Characterization of ion channels on the surface membrane of adult rat skeletal muscle

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ABSTRACT The channels present on the surface membrane of isolated rat flexor digitorum brevis muscle fibers were surveyed using the patch clamp technique. 85 out of 139 fibers had a novel channel which excluded the anions chloride, sulfate, and isethionate with a permeability ratio of chloride to sodium of <0.05. The selectivity sequence for cations was $Na^+ = K^+ = Cs^+ > Ca^{++} = Mg^{++} >$ *N*-Methyl-D-Glucamine. The channel remained closed for long periods, and had a large conductance of ~320 pS with several subconductance states at ~34 pS levels. Channel activity was not voltage dependent and the reversal potential for cations in muscle fibers of ~0 mV results in the channel's behaving as a physiological leakage conductance. Voltage activated potassium channels were present in 65 of the cell attached patches and had conductances of mostly 6, 12, and 25 pS. The voltage sensitivity of the potassium channels was consistent with that of the delayed rectifier current. Only three patches contained chloride channels. The scarcity of chloride channels despite the known high chloride conductance of skeletal muscle suggests that most of the chloride channels must be located in the transverse tubular system.

INTRODUCTION

Skeletal muscle membrane has a large resting conductance. This conductance is mostly due to potassium and chloride conductances (Hogkin and Horowicz, 1959; Adrian and Freygang, 1962). Resting or weakly voltage dependent conductances play an important role in maintaining the resting potential, and also in determining the passive membrane properties of the sarcolemma. Such conductances play an important role in action potential repolarization in the restricted space of the transverse tubular system (Adrian and Freygang, 1962; Hutter and Nobel, 1970; Hutter and Warner, 1972).

In mammals the major component of the resting conductance, measured using macroscopic techniques, is due to chloride permeability (Palade and Barchi, 1977; Dulhunty, 1978). We investigated the channels that may contribute to the resting membrane conductance of mammalian skeletal muscle using the patch clamp technique (Hamil et al., 1981) with inside out and cell attached patches with slowly changing voltages.

Surprisingly the most frequent encountered ion channel on the surface membrane was not chloride selective, but rather nonselectively permeant to cations. Potassium channels were also common; chloride channels were rarely found. The scarcity of chloride channels on the surface membrane confirms a previous suggestion that they are mostly located in the transverse tubular system (Dulhunty, 1979), which is a part of the membrane system that is inaccessible to the patch clamp electrode.

The role of the nonselective cation channel described in this study may be that of a passive leakage resistance. However, because cations are out of equilibrium (e.g., Lewis, 1979), this leakage conductance will contribute to the resting membrane potential and augment the positive shift in membrane potential away from the potassium equilibrium potential that is caused by the chloride permeability because chloride distribution across the sarcolema is out of equlibrium (Bolton and Vaughan-Jones, 1977; Dulhunty, 1979; Harris and Betz, 1987; Aickin et al., 1989).

METHODS

Preparation

Fibers were isolated from the flexor digitorum brevis muscle of adult rats using an adaptation of the technique of Bekoff and Betz (1977). Muscles were digested with 0.3–0.6% collagenase in oxygenated calcium-free Krebs solution (NR, see Table 1) for 1–3 h at 30°C and then transferred to fresh Krebs solution. Fibers were separated by tituration and allowed to settle and adhere to a clean, glass bottom chamber. The cells were maintained in calcium-free Krebs solution at 22–25°C.

Solutions

Solutions for the electrode usually contained 136 mM NaCl with 5 mM KCl, which mimicked the normal extracellular concentration of these ions. The internal (intracellular) side of ripped-off patches were usually placed in a solution containing 141 mM of a cation, 141 mM

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TABLE 1 **Solutions**

Solution	Major cation	[C+]	K	Ca	Mg	Major anion	[A−]	TES	SUCROSE
		mM	*mM*	*mM*	*mM*		*mM*	*mM*	*mM*
NR	Na	136	5	—	1	Cl	141	2	—
AE	Na	136	5	0.4	—	Cl	141	2	—
NA1	Na	141	—	0.4	—	Cl	141	2	—
NA1-75	Na	106	—	0.4	—	Cl	106	2	73
NA1-50	Na	71	—	0.4	—	Cl	71	2	145
NA1-25	Na	35	—	0.4	—	Cl	35	2	218
K	K	—	141	0.4	—	Cl	141	2	—
CS	Cs	141	—	0.4	—	Cl	141	2	—
NMDG	NMDG	141	—	0.4	—	Cl	141	2	—
CA	Ca	141	—	141	—	Cl	282	2	—
MC	Mg	—	—	—	141	Cl	282	2	—
SO_4	Na	188	—	0.4	—	SO4	94	2	—
ISE	Na	141	—	0.4	—	Ise	141	2	—

All solutions were pH 7.4 and contained 0.5 mM EGTA. NaOH was used to adjust the pH of solutions containing Na, Ca or Mg as the major cation. KOH was used for the K solution and HCl for the *N*-Methyl-D-Glucamine solution. The NR solution also contained 11 mM of glucose. Ise = isethionate.

chloride, and pH and calcium buffers. When chloride was replaced, an equal molar amount of sodium isethionate, or an isoosmotic amount of sodium sulphate was used. The exact composition of solutions used are listed in Table 1. In the solutions with 0.5 mM EGTA and 0.4 mM $CaCl_2$ the theoretical free ionized calcium concentration was 0.4 uM (Blatz and Magleby, 1983).

Recording techniques

To study ionic currents that may have been weakly or not voltage dependent the tight patch recording technique was used (Hamill et al., 1981). Patch electrodes (2–12 MOhm) were coated with silicone elastomer (Sylgard; Dow Corning) and connected to a conventional patch clamp amplifier (Axopatch 2; Axon Instruments).

A double chamber recording dish was used, which allowed recordings to be made in the cell attached and inside out configuration. Inside out patches were formed and transferred without exposure to air via a narrow connecting channel to a side chamber. The main chamber was continuously perfused with Krebs solution with no added calcium (solution NR, Table 1). The solution in the side chamber could be independently changed while maintaining the patch. Mixing between the chambers was minimal, dye added to one chamber could not be detected in the other.

The reference Ag/AgCl electrode was placed in the chamber containing Krebs solution, and was always in contact with chloride ions. Electrical continuity to the side chamber was through the solution in the connecting channel. Potentials were measured relative to the reference Ag/AgCl electrode, and quoted relative to zero potential in the solution bathing the extracellular face of the membrane. The patch electrode was initially zeroed in Krebs solution in the main chamber, and then moved to the side chamber. The change in potential with the various internal solutions in the side chamber was <1 mV, except for the sulphate solution, which produced a 2 mV change. No correction was made for these small potentials. The patch electrode solutions always contained chloride ions. Positive current refers to current flowing outward across the cell membrane.

Slowly depolarizing ramps from -90 to 90 mV, each lasting ~ 2 s and with a rapid return to -90 mV were used to measure channel conductance, reversal potential, and seal resistance. Ramps allowed the rapid determination of channel characteristics, and coveniently showed whether channel currents were an open channel shutting or a shut channel opening. Also activity from a specific channel could be followed over a large potential range, even in the presence of other channel currents. Channel activity at steady voltages was also recorded.

Analysis

Current and voltage measurements were stored on an FM tape recorder with a bandwidth of 5 Khz. Recorded data were passed through an eight pole low pass Bessel filter and digitized with a digital oscilloscope (Nicolet 209; Nicolet Instr. Corp., Madison, WI). The filter cut off frequency (f_{-3db}) was generally 800 Hz when analyzing nonselective cation or chloride currents and 400 Hz for potassium current. The sampling rate was 2 KHz. For fast events a cut off frequency of 1,600 Hz and a digitization rate of 5 KHz was used. A 9816 model (Hewlett-Packard, Palo Alto, CA) computer system was used to store and analyze the digitized data.

RESULTS

Cell attached recordings with 136 mM NaCl and 5 mM KCl (solution AE, Table 1) in the pipette were made from 139 cells. Slowly depolarizing ramp potentials applied to the patches revealed activity from potassium, chloride, and nonselective cation ion channels (Table 2). Seal resistances were generally 4–22 GOhm, and averaged 16 GOhm. Voltage dependent sodium channels were not observed. The procedures used would have inactivated these channels. Voltage steps and perhaps veratridinic drugs to extend channel lifetime are required to study these channels (e.g. Aldrich et al., 1983; Nowak et al., 1987).

12 patches had no observable channel activity. In these patches activity could not be evoked by applying ramp potentials for at least 5 min, steady-state potentials

TABLE 2 **Channel types in 139 cell attached patches**

Channel type	Conductance	Number of patches with channel	Total number of patches
	pS (multiple levels)		
Nonselective cation	34...320...	85	85
K^+	6.6 ± 0.7	8	65
	12 ± 0.3	34	
	25 ± 0.4	41	
	120	1	
	211, 265	2	
Cl^-	58 ± 1	3	

in the range of −80–+80 mV, and voltage steps between 0, + −30, and + −60 mV. Excising these patches generally did not reveal channel activity.

Nonselective cation channels

Typical activity from the most commonly found type of channel is shown in Fig. 1. These currents were recorded with steady application of potential to a patch of the inside out configuration with sodium as the major cation and chloride as the anion (solution NA1 applied to the internal [intracellular] surface and solution AE to the external [extracellular] surface of the patch). The maximum conductance of this activity was often larger than 270 pS. When the channel was open much flickery activity was present, which was not well resolved and was much larger than the baseline noise present when the channel was shut. This noise appeared to be closing noise. Openings were infrequent when the channel was shut and were not sensitive to positive or negative pressure applied to the pipette. This current activity was present in the cell attached configuration in 85 patches. Often several minutes would elapse before the activity appeared or disappeared. When the channel was open the flickering shut did not always return to the fully closed state. Frequently the channels were in these apparent subconductance states for a sufficient time to suggest that it was not due to unresolved buzzing activity (Fig. 1). The overall gating of the channel or channel complex was clearly demarcated by the turning on or off of the flickery channel activity, which together with long silent periods suggests that this activity was the result of an ion channel and not dielectric breakdown of the membrane.

The voltage dependence of this channel can be easily measured by averaging a number of currents produced during voltage ramps, as illustrated in Fig. 2. Fig. 2*A* shows a single ramp from an inside out patch with AE

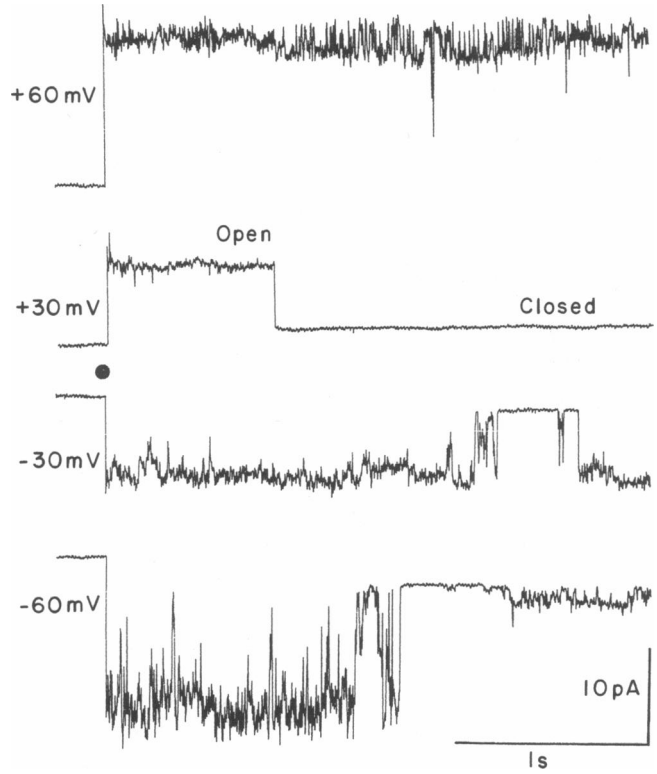
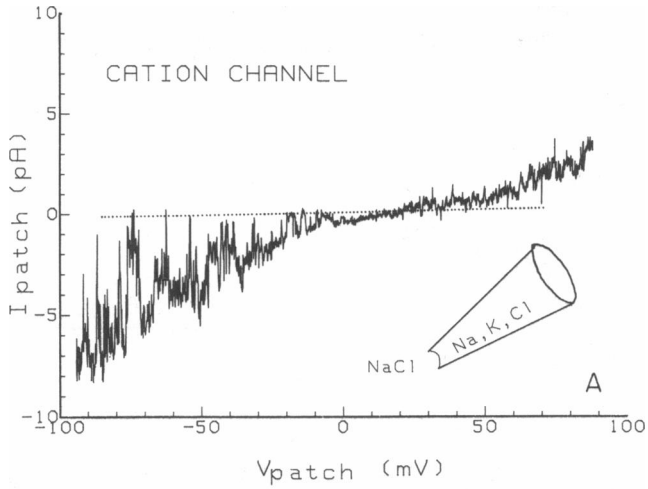


FIGURE 1 Channel activity recorded from an inside out patch bathed in symmetrical NaCl (141 mM). At the times marked by the solid circle, the potential was stepped to the levels shown on the right. In this and all other figures, voltage sign is with respect to the intracellular surface of the membrane.

(see Table 1) in the pipette and Na1 in the bath. Seal current has been subtracted so that displacement from the horizontal axis passing through zero is channel current. When 32 such sweeps from the same cell were averaged a macroscopic current I–V curve was generated (Fig. 2 *B*). In this example the linear relationship of voltage and current shows that this channel is not voltage dependent.

Initially we thought that these channels might have been chloride selective. For example, they were one of the most frequently observed channel types (and macroscopic membrane chloride conductance is higher than that of any other ion), they had high single channel conductance with a great deal of flicker (as do chloride or anion channels in some other cell types), the reversal potential for inside out patches in symmetrical NaCl was 2 mV (±1, SE, n = 25), and, as described in more detail below, replacing Na^+ with K^+ has a negligible effect on channel reversal potential.

The first indication that these were not chloride channels came when we replaced Cl^- with$SO_4^=$. The conductance and reversal potential of excised patches



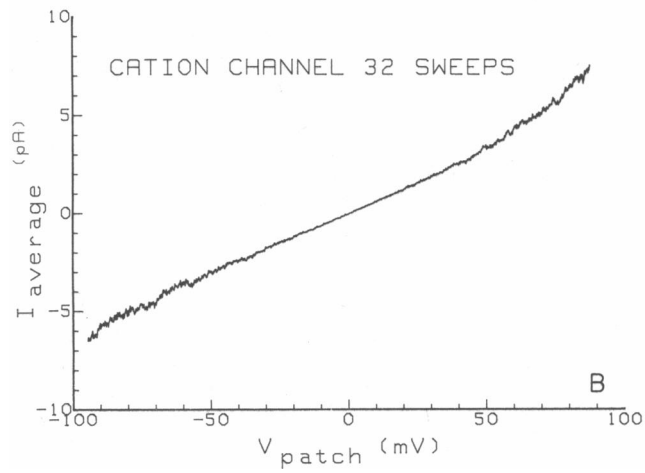
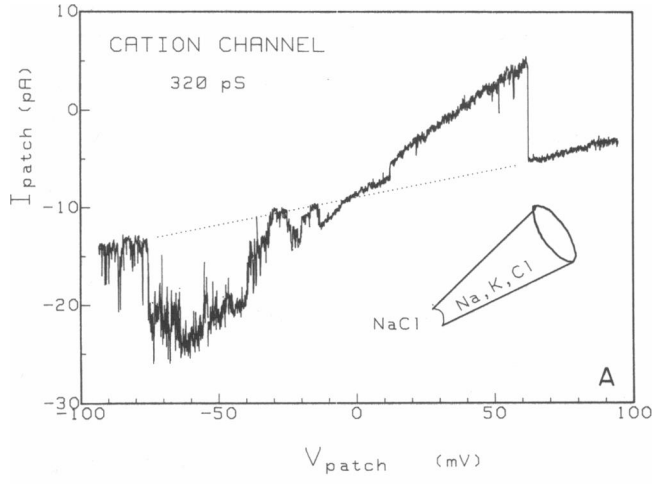


FIGURE 2 (*A*) Channel activity plotted as a function of membrane voltage, which was changed continuously (ramped) over a 2 s period. As shown in the cartoon, this was an inside out patch with the extracellular surface of the membrane exposed to Na^+, K^+, and Cl^- (solution AE), and the internal surface to NaCl (solution NA1). The dotted line marks the closed state. (*B*) Average of 32 sweeps. The nearly linear relationship shows that the channel exhibited little if any voltage dependence.



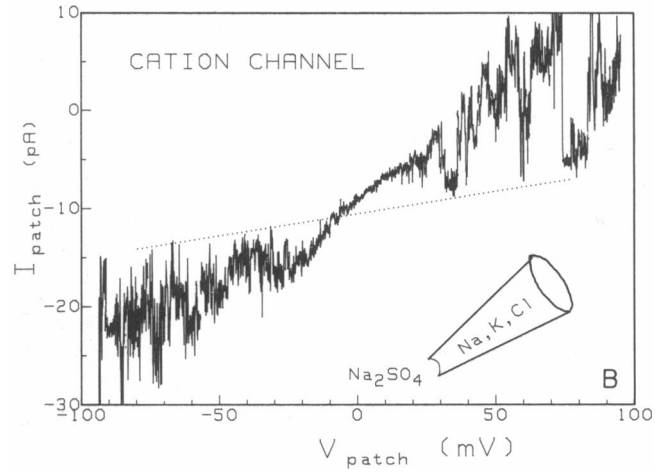


FIGURE 3 Effect of replacing bath Cl^- with $SO_4^=$ (pipette contained solution AE). (*A*) Channel activity in control bathing solution (NA1). (*B*) Cl_- replaced with $SO_4^=$ (solution SO). The reversal potential, indicated by the intersection of the open channel current and dotted line, changed relatively little, indicating little difference in the channel permeability to Cl^- and $SO_4^=$.

with the internal side of the patch exposed to isotonic sulfate solution (SO, Table 1) were measured using voltage ramps (Fig. 3). The reversal potential, which should have undergone a large positive shift if this were a chloride channel, actually shifted negatively by ~6 mV (to −4 mV ± 1, SE, n = 8). This difference agrees with the theoretical Nernst potential of −7 mV for the electrochemical gradient difference in sodium ion concentration (141 mM outside and 188 mM inside). The measurements were repeated with 141 mM sodium and isethionate (solution ISE, Table 1) replacing chloride in the internal (bath) solution. The reversal potential was 0 mV (±1, SE, n = 10), which was not significantly different from symmetrical NaCl. Therefore, either isethionate was passing through the channel or the channel was not an anion channel.

The definitive experiment to determine the relative cation and anion selectivities of the channel involved simply reducing the salt concentration on one side of the patch (osmolarity was maintained by the addition of sucrose). For example, switching from symmetrical NaCl solutions (reversal about 0 mV) to 50% NaCl on the internal side of the membrane would shift E_{Na} positive and E_{Cl} negative by equal amounts. The magnitude and polarity of the observed shift in channel reversal potential would provide a measure of relative channel selectiv-

ity to the two ions. Sodium chloride concentration was reduced to 75, 50, and 25% of normal internal solution with osmolarity being maintained by the addition of sucrose (solutions NA1-75, NA1-50, NA1-25, and NA1, see Table 1). The reversal potentials became positive when the internal (bath) salt concentration was reduced, consistent with a channel that is permeant mostly to sodium or cations. The plot of reversal potential versus sodium chloride concentration was nearly linear (Fig. 4), indicating that the channel did not change its selectivity when the internal ionic strength was reduced. If the constant field assumption is valid in this system, the Goldman-Hodgkin-Katz equation shows that this channel has a chloride to sodium permeability ratio of <0.05. The 1.7 mV reversal in symmetrical NA1 was subtracted from the other reversals to correct for what may have been a junction potential error. This correction is likely to give an underestimate of the permeability ratio.

Having established that this channel is a cation selective channel, we measured the permeabilities of other monovalent and divalent cations using inside out patches, and replacing internal sodium with K^+, Cs^+, Ca^{++}, and Mg^{++} ions (solutions K, CS, CA, and MC, respectively, see Table 1). The measured reversal potentials with sodium substitutions are given in Table 3. Permeability ratios were calculated using the Goldman-Hodgkin-Katz (GHK) equation which, for permeant divalent cations, was modified according to Lewis (1979; Appendix A). For monovalent cations (K^+ and Cs^+), calculated permeability ratios relative to sodium were 1.0; for Ca^{++} and Mg^{++}, permeability ratios were ~ 1.3 (using activity coefficients of 0.52 for divalent cations; Robinson and Stokes, 1971). As noted by Lewis (1979),

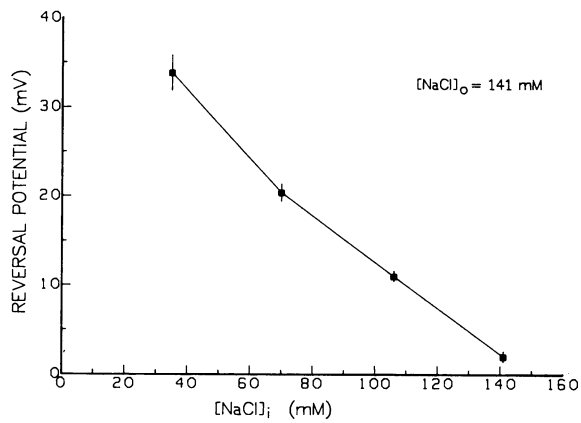


FIGURE 4 Effect of diluting NaCl in the solution facing the intracellular membrane face on channel reversal potential (osmolarity was maintained with sucrose addition). The positive shift in reversal potential shows that the membrane was more permeable to Na^+ than to Cl^-; the ratio P_{Cl}/P_{Na} calculated from the slope of the line is <0.05.

TABLE 3 **Reversal potential with cation substitutions**
Nonselective cation channel external solution 141 mV NaCl

	Internal	Vrev	*n*
	mM	*mV*	
Cation substitutions			
NaCl	141	2 ± 1	25
KCl	141	2 ± 2	6
CsCl	141	4 ± 4	3
$CaCl_2$	141	1 ± 2	7
$MgCl_2$	141	−1 ± 2	2
N-Methyl-D-Glucamine Chloride	141	23 ± 8	7
		mean ± SE	

this calculation ignores surface charge screening effects by divalent ions. In the present case, charge screening at the internal membrane surface by divalent cations would make the membrane potential more positive than the measured value, which would lead to an overestimate of P_{Ca}/P_{Na}. For example, a positive shift of the reversal potential of 20 mV in high [CA^{++}] due to surface charge screening (cf. Lewis, 1979) would reduce P_{Ca}/P_{Na} to a value of ~ 0.5. Thus, there appears to be little if any selectivity by the channel for either monovalent or divalent cations. Gating activity did not appear to be sensitive to internal Ca^{++} over the range 0.01–141 mM; the channel could still be observed when 1 or 141 mM Ca^{++} was present in the external solution (Pipette). Examples are shown in Fig. 5. To further confirm that

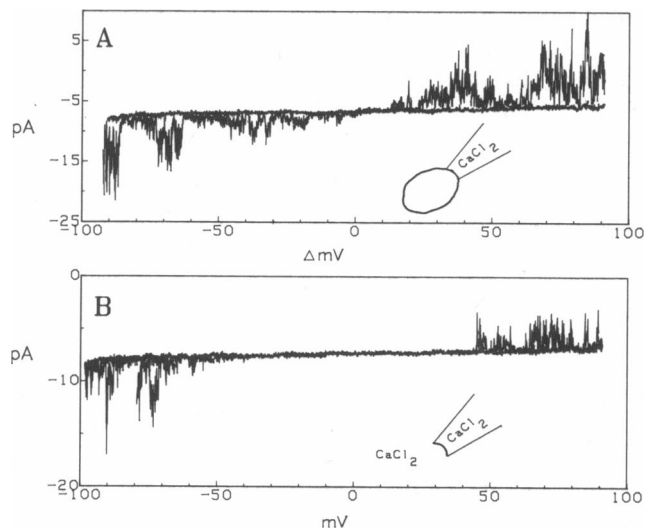


FIGURE 5 Nonselective cation channels are present in the presence of calcium ions. In this experiment, the patch electrode contained 141 mM $CaCl_2$ plus 2 mM TES at pH 7.5. Each panel shows two superimposed sweeps, one sweep with little activity, and one with channel openings. (*A*) Cell-attached patch. (*B*) Inside out patch.

the channel activity was indeed a cation channel, a large and generally less permeant cation, *N*-methyl-D-glucamine (NMDG) was tested (Bevan et al., 1984; Friel and Bean, 1988). The reversal shifted +25 mV relative to sodium (Table 3). If it is assumed that chloride is not significantly permeant under these conditions, then NMDG has a permeability ratio of ~0.35 relative to sodium.

K channels

65 of the cell attached patches possessed potassium channel activity, and more than 132 potassium channels could be discerned. Most of the potassium channels displayed voltage dependence, opening when the patch was depolarized by ~20 mV (membrane potential across the patch decreased by 20 mV). A typical record of the current produced by a voltage ramp of −70–+70 mV applied to the patch is shown in Fig. 6. In the cell attached mode currents were outward and the average reversal potential was −59 mV ±4.8 (SE, n = 90). The channels were voltage dependent, opening when the patch was depolarized by 20 mV. Such channel activity was abolished or greatly reduced by the replacement of internal potassium with sodium.

Based on conductance measurements five different types of potassium channels were identified. The largest was seen only twice and had conductances of 211 and 265 pS, and opened and closed rapidly. It was similar to the "big K channel" present in various tissues (Pallota et al., 1981; Latorre et al., 1982; Marty, 1983; Marty and Neher, 1985; Simmonneau et al., 1987; Nowak et al., 1987). A second large conductance potassium channel, 120 pS, was observed only once and had a similar conductance to the sarcoplasmic reticulum potassium channel (Labarca and Miller, 1981).

Smaller conductance potassium channels were common. Fig. 7 shows a frequency distribution histogram of the conductances of these channels measured from ramps records made in the cell attached configuration. Conductances were centered around 25, 12, and 6.6 pS in 42, 34, and 8 patches, respectively (see Table 2). Some patches contained all three types, whereas others contained only one type, indicating that the smaller channels were not necessarily subconductance states of a larger channel. However the possibility of some of these currents being subconductance states cannot be ruled out conclusively with the data gathered in this study.

The potassium channels could not be seen when inside out patches were in Krebs solution ($[K]_i$ = 5 mM), or when the patches were transferred to potassium-free solutions (e.g. NA1). The reversal potential of the 25 pS channel was −58 ± 6.5 mV (SE, n = 6) with inside out recordings with 5 mM potassium in the pipette (AE solution) and 141 mM potassium in the bath. This is consistent with a potassium selective channel with a reversal potential of −83 mV under these conditions, if it were perfectly selective for potassium ions. Therefore, these channels are most probably potassium channels.

Chloride channels

The least common channel found in the cell attached patches (3 patches out of 139) was most likely to have been chloride selective. Fig. 8 shows the ramp potential current activity due to such a channel. This channel had well resolved current transitions, could open at all potentials, but was open more often at positive potentials. Activity could be recorded with cesium chloride in

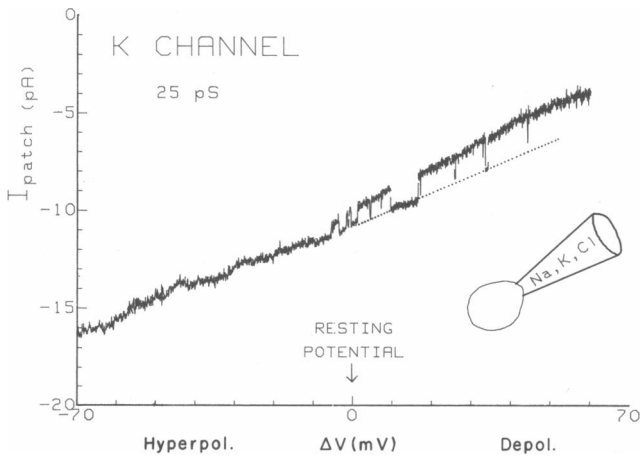


FIGURE 6 A potassium channel recorded in the cell attached mode (solution AE in pipette). The dotted line marks the closed state.

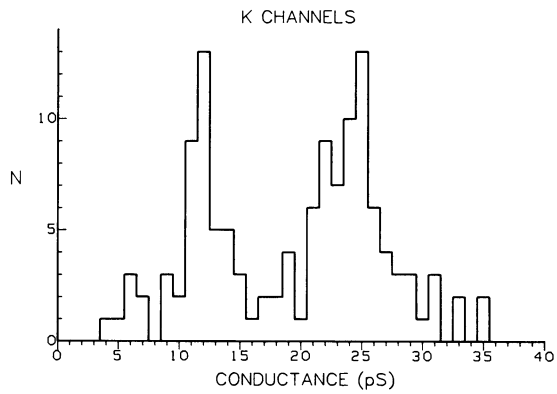


FIGURE 7 Frequency distribution of K^+ channels recorded in cell attached mode. The most commonly encountered channels had conductances of ~12 and 25 pS. A third type, with a conductance of about 6 pS, is also suggested.

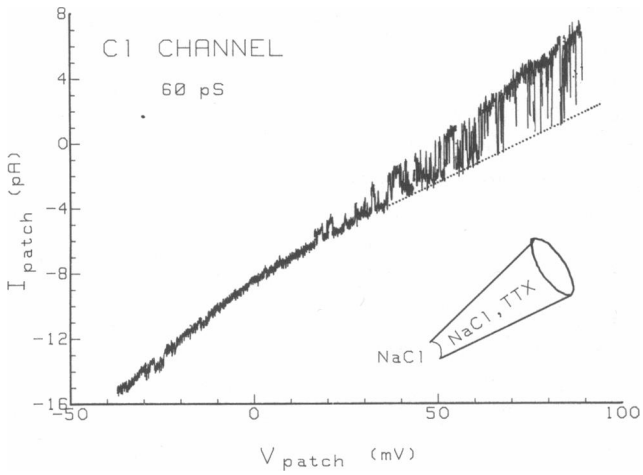


FIGURE 8 An example of one of the few Cl^- channels encountered. This channel showed clear voltage sensitivity, opening with depolarization. The dotted line marks the closed state.

the pipette (CS solution, Table 1) and in symmetrical cesium chloride in the inside out configuration (Fig. 9). Sodium chloride solution (NA1) with 10 mM $CoCl_2$ and 3 μm TTX in the pipette and the bath did not affect the conductance or abolish the current transitions. Therefore, it is unlikely that this activity was due to sodium or calcium channels. The average conductance in the cell attached mode was 58 ± 1 pS (n = 3) and the average reversal potential was +4 mV. This agrees with the reversal potential for chloride being positive from rest in intact mammalian skeletal muscle (Dulhunty, 1978; Harris and Betz, 1987; Aickin et al., 1989).

With excised patches in solutions with symmetrical chloride, the conductances of two such patches were 55 and 60 pS, whereas the reversal potential was −4 and 6 mV. This is close to the expected reversal of 0 mV in equal chloride concentrations across the patch. Also the mean open time (13 ms) and mean closed time (39 ms) of one patch (Fig. 8) is similar to a chloride channel reported in cultured myotubes reported by Blatz and Magleby (1985). However, it was not possible to fully characterize the kinetic and permeability properties of these channels because these channels were rarely encountered.

DISCUSSION

Using patch clamp techniques we have surveyed the population of ion channels present in the surface membrane of collagenase dissociated muscle fibers of adult

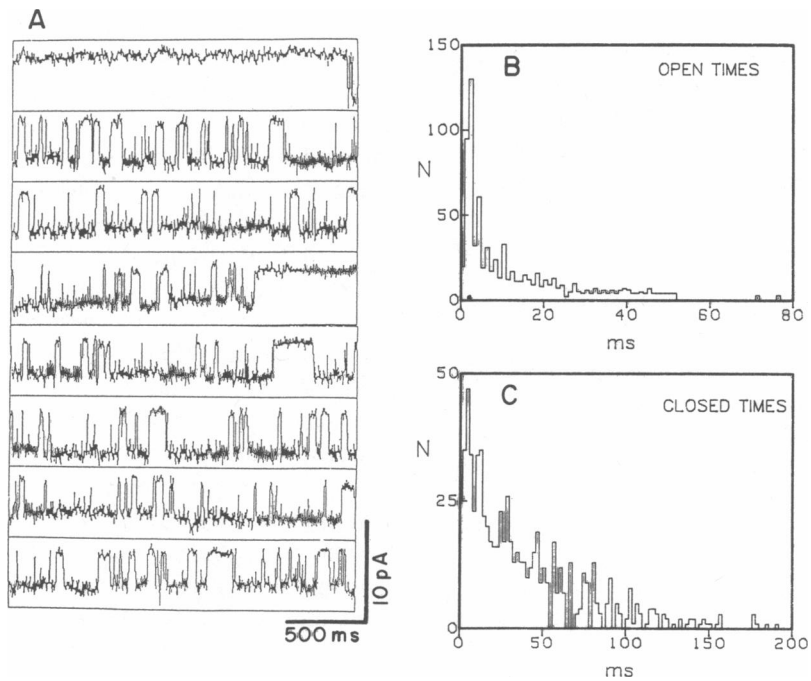


FIGURE 9 Chloride channel characteristics. Inside out patch bathed in symmetrical 141 mM CsCl. (*A*) Sample channel activity recorded at constant membrane potential (60 mV, intracellular surface negative). (*B*) Open time histogram. Mean open time was 13 ms. (*C*) Closed histogram. Mean closed time during the period of recording was 39 ms.

rats. Two unexpected results were obtained. First, we rarely observed chloride channels, despite the high macroscopic chloride conductance of mammalian skeletal muscle. As discussed below, this suggests that chloride channels are restricted to the transverse (t) tubular membrane, whose channels we could not sample. Second, an unexpected channel type was frequently encountered. This channel excluded anions, but was relatively nonselective for cations.

Nonselective cation (NSC) channel

NSC channels were observed in most patches, in both cell attached and inside out configurations. We cannot rule out the possibility that the NSC channels we observed represent a population of channels which were artifactually altered by the enzyme treatment. Different enzyme concentrations and lengths of enzyme incubation did not noticeably alter the incidence or properties of these channels, suggesting that if they formed artifactually, they did so early in the enzyme incubation and at low collagenase concentrations, which seems unlikely. Moreover, a similar current has recently been reported in frog skeletal muscle which was not exposed to collagenase; in that study, macroscopic currents were recorded with the vaseline gap voltage clamp technique (Godinez et al., 1988).

The NSC channel showed periods of flickering activity alternating with silent periods, which is indicative of a single controlling process which overrides all of the open subconductance states in the channel. Such a mechanism has been proposed for a large conductance chloride channel, which has its subconductances currents gated off by one controlling process (Krouse et al., 1986).

The rapid noise during the open time of the channel could not be subjected to quantitative study due to limited temporal resolution. Similar noise has been reported in calcium activated nonselective cation channels in lymphocytes (Lipton, 1986; Partridge and Swandulla, 1987). Several processes could account for the open channel noise. Firstly the channel could inherently have fast kinetics. This is not true of the nonselective cation channel reported in other tissues, which have slow kinetics (Colquhuon et al., 1981; Yellen, 1982; Maruyama and Peterson, 1982; Simonneau et al., 1987). It is possible that the channel in rat skeletal muscle is inherently slow but is subject to rapid blocking and unblocking by other ions (Hille, 1983) such as anions or protons. Proton block has been described in other channels (Prod'hom et al., 1987), but the channel activity in our study was not pH dependent (unpublished observations). Similarly block by anions should be voltage dependent and affected by substitution of chloride by sulfate. Little or no voltage dependence of channel activity was found and the channel conductance was not changed by internal sulfate. An alternative source of the noise is from the channel itself. Conformational transitions in the channel protein may alter ion permeation and cause fluctuations (Sigworth, 1985). The rapid current fluctuations made the measurement of channel conductance difficult. However, occasionally prolonged openings occurred and were easily measured; the maximum conductance was 320 pS. Larger conductances measured from single patches could be due to the presence of multiple channels or channel clusters; however, 320 pS may not be the fully open state of the channel complex. The apparent subconductance levels were at ~34 pS intervals with the smallest conductance being 34 pS, and is similar to the conductance of the acetylcholine activated cation channel (Horn and Patlak, 1980; Hamill and Sakmann, 1981). The acetylcholine activated cation channel also has similar ion permeability as the nonselective cation channel in that neither discriminates between monovalent cations and both readily pass calcium and magnesium ions. Stretch-activated channels are also not very selective between cations (Guharay and Sachs, 1984; Erxleben, 1989) but the nonselective cation currents in the present study displayed no pressure sensitivity.

The relatively high conductance of this channel to calcium is not seen in the calcium activated nonselective cation channels reported by others (Yellen, 1982; Bevan et al., 1984). Also the activity is not affected by calcium in the internal side of the patch in the range of 10^{-5}–141 mM. This is in contrast to the nonselective cation channel in other tissues, which is sensitive to internal calcium concentrations as low as 100 nM (Simonneau et al., 1987; Partridge and Swandulla,, 1987). Loss of selectivity of calcium channels probably did not significantly contribute to the nonselective current recorded because calcium channels require extremely low concentrations of external calcium to become nonselective (Almers et al., 1984). In this study the nonselective current could still be recorded when 1 or 141 mM calcium was present in the pipette and/or solution bathing the intracellular face of the patch.

The physiological role of the NSC channels is unknown. With a reversal potential near 0 mV, channel openings would depolarize muscle fibers and reduce their input resistance. Both actions could have important implications for a muscle fiber's excitability properties, as discussed for a NSC channel in snail neurons by Swandulla and Lux (1985). Further characterization of the role of this channel would of course benefit from the identification of a selective blocking agent.

Potassium channels

The detection of several types of potassium channels is not unexpected because potassium ion channels are heterogeneous (see Schwarz and Passow, 1983; Latorre et al., 1984). Under the physiological concentrations of sodium and potassium in the pipette and cell attached recordings only voltage dependent outward potassium currents flowed. The 6.6 and 12 pS channels were similar to voltage activated potassium channels in chromaffin cells (Marty and Neher, 1985). The 25 pS channel was similar to a calcium dependent voltage activated in astrocytes (Quandt and Vicar, 1986). These channels would repolarize the cell membrane in response to a depolarization. The less common channels (120 and ~230 pS) were probably larger types of calcium dependent potassium channels (Pallota et al., 1981; Latorre et al., 1982; Marty, 1983; Marty and Neher, 1985; Simonneau et al., 1987).

Chloride channels

The scarcity of chloride channels was surprising when the high conductance to chloride in mammalian skeletal muscle is considered (Palade and Barchi, 1977; Dulhunty, 1978). One possible explanation is that the chloride channels were not activated by the voltage protocols used. However, the few chloride channels recorded were similar to the slow 60 pS channel reported by Blatz and Magleby (1985). Such channels would have opened with the recording modes and voltages used in this study.

However, the simplest explanation for the paucity of chloride channels is due to the large area of membrane in the transverse tubular system, which is in connection with the surface membrane. In proportion to the relative membrane areas of the surface and the transverse tubular system, most of the chloride conductance has been suggested to be in the tubular system (Dulhunty, 1979, 1982). The low occurrence of chloride channels on the surface membrane confirms that the majority of chloride channels must be located in the transverse tubular system.

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