The crescents and spindle fibers average 60 μm and 25 μm in length respectively and also have characteristic morphologies. It is still unclear how these three categories of muscle fibers relate to the ultrastructural observations. Because of the prevalence of the frayed fibers in the preparation and the bulk of longitudinal muscle mass, the frayed fibers may represent longitudinal muscle fibers.

A fourth kind of fiber also appears constantly in the preparation and makes up approximately 10 to 20 percent of the muscle fibers present. In this thesis this muscle fiber type will be referred to as the large muscle fiber because it is generally 200 um in length, much larger than the fibers in any of other three categories. The large fibers are compared with frayed fibers in figure 2.

FIGURE 1. Large muscle fibers appear less frequently than frayed muscle fibers in the preparation. Example of two fields of cell dispersion of the large fibers and frayed fibers under the optical microscope. Frayed fibers have frayed ends and more constant shape (top). Large fibers usually have two single halves and twisted ends (bottom).

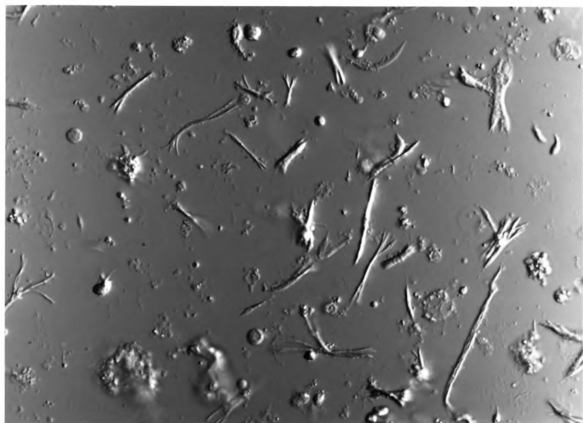
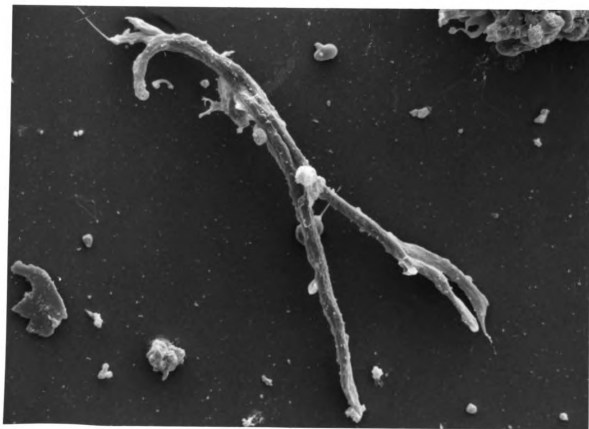
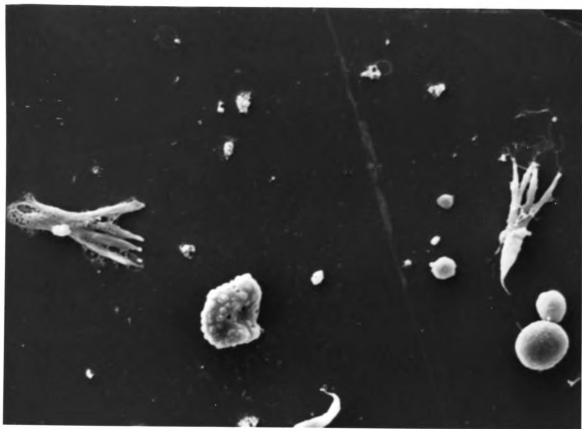


FIGURE 2. The morphological comparison between the large muscle fibers and the frayed muscle fibers.

Scanning electron micrographs of a frayed muscle fiber with a length of 23 μm (top) and a large muscle fiber with a length of 75 μm are presented here (bottom). The magnification for these two photographs are both 1000.



2. General physiology and electrophysiology of frayed muscle fibers

Previous studies in this laboratory had been involved in electrophysiological and molecular biological studies on the frayed fibers. Using the whole-cell clamp intracellular recording technic, two outward voltage-dependent potassium currents have been detected in the frayed fibers (Day, et al, 1993). One current fits in the delayed voltage-dependent rectifier potassium current category, which activates slowly after depolarization and also inactivates slowly. The other current is a fast-activating, transient, voltage-dependent K^+ current which is characteristic of "A" currents. (Gettings, 1983)) This current activates quickly after depolarization and also inactivates quickly. The electrophysiological properties of both currents are listed in Table 1.

Both currents are K^+ selective, which is shown by the reversal potential of both currents and that both are blocked by the K^+ channel blocker, 4-aminopyrimidine. Both currents are independent of extracellular Ca^{++} . Another K^+ channel blocker TEA demonstrates a small effect when applied

in the extracellular solution. (Day et al., 1993)

Table 1. Properties of the two voltage-dependent K^+ currents of schistosome frayed muscle fibers.

	Delayed rectifier current	"A" current
Activation threshold	-10 mV	-40 mV
$\tau_{act,+50mV}$	16.8 ± 4.2 ms	1.1 ± 0.4 ms
$\tau_{inact,+50mV}$	3423 ± 782 ms	13 ± 6 ms
I_{max}	413 ± 158 pA	165 ± 87 pA
G_{max}	3.13 ± 1.20 ns	1.32 ± 0.71 ns
10 mM 4 ⁻ AP blockage	48%	45%
30 mM TEA blockage	12%	31%

Another calcium-dependent K^+ channel has also been identified in the muscle cells. The selectivity of this channel is greater than 10:1 for K^+ over Na^+ , Cs^+ or NH_4^+ . The percentage open time is dependent on Ca^{++} concentration

at the intracellular face (Blair et al., 1991)

No inward current has been detected yet on the frayed muscle fibers.

3. Effects of Neurotransmitters on Isolated Frayed Muscle Fibers

a. Serotonin

Serotonin has excitatory effects on motor activity when applied to the whole *S. mansoni* (Blair, et al., 1993).

It has long been considered as a possible excitatory neurotransmitter in these parasites. By activating G- proteins, serotonin can activate adenylate cyclase and elevate cAMP in homogenate from *S. mansoni* (Northrup & Mansour, 1978). Phosphofructokinase, the rate-limiting enzyme for energy production in these parasites, is one of the proteins which is phosphorylated by cAMP (Mansour, 1984).

Serotonin shows no contractile effect when applied to individual muscle fibers, but it is required for maintenance of contractility (Day et al., 1994). Serotonin is required at a concentration of 300 nM or higher to obtain

contractions in response to exposure to high K^+ concentrations. The response is dose-dependent with concentrations of serotonin less than 300 nM. For isolated *S. mansoni* muscle fibers, the adenylate cyclase activator forskolin can mimic the effect of serotonin and the protein kinase A inhibitor H89 can block the response in the presence of serotonin. One explanation of this phenomenon is that serotonin is needed for sufficient energy production within the cells to give contraction. Because of this, 10^{-6} M serotonin is applied to all experimental extracellular solution used in the experiments described here.

b. Elevated extracellular K^+ .

The individual frayed muscle fibers isolated from *S. mansoni* contract in a dose-dependant manner when exposed to elevated extracellular K^+ . The contraction is dependent on extracellular Ca^{++} . Ca^{++} channel blockers such as Co^{++} or nifedipine can block the high K^+ induced contractions (Day et al., 1994). The possible mechanism of the high K^+ induced contraction is that the high K^+ depolarizes the muscle membrane and causes influx of Ca^{++} through voltage-gated Ca^{++} channels and that the Ca^{++} influx can induce the

contraction of individual muscle fibers.

c. FMRFamide and its related peptides

Molluscan FMRFamide and two recently discovered platyhelminth FMRFamide-related peptides, GNFFRFamide from the cestode *Moniezia expansa* and RYIRFamide from the terrestrial turbellarian *Artioposthia triangulate*, can cause dose-dependent contractions of individual muscle fibers of *S. mansoni* (Day, et al., 1994). The most potent is the turbellarian peptide RYIRFamide, which produces a contraction of the frayed muscle fibers at concentrations between 10^{-9} - 10^{-7} M. This FMRFamide-related peptide-induced contraction is dependent on extracellular Ca^{++} , but the Ca^{++} channel blockers nicardipine, verapamil or diltiazem do not block the contraction.

d. Glutamate.

In the central nervous system of vertebrate and mammalian species, glutamate receptors mediate fast synaptic action. According to pharmacological features, the glutamate receptors are subdivided into one class which is

specifically activated by N-Methyl-D-aspartate (NMDA), and a second class which is not activated by NMDA.

It has been found that frayed muscle fibers isolated from *schistosoma* contract when glutamate is present in the extracellular solution. (Day et al., 1994) Aspartate, which has a similar molecular structure, has the same effect on these muscle fibers and is more efficient in inducing contractions than glutamate at the same concentration.

OBJECTIVE

Though K^+ channels with different characteristics have been found in nearly all kind of organisms, there is still limited knowledge of the difference and distribution of these channels on different muscle fibers within one organism. Research on the different electrical properties of the channels from different kinds of muscle fibers in the same organism could improve our understanding of the functions of the different kinds of channels within the various muscle types within one organism.

The purpose of the research presented here was to reveal the electrophysiological and pharmacological properties of the large muscle fiber described earlier. As studies have been done on the frayed muscle fibers which are obtained using the same enzyme treatment from the adult *Schistosoma*, the research will reveal more about the difference between different muscle fibers and their ion channels in the same organism.

For electrophysiological studies, the whole-cell patch clamp method was used to test the activation and

inactivation of voltage-activated currents. Different sets of voltage protocols were selected and used for testing the "A" current and delayed current on large fibers. K^+ channel blockers were also tested in the extracellular solution to test the ions which composed the currents.

For pharmacological research, the microperfusion method was used to test the reaction and sensibility of the large fibers to the neurotransmitters. The same enzyme treatment as that used for preparing frayed fibers was used to obtain the large fibers.

MATERIALS AND METHODS

I. Enzyme Treatment

The incubation medium (DMEM⁺) used in this study was Dulbecco's Modified Eagle's Medium (DMEM, Sigma). The DMEM was diluted by 33% and 2.2 mM CaCl₂, 2.7 mM MgSO₄, 0.04 mM Na₂HPO₄, 61.1 mM glucose, 1.0 mM dithiothreitol (DTT), 10 μ M serotonin, 10 mg/ml pen-strep and 15 mM HEPES were added. The final osmolality was 290 mosm and pH at 7.4. The actual ion concentration of the DMEM⁺ medium is 4.1 mM K⁺, 82.6 mM Na⁺, 3.6 mM Ca²⁺, 93.7 mM Cl⁻, 3.3 mM SO₄²⁻, and 0.04 mM PO₄²⁻.

About 26-45 adult *S. mansoni* were harvested from the portal and mesenteric vein of female Swiss Webster mice and stored in the DMEM⁺ at 37°C. After rinsing the worms with the DMEM⁺ thoroughly 4 times, the worms were placed on a sterilized glass slide and cut into small pieces with a razor blade. The pieces of the worms were transferred to a papain solution and treated three times for 15 minutes each time on a shaker bed. The papain solution was made by adding 1 mM EGTA, 1 mM EDTA, 0.1% bovine serum albumin, and 1 mg/ml papain (Boehringer Mannheim, Germany) to the DMEM⁺

medium. The worm pieces were rinsed gently with DMEM+ medium for 4 times after the papain treatment, and incubated for 10 min in 37°C DMEM+ on a shaker in medium free of enzyme. To break the worm pieces into individual muscle fibers, the medium with the small pieces of worms was pipetted up and down 30-50 times with a normal Pasteur pipet, which yielded individual muscle fiber. The suspension of isolated muscle fibers was then transferred to a small container made with a cover glass for patch-clamp experiments, or into a 35 mm Corning tissue culture dish for microperfusion experiments.

II. Electrophysiological Recording

A List EPC-7 patch clamp amplifier (List Electronic Darmstadt/Eberstadt, Germany) was used for current to voltage conversion and amplification. An Axon TL-1 DMA interface (Axon Instruments, Foster City, CA, USA) was used to convert the voltage signal from analogue to digital. All the data were acquired and analyzed with the help of P-Clamp 5.5 software (Axon Instruments).

An inorganic incubation medium was used as a standard extracellular bathing solution for whole-cell patch clamp

studies. This solution consisted of 110 mM NaCl, 5.4 mM KCl, 0.4 mM MgCl₂, 2 mM EGTA and 20 mM Na-Hepes (ph=7.4). The intracellular solution in the pipet consisted of 120 mM KCl, 1 mM CaCl₂, 10 mM EGTA and 20 mM K-HEPES (ph=7.4).

Recording pipets were pulled from 1.2 mm outer diameter borosilicate glass (World Precision Instruments, INC) using a two-stage vertical puller (NARISHIGE PP-83, Japan). Fire polishing was applied to the tips to make sure that high-resistance seals could be achieved. All the experiments were done at room temperature. After whole-cell clamp was achieved the muscle fibers were held at -40 mV as a standard holding potential.

III. Microperfusion Setup

Before the microperfusion experiments, the DMEM+ medium was exchanged with an inorganic medium (DMI). This medium consists of 4.1 mM K⁺, 82.6 mM Na⁺, 3.6 mM Ca²⁺, 100.4 mM Cl⁻, 79.9 mM glucose and 15 mM HEPES (N-[2-Hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]). Microperfusion experiments were carried out in 35 mm tissue culture dishes.

Micropipets filled with the medium containing different concentrations of different neurotransmitters were also used in this method. With the help of a micromanipulator, the tip of micropipets filled with different neurotransmitters

were moved close to isolated individual muscle fibers in the DMI solution. Then higher air pressure was applied from the other end of the pipet. The medium containing different neurotransmitters was forced out of the pipet from the tip and perfused onto the individual muscle fibers. The responses of the muscle fibers were recorded by video camera and analyzed later.

For each 35 mm tissue culture dish, about 10-20 frayed fibers and 5-15 large fibers were tested. Frayed fibers and large fibers were chosen in the same dish and microperfused with the same solution to minimize temperature and other differences. The data are expressed as the percentage of all the cells that were chosen and microperfused which contracted. For all the microperfusion experiments, DMI with 4.1 mM K^+ was used as control solution. Because the large fibers appear less frequently than the frayed fibers, average 76 of large fibers were tested for each different concentration of different neurotransmitters, while the average number of frayed fibers tested for each different concentration was 103.

RESULTS

I. ELECTROPHYSIOLOGICAL RESULTS FROM WHOLE-CELL CLAMP RECORDING

Because the large muscle fibers are much larger than the frayed fibers, and their surface membrane is more regular and smoother than the frayed fibers, it was easier to be successful in obtaining a good tight-seal whole-cell clamp on the large fibers than on the frayed fibers. In these studies, 80 percent of attempts to get tight-seals were successful.

The resting potential of the large fibers was measured by determining the membrane potential present when there was zero current across the membrane. The average resting potential determined in this way for 21 large fibers was -24 ± 5 mV. This resting potential of the large fibers was very close to the resting potential of -22 ± 3 mV reported previously for the frayed fibers (Day, et al., 1993). The capacitance of the large fibers was 5.6 ± 1.4 pF, this is also nearly the same as the capacitance of 5.7 ± 1.4 pF for the frayed fibers .

1. Two Outward Rectifying Currents on Large Fibers

Whole-cell voltage-clamp was used on the large fibers

and 150 ms test potentials from -70 mV to +50 mV were applied. More than 60 percent of the large fibers tested showed two different outward rectifying currents (Figure 3).

One current had a large amplitude, which activated and inactivated slowly and incompletely in the span of the 150 ms test pulse. The average amplitude of the slow current elicited with a +50 mV test pulse ranged from 2745 ± 1743 pA. The value are the average of the current levels from 130-140 ms after the application of the test pulse. About 40 percent of the large fibers tested showed only the delayed large current.

There was another outward current which was about one fourth the size of the large current with an average of 695 ± 328 pA current when a test pulse of +50 mV was applied. This small current activated and inactivated quickly within 20 ms (figure 4). The small current was totally inactivated with a -10 mV prepulse but was not inactivated with a -90 mV prepulse. So the fast small current could be isolated from the slow large current by subtracting a family of test potentials with a -10 mV prepulse form the same family of test potential with a -90 mV prepulse (Figure 4).

Figure 3. Two examples of voltage-dependent currents in the large muscle fibers from *S. mansoni*.

Sixty percent of the fibers showed two different voltage-dependent outward currents. The other forty percent showed only a slow large current.

On the left is an example of a recording on a large muscle fiber in which the two currents are present. The beginning part of the recording rises more quickly than in the right example which shows only the large current.

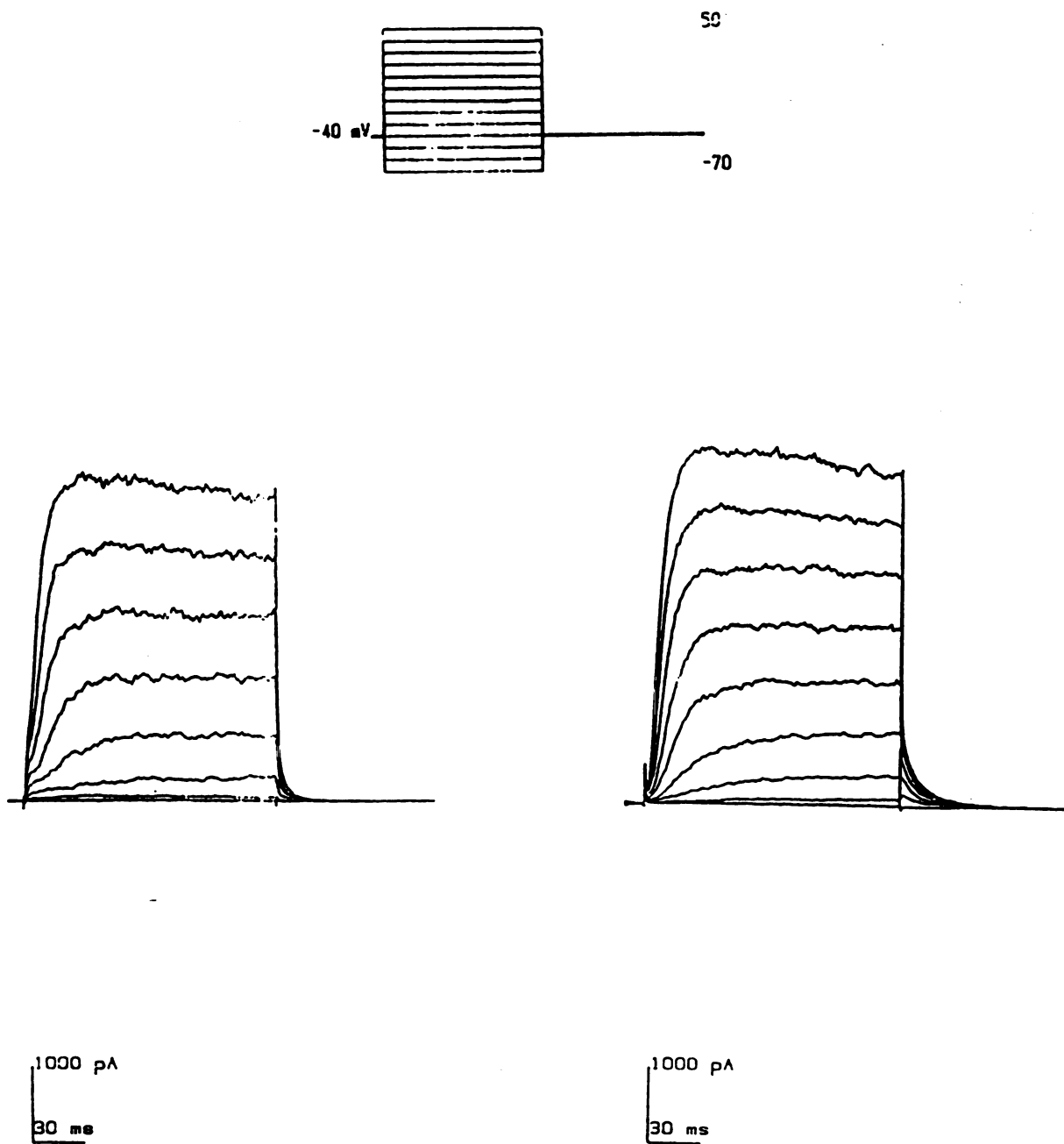
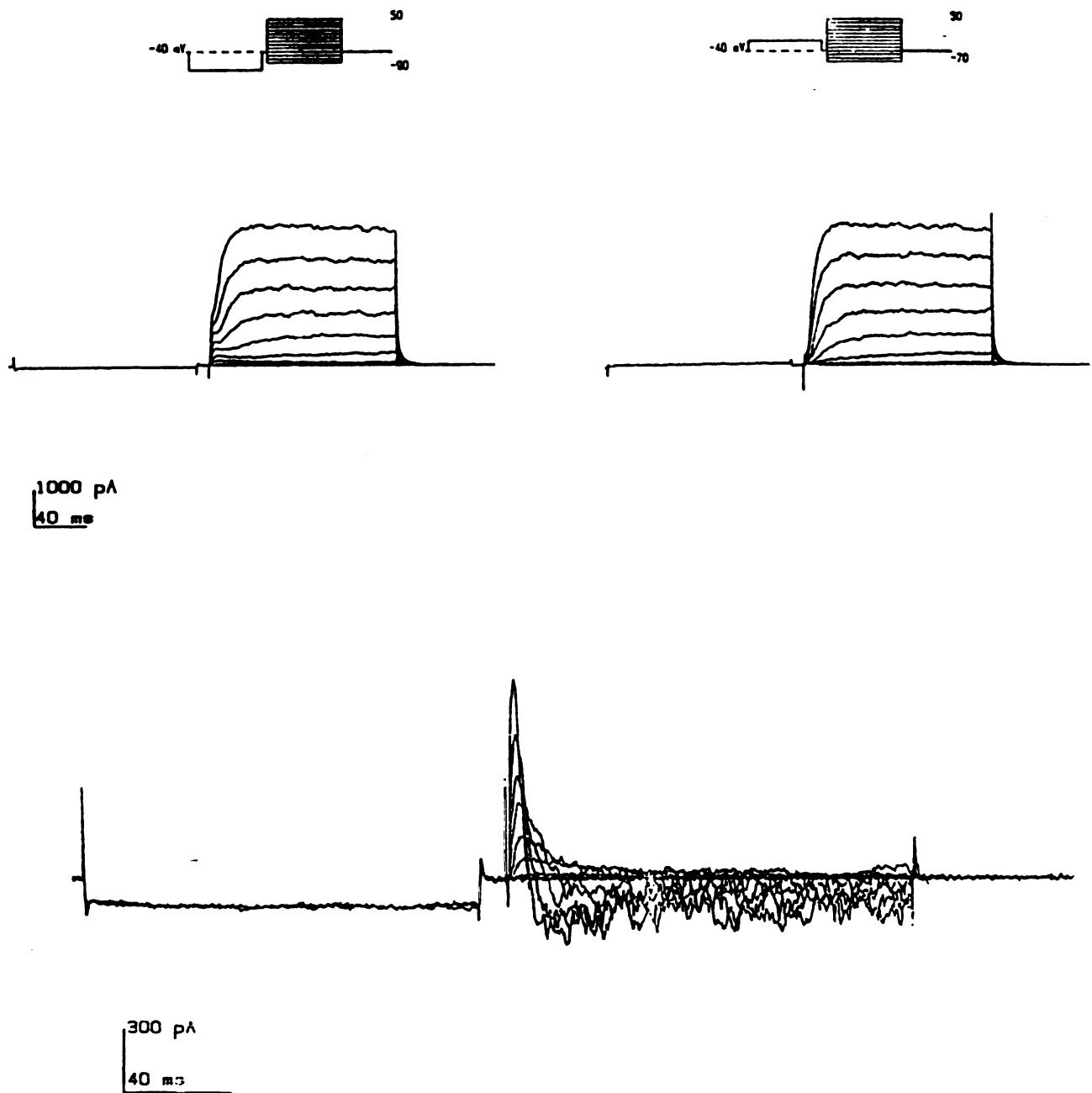
Figure 3

Figure 4. Separation of the small fast current from the total current in the large muscle fiber of *S. mansoni*.

The top two recordings were recorded while applying two different sets of test pulses to the same large muscle fiber, in which two currents were present: a small fast current and delayed current.

On the left is a recording from the fiber in which test pulses ranging from -70 to +50 mV were applied following a short pre-pulse to -90 mV (see voltage protocol at top left). On the right, the voltage protocol applied to the same fiber was identical to the one on the left with the exception that a short pre-pulse to -10 mV was used (see voltage protocol at top right). The pre-pulse to -10 mV causes the inactivation of the small, fast current but does not affect the large current. The pre-pulse to -90 mV (recording on the left) does not inactivate the small, fast current. Subtraction of the currents on the left from those on the right gives the result in the traces shown at the bottom.

Figure 4

2. Voltage Dependence of The Two Potassium Currents

Both of the outward currents present in the large fibers are voltage-dependent. This voltage-dependent activation was shown by the increase in conductance with increasing voltage pulses. Because the currents were K^+ selective currents, the conductance was calculated by:

$$G = \text{Current Amplitude} / (K^+ \text{ equilibrium potential} + \text{test potential})$$

The K^+ equilibrium potential in the case of the intracellular and extracellular solutions used in these experiments was -82 mV. At a test potential of +50 mV the conductance of the slow current was 20.8 ± 13.2 ns and that for the fast small current was 5.3 ± 2.5 ns. The relationship of these two currents and different test pulses are shown in figure 5 and figure 6.

FIGURE 5. The current-voltage (I-V) relationship for the large slow current.

Top: Example of voltage-dependent K^+ current in a large fiber with only the slow-large current. A series of voltage pulses ranging from -70 to +50 mV were applied.

Bottom: The relation between voltage applied and slow large current elicited from the large fibers.

The delayed current becomes activated at around -20 mV and current amplitude increase with the size of test potential. The current measured is the average of the current levels from 130-140 ms after the beginning of the 150 ms test pulse. The current present with a +50 mV test pulse is 2744.6 ± 1742.5 pA. (n=16)

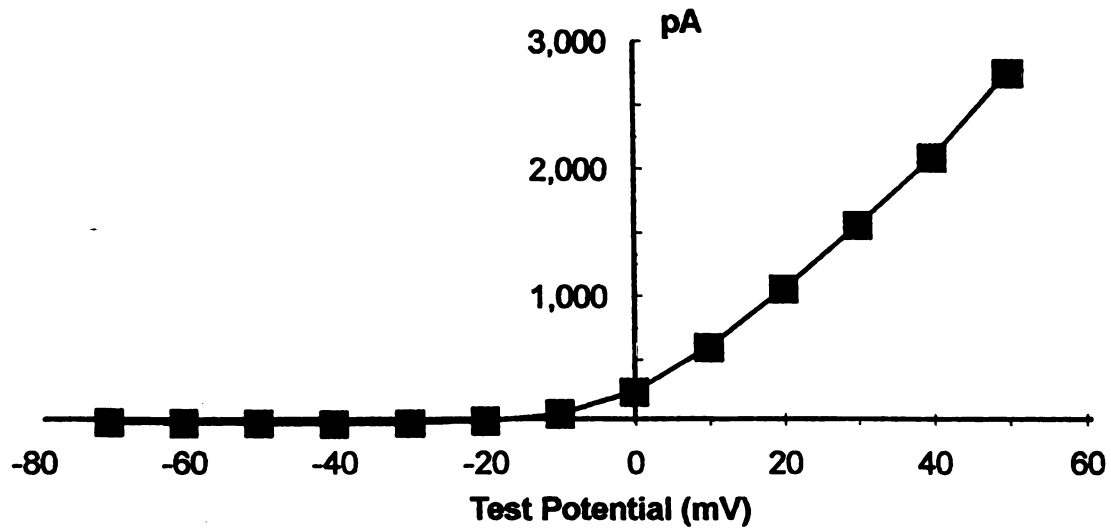
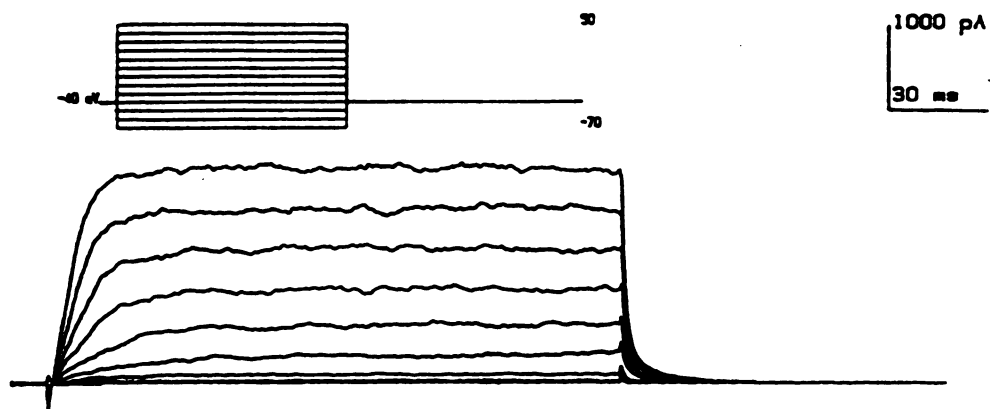
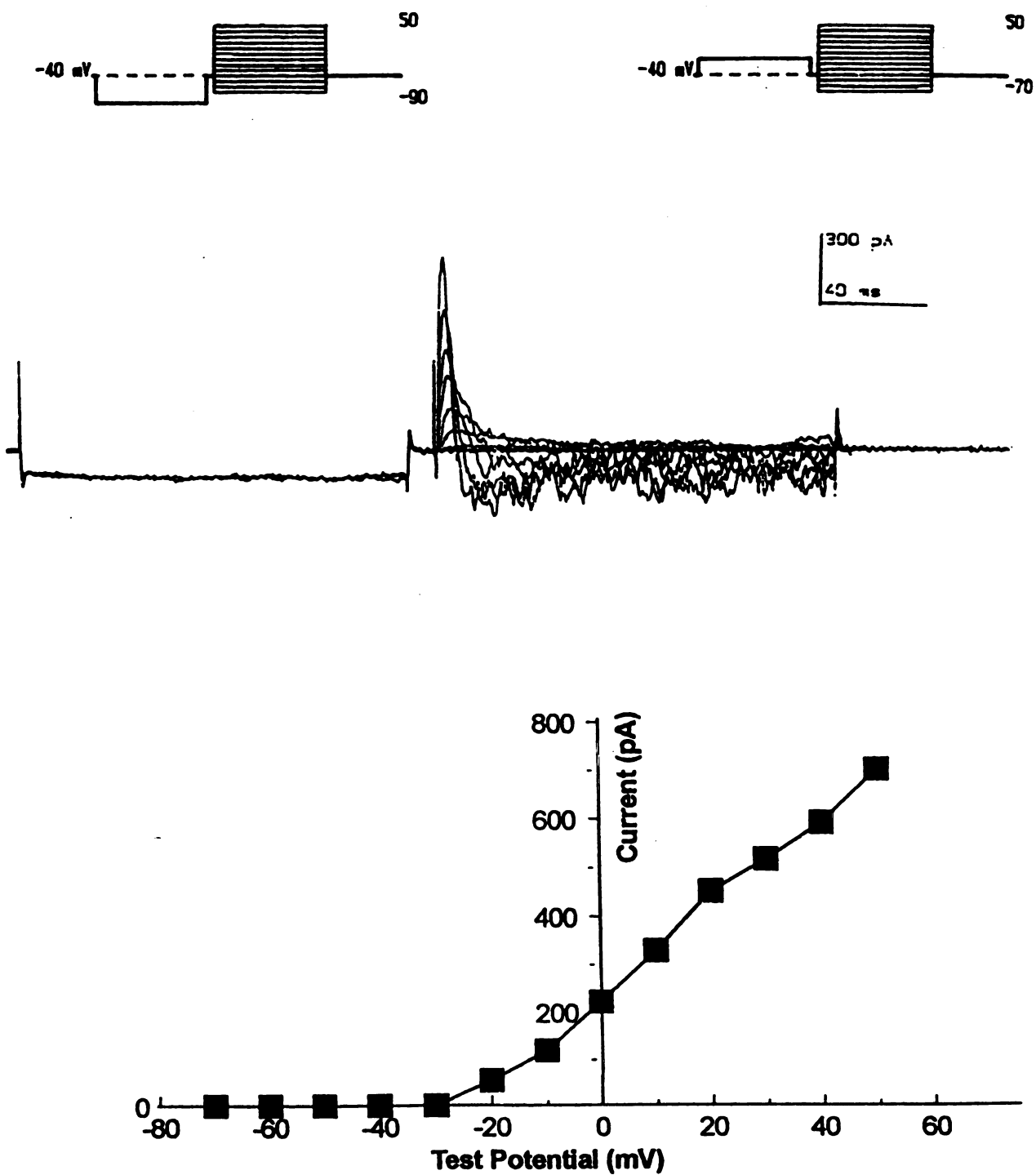
Figure 5

FIGURE 6. The current-voltage (I-V) relationship for the "A" current as derived by the subtraction method described in Figure 4.

Top: An example of the currents in response to a series of voltage pulses ranging from -70 to -50 mV.

Bottom: The average maximum current present in 14 large fibers.

Figure 6



3. The Kinetics of The Two K⁺ Currents

a. Activation kinetics:

The activation of both the potassium currents is voltage-dependent. The greater the depolarization the faster the activation is. The time course of activation for both currents was fitted with the Hodgkin-Huxley-type kinetics equation:

$$I = A + A_1 (1 - e^{-t/\tau})^n$$

τ (the time constant of activation)

A (the amplitude of current fitted by the equation)

A_1 (the amplitude of the offset from zero of the current fitted by the equation)

The delayed current in the large fibers showed a time constant of activation (τ_{act}) of 10.7 ± 1.0 ms ($n=15$) for an activating potential of +50 mV and an e-fold change for every 30 mV change in activating potential (Figure 7).

The fast current was obtained by subtracting the current resulting in response to each of the 150 ms test pulses when -10 mV pre-pulse was given and from the currents when -90 mV prepulses were applied. The fast current in the large

fibers had a time constant of activation (τ_{act}) of 3.9 ± 0.6 ms ($n=6$) for a test pulse to 50 mV. An e-fold change for every 30 mV change in pulse potential are shown by the graph (Figure 7).

Both the currents were described best when the exponent $n=1$ was used in the kinetics equation.

b. Inactivation kinetics:

The inactivation of the current was fitted by a single exponential, utilizing the equation below:

$$I = A + A_1 e^{-t/\tau}$$

Where A is the amplitude of the non-inactivating current, A_1 is the amplitude of the inactivating current and τ is the single time constant of inactivation.

The inactivation for the "A" current was voltage-dependent, which is showed on figure 8 (top). The time constants of inactivation (τ_{inact}) of the "A" current were in the range of 20 ms to 2 ms corresponding to -70 to 20 mV test pulse.

The inactivation for the slow delayed current did not show any voltage-dependence (Figure 8 bottom). The time

constants of inactivation (τ_{inact}) of the slow delayed current was in the range of 2000 ms to 4300 ms corresponding to 10 to 70 mV test pulse. The delayed current's inactivation was nearly 1000 times slower than that of the "A" current.

FIGURE 7. The relationship between the time constant of activation and amplitude of test pulses applied for the delayed current (squares) and of the "A" current (diamonds)
The Hodgkin-Huxley-Type kinetics equation was used in the fitting of the activation constants of both currents.

Figure 7

**Time constant of activation of slow current and A current
is voltage-dependent**

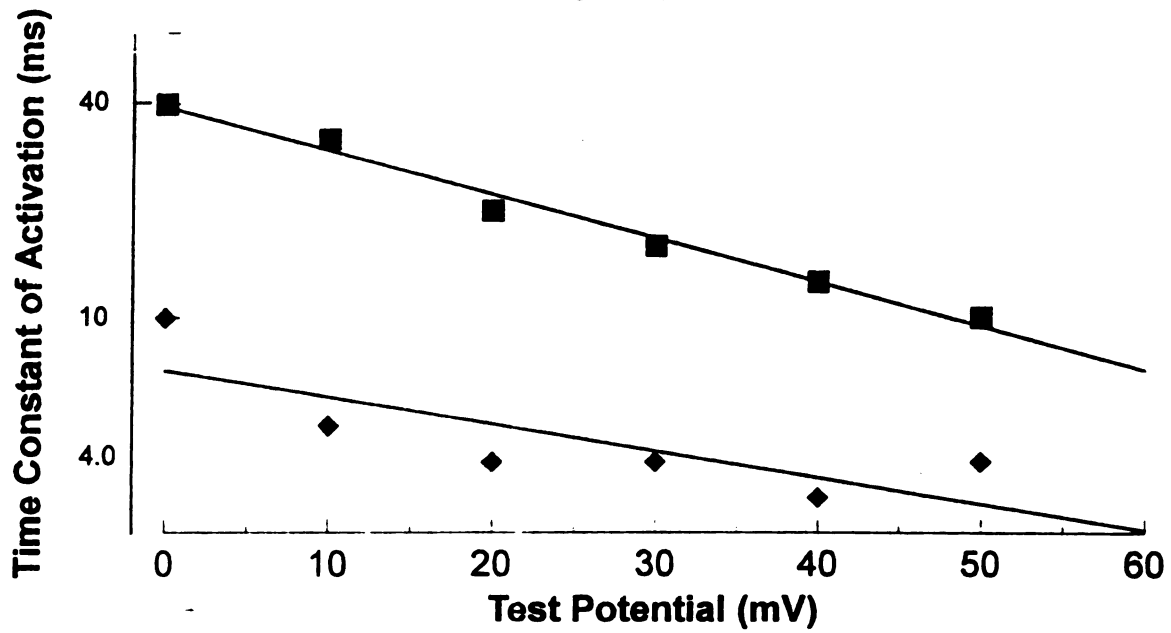
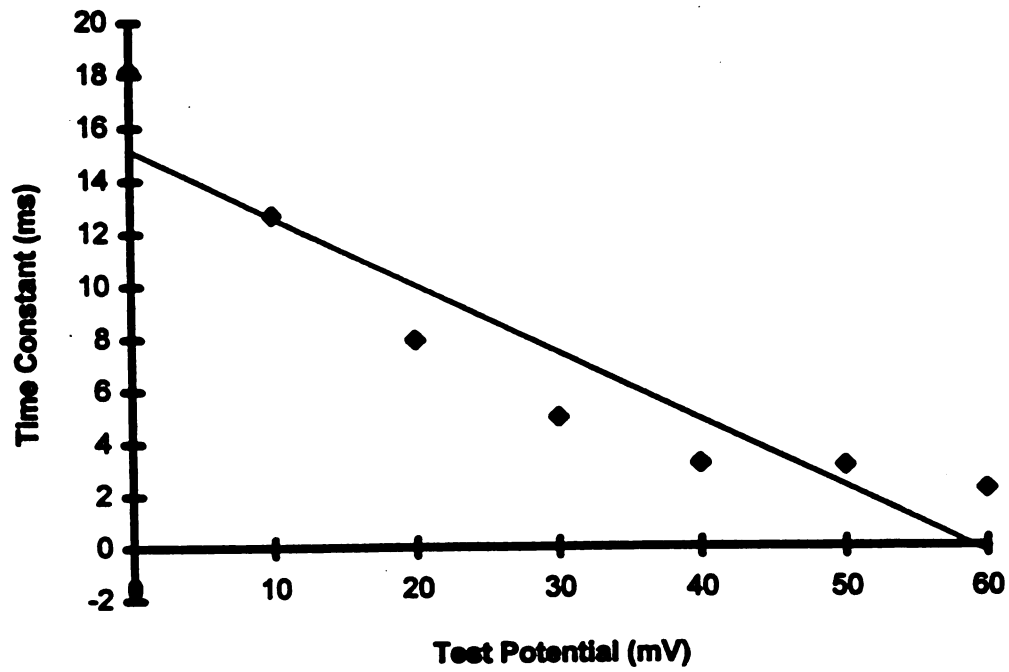
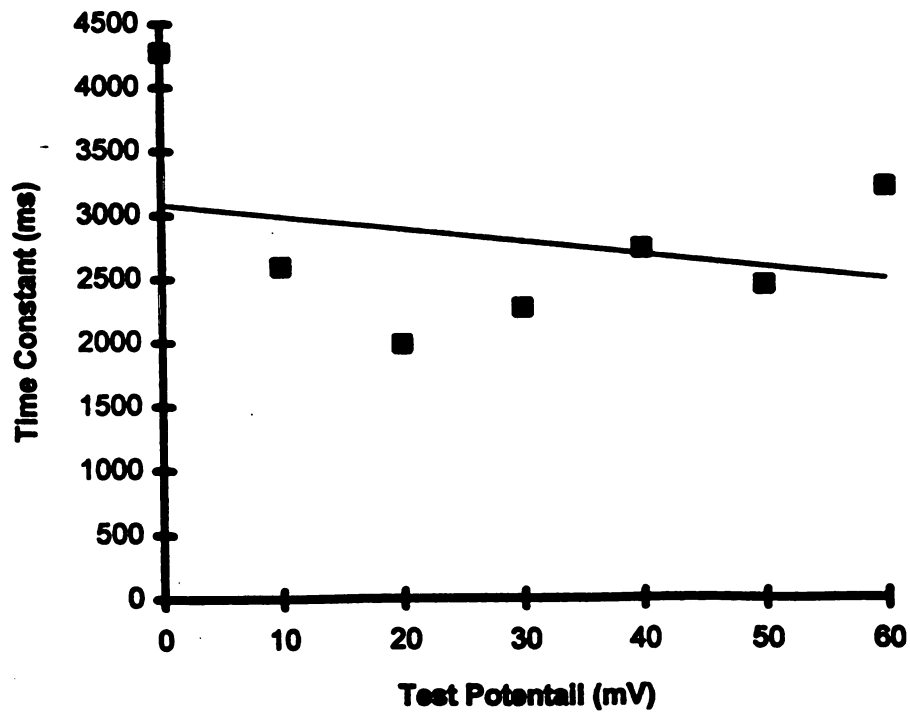


FIGURE 8. The time constant of inactivation of the "A" current (top) and the delayed current (bottom)

The time constant of inactivation of the "A" current was voltage-dependent, but that of the delayed current was not.

Both currents were fitted with superimposed mathematical approximation of the region that spanned from the peak of the current to the end of test pulse. Inactivation of the "A" current was nearly 1000 times faster than that of the delayed current.

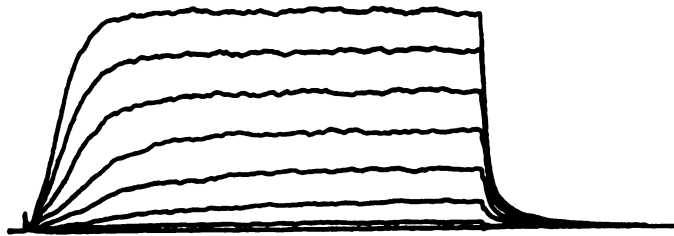
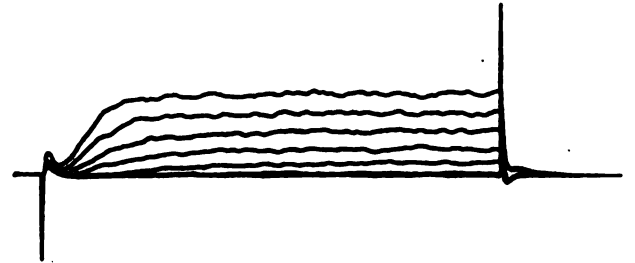
Figure 8**Time Constant of Inactivation of "A" Current****Time Constant of Inactivation of Slow Currents**

4. Pharmacological Characters of the Channels Conducting the Two Outward Currents.

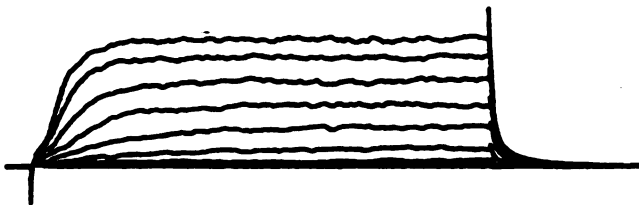
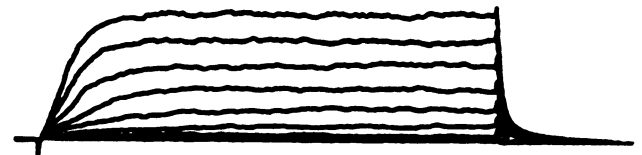
Two well known potassium channel blockers tetraethylammonium (TEA^+) and 4-aminopyrimidine (4-AP), were tested by applying them to the extracellular solution bathing cells in the whole-cell clamp. The external application of 30 mM TEA^+ showed little blockage of the delayed current (Figure 9), but the blockage of the delayed current when 10 mM 4-AP was applied to the extracellular solution was obvious (Figure 9). The amplitude of induced currents was reduced to 38% of the control values at all voltages applied. Due the physical disturbances to the muscle fibers under the patch clamp when blocker solution was added, it was not possible to test accurately the blockage of the fast current.

FIGURE 9. Effect of 10mM 4-aminopyrimidine (4-AP) (Top) and 30 mM tetraethylammonium (TEA) on the delayed outward current.

The left traces in each case represent the currents prior to addition of drug; the right traces, after addition of drug.

Figure 9**Control****With 4-AP**

1000 pA
30 ms

**Control****With TEA**

II. RESULTS OF MICROPERFUSION OF DIFFERENT LIGANDS

1. K^+ solution:

Both the large and the frayed muscle fibers contracted when exposed to high potassium solutions (4.1 mM - 50 mM), but the large muscle fibers were less sensitive to high potassium solution than the frayed muscle fibers (Figure 10). With 50 mM potassium, 83% of the frayed fibers contracted while 55% of the large fibers did at this concentration. Otherwise the shapes of the dose-response relation curve are similar in the two muscle fibers.

2. Glutamate:

Fifty percent of the large muscle fibers contracted when 10^{-3} M glutamate was perfused onto them (Figure 11). Only 20 percent showed contraction when exposed to 10^{-5} M glutamate. Both these percentages are lower compared to the frayed muscle fibers which showed 90% contraction at 10^{-3} M and 45% at 10^{-5} M glutamate.

3. FMRFamide:

Microperfusion experiments have also been carried out using the molluscan cardio-excitatory peptide, FMRFamide. The large muscle fibers were less sensitive to this neuropeptide than the frayed fibers (Figure 12). At 10^{-6} M FMRFamide, 64 % of the large fibers showed a contraction

response, while 92 % of the frayed fibers contracted at the same concentration. FMRFamide at 10^5 M gave a response no greater than that at 10^6 M.

FIGURE 10. The percentage of fibers' contracting in response to exposure to elevated external K^+ .

The squares represent responses in the frayed fibers; the diamonds responses in the large fibers. Each point on the graph is the average $\pm$ SEM for a minimum of 76 replications for the large fibers and 100 for the frayed fibers.

Figure 10

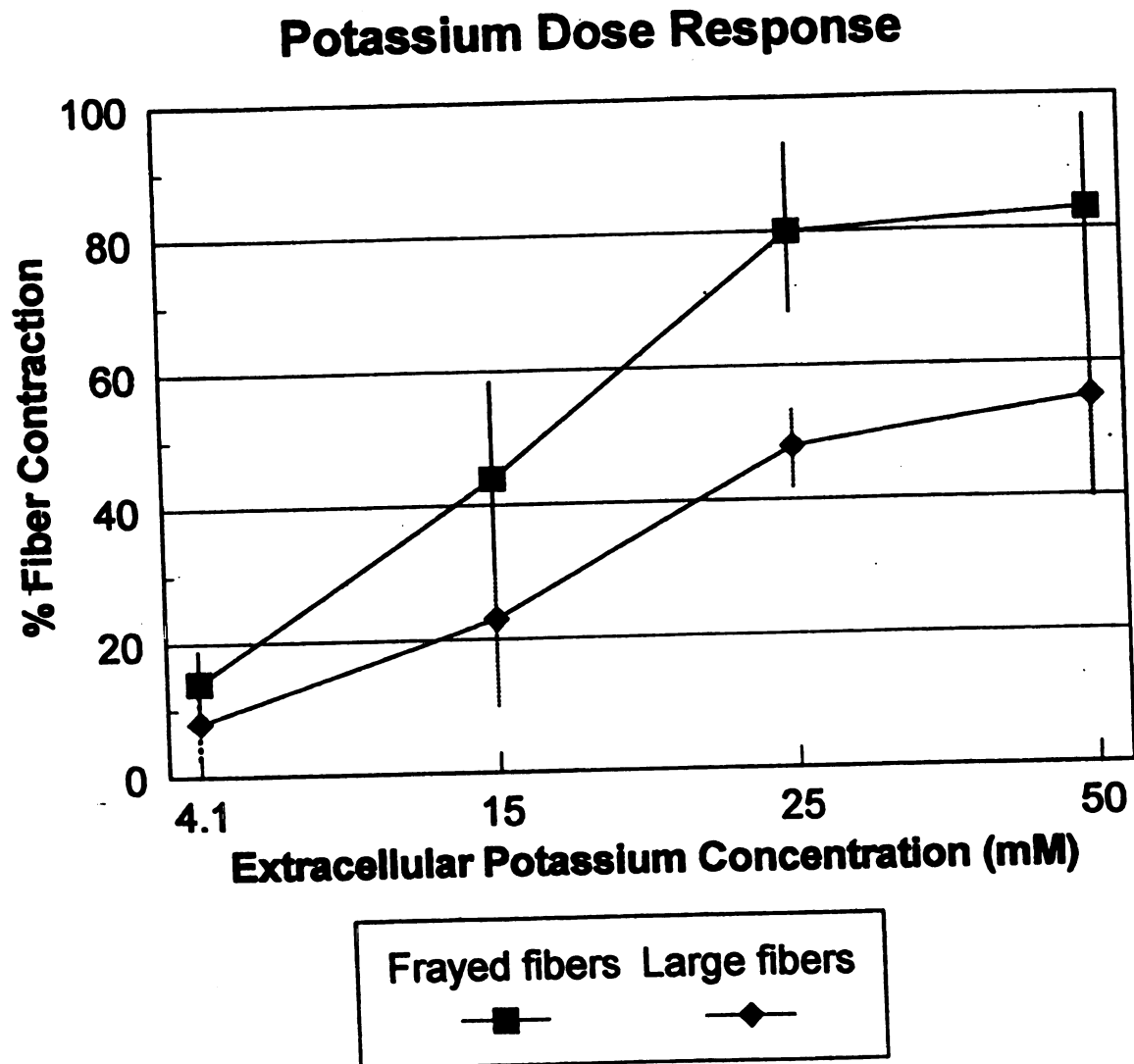


FIGURE 11. The percent fibers contracting in response to exposure to various concentrations of L-glutamate.

The square represent responses of the frayed fibers; the diamonds, the responses of the large fibers. Each point on the graph is the average $\pm$ SEM for a minimum of 76 replications for the large fibers and 100 for the frayed fibers.

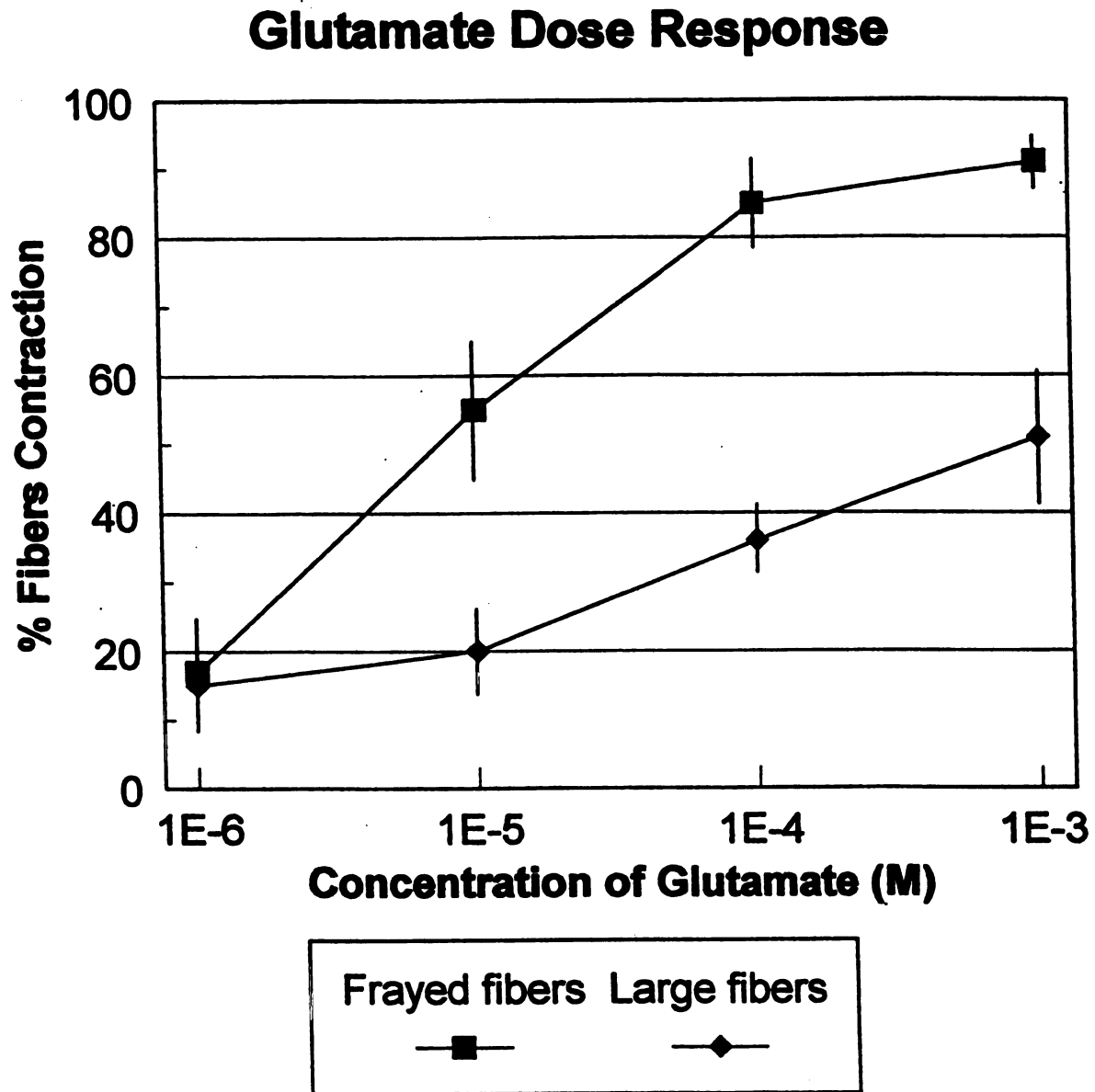
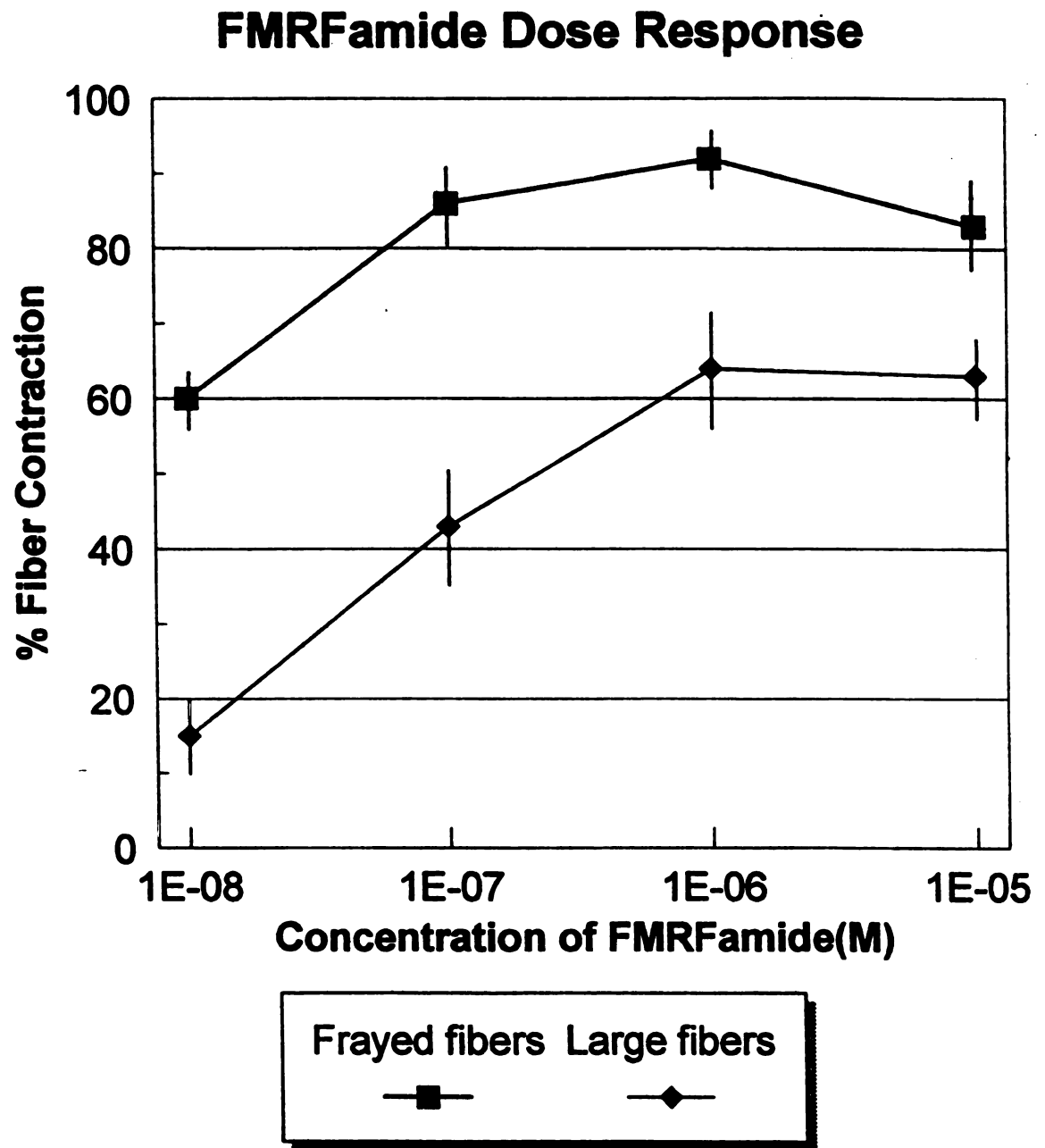
Figure 11

FIGURE 12. The percent fibers contraction in response to exposure to various concentrations of FMRFamide.

The squares represent responses of the frayed fibers; the diamonds, the responses of the large fibers. Each point on the graph is the average $\pm$ SEM for a minimum of 76 replications for the large fibers and 100 for the frayed fibers.

Figure 12

DISCUSSION

I. RECEPTORS PRESENT ON SCHISTOSOMA MUSCLE FIBERS

1. The Two Kinds of Muscle Fibers are Differentially Sensitive to Elevated K^+ .

Elevated potassium concentrations induce contractions in both of the frayed fibers and the large fibers. When the fibers are exposed to excess extracellular potassium, potassium will enter the cell through open potassium channels on the cell membrane and the potassium entering the cell will depolarize the cell. This would be expected to open the voltage-dependent calcium channels and the entrance of calcium will then induce contractions.

Frayed fiber muscles are more sensitive than the large frayed fibers to the elevated extracellular potassium. The frayed fibers can reach a 90% response at 25 mM K^+ concentration, while the large fibers reach only a 50% response rate at the same K^+ concentration. There may be several reasons for this. One reason may be that the calcium channels in these two kinds of muscle fibers have different properties and the calcium channels on the membrane of the large muscle fibers may require greater depolarization to open. Another reason may be that the potassium channels in the large muscle fibers are different

from those in the frayed fibers. They may need higher K^+ concentrations to induce the calcium influx. Also, a greater calcium influx may be required in the large fibers to produce contractions in these fibers.

2. Glutamate Receptors on Schistosome Muscle Fibers.

The results of the glutamate microperfusion test indicate that both the large fibers and the frayed fibers of *S. mansoni* have glutamate receptors in their membranes. They both show similar dose-response pattern, but the results do show there is difference in the sensitivity of the two kinds of muscle fibers to the same glutamate concentrations.

The differences in the sensibility of these two kinds of *S. mansoni* muscle fibers may be caused by differences in the density, receptor distribution, molecular structure or other functional properties of the glutamate receptors on these two kinds of muscle fibers. To determine the reasons for the differences on the two kinds of *S. mansoni* muscle fibers, more electrophysiological, pharmacological and molecular biological studies would be needed.

3. FMRFamide Receptors Exist on Both Types of Muscle Fibers.

The results of the microperfusion experiments of

molluscan cardio-excitatory peptide, FMRFamide, indicate that both the large muscle fibers and the frayed muscle fibers have receptors for this neurotransmitter on their membranes. Both types of fibers show the same dose-response pattern, but the results also show there are differences in the sensitivity of the two kinds of muscle fibers to FMRFamide. Both the large and the frayed muscle fibers reach their highest response at 10^{-6} M concentration. However sixty percent of frayed fibers contract with 10^{-8} M FMRFamide, while only 15% of the large fibers do at the same concentration.

II. THE SUBTLE PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL DIFFERENCES IN THE K^+ CHANNELS ON THE LARGE AND THE FRAYED FIBERS.

1. Both the Currents on Large Muscle Fibers Are Mediated by K^+ Channels.

The pharmacological and electrophysiological properties of both currents detected in the large fibers demonstrate that they are K^+ currents. The reversal potential and the equilibrium potential for both kinds of currents in both types of muscle fibers show that they are K^+ -selective. They are also both partially blocked by K^+

channel blockers.

The amplitude of both the currents recorded in the large muscle fiber are larger than these currents in the frayed fibers (Table 2). This may be due to the larger membrane surface in the large fibers, but there may also be dissimilarities with respect to density and distribution of the K^+ channels. The large, delayed currents in the large fibers and the frayed fibers become activated and inactivated at nearly the same test voltage of test pulse (Table 2). They also show similarities in the kinetics of their activation and inactivation.

It is also the same for the fast, transient currents in these two muscle fiber types (table 2). The small fast current is very similar to "A" current found in many other animals.

The kinetics of activation and inactivation of the fast, transient current is similar to the voltage-gated current carried by the shaker subfamily of K^+ channels first discovered on *Drosophila* (Salkoff et al., 1992). It activates rapidly with a steep current-voltage relationship, and it inactivates quickly.

Table 2. Comparison of the Two Outward K⁺ Currents on the Two Different Muscle Fibers

<u>DELAYED RECTIFIER CURRENT</u>		
	<u>Large fiber</u>	<u>Frayed fiber</u>
I _{max+50}	2744 pA	413 pA
G _{max+50}	20.8 ns	3.13 ns
Activation threshold	-20 mV	-10 mV
Activation Tau ₊₅₀	10.7 mS	16.6 mS
Inactivation Tau ₊₅₀	2754 mS	3423 ms
<u>"A" CURRENT</u>		
	<u>Large fiber</u>	<u>Frayed fiber</u>
I _{max+50}	659 pA	165 pA
G _{max+50}	5.3 ns	1.32 ns
Activation threshold	≈-30 mV	≈-40 mV
Activation Tau ₊₅₀	3.9 ms	1.1 ms
Inactivation Tau ₊₁₀	18.2 ms	17 ms

2. Comparison of the Schistosome K⁺ Channels to K⁺ Channels in Other Animals.

Potassium channels are the largest and most diverse of the known ion channels, and the voltage-gated K⁺ channels constitute the largest group among them. Since K⁺ channels were first detected by Hodgkin and Huxley (Hodgkin et al., 1952), K⁺ channels with different properties have been found in all kinds of organisms and they play a vital role in the functioning of diverse cell types.

The explosion in the number of cloned genes and cDNAs over the past decade has significantly improved our understanding of the structure of the voltage-gated K⁺ channels. Using genetic techniques in combination with molecular strategies, four related *Drosophila* genes subfamilies: shaker, shab, shaw and shal subfamily have been isolated. Each of them produces functionally distinct channels (Salkoff et al., 1992). One *S. mansoni* shaker-related K⁺ channel has been cloned and functionally expressed in *Xenopus* oocytes (Kim et al., 1995).

Of all the K⁺ channels found in different organisms, most of them show a high level of inter-species conservation. On the other hand, the channels of a specific type expressed in diverse cells within a single organism may show variable inactivation times. For example, channels

from *Drosophila* muscle and photoreceptor have different inactivation times. This suggests the existence of subtle difference in tissue-specific properties of K⁺ channels.

The results of these pharmacological and biophysical experiments on the two types of muscle fibers isolated from *S. mansoni*, for the first time, show that there are subtle differences in the voltage-gated K⁺ channels present on the large muscle fibers compared to those present on the frayed fibers. They are different in their toxin sensitivity, kinetics of activation and inactivation (Table 2). This subtle variability of the K⁺ channels in the two kinds of muscle fibers may indicate different functions for these channels in these two groups of muscle fibers.

Since *S. mansoni* is a primitive invertebrate, a Trematode, the more understanding of the subtle differences in the K⁺ channels in the different muscle fibers may also advance our knowledge about the origin and diversity of the subfamilies of K⁺ channels in the animal kingdom.

SUMMARY

In the electrophysiological research, both the large muscle fibers and the frayed muscle fibers have shown both "A" current and delayed current. The "A" currents found on these two kinds of muscle fibers demonstrate subtle differences in their activation and inactivation kinetics, and their sensitivity of K^+ channel blockers. So does the delayed currents. The large differences of currents amplitudes of these two currents on these two muscle fibers may be due to the density of K^+ channels and the surface membrane area of these two muscle fibers.

In the pharmacological research, both kinds of muscle fibers displayed the same pattern of dose-response to a series concentration of glutamate, FMRFamide and high extracellular K^+ . But these two kinds of muscle fiber also demonstrated different sensitivity to glutamate, FMRFamide and high extracellular K^+ .

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