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Patch-clamp studies on
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presented by

Timothy Allen Day

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Ralph A Pax
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PATCH-CLAMP STUDIES ON *SCHISTOSOMA MANSONI*

By

Timothy Allen Day

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ABSTRACT

PATCH-CLAMP STUDIES ON SCHISTOSOMA MANSONI

By

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Patch-clamp techniques, particularly those utilizing excised, inside-out patches, were used to study ion channels in the outer membrane of the schistosome tegument and in the sarcolemma of dispersed schistosome muscle cells.

Membrane patches pulled from the outer membrane of the tegument displayed a 295 pS channel that allowed the passage of cations but not anions. This channel had a much greater percent open time when held at negative membrane potentials and the opening and closing behavior could be modulated by membrane voltage.

Membrane patches excised from the sarcolemma of dispersed muscle displayed a 195 pS Ca^{2+}-dependent K^+ channel. This channel had a > 10:1 permeability ratio for K^+ over Na^+, NH_4^+ and Cs^+. 10 mM intracellular Ba^{2+} blocked the channel. This Ca^{2+}-activated K^+ channel in the schistosome muscle membrane is similar in most respects to the maxi-K^+ channels which have been described in a variety of cells from more highly evolved animals.

ACKNOWLEDGEMENTS

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INTRODUCTION

General Background on *S. mansoni*

World health problem

The schistosome, an endoparasitic blood fluke, is the etiological agent of schistosomiasis. Schistosomiasis affects humans in a large part of the world, being ranked by the World Health Organization as the second most important disease in the world in terms of socioeconomic and public health importance. 200 million people are currently infected with schistosomes and 600 million people are in danger of infection. Four species of *Schistosoma* are primarily responsible for human schistosomiasis: *S. mansoni*, *S. japonicum*, *S. heamatobium*, and *S. mekongi*.

Life-cycle

The four schistosome species that cause human disease vary in some important ways, including geographic distribution, gross morphology, intermediate hosts, reservoir hosts, disease patterns, and responses to antischistosomal agents. Despite these differences, all the species have the same basic polymorphic life-cycle.

Eggs pass out of the body of the definitive host via feces or urine and, if the eggs reach a fresh water environment, hatch into a free-swimming larval stage called the miracidium. Each miracidium then has a few hours to find and penetrate an appropriate intermediate host, a

freshwater snail. Within the snail, the miracidia undergo asexual reproduction, developing into primary sporocysts and then into secondary sporocysts. The secondary sporocysts release large numbers of free-swimming cercariae, which begin to emerge from the snail approximately 30 to 40 days after infection. Each cercaria then has about 24 hours to locate an appropriate definitive host and subsequently penetrate by burrowing through the skin of the host. Upon penetration, the cercariae lose their tails and are referred to as schistosomules. The schistosomules migrate to the venous system and journey through the lungs until they eventually reach their final destination, the mesenteric veins. In the mesenteric veins, the worms pair and mature to adults in 5 to 6 weeks. The female schistosome lives most of her life almost completely surrounded by the male, leaving only for the purpose of egg-laying. The eggs, which are deposited in the capillary beds of the mesentery, secrete enzymes which allow them to dissolve tissue and pass into the intestine or urinary bladder where they are excreted by the host.

Phylogeny

Schistosomes are trematodes, which along with cestodes (parasitic tapeworms) and turbellarians (non-parasitic worms) comprise the phylum Platyhelminthes. Platyhelminths

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are acoelomates and are among the most primitive phyla of bilaterally symmetrical animals.

The Tegument of *S. mansoni*

Tegument anatomy

The flat body of the male schistosome folds to form the ventral gynecophoral canal in which the female lives. The male worms are approximately 1 cm long and 0.1 cm wide, with the females being more rounded as well as slightly longer and thinner.

Trematodes, like cestodes, have a living, metabolically-active structure called a tegument as an outer surface. The tegument consists of an outer and an inner lipid bilayer which encloses a cytoplasm that is syncytial around the entire animal. The cytoplasm of the tegument is anucleate, with the nuclei servicing this living layer located in cytons that are buried beneath the muscle layers and connected to the tegumental cytoplasm by thin cytoplasmic bridges. Beneath the tegument lie layers of circular and then longitudinal muscle.

Tegument physiology

The tegument of both cestodes and trematodes is adapted to a greater or lesser degree to serve as an absorptive surface. All cestodes are completely without a digestive tract and their tegumental surfaces are highly endowed with

microtriches (Rothman, 1959, 1960), surface projections somewhat analogous to microvilli, that serve to increase the tegumental surface area. Trematodes, on the other hand, possess a very primitive digestive tract, and the relative importance of the tegument *vs.* the digestive tract as the location of nutrient absorption is different among different species. There is an inverse correlation between the presumed functionality of the digestive tract and the amount of tegument surface area amplification in trematodes (Isseroff *et al*, 1972). Among species with a less functional digestive tract, tegument topography tends to be more irregular due to surface invaginations and channelizations (Bogitsh, 1968; Bogitsh & Krupa, 1971). Schistosomes are among the trematodes which possess a digestive tract with very limited function and, thus, a highly absorptive tegumental surface.

Schistosomes, like all of the trematodes, are totally dependent on carbohydrate degradation for energy metabolism (Tielens, van Oordt & van den Bergh, 1989). Except for relatively small amounts of glycogen that are rapidly turned over, the adult worms metabolize glucose obtained directly from the host's bloodstream (Teilens, van den Heuvel & van den Bergh, 1990). The weight-specific metabolic rate of the schistosome is extremely high ($\approx$ 1 μmol/(g WW)/min) (Shapiro & Talay, 1982) and the glucose fluxes across the absorptive surfaces must be huge, around 10^{-7} mol/cm^2/sec, based on

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estimates of the epidermal surface area that include both the gut and the tegument (Gomme & Albrechtsen, 1988). Classically, the dominant role in glucose uptake has been given to the tegument (Rogers and Bueding, 1975). Bogitsh (1989) has speculated that material entering the gut may require as long as 24 hours to traverse the tract (which is less than 1 cm long), a rate of digestion that certainly could not accommodate the large glucose uptake required by the schistosome. The most critical function of the gut appears to be the ingestion of hemoglobin-containing red blood cells and the absorption of iron.

The tegument also has specific mechanisms for the uptake of amino acids (Mercer & Chappell, 1985), purine and pyrimidine compounds (Levy & Read, 1975) and choline (Young & Podesta, 1985). Thus, the schistosome tegument is a uniquely adapted absorptive epithelium that is critical to the nutritional well-being of the parasite.

Tegument electrophysiology

The tegumental cytoplasm of the schistosome maintains a -60 mV potential difference with reference to the external environment. This potential can be depolarized by alterations of the external medium, such as increased K^+, ouabain, reduced Ca^{2+} or reduced temperature. The ouabain-sensitive component of the potential is uncommonly large. As little as 0.1 mM can reduce the potential by 20 mV

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(Fetterer, Pax & Bennett, 1980), indicating that some form of active Na^+-K^+ transport is involved in maintaining an ion gradient across the parasite's tegument. The active maintenance of this potential across the outer membrane of the tegument means that the membrane is selectively permeable to ions and implies the presence of specific ion channels.

To date, all of the studies on tegumental electrophysiology have been on the aggregate fluxes of ions and particles across the tegumental membranes. Except for some ATPases (Cesari et al., 1989; Fetterer, Pax & Bennett, 1981), no specific mechanisms of facilitated ion permeability have been directly identified.

The Neuro-muscular system of *S. mansoni*

Muscle anatomy

Schistosome muscles are located directly beneath the inner membrane of the tegument and are arranged as a thin layer of circular muscle and a deeper, thicker layer of longitudinal muscle. The muscle of the schistosome, like that of most invertebrates, is smooth muscle (Lowry & Hanson, 1962). In both longitudinal and circular muscle, the ovoid nuclei are separate from the contractile elements, located deeper in the animal in cell bodies that are connected via thin cytoplasmic bridges to the muscle (Silk & Spence, 1969a). The opposing sarcolemmas of adjacent

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muscles often display junctional complexes, being apposed by distances of only 7 to 9 nm (Silk & Spence, 1969a), and low resistance pathways make the muscle compartment an electrical syncytium (Thompson, Pax & Bennett, 1982). Similar junctional complexes are observed between the muscles and tegumental cytons as well (Silk & Spence, 1969a) and these two compartments are also joined by low-resistance pathways (Thompson, Pax & Bennett, 1982).

Neuro-anatomy

The schistosome, like all trematodes, has two pairs of anterior central ganglia which lie on either side of the esophagus and are joined by circumesophageal commissures (Bullock & Horridge, 1965). Two major pairs of nerve chords, a dorsal pair and a ventral pair, extend longitudinally from the ganglia (Fripp, 1967). The longitudinal nerve chords are joined at intervals by dorsal and ventral transverse commissures. Small branches of the chords project peripherally and terminate in the muscle layers below the tegument (Silk & Spence, 1967b).

Acetylcholine, serotonin, dopamine and norepinephrine have each been identified in the schistosome and are suspected to serve as neurotransmitters.

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Whole-animal studies

Many studies on how various ions, putative neurotransmitters and anitschistosomals affect muscle activity have been performed on whole schistosomes. The exogenous application of serotonin on an intact schistosome causes a marked increase in the level of contractile activity of the parasites (Barker, Bueding & Timms, 1966). The application of cholinergic agonists, such as carbachol, or of cholinesterase inhibitors, such as physostigmine, inhibit spontaneous contractions of the worm and reduce both circular and longitudinal muscle tone (Barker, Bueding & Timms, 1966; Pax, Siefker & Bennett, 1984). Catecholamines such as dopamine, epinephrine and norepinephrine cause a relaxation of the parasite's musculature (Tomosky, Bennett & Bueding, 1974).

To date, all of the studies investigating the effects of neurotransmitters or various ions on schistosome muscle activity have been performed at the whole-animal level. As such, it is impossible to discern if the observed effect on the contractile state of the parasite is due to a direct action on the muscle. The effects could be indirect to the muscle via sensory neurons, interneurons, or neurons pre-synaptic to the muscle. Consequently, there is no information about the precise mechanisms by which the muscle activity in this parasite is modulated. In fact, very little experimental evidence exists that describes the

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neuro-muscular control of any animal in the phylum Platyhelminthes.

Rationale and Objectives

The general goal of these studies was to investigate the feasibility of using patch-clamp techniques to study and characterize specific ion channels of the outer tegumental membrane and the muscle membrane of the schistosome.

Tegument studies

The tegument is the key absorptive surface for nutrients in the schistosome, and as such the outer membrane's ability to maintain and utilize electrochemical gradients is critical to the parasite. The potential of the tegumental cytoplasm has been studied in aggregate, however neither the permeabilities of specific ions nor the actual mechanisms of ionic permeability have been established for the outer membrane. Therefore, it was of interest to see if patch-clamp techniques could be applied to the direct study of any channels that might be present in this membrane.

Dispersed muscle studies

Coordinated muscle control is critical for the parasite's ability to maintain position within the host and also for the female's ability to migrate to appropriate locales for egg-laying. The contractile response of whole

parasites has been studied but, as mentioned earlier, no studies have been able to identify the site of any response to any putative neurotransmitter or antihelminthic. In order to definitively localize neuro-muscular responses in the schistosome, it is necessary to dissect the components of this system. Protocols to disperse spindle-shaped contractile muscle fibers from the schistosome have recently been developed in this lab.

My goal was to investigate the feasibility of using patch-clamp techniques for the direct study of channels present in the schistosome muscle membrane. This would afford the opportunity to examine the characteristics of the schistosome muscle fibers themselves, apart from the influence of other mechanisms present in the intact animal.

MATERIALS AND METHODS

Source and Maintenance of Animals

Adult paired S. mansoni (Puerto Rican strain) were obtained from the portal and mesenteric veins of female Swiss Webster laboratory mice. Once extracted from the host, the parasites were maintained at 37°C in RPMI 1640 (GIBCO) with 100 units/ml of penicillium-streptomycin (GIBCO) added.

Patch-Clamp Methods

Patch pipets were pulled using a Narashige (PP83) two-stage vertical puller from 1.2 mm outer diameter borosilicate glass (World Precision Instruments, 1B120F-4) and fire-polished with a heated nichrome filament. Patch-clamp data were acquired with the use of a List model EPC-7 (Medical Systems Corporation) for current-to-voltage conversion. Voltage records were digitized with an Axolab A-D converter system (Axon Instruments, model TL-1) controlled by PClamp (Axon Instruments, version 5.5) or Axotape (Axon Instruments, version 1.2) data acquisition software. Analysis of records was performed using PClamp (Axon) and Patch (J. Dempster, University of Strathclyde) data analysis software.

Procedures for Tegument Studies

For clarity, the solutions used in the tegument experiments will be referred to in the text by the title at the top each of the columns in Table I. These titles summarize the concentrations, in m$\underline{M}$, of the three principal ions under investigation. All media was adjusted to pH 7.4.

Paired parasites were removed from their hosts and incubated at 37°C in the $5K^+/139Na^+/132Cl^-$ culture medium to which had been added 100 $\mu\underline{M}$ carbachol. Carbachol served to relax the parasites, inducing the males to release the females from their gynecophoral canals and ensuring a minimal amount of movement from the females during the remainder of the procedure. The female worms were separated and rinsed approximately ten times. Individual rinsed females were then placed in 35 mm Petri dishes containing the $5K^+/139Na^+/132Cl^-$ culture medium with 100 $\mu\underline{M}$ carbachol and maintained at 37°C until used.

Pipets pulled to resistances of approximately 15 MΩ were then brought into contact with the outer surface of the intact worms in an attempt to obtain a high resistance seal. Upon formation of a seal of one-half GΩ or better, the pipet was withdrawn from the parasite, exposing the intracellular face of the patch of excised tegumental membrane to the bath solution in an inside-out, cell-free configuration. If the resistance and capacitance dramatically increased at this

TABLE I A summary of solutions used in the tegumental experiments. All media were adjusted to pH 7.4 before use.

ion	concentrations in pipet solutions (mM)		concentrations in bath solutions (mM)	
	$140K^+/4Na^+/132Cl^-$	$70K^+/74Na^+/132Cl^-$	$5K^+/139Na^+/132Cl^-$	$10K^+/9Na^+/7Cl^-$
K^+	140	70	5	10
Na^+	4	74	139	9
Cl^-	132	132	132	7
Ca^{2+}	0.4	0.4	0.4	0.4
Mg^{2+}	0.4	0.4	0.4	0.4
HEPES	20	20	20	20
ATP	2	2	2	2

step, it was hypothesized that the membrane had vesiculated and that particular patch was not used for further study.

For experiments with tegumental patches, the intracellular medium was changed by a complete change of the bath solution. A 50 ml media reservoir fed to a glass tube with inner diameter of approximately 1 mm that was fixed on the stage and placed into the recording chamber. A 50 ml syringe was attached to another glass tube of equal size, fixed to the stage, and placed in the chamber. When a clamp was removed from the incoming line, gravity fed the medium into the recording chamber while at the same time, the syringe was manually manipulated to withdraw medium from the chamber. The 2 ml volume of the recording chamber was replaced by the new medium five times.

Procedures for Dispersed Muscle Studies

For all the steps listed below, procedures were carried out under a sterile hood using autoclaved glassware and pipets and all media were filtered at 45 μm. After removal from the hosts, 8-25 paired parasites were placed directly into 37°C RPMI-1640 to which had been added 5 mM L-cysteine, 0.1 mM carbachol, 0.01 mM serotonin, 20 mM Na-HEPES (pH=7.4) and 100 units/ml penicillium-streptomycin. The worms were then rinsed 5-10 times in the same medium to which had been added 0.1% bovine serum albumin (BSA). The worms were then placed on a glass slide where they were roughly chopped with

a razor blade. The resultant pieces were incubated in the medium with BSA at 37°C for 15 minutes. This medium was then drawn off and replaced with 4 ml of the same medium to which had been added 6 mg/ml papain (E.C. 3.4.22.2)(12 units/mg, Sigma).

This preparation was incubated for 30-45 minutes and then gently triturated by several passages through a Pasteur pipet. At this point, the desired result was for the larger pieces to begin to show signs of disintegration and the medium to be somewhat cloudy. If this had not occurred after 30 minutes and a gentle trituration, the pieces were allowed to incubate longer in this medium with a papain concentration of 6 mg/ml. Once the desired results were obtained, the preparation was allowed to settle for approximately 5 minutes following the trituration. At this point, 8 ml of the original medium (w/o BSA or papain) was added, thus reducing the papain concentration by 67%. This preparation was then incubated for 90 minutes and then triturated gently but extensively by many passages through a Pasteur pipet (1 mm original inner diameter) that had been fire-polished to close the end to approximately one-third of its original diameter. The preparation was then allowed to incubate for an additional 10 minutes and then triturated a final time with an unmodified Pasteur pipet.

Before the suspension had settled, aliquots of 100-400 µl were plated as droplets onto 30 mm plastic Petri dishes.

The amount of suspension plated depended on the cloudiness of the suspension (presumed density of the cells) and the desired final density of cells on the plates. These droplets were allowed to remain on the dishes undisturbed for 5 minutes in order to facilitate the attachment of the cells. The dishes were then gently filled with the medium and allowed to settle for another 5 minutes. All plates were then gently rinsed 3 times by drawing off the supernatant with a Pasteur pipet and replacing with the same medium. The plated cells were stored at 37°C until used, usually within six hours.

The solutions utilized in the dispersed muscle patch-clamp experiments will be referred to by the title at the top of each of the columns in Table II, which in all cases except "culture medium" is the dominant cation of that solution. All media were adjusted to pH 7.4. Immediately before use, the cells were rinsed 15 times in the culture medium. This is the bath medium in which all of the patches were obtained. For experiments with dispersed muscles, the pipet (extracellular) solution was the KCl pipet solution which contained 1 mM EGTA (see TABLE II). The solutions used for the calcium-sensitivity determinations were prepared using a calibrated calcium electrode as per the protocols described by Tsien and Rink (1983).

TABLE II A summary of solutions used in the dispersed muscle experiments. All media were adjusted to pH 7.4 before use.

	[]'s in pipet sol'ns (extra)	concentrations in bath solutions (intracellular)				
ion	KCl pipet	KCl	NaCl	CsCl	NH_4Cl	culture medium*
K^+	140	140	-	-	-	5
Na^+	-	-	140	10	10	125
Cs^+	-	-	-	130	-	-
NH_4^+	-	-	-	-	130	-
Cl^-	131	132	132	132	132	132
Ca^{2+}	-	0.4	0.4	0.4	0.4	0.4
Mg^{2+}	0.4	0.4	0.4	0.4	0.4	0.4
HEPES	20	20	20	20	20	20
EGTA	1	-	-	-	-	-

* Variations of the culture media were used to make the solutions with varying Ca^{2+} levels.

When viewed through an inverted microscope, the preparation obtained using the methods described above contained a variety of cell types including numerous spindle-shaped muscle fibers. Only spindle-shaped cells which shortened and then relaxed upon brief exposure to extracellular KCl (30 m$\underline{M}$) were used.

After selection of an appropriate cell, the pipet was put into contact with the cell surface and a slight suction applied through the pipet, facilitating the formation of a seal between the membrane and the pipet. Pipets with resistances between 5 and 10 MΩ were most often used, but due to the small size of these cells, pipets with resistances as high as 15 MΩ were sometimes useful. Seals of 10 GΩ were routinely obtained and only patches in which the seal quality was of this magnitude or greater were used for further analysis. After the seal was established, the pipet was withdrawn from the cell, exposing the intracellular face of the patch of muscle membrane to the bath solution in an inside-out, cell-free patch configuration. The membrane occasionally formed a vesicle upon withdrawal. If this occurred, the patch was discarded.

For the manipulation of the media to which the intracellular face of the patch was exposed, cell-free patches were moved into the stream of a microperfusion system with a flow rate of 100 µl/min. The microperfusion system consisted of 10 ml media reservoirs that fed to a

common polyethylene micropipet (100 μm I.D. tip). Flow rate was controlled by manipulation of the height of the media reservoirs. The patch was positioned at the mouth of the micropipet tip in the center of the plume created by the flowing media.

Analysis of the data obtained from the muscle membrane was designed primarily to determine channel amplitude and percent of time channels were in the open state. Channel current amplitude was determined by constructing a current amplitude histogram of a 1 min digitized record and measuring the difference between the mean of a Gaussian fit to the peaks of the closed and open current levels. The event discriminator was then set to half of the estimated channel current amplitude and the record was analyzed using this threshold to determine the percentage of the 1 min record in which the channel was in the open state (percent open time).

Relative permeabilities of test ions were determined by using the Goldman-Hodgkin-Katz equation for the biionic condition,

$$E_{rev} = RT/zF * \ln \{P_{K+}[K^+]_o/P_{X+}[X^+]_i\}$$

when K^+ is the only cation at the extracellular face and X^+ is the only cation at the intracellular face and where E_{rev} is the observed membrane potential at which current reverses, the "biionic potential". According to this equation, under our conditions, if current reversal through

the channel could not be observed at or extrapolated to membrane potentials less than +60 mV, the permeability ratio of $K^+:X^+$ would be greater than 10:1.

RESULTS, Part I: THE TEGUMENT

Most attempts at obtaining a high-resistance seal with the outer membrane of the tegument resulted in failures. Even when high-resistance seals of sufficient quality were formed, channel activity was rarely observed while the patch remained attached to the animal ("cell-attached" configuration). However, upon patch excision to the cell-free, inside-out configuration, discrete conductances could be observed (FIGURE 1).

Conductance

On the basis of amplitude and the characteristics of the conductance transitions, there was clearly more than one conductance commonly present in successfully pulled inside-out patches. The most commonly observed conductance had a slope-conductance of 295 ± 19 pS (n=4) with $140K^+/4Na^+/132Cl^-$ in the pipet (extracellular face) and culture medium, $5K^+/139Na^+/132Cl^-$, in the bath (intracellular face) (FIGURE 2). The channel displayed some distinctive responses to voltage stimulations (see section 3 for further description) that were used to identify the channel. The current reversed at approximately -5 mV. Under these conditions, the reversal potential for K^+ was 84 mV; for Na^+ was -89 mV; and for Cl^- was 0 mV.

The potential at which an observed current reverses will correspond with the calculated reversal potential (or

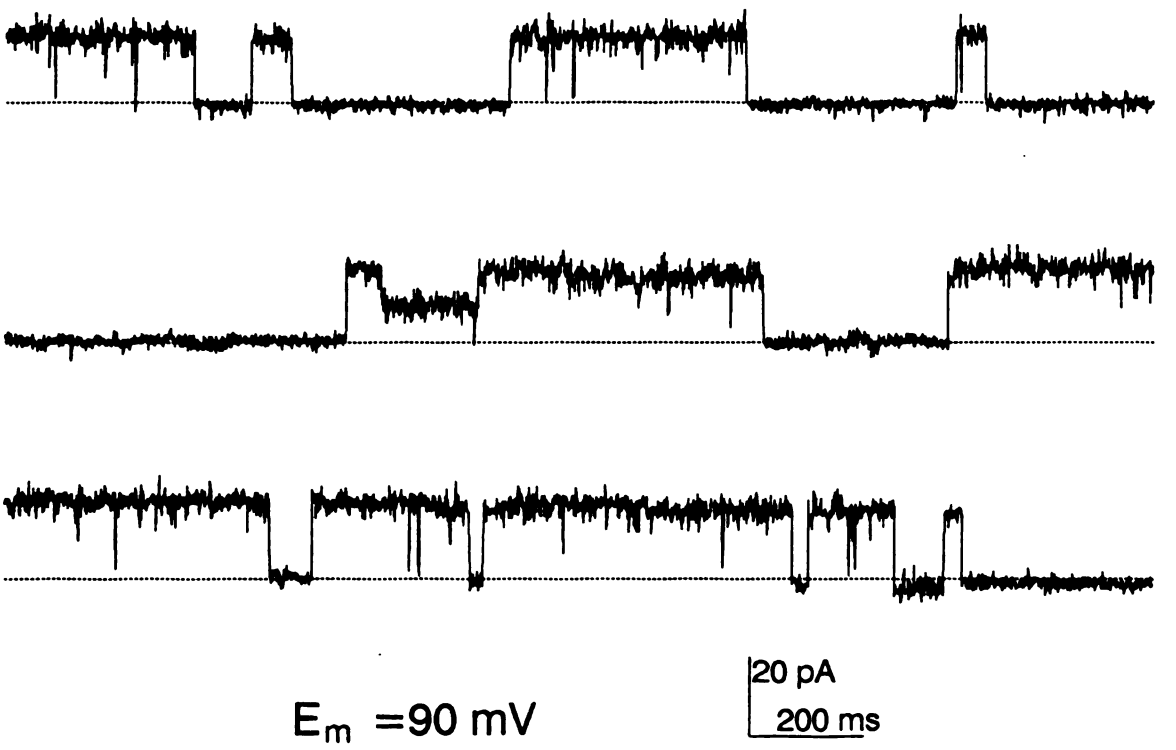


FIGURE 1 Continuous current traces through an inside-out patch of outer tegumental membrane pulled from a female schistosome. $140K^+/4Na^+/132Cl^-$ was the extracellular medium and $5K^+/139Na^+/132Cl^-$ was the intracellular medium.

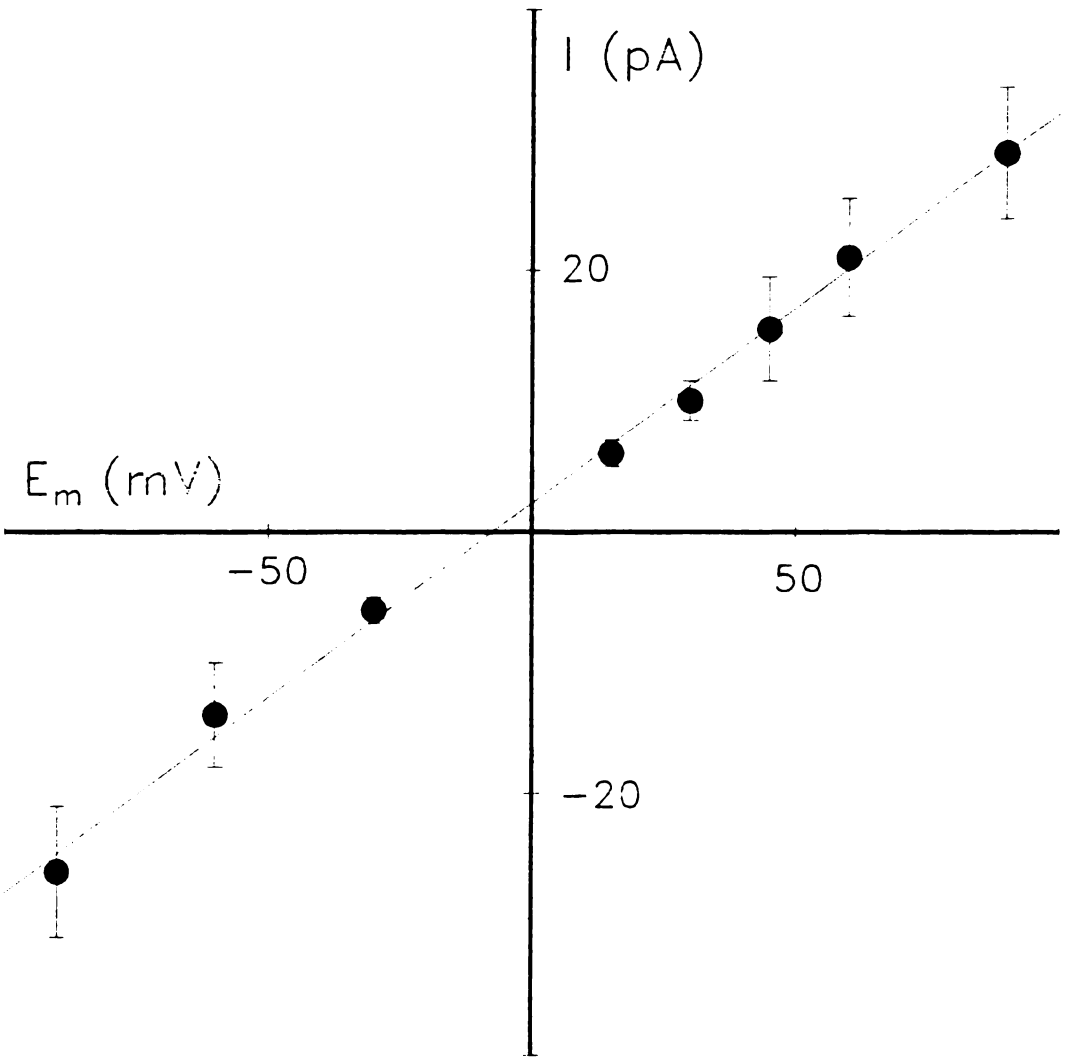


FIGURE 2 Current vs. membrane potential relationship for the most prominent conductance through cell-free, inside-out patches of outer tegumental membrane. The line represents a linear regression through the mean of the data points (n=4, ± s.d.) with a slope-conductance of 295 ± 19 pS. This current reversed near -5 mV under these conditions in which the reversal potentials were 84 mV for K^+, -89 mV for Na^+ and 0 mV for Cl^-. The extracellular solution was $140K^+/4Na^+/132Cl^-$ and the intracellular solution was $5K^+/139Na^+/132Cl^-$.

potentials) for the ion (or ions) responsible for carrying that current. Since the reversal of current, -5 mV, was conspicuously close to the calculated reversal potential for Cl^-, it was easy to speculate that Cl^- could be the ion carrying the current. This observed reversal potential, however, could also have been indicative of a current carried by cations, that is K^+ and Na^+ whose combined reversal potential would fall near 0 mV. Another prospect is that the current could be non-specific for anions and not be specifically limited to Cl^-. It was also possible that the current was completely non-specific and created by the flow of all ions.

The channel was greatly predisposed to the open state, so much so that it would remain open for minutes at a time. It was an early observation that when ATP was added to the intracellular medium the channel displayed more closures. For this reason, 2 mM ATP was included in all media for all reported trials.

Cation selectivity

The possibility that the current was Cl^- specific was the easiest to test. Experiments were performed in which the chloride in the medium exposed to the intracellular face of the patch was replaced by $gluconate^-$, $sulfate^{2-}$ or $citrate^{3-}$. The hydrated size, relative to Cl^-, is 2.50 for $gluconate^-$, 1.92 for $sulfate^{2-}$ and 3.20 for $citrate^{3-}$

(Edwards, 1982). Thus, each substitution placed an anion larger than Cl^- at the intracellular face. This substitution caused an alteration in the calculated reversal potential of Cl^-, shifting it from 0 mV to a large positive value. As a consequence, one would expect that if the channel were selective only for Cl^-, the current reversal would change to reflect the change in the reversal potential for Cl^-.

However, none of the anion replacements produced a significant change in the reversal of the current or the slope-conductance of the channel. This indicates that the channel is not selective for Cl^-, but does not rule out the possibility that the current could be carried by any anion and that the channel is simply large enough to allow the passage of the substitute anions as well as Cl^-.

In order to test if the channel was specific for anions or cations, it was necessary to create experimental conditions under which the reversal potential for all cations and the reversal potential for all anions could be made to segregate. In order to accomplish this, it was necessary to change the pipet solution to a medium that contained approximately equal concentrations of K^+ and Na^+, $70K^+/74Na^+/132Cl^-$. Once again, the initial recordings were performed in the $5K^+/139Na^+/132Cl^-$ bath, under which conditions the reversal potentials were 66 mV for K^+, -16 mV for Na^+ and 0 mV for Cl^-. Both reversal potential, + 5 mV,

and single-channel slope-conductance, 299 ± 17 pS (FIGURE 3), were very similar under these new control conditions to what they had been under the original control conditions (-5 mV and 290 pS). After this control, the intracellular face of the patch was perfused with the $10K^+/9Na^+/7Cl^-$ bath. Under these conditions, the reversal potential for Cl^-, the only anion, was -60 mV and the reversal potential for all cations was between the reversal potentials for K^+, +49 mV, and Na^+, +53 mV. Upon perfusion with this medium, reversal of the current through the inside-out patch shifted to +54 ± 8 mV (FIGURE 3 & FIGURE 4). The slope-conductance was also altered, shifting to 361 ± 17 pS.

The observed change in reversal potential indicates that the channel is allowing the passage of cations and not anions. It rules out the possibility that the current is completely non-specific.

Voltage Responses

The channel displayed some distinctive responses to voltage stimulations applied to the patch. These distinctive behaviors were used to identify the channel.

When the channels were held at positive potentials percent open times were between 0 and 50 for any 60 second interval. But, channels held at negative potentials typically displayed percent open times greater than 99 and

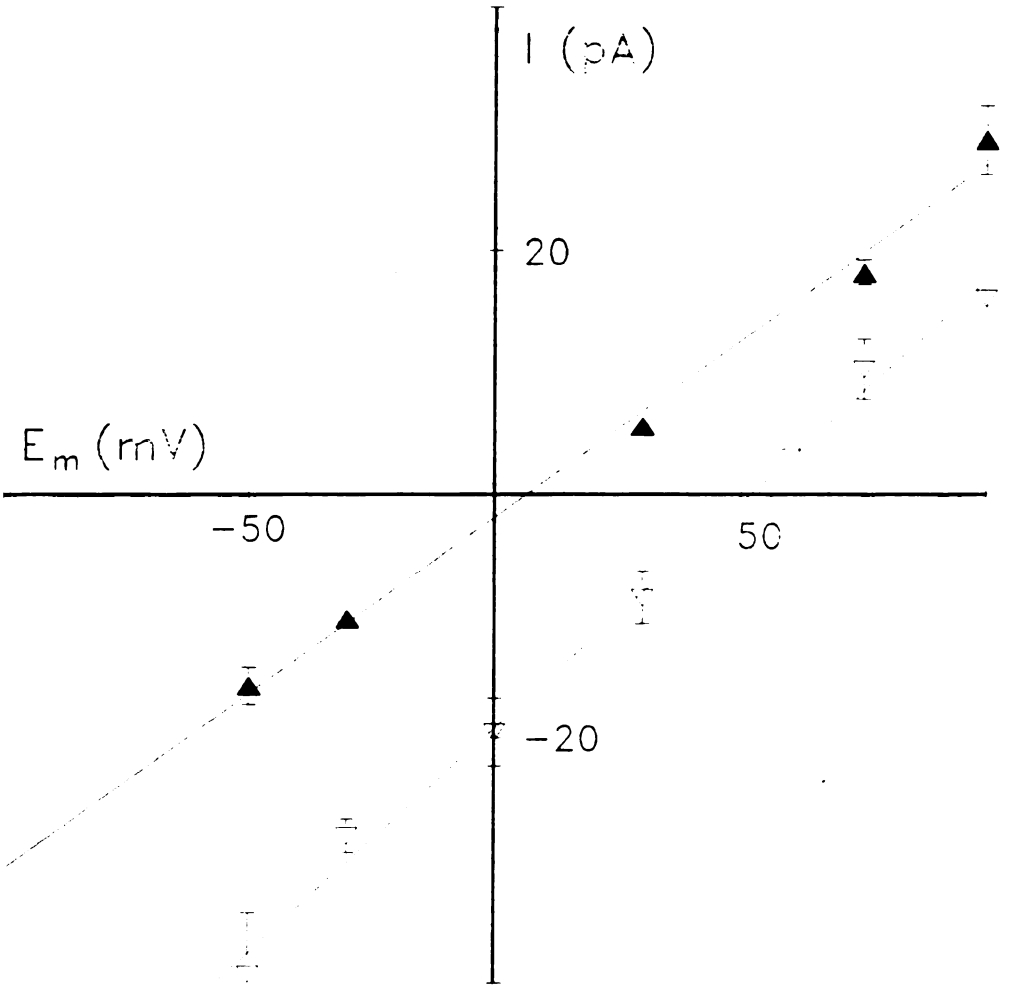


FIGURE 3 Current vs. membrane potential relationships for the large conductance in different ionic conditions showing that the reversal of current through the large channel was dependent on the reversal potential for all cations. In both cases, the extracellular solution is $70K^+/74Na^+/132Cl^-$. The filled triangles are the mean (n=3, ± s.d.) of the data with $5K^+/139Na^+/132Cl^-$ as the intracellular solution. Under these conditions, the reversal potentials were 66 mV for K^+, -16 mV for Na^+ and 0 mV for Cl^-. The hollow triangles are the mean of the data from the same patches when the intracellular solution was exchanged for $10K^+/9Na^+/7Cl^-$ and the reversal potentials were shifted to 49 mV for K^+, 53 mV for Na^+ and -74 mV for Cl^-. The calculated reversal potential for all cations was approximately 45 mV after the change. The reversal of the current was accordingly shifted from 5 mV to 54 ± 8 mV by the exhange. The slope-conductance was also altered, shifting from 299 ± 18 pS to 361 ± 17 pS.

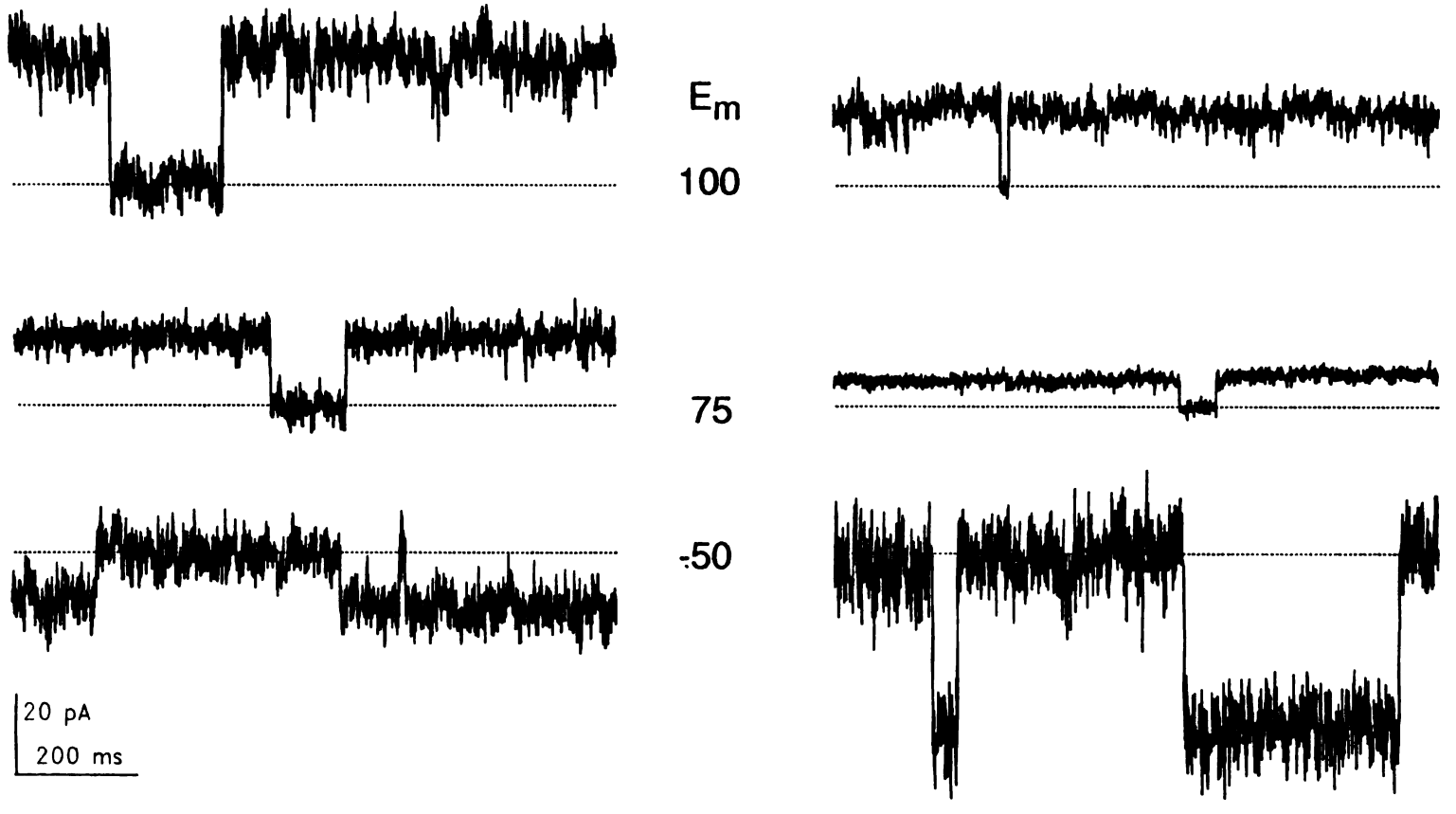


FIGURE 4 Current traces from the large conductance in different ionic conditions showing that the reversal of current through the large channel was dependent on the reversal potential for all cations. The extracellular solution is 70K+/74/Na+/132Cl- for all of the traces. The intracellular solution is 5K+/139Na+/132/Cl- for the traces on the left and 10K+/9Na+/7Cl- for the traces on the right. Current through through the channel reverses around the reversal potential for all cations present in either situation, approximately 0 mV on the left and +50 mV on the right.

the channels often would remain in the open state for minutes without any closures (FIGURE 5).

An experiment which illustrates this is given in Figure 5. In this experiment, the membrane potential of the patch was held for alternating 10 second intervals at +45 and -45 mV. When the patch was held at positive membrane potentials, an all-points histogram showed two prominent peaks of positive current, a closed channel current level and an open channel current level. However, at negative potentials, the peak associated with the closed channel current level was very small, while the open channel current level peak was still prominent.

During these experiments it was noticed that even if the channel was in a closed state while being held at a positive membrane potential, the step to a negative potential would result in the channel arriving at the negative potential in an open state (FIGURE 5, top). From this consistently observed behavior, it appeared that the channel opening was occurring during the transition to the negative membrane potential.

Further evidence to support this was obtained by the application of negative-going voltage ramps to the channels. If the membrane potential was ramped from $\geq$ 0 mV to -100 mV at a rate of -0.05 mV/ms, the channel would typically open at some point during the application of the ramp, usually at a potential less than -30 mV (FIGURE 6).

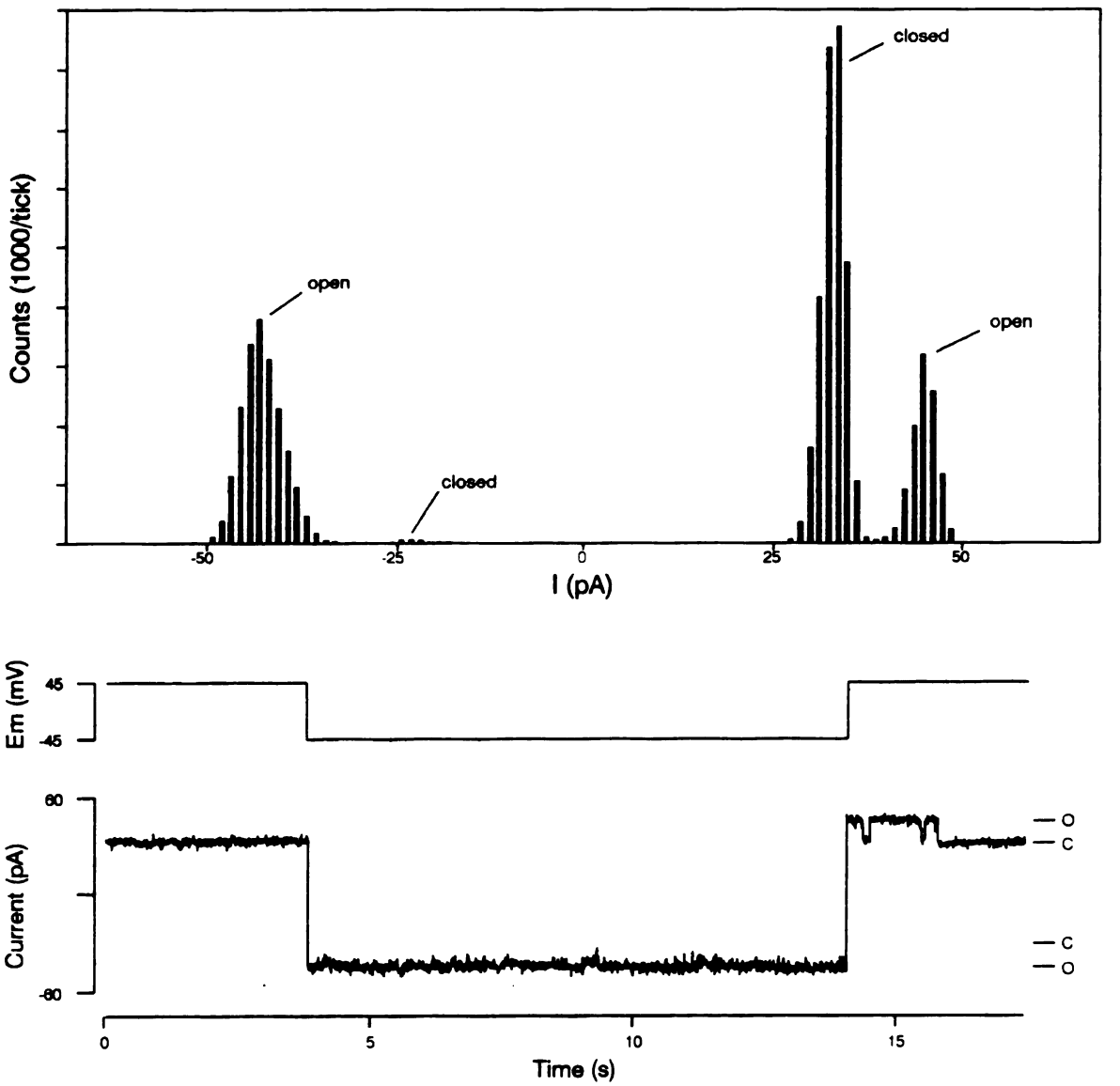


FIGURE 5 An all-points histogram and a sample trace of currents through the large channel when the membrane potential is held for alternating 10 s intervals at +45 and -45 mV. The all-points histogram (top) shows two distinct peaks associated with the period when the membrane is held at positive potentials. When the membrane is held at negative potentials, the "closed" peak is discernable, but small. This reflects the fact that the channel is greatly disposed to the open state when held at positive potentials. The histogram represents the data from one 2 min record sampled at 5 KHz. The current trace (bottom) begins with the channel held at +45 and the channel in the closed state. The channel arrives at -45 in the open state. Upon return to +45, the channel is still open.

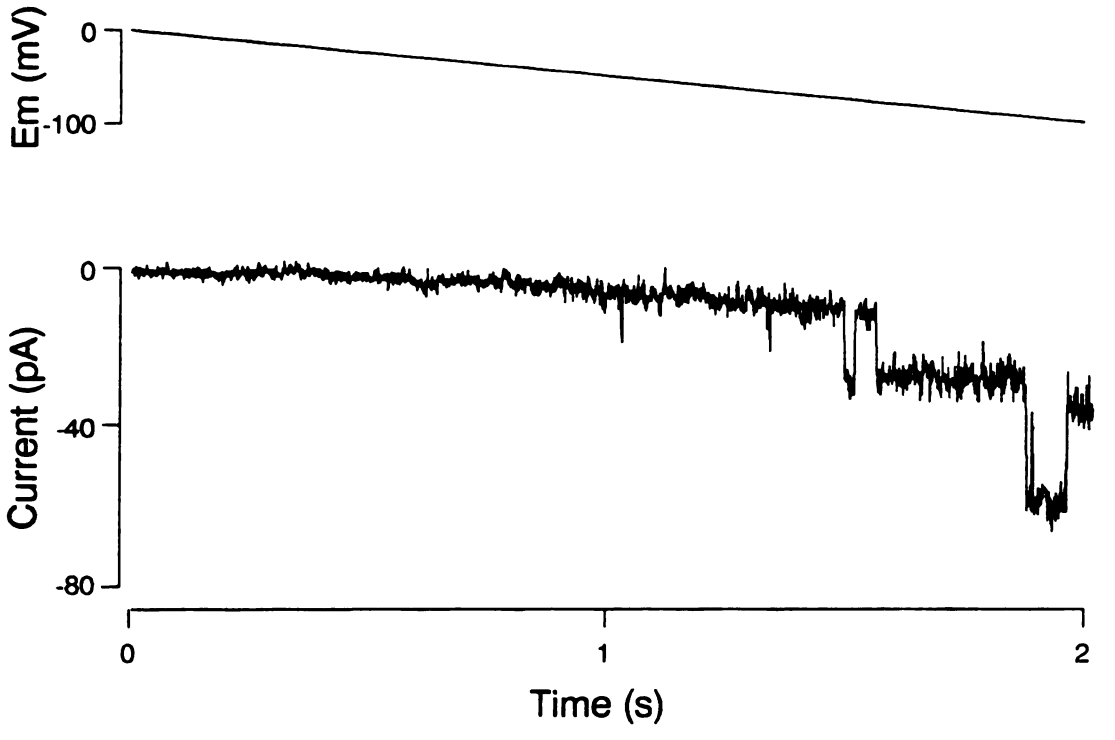


FIGURE 6 Current trace from a patch of inside-out membrane with the application of a negative-going voltage ramp to demonstrate channel opening during the ramp application. There are two channels present in the patch, one that first opens at ≈ -75 mV and one that opens at ≈ -90 mV. The membrane was depolarized from 0 to -100 mV at a rate of -0.05 mV/ms.

If the channel was actually opening during the negative-going voltage transition, then it would follow that one might be able to induce the closed channel to open with a short negative-going pulse. Experiments confirmed that if the channel was closed while being held at a positive potential, a 1 ms pulse to -45 mV would typically result in the channel returning to the positive potential in the open state (FIGURE 7). These experiments also revealed that after the channel was induced to open by a 1 ms pulse to -45 mV, the initial opening would be followed by a period in which the channel would remain open for a variable time, then display an increased frequency of opening and closing events and eventually return to the predominantly closed state.

When the period of increased frequency of opening after induced opening was examined, it was determined that the period of increased channel openings decayed with a $t_{\frac{1}{2}}$ of approximately 8 seconds (FIGURE 8).

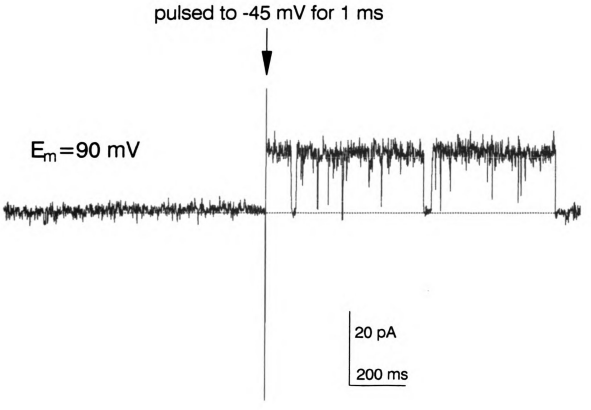


FIGURE 7 Current trace from a patch of inside-out membrane with the application of a 1 ms negative-going pulse to demonstrate channel opening induced by negative-going pulses. The membrane was held at 90 mV and pulsed to -45 mV. As in this record, the channel typically returned from the depolarization in the open state and showed an increase in activity for a variable period of time immediately after the pulse.

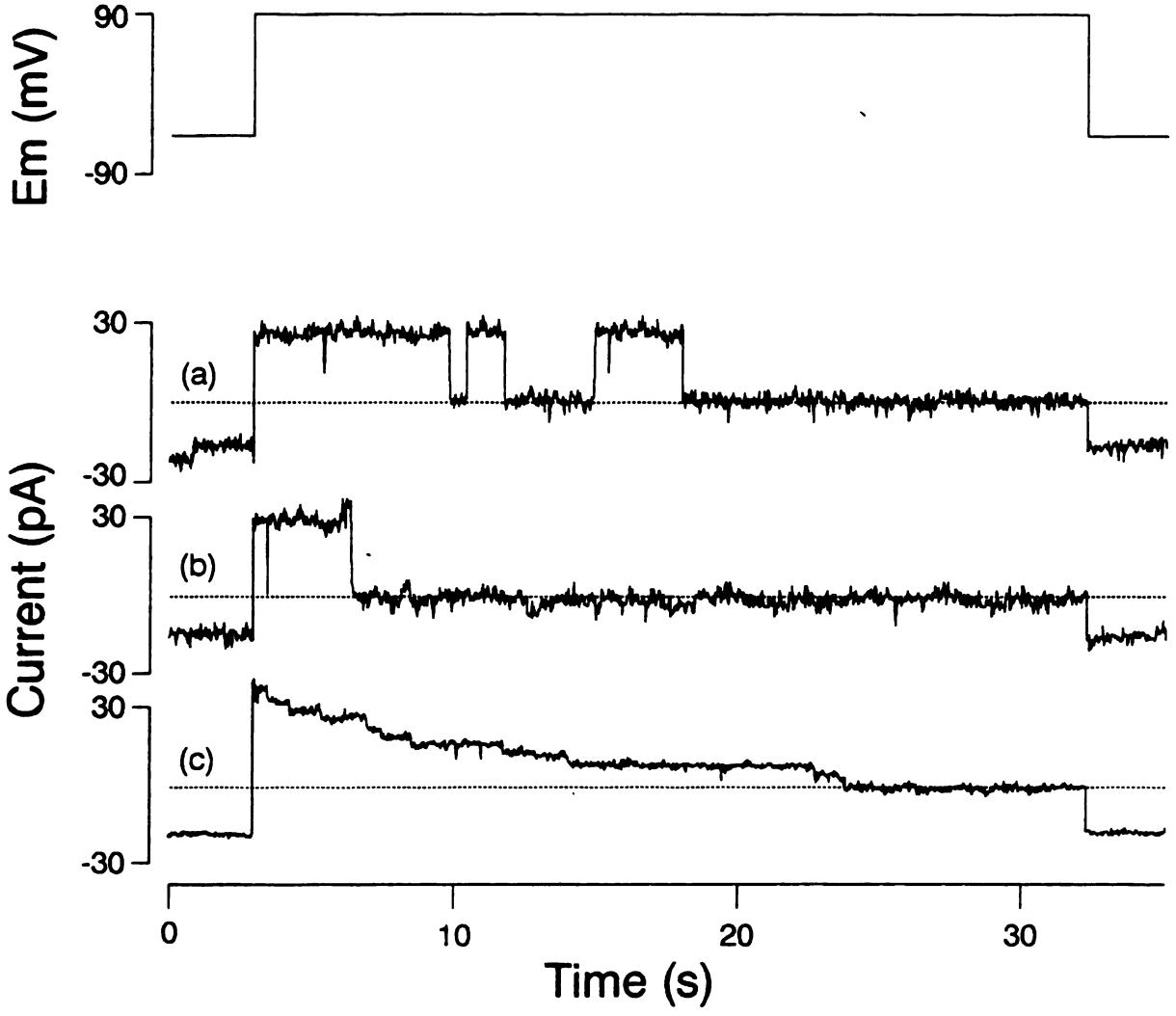


FIGURE 8 Current traces from a patch of inside-out membrane that demonstrate the decrease in channel openings that occur over time after opening is induced by a negative-going pulse. Prior to the part of the trace that is shown, each patch is held at +90 mV until channel openings are very infrequent and then pulsed to -45 mV. The trace shown displays the last three seconds of the pulse to -45 mV and the return to +90 mV. Current traces (a) and (b) are examples of single trials while (c) is a summed average of 10 such trials.

DISCUSSION, Part I: THE TEGUMENT

Comparative Aspects

The tegument should be considered a transporting epithelium and the cation channel characterized from the outer membrane of the tegument of the schistosome should probably be considered an epithelial ion channel. Although several epithelial ion channels have been identified, very little is actually known about the diversity of channels that may exist in epithelial tissues. It is worthy to note the extremely high diversity of transport mechanisms that are known to exist in different epithelia. This variety may well be expressed in the array of channel types that epithelia contain as well. It should not be ignored, however, that the tegument of the schistosome may have critical functions that are not at all typical of classical epithelia.

Surprisingly, the tegumental channel described here is most similar to a type of non-selective cation channel that has previously been described only in the neurons of crustaceans (McClintock & Ache, 1990) and molluscs (Chesnoy-Marchais, 1985 & Yazejian & Byerly, 1989). These cation channels are characterized by the fact that they are not active in cell-attached patches and activate only upon patch excision. The channel in the tegument of the schistosome was also never observed until patch excision.

Yazejian & Byerly (1989) have proposed that high intracellular calcium levels and decreased intracellular ATP levels are involved in the activation of these crustacean channels upon excision. Although the schistosome channel was not tested for calcium sensitivity, there was an increase in closures when ATP was added to the intracellular media. This would be in accord with hypotheses that exposure to ATP-free intracellular media allows maximum activation. The tegumental cytoplasm has high concentrations of ATP and this may explain why the channels are not active in the cell-attached (animal-attached) configuration.

The cation channel from lobster olfactory receptor neurons has a conductance of 320 pS (McClintock & Ache, 1990) as compared to the 295 pS conductance of the schistosome tegument channel. This channel showed a voltage dependence of percent open time, as does the schistosome tegument channel, however the relationship between percent open time and membrane potential was inverse to that of the schistosome tegument channel.

Possible Function of the Channel

Assigning any physiological function to the tegument cation channel is virtually impossible with the relatively small amount of information known about this channel and no knowledge of the array of other channels that might be

present in the outer tegumental membrane. The excision-activated cation channels that have been identified in neurons have been hypothesized to be involved in processes responding to cell damage (McClintock & Ache, 1990).

RESULTS, Part II: DISPERSED MUSCLE

Conductance

Patch-clamp recordings from inside-out cell-free patches of muscle membrane pulled from isolated muscle fibers displayed the presence of a variety of discrete conductances. When exposed to symmetrical KCl solutions, the current most frequently observed was a large 195 ± 13 pS (n=6) conductance that reversed when the membrane potential was at 0 mV (FIGURE 9). In these studies, the KCl pipet solution was at the extracellular face and the KCl bath solution was at the intracellular face. Current reversed at approximately 0 mV under these conditions in which the reversal potential for both K^+ and Cl^- is 0 mV. Therefore, this reversal could be indicative of a current carried by either K^+ or Cl^-.

When NaCl was substituted for the KCl at the intracellular face, only inward currents could be observed in the range from -60 mV to +60 mV and the reversal potential shifted to a potential greater than +60 mV (FIGURE 10). Under these conditions the reversal potential for Cl^- remained at 0 mV, so it appears clear that the current carrier for this conductance was not Cl^-. However, the reversal potential for K^+ with the NaCl solution at the intracellular face was a large positive potential and the reversal potential of the current observed through the channel had shifted accordingly. Therefore, it can be

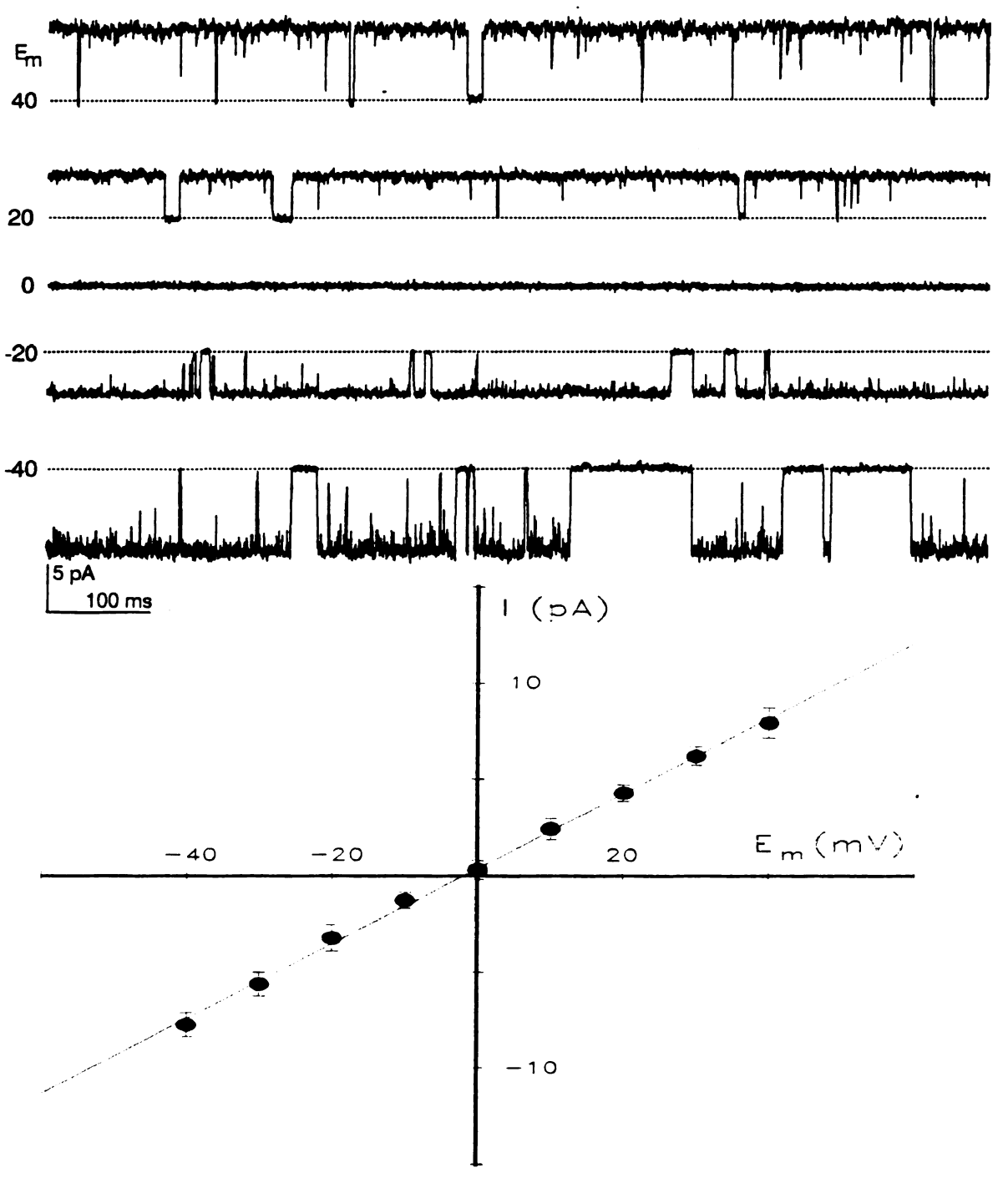


FIGURE 9 Current traces and current vs. membrane potential relationship of the most prominent conductance observed in cell-free, inside-out patches exposed to symmetrical 140 mM KCl. For the current traces, the dashed line represents the closed channel current level. Current was able to flow in either direction in the symmetrical KCl solution and it reversed around 0 mV. For the IV relationship, the line represents a linear regression through the data that has a slope of 195 ± 13 pS.

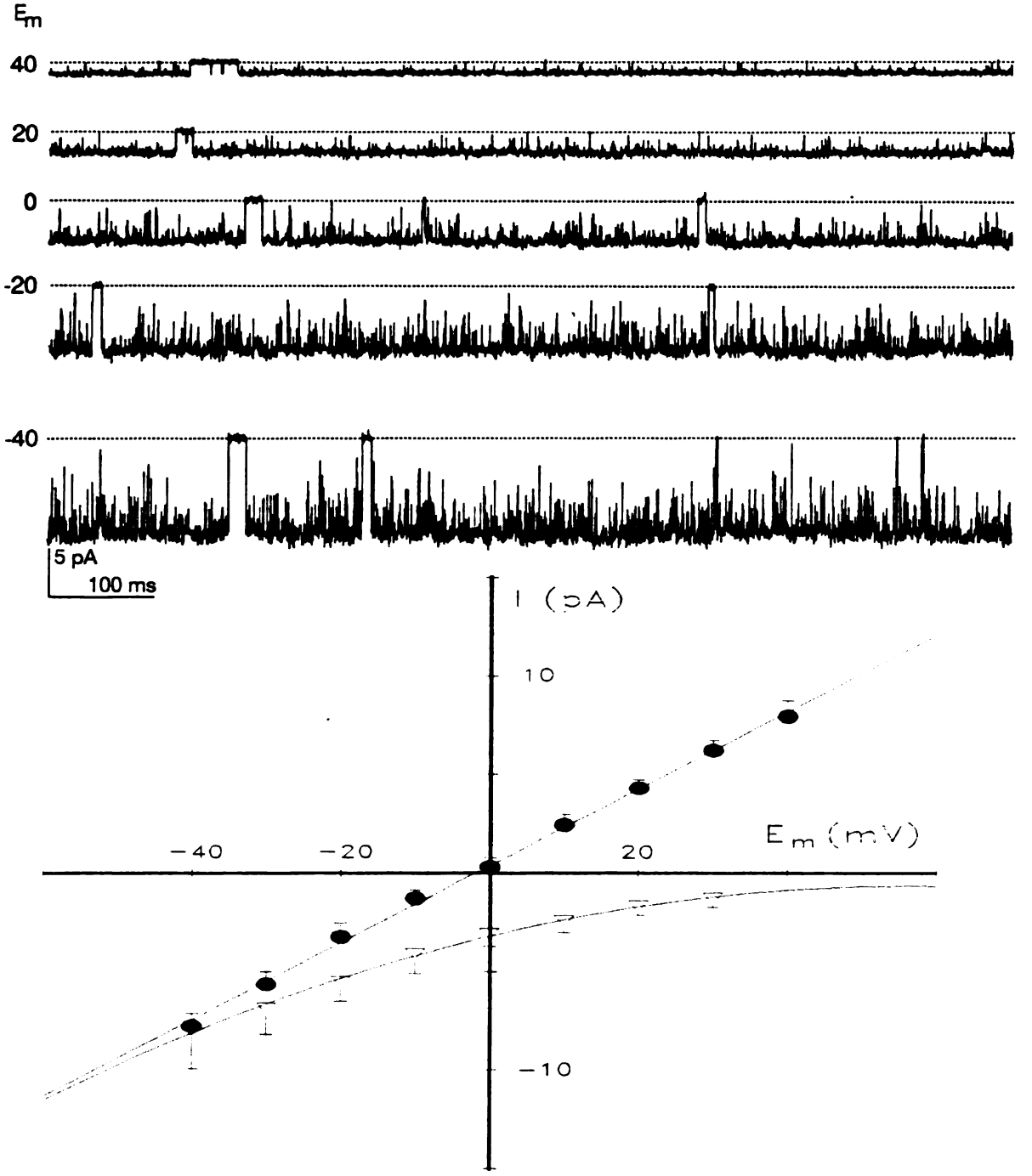


FIGURE 10 Current traces and current vs. membrane potential relationship of the channel with NaCl at the intracellular face. The current traces show that currents were always inward in the range of membrane potentials from -60 to 40 mV. No outward currents could be observed even with membrane potentials at 60 mV. The data for the IV relationship are the average and standard deviations from six patches, each of which contained a single channel and was exposed to both intracellular KCl (●) and NaCl (▽).

concluded that the current carrier was K^+ and that the conductance was due to activity of a channel that is selective for K^+ over Na^+ and Cl^-.

Selectivity

The K^+ channel showed a marked selectivity for K^+ over the other cations Na^+, Cs^+, and NH_4^+ (FIGURE 10 & FIGURE 11). In these experiments, the KCl pipet solution was at the extracellular face and the intracellular face of the patch was first exposed to the KCl bath solution. Under these conditions, the parameters of current reversal potential, slope-conductance and percent open time were measured. Then, the KCl bath solution was replaced by a solution with a different dominant cation and the parameters were re-measured.

All three of these ions, when present as the dominant cation at the intracellular face of the channel, shifted reversal of the current through the channel from 0 mV to a membrane potential greater than +60 mV, indicating a permeability ratio of greater than 10:1 for K^+ over these ions. Another result of the relative impermeability of the substitute cations was that all three of them altered the current *vs.* membrane potential relationship from a linear function to a curvilinear function. None of these cations affected the percent open time of the channel when they were

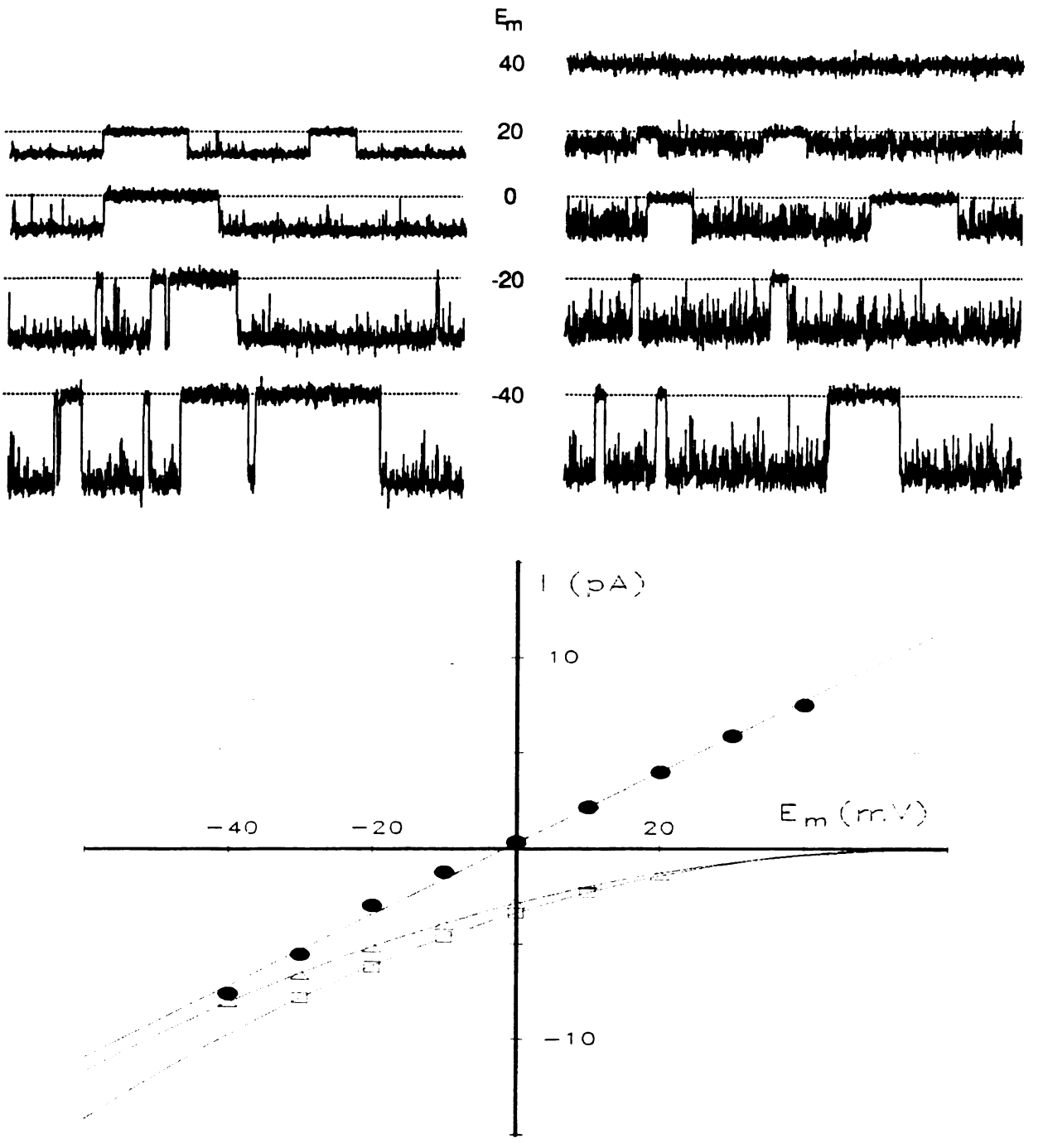


FIGURE 11 Current traces and current vs. membrane potential relationship of the channel with NH_4Cl and CsCl at the intracellular face. The current traces with NH_4^+ (left) and Cs^+ (right) as the dominant intracellular cation show in both cases that currents were always inward in the range of membrane potentials from -60 to 40 mV. No outward currents could be observed even with membrane potential at 60 mV. The data for the IV relationship are the average from three patches, each of which was exposed to intracellular KCl (●), NH_4Cl (□) and CsCl (△).

substituted for K^+ as the dominant cation at the intracellular face.

Na^+ and Cs^+ produced a marked increase in the fast "flicker" activity of the channel when they were substituted for K^+ as the dominant cation at the intracellular face. The apparent "noise" through the channel in the open state was noticeably increased, in rank order, by $Cs^+ > Na^+ > NH_4^+ \geq K^+$ (FIGURE 12).

The results of these experiments when Rb^+ was substituted for K^+ as the dominant intracellular cation were different from those obtained with the other test cations (FIGURE 13). When Rb^+ was at the intracellular face and when the membrane was held at negative potentials, the apparent current amplitude and conductance was similar to that observed in symmetrical K^+. These data obtained at negative membrane potentials, if taken alone, imply that the current would reverse at 0 mV and, thus, that Rb^+ and K^+ were equally permeant. However, when the membrane was clamped at positive potentials, no discernable channel events were ever observed. From this one would conclude that either percent open time had gone to zero or the channel did not allow outward current to flow if Rb^+ was at the intracellular face.

In these ion substitution experiments the electrochemical gradient for potassium was inward at all potentials, since there was no potassium in the

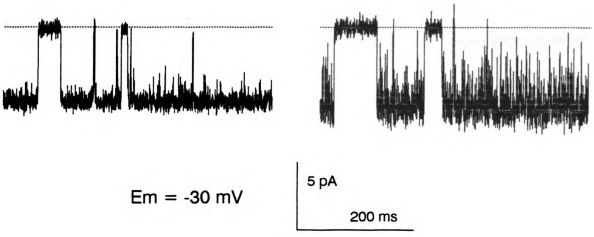
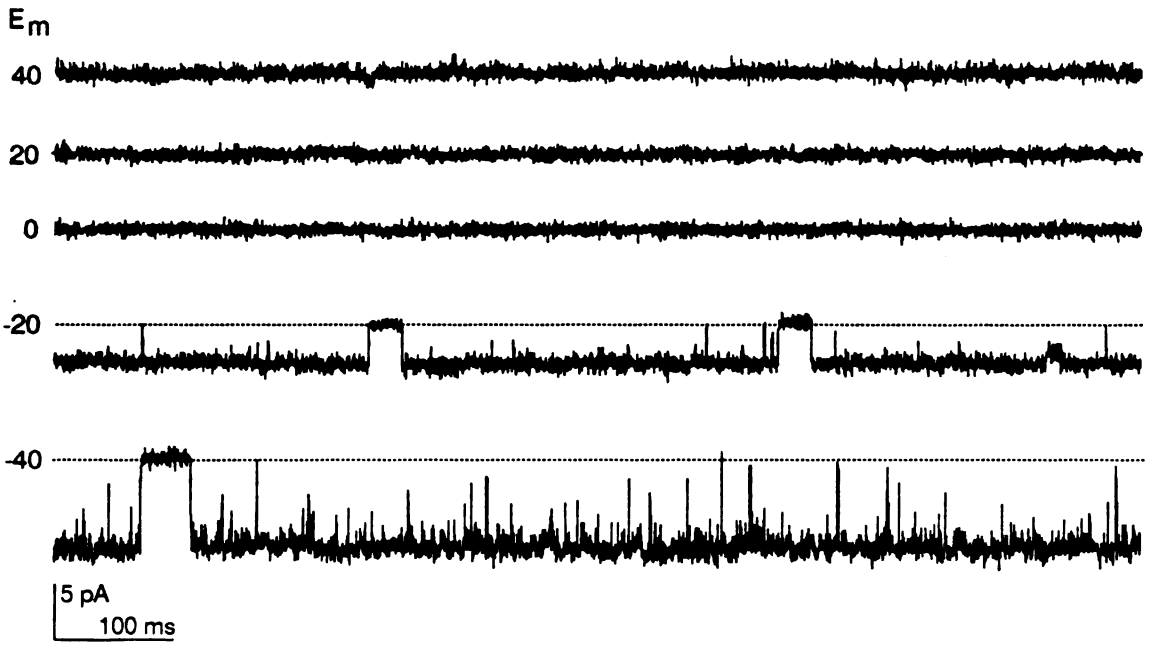


FIGURE 12 Current traces that demonstrate the increase in fast "flicker" activity that occurs with Cs^+ as the dominant cation at the intracellular face. The trace on the left is from a patch in symmetrical KCl and the trace on the right is from the same patch after CsCl was substituted for KCl in the intracellular medium. This increase in flicker activity was also seen with NaCl at the intracellular face.



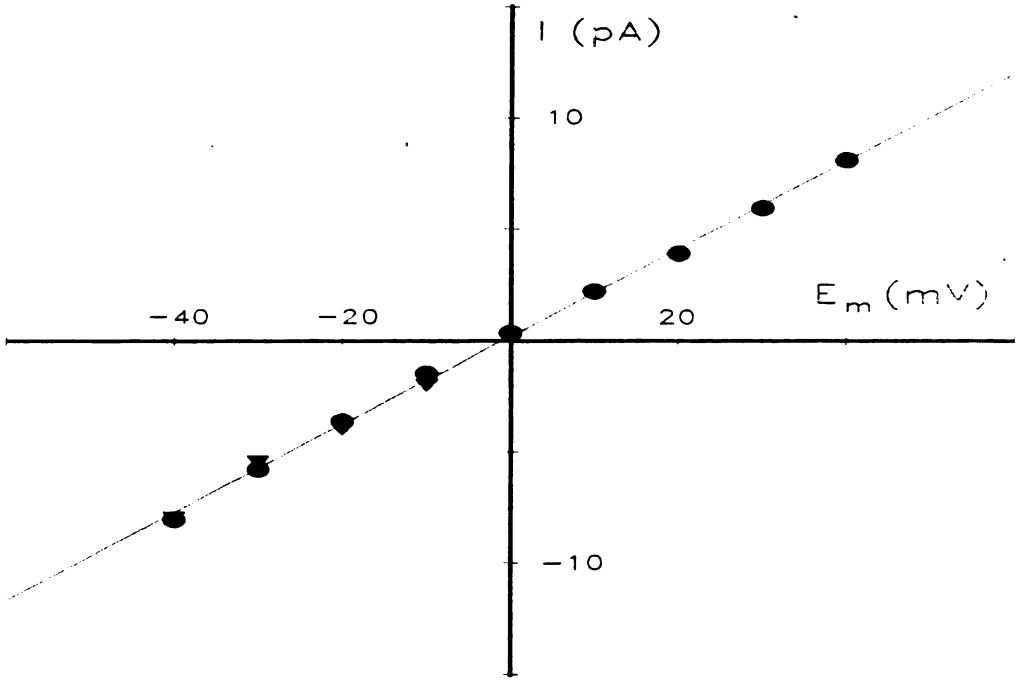


FIGURE 13 Current traces and current vs. membrane potential relationship for the channel with RbCl at the intracellular face. The current traces show that inward currents pass through the channel at potentials less than 0 mV, but that no currents flow at potentials greater than 0 mV. The data for the IV relationship are the average of four patches, each of which was exposed to intracellular KCl (●) and RbCl (▼).

intracellular medium. If the channel were essentially impermeable to Rb^+, one would expect a current *vs.* membrane potential relationship similar to that for Cs^+, NH_4^+ and Na^+, where inward potassium currents are measurable from -60 to +40 mV and the current *vs.* membrane potential relationship appears to become asymptotic to 0 pA as membrane potential goes to $+\infty$. What was observed was that inward currents flowed but not at all potentials which created an inward driving force for K^+, rather only at potentials that created an inward driving force for the summed pool of K^+ and Rb^+ (<0 mV). The current *vs.* membrane potential relationship appeared as though it would intersect 0 mV and showed no signs of curvilinear behavior. Outward currents were not able to flow at any potentials, although they might have been expected at potentials that created an outward driving force for the summed pool of K^+ and Rb^+ (>0 mV).

Ca^{2+}-sensitivity

The percent open time of the channel was dependent on the concentration of Ca^{2+} at the intracellular face. For these experiments, the first intracellular medium to which the patch was exposed was the culture medium bath with a 200 μM calcium activity and three variables were determined: 1) percent open time at -50 mV membrane potential, 2) slope-conductance and 3) reversal potential. Following these

determinations, the patch was sequentially exposed to one or more intracellular Ca^{2+} levels ranging from 3 μM to 0.1 μM and the same variables re-examined.

Percent open time, but neither slope-conductance nor reversal potential, was affected by the level of Ca^{2+} at the intracellular face. When the membrane potential was clamped at -50 mV with 200 μM Ca^{2+}, the channel exhibited an average 99% open time with the minimum being 97%, n=15 (FIGURE 14). Open time was still greater than 95% with a Ca^{2+} level of 1 μM, but it was less than 2% with 0.1 μM Ca^{2+}. The cytoplasmic Ca^{2+} concentration at which the open time was 50% fell in the range from 0.2 μM to 0.6 μM. Once channel activity had been decreased to near zero by exposure to low Ca^{2+} activity, re-exposure to high Ca^{2+} did not result in full restoration of channel activity.

To determine the voltage sensitivity of percent open time, percent open time was determined at potentials ranging from -50 to -10 mV for all the calcium concentrations tested. At no calcium concentration from 200 μM to 0.1 μM was there a significant difference between percent open times at two different holding potentials.

Blocker-sensitivity

The channel was blocked by high concentrations (10 mM) of Ba^{2+} at the intracellular face, but not by the same concentrations of tetraethylammonium (TEA) or 3,4-

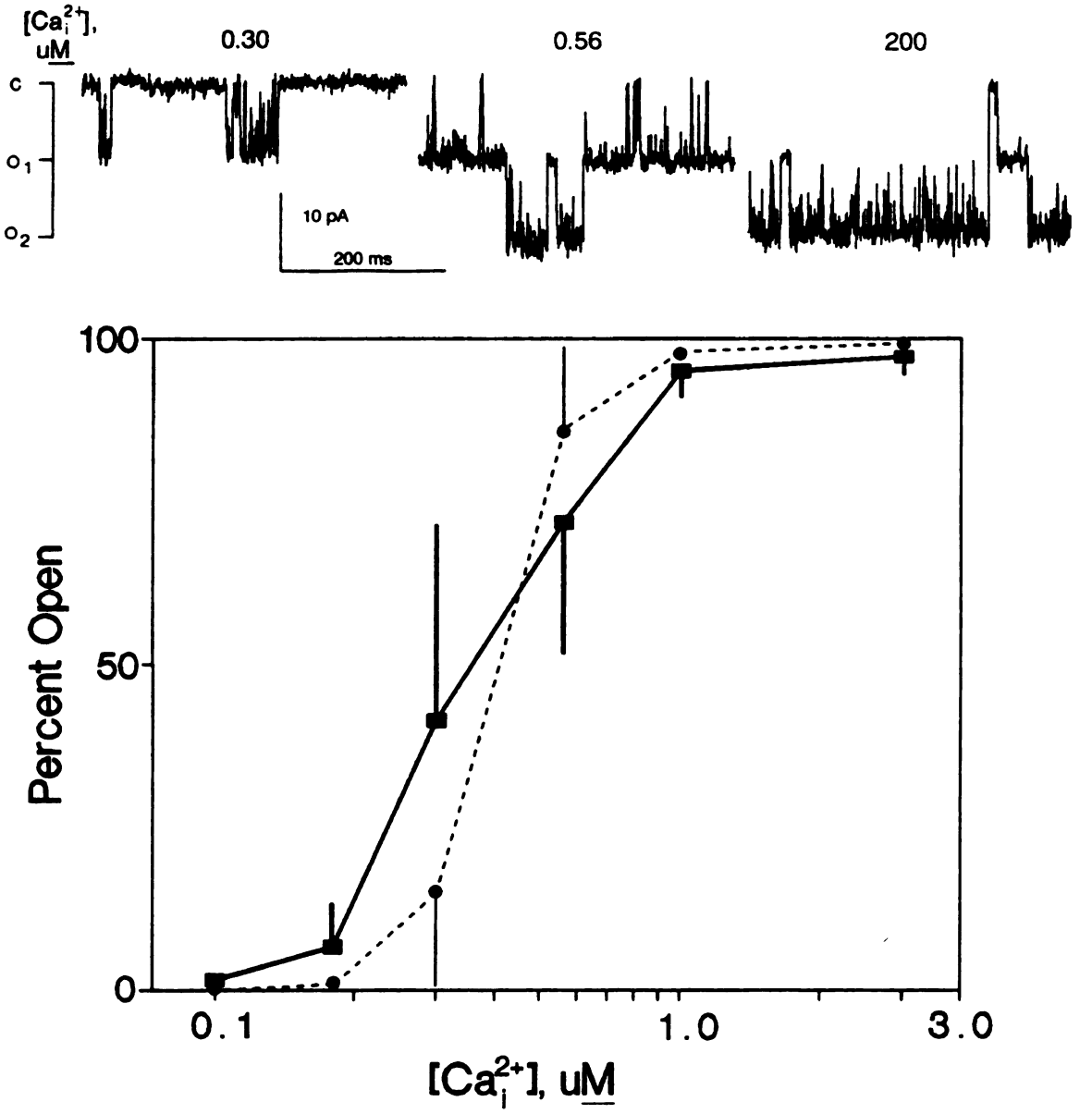


FIGURE 14 Current traces at different calcium levels and the relationship between percent open time of the K^+ channel and the $[Ca^{2+}]$ at the intracellular face. (Top) The current traces are from a single patch containing two channels that was exposed to three levels of intracellular $[Ca^{2+}]$. The mebrane potential is -50 mV for all of the traces shown. (Bottom) Shown are percent open times (mean ± s.d.) calculated from records taken with the membrane potential at -50 mV (■) and -10 mV (●). The intracellular solution was the culture medium with varying Ca^{2+} concentrations buffered with 10 mM EGTA. The data at -50 mV were taken from 15 patches, each of which was exposed to several $[Ca^{2+}]_i$. The data at -10 mV is taken from 9 of the 15 patches, each of which was exposed to several $[Ca^{2+}]_i$.

diaminopyridine (DAP). Each patch used for these experiments contained a single channel and was first exposed to symmetrical KCl with 200 μM Ca^{2+} activity at the intracellular face, and then exposed to the same medium to which had been added 10 mM of the test agent.

10 mM Ba^{2+} at the intracellular face decreased both the conductance and the percent open time. No outward currents were ever observed in any experiments after the addition of 10 mM Ba^{2+} to the perfusing bath (n=4), indicating a percent open time of zero or a conductance of zero through the open channel. No inward currents were observed in two of the Ba^{2+} experiments, however smaller currents (67% of current amplitudes before Ba^{2+}) with significantly decreased percent open times (i.e. 99% before Ba^{2+} as compared to 13% after) were observed in two experiments.

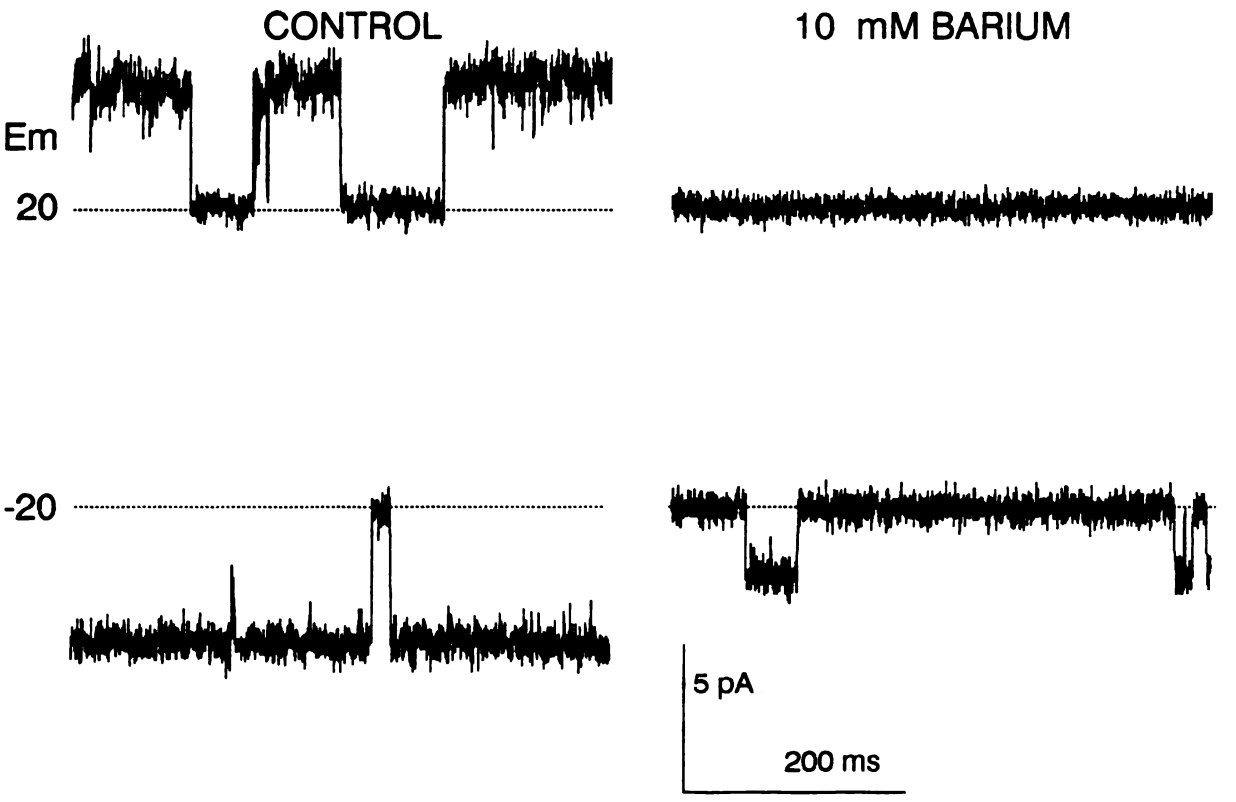


FIGURE 15 Current traces displaying the ability of 10 m<u>M</u> Ba^{2+} to block the channel from the intracellular face. The current traces on the left are from a patch exposed to symmetrical KCl solutions. The current traces on the right are from the same patch after the intracellular medium is exchanged for the same KCl solution with 10 m<u>M</u> Ba^{2+} added. The block was complete at positive membrane potentials. At negative membrane potentials smaller currents with reduced percent open times were sometimes observed.

DISCUSSION, Part II: DISPERSED MUSCLE

Comparative Aspects

Conductance

Under the conditions of my experiments, the most prominent conductance appears to be due to the presence of Ca^{2+}-dependent K^+ channels in the muscle fiber membranes. Such channels have been described in many invertebrate as well as vertebrate cell types (Latorre et al., 1989). They are commonly categorized as either small potassium conductance channels (SK, ≤80 pS in symmetrical 100-140 mM KCl) or large potassium conductance channels (BK, or maxi-K^+, 130-300 pS in symmetrical 100-140 mM KCl). According to this criterion, the channel described here appears to be best categorized as a maxi-K^+ channel.

Maxi-K^+ channels have been found in a variety of vertebrate cell types, including neurons (Krueger et al., 1982), pituitary cells (Wong & Adler, 1986), chromaffin cells (Marty, 1981), acinar cells (Maruyama, Gallacher & Peterson, 1983), pancreatic cells (Findlay, Dunne & Peterson, 1985) and many kidney cell types (Guggino et al., 1987) as well as both smooth (Singer & Walsh, 1987) and skeletal muscle (Latorre et al., 1982). The conductance of the channel in schistosome muscle, 195 pS in symmetrical 130 mM KCl, is within the range of that described for vertebrate smooth muscle, which is generally between 150-250 pS under comparable conditions (Latorre et al., 1989).

Selectivity

The schistosome muscle channel combines a greater than 10:1 selectivity for K^+ over Na^+ with its large conductance, like nearly all maxi-K^+ channels thus far described. The schistosome channel also resembles other maxi-K^+ channels in that it is relatively impermeable to Cs^+ (Cecchi, Alvarez & Wolff, 1986).

The schistosome channel varies from maxi-K^+ channels of other smooth muscle in that it is relatively impermeable to NH_4^+ (Hu, Yamamoto & Kao, 1989). A smaller Ca^{2+}-activated K^+ channel (72 pS) in Paramecium, however, has also been shown to be impermeable to NH_4^+ (Saimi & Martinac, 1989).

The seemingly discordant results obtained from the Rb^+ permeability experiments have been reported in mammalian exocrine acini (Gallacher, Maruyama & Peterson, 1984). K^+ and Rb^+ are generally thought to have very similar physical and chemical properties and K^+ channels are typically thought not to discriminate between K^+ and Rb^+. Maxi-K^+ channels from vertebrate muscle preparations typically display P_{K+}/P_{Rb+} ratios between 0.5 and 1 (Cecchi, Alvarez & Wolff, 1986; Hu, Yamamoto & Kao, 1989). For the schistosome channel, a straight line drawn through the data in Figure 14 would result in an x-intercept near zero, which would indicate that P_{K+}/P_{Rb+} is near one according to the biionic equation. This conclusion, however, seems at odds with the observation that Rb^+ has, at best, a very low conductance

through the channel. According to the electrodiffusional model of Barry and Gage (1984), the ratio of permeabilities for two cations under biionic conditions is:

$$P_x/P_y = u_xK_x/u_yK_y$$

where K is the equilibrium constant for binding and u is the mobility. Therefore, it can be hypothesized that the channel's permeability to Rb^+ and K^+ may be equal but that the mobility (i.e., conductance) of Rb^+ is much less than that of K^+, providing that Rb^+ has an equilibrium constant that is much higher than that of K^+.

Calcium sensitivity

The activity of the maxi-K^+ channel in schistosome muscle is modulated by the concentration of Ca^{2+} at its intracellular face, as is the activity of maxi-K^+ channels in other smooth muscle cells (Singer & Walsh, 1987). In the schistosome channel, the Ca^{2+} concentration range over which percent open time is modulated is 0.1 μM to 1.0 μM. This range is comparable to the calcium concentrations over which maxi-K^+ channels are modulated in vertebrate smooth muscle (Latorre, et al., 1989). In contrast to most other maxi-K^+ channels, however, no voltage sensitivity in the schistosome muscle channel was detected. The recording conditions may simply not have covered a sufficiently wide range of

voltages over the calcium concentrations tested or it could reflect a real difference between this type of channel in a primitive animal such as the schistosome and more highly evolved organisms.

Blocker sensitivity

Intracellularly applied Ba^{2+} blocked the schistosome muscle channel, as is the case for the maxi-K^+ channel in other smooth muscle preparations (Benham *et al.*, 1985). In fact, the characteristics of the Ba^{2+} block were nearly identical to those observed in arterial and intestinal smooth muscle maxi-K^+ channels. The channel block was stronger when the membrane potential was such that it drove the Ba^{2+} into the channel. This is consistent with the hypothesis that Ba^{2+} enters the open channel from the intracellular face and blocks at a site that is most of the way through the channel (Benham, *et al.*, 1985).

TEA block of maxi-K^+ channels in other smooth muscle preparations and other tissue types is most often mediated by a low affinity site at the intracellular face (K_d's range from 20-60 m**M**) and by a high affinity site at the extracellular face (K_d's 0.3 m**M** and lower) (Latorre *et al.*, 1989). The high affinity block of the maxi-K^+ can occur at the intracellular face (Wong & Adler, 1986), but only in exceptional cases, and not in any muscle tissue. The schistosome muscle channel did not show a high-affinity TEA

binding site at the intracellular face. It was not possible to test the extracellular face for TEA sensitivity using these inside-out patches.

The schistosome muscle channel resembled other maxi-K^+ channels in that Na^+ and Cs^+ increased the fast "flicker" activity of the channel when they were present in high concentrations at the intracellular face. This has been called Na^+ or Cs^+ "fast block" (Yellen, 1984). It is hypothesized that these cations are small enough to enter the pore of the channel and, although they can not be conducted through, they can occupy the pore temporarily and interrupt the flow of K^+ ions into the cell (Cornejo et al., 1989; Cecchi et al., 1987).

Possible Function of this Channel

Ca^{2+}-dependent K^+ channels have been described in a variety of cell types in a wide range of organisms from protozoa (Saimi & Martinac, 1989) to mammals (Latorre et al., 1989). In vertebrate smooth muscle one proposed function for these channels is control of slow wave electrical activity (Latorre et al., 1989). Since elevated intracellular Ca^{2+} activates these channels, they may serve to terminate periods of voltage-dependent Ca^{2+} entry into cells by repolarizing the cell (Hu, Yamamoto & Kao, 1989).

In intact schistosomes the musculature shows continuous, more or less rhythmic activity (Barker, Bueding

& Timms, 1966; Fetterer, Pax & Bennett, 1977). Much of this activity appears to be myogenic (Pax et al., 1981). Ca^{2+}-activated K^+ channels in the muscle membranes of the schistosome could be intimately involved in bringing about the rhythmic muscle activity one observes but, obviously, much more detailed studies on these isolated muscle fibers are required. Only after there is a more complete picture of the kinds of ion channels and their pharmacological profiles will it be possible to make definitive statements about the functional characteristics of schistosome muscle fiber membranes.

GENERAL CONCLUSIONS

Application of Patch-Clamp to Tegument

This research shows that it is possible to use patch-clamp techniques to study individual ion channels present in the schistosome outer tegumental membrane. Unfortunately, these studies are limited by the extremely low success rate of obtaining high resistance seals. A rather substantial glycocalyx covers the outside surface of the worm (Sher, et al., 1989). This glycocalyx is probably the greatest hinderance to successful seal formation. There are two possible approaches by which one may be able to overcome this limitation. Firstly, one might apply enzymes to isolated worms to selectively remove known components of the glycocalyx. The disadvantage to this approach would be that one would not know what effect the enzymes might have on the protein channels. Secondly, one may be able to induce the formation of tegument vesicles through ionic stress. One of these strategies may prove the most fruitful for the continued study of the outer tegumental membrane with patch-clamp techniques.

Application of Patch-Clamp to Dispersed Muscle

These studies are the first to study directly the physiological properties of a dissected component of the schistosome neuro-muscular system. They show that patch-clamp techniques offer a means by which the properties of

individual muscle fibers can be studied apart from the effects of the nervous system or other complications present in whole-animal studies. Only through these types of direct studies will it be possible to localize the responses observed in these parasites to specific mechanisms at specific locations.

It is clear that there are other channels present in the muscle membrane that could be biophysically characterized using the inside-out cell-free patch techniques described here. It is now also possible to study the muscle membrane in the outside-out configuration, using the presence of the Ca^{2+}-activated K^{+} channel described above as an assay for the outside-out configuration. If the pipet contained high calcium concentrations and the bath contained no calcium, only patches in the outside-out configuration would display the Ca^{2+}-activated K^{+} channel. However, the most fruitful application of patch-clamp studies to these isolated muscles would most likely be the whole-cell configuration.

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