SINGLE CHLORIDE-SELECTIVE CHANNELS ACTIVE AT RESTING MEMBRANE POTENTIALS IN CULTURED RAT SKELETAL MUSCLE

ANDREW L. BLATZ AND KARL L. MAGLEBY
Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, Florida 33101

ABSTRACT The patch-clamp technique was used to characterize channels that could contribute to the resting Cl^- conductance in the surface membrane of cultured rat skeletal muscle. Two Cl^--selective channels, in addition to the Cl^--selective channel of large conductance described previously (Blatz and Magleby, 1983), were observed. One of these channels had fast kinetics and a conductance of 45 ± 1.8 pS (SE) in symmetrical 100 mM KCl. The other had slow kinetics and a conductance of 61 ± 2.4 pS. The channel with fast kinetics typically closed within 1 ms after opening and flickered between the open and shut states. The channel with slow kinetics typically closed within 10 ms after opening and displayed less flickering. Both channels were active in excised patches of membrane held at potentials similar to resting membrane potentials in intact cells, and both were open a greater percentage of time with depolarization. Under conditions of high ion concentrations, both channels exhibited nonideal selectivity for Cl^- over K^+ with the permeability ratio P_K/P_{Cl} of 0.15–0.2. Additional experiments on the fast Cl^- channel indicated that its activity decreased with lowered pH_i and that SO_4^{-2} and $CH_3SO_4^-$ were ineffective charge carriers. These findings, plus the observation that the fast Cl^- channel was also active in membrane patches on intact cells, suggest that the fast Cl^- channel provides a molecular basis for at least some of the resting Cl^- conductance. The extent to which the slow Cl^- channel contributes is less clear as it was typically active only after excised patches of membrane had been exposed to high concentrations of KCl at the inner membrane surface.

INTRODUCTION

In skeletal muscle 65–85% of the resting membrane conductance is due to Cl^- ions (Hodgkin and Horowicz, 1959; Hutter and Noble, 1960; Palade and Barchi, 1977). A large Cl^- conductance stabilizes the resting membrane potential in mammalian muscle, as muscles with reduced Cl^- conductance can become hyperexcitable and produce trains of action potentials characteristic of myotonia (Adrian and Bryant, 1974). Resting Cl^- conductance increases with depolarization (Hutter and Warner, 1972; Palade and Barchi, 1977), decreases with lowered pH_o (Hutter and Warner, 1972; Woodbury and Miles, 1973; Palade and Barchi, 1977), and is decreased when SO_4^{-2} or $CH_3SO_4^{-1}$ is substituted for Cl^- (Hutter and Noble, 1960; Palade and Barchi, 1977). The single-channel recording technique (Hamill et al., 1981) provides a means to investigate directly the molecular mechanism underlying the resting Cl^- conductance. Using this technique, we have previously described a Cl^--selective channel of large conductance (440 pS), but this channel would contribute little to resting conductance as it is typically closed at resting membrane potentials (Blatz and Magleby, 1983). We now describe two additional Cl^- channels, one with fast kinetics and one with slow. These channels are active in patches of membrane held at resting membrane potentials, and their conductance is only ~15–18% that of the Cl^- channel of large conductance. The channel with fast kinetics has many properties consistent with the resting Cl^- conductance in mammalian muscle. The function of the slower channel is less clear.

METHODS

Currents flowing through patches of surface membrane of primary cultures of rat skeletal muscle were obtained with the single-channel recording technique (Hamill et al., 1981). Specific recording and solution changing details are in Barrett et al. (1982), and tissue culture details are in Blatz and Magleby (1984). Unless indicated, experiments were performed on excised inside-out patches of membrane where the normal intracellular side of the membrane (i) was exposed to the perfusion solution and the normal extracellular side (o) was exposed to the solution in the pipette. In addition to the ions listed in the figure legends, intra- and extracellular solutions were buffered with 5 mM *N*-Tris(hydroxymethyl)-methyl-2-aminoethane sulphonic acid (TES) and contained 1 mM

Address all reprint requests to Dr. Karl L. Magleby.

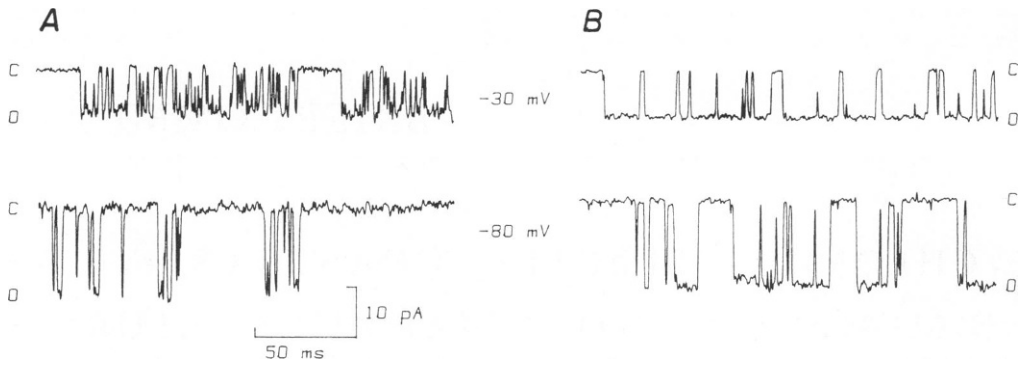

FIGURE 1 Single-channel currents recorded from excised patches of plasma membrane from cultured rat skeletal muscle are shown. Currents from Cl^--selective channels with fast (*A*) and slow (*B*) kinetics are shown at the indicated membrane potentials. Closed (c) and open (o) channel current levels are indicated. Inward currents are plotted downward. For *A* there was 140 mM KCl_o, 1.6 M KCl_i, and $<10^{-9}$ M free Ca^{2+} in both solutions. For *B* there was 1.4 M KCl_i, 100 mM KCl_o, and 20 μM free Ca^{2+} in both solutions. The difference in kinetics was still observed when the $[Ca^{2+}]$ was the same for both channels. Low-pass active filtering of 1.6 kHz, 24 dB/octave. Different membrane patch for *A* and *B*.

EGTA and sufficient Ca^{2+} to give the indicated free Ca^{2+} concentrations. pH was adjusted to 7.2 and experiments were performed at room temperature, ~23 °C. High concentrations of Cl^- were typically used to increase single-channel current amplitudes. Membrane potentials refer to the inner membrane surface ($V_i - V_o$), as is the convention for intact cells.

RESULTS

Fig. 1 shows single-channel currents recorded from the two new types of Cl^--selective channels observed in this study. At a membrane potential of −80 mV the Cl^- channel with fast kinetics (*A*) opened only occasionally (indicated by the downward-going currents) and then shut within a few milliseconds. In contrast, at −30 mV the channel typically reopened shortly after closing, flickering between the open and shut states for extended periods of time. The percent of time spent in the open state increased over 10-fold for the 50 mV depolarization, from 0.04 at −80 mV to 0.55 at −30 mV. These voltage-dependent changes in channel activity appeared to occur rapidly (<1 s) with changes in membrane potential.

In contrast, the Cl^- channel with slow kinetics (*B*) was open a greater percentage of time at −80 mV than the fast one was, remained open for longer periods (~10 ms) before shutting, and was less voltage sensitive over the same range of membrane potentials. The percent of time the slow channel spent in the open state increased only 1.7 times for the same 50 mV depolarization, from 0.45 at −80 mV to 0.78 at −30 mV.

Channel activity of both fast and slow Cl^- channels appeared to occur in bursts, with bursting more apparent for the fast Cl^- channel, as can be seen in Fig. 1. Both channels also occasionally entered inactived shut states with lifetimes of seconds. Bursting kinetics suggest more than one shut state (Colquhoun and Hawkes, 1982).

The conductance of these two channels was determined from plots of single-channel current amplitude vs. membrane potential. Fig. 2 presents data for the fast Cl^- channel. The single-channel records in part *A* were recorded with 2 M KCl_i and 140 mM KCl_o. A clear reversal of current through the channel was observed, as indicated by the inward currents at −40 mV and the outward currents at 60 mV. This and additional data from the same experiment are plotted as filled circles in part *B*,

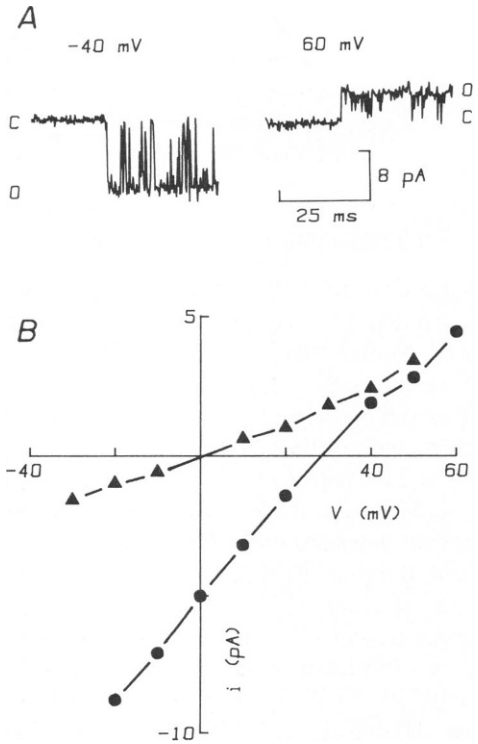

FIGURE 2 Conductance and selectivity of the fast Cl^- channel. (*A*) Single-channel recordings with 2 M KCl_i and 140 mM KCl_o. (*B*) Single-channel current amplitude vs. membrane potential is plotted as filled circles for the experiment in *A*. Slope conductance (0–40 mV) of 177 pS. With symmetrical 140 mM KCl (filled triangles), the conductance was 59 pS.

and give a (slope) conductance of 177 pS between 0 and 40 mV with a reversal (zero current) potential of 29 mV. Decreasing the $[KCl]_i$ to 140 mM while maintaining KCl_o at 140 mM decreased conductance to 59 pS, as indicated by the filled triangles. In seven patches, the slope conductance of the fast Cl^- channel in symmetrical 140 mM KCl was 64.1 ± 1.8 pS (mean ± SE). The conductance was reduced to 45 ± 1.8 pS in symmetrical 100 mM KCl ($n = 3$).

Fig. 3 presents data for the slow Cl^- channel. The filled squares in part *B* give a conductance of 142 pS and a reversal potential of 34 mV with 1.4 M KCl_i and 100 mM KCl_o. Decreasing the $[KCl]_i$ also decreased the conductance of the slow Cl^- channel. In six patches the conductance with 100 mM symmetrical KCl was 61.2 ± 2.4 pS, 36% greater than for the fast Cl^- channel under similar conditions.

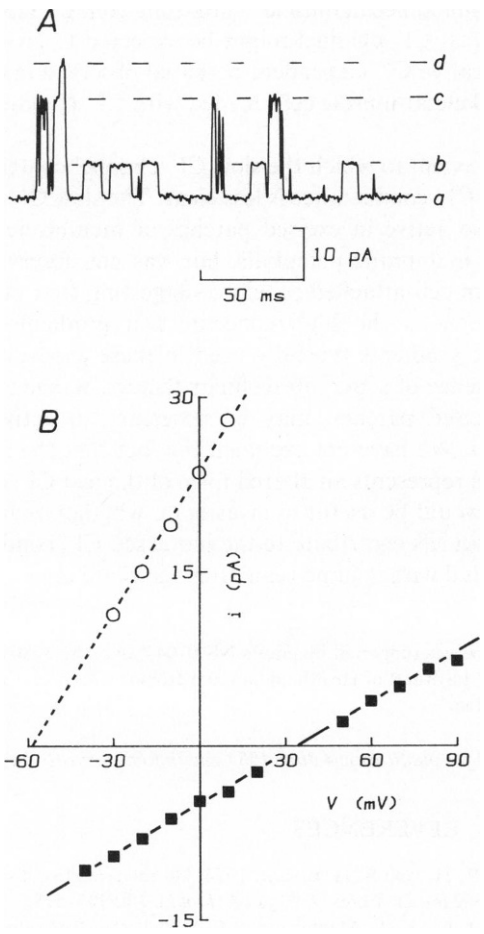

FIGURE 3 Conductance and selectivity of the slow Cl^- channel. (*A*) Single-channel recording with 1.4 M KCl_i and 100 mM KCl_o. Both a Ca-activated K^+ channel and slow Cl^- channel are active in the patch of membrane. Current through the Cl^- channel is inward (downward) and current through the Ca-activated K^+ channel is outward (upward) at the −10 mV membrane potential for the recording. At current level *a* only the Cl^- channel is open; at level *b* no channels are open; at level *c* both the Cl^- and Ca-activated K^+ channels are open; at level *d* only the Ca-activated K^+ channel is open. (*B*) Single-channel current amplitude vs. membrane potential is plotted for the experiment in *A*. The dashed line through the open circles indicates the current/voltage relationship for the Ca-activated K^+ channel, which had a conductance of 405 pS. The dashed line is a least-squares fit through the plotted open circles and eight additional data points that extend off scale up to 60 pA. The line through the filled squares indicates the current/voltage relationship for the slow Cl^- channel, which had a conductance of 142 pS.

The observation in Figs. 2–3 that single-channel currents are inward at 0 mV under conditions where the concentration gradient for Cl^- is outward suggests that Cl^- is the major charge carrier through the channels. If K^+ were the major charge carrier, then the currents at 0 mV should have been outward. If the channels were exclusively permeable to Cl^-, then the reversal potential calculated with the Nernst equation would be 61 mV for the experiment plotted as filled circles in Fig. 2 *B* and 60 mV for the experiment plotted as filled squares in Fig. 3 *B*. The observed reversal potentials of 29 and 35 mV indicate that these Cl^- channels are not exclusively selective for Cl^-. Since K^+ is the only other major ion in the solution, then K^+ also passes through these channels. In a series of experiments like those shown in Figs. 2 and 3, including some with 140 mM KCl_o and 600 mM KCl_i, calculations with the Goldman, Hodgkin, and Katz equation (Goldman, 1943; Hodgkin and Katz, 1949) indicated that K^+ is ~15–20% as permeant as Cl^- through both the fast and slow Cl^- channels.

The selectivity of these Cl^- channels thus appears to differ from that of Cl^- channels in heart (Coronado and Latorre, 1982) and electroplax (Miller and White, 1980) that exclude K^+. To determine whether the apparent K^+ permeability that we observed for the fast and slow Cl^- channels might arise from some form of experimental error, we examined the reversal potentials of Ca-activated K^+ channels that were active in the same patches of membrane as the Cl^- channels. A recording of currents through both a Ca-activated K^+ channel and a slow Cl^- channel in the same patch of membrane is shown in Fig. 3 *A*. At the membrane potential of −10 mV for this record, single-channel currents through the Ca-activated K^+ channel were outward and 19 pA in magnitude and currents through the slow Cl^- channel were inward and −6.5 pA in amplitude.

These and additional data points obtained from the same experiment are plotted in Fig. 3 *B* as open circles for the Ca-activated K^+ channel and filled squares for the slow Cl^- channel. The projected reversal potential for the Ca-activated K^+ channel was −58 mV, near the −60 mV equilibrium potential for K^+. This observation, that the Ca-activated K^+ channel reversed as expected near the Nernst potential for K^+ (Latorre and Miller, 1983; Blatz and Magleby, 1984), excludes the possibility that incorrect solutions, drift, or junction potentials give rise to the apparent K^+ permeability of the slow Cl^- channel. Similar experiments, analyzing Ca-activated K^+ channels and fast Cl^- channels in the same patches of membrane gave similar results.

Larger conductance Cl^- channels also allow some passage of K^+ (Schein et al., 1976; Blatz and Magleby, 1983; Bevan, Gray and Ritchie, 1984). Thus, the selectivity filters in the fast and slow Cl^- channels and larger conductance Cl^- channels may differ from those in Cl^- channels in heart and electroplax. However, since permeability ratios as measured by shifts in reversal potential are determined at zero net current and are dependent on the experimental conditions, we have not ruled out that the fast and slow Cl^- channels exclude K^+ under different ionic conditions.

A typical excised patch of membrane contained 1–2 fast Cl^- channels, with a range from 0 to 5. Slow Cl^- channels were observed much less frequently. When several Cl^- channels were present, they were usually either all fast or all slow. Patches with odd numbers of active Cl^- channels, either fast or slow, were observed, indicating that these Cl^- channels are not active in obligatory protochannels, as is the case for Cl^- channels in electroplax (Miller, 1982). While the number of active Cl^- channels in a patch often remained constant, both increases and decreases were observed. Both fast and slow Cl^- channel activity was present in $<10^{-8}$ M free Ca^{2+}, and channel activity appeared relatively independent of $[Ca]_i$ over the range of 0.01–20 μM.

Fast Cl^- channels were also observed in cell-attached patches held at resting membrane potentials (-40 to -70 mV). In these experiments there was 140 mM KCl in the pipette and the cells were bathed with either Tyrode's solution or 140 mM KCl. The activity and percent open time of the fast Cl^- channels were usually greater in cell-attached patches than in excised patches held at the same transmembrane potential, suggesting some form of modulation by intracellular factors. Slow Cl^- channels were not observed in cell-attached patches. We do not know whether this reflects sampling error (we did not always look for channels before excising the patch of membrane) or whether these channels are inhibited by intracellular factors or initially activated by the high concentrations of KCl at the inner membrane surface and/or osmotic gradients usually present in these experiments.

Further experiments on the fast Cl^- channel indicated that single-channel currents were reversibly decreased to near the noise level when Cl^- was replaced by SO_4^{-2} or $CH_3SO_4^-$, suggesting the fast Cl^- channel is not very permeable to these anions. Decreasing the pH at the inner membrane surface decreased the percent of time spent in the open state. At -40 mV lowering pH_i from 7.0 to 5.0 decreased open probability over five times, from 0.18 to 0.035. Single-channel current amplitude was little affected by this change in pH.

DISCUSSION

Here we describe two voltage-dependent Cl^--selective channels (or two states of a single channel) in surface membrane of cultured rat skeletal muscle. The observations that the Cl^- channel with fast kinetics is active in both cell-attached and excised patches of membrane held at resting membrane potentials, that SO_4^{2-} and $CH_3SO_4^-$ are ineffective charge carriers through this channel, and that the percent of time spent in the open state increases with depolarization and decreases with lowered pH_i are all consistent with the properties of the resting Cl^- conductance in intact skeletal muscle (Hutter and Noble, 1960; Hutter and Warner, 1972; Woodbury and Miles, 1973; Palade and Barchi, 1977). Thus, the fast Cl^- channel provides a molecular basis for at least some of the resting Cl^- conductance in muscle. The strong voltage dependence of the fast Cl^- channel might be expected to produce the regenerative Cl^--dependent potential observed in cultured chick skeletal-muscle cells loaded with Cl^- (Fukuda et al., 1976).

The extent to which the slow Cl^- channel contributes to resting Cl^- conductance is less clear. The slow Cl^- channel was also active in excised patches of membrane held at resting membrane potentials, but was not observed to be active in cell-attached patches, suggesting that other factors, such as the high concentration gradients and/or osmotic gradients typically used in these experiments, or the absence of some intracellular factors, washed away in the excised patches, may be necessary to activate this channel. We have not excluded the fact that the slow Cl^- channel represents an altered form of the fast Cl^- channel, and it would be useful to investigate whether or not these Cl^- channels contribute to the increased Cl^- conductance associated with volume regulation (Sarkadi et al., 1984).

This work was supported by grants NS 07044 and AM 32805 from the National Institutes of Health and a grant from the Muscular Dystrophy Association.

Received for publication 4 June 1984 and in final form 10 August 1984.

REFERENCES

Adrian, R. H., and S. H. Bryant. 1974. On the repetitive discharges in myotonic muscle fibres. *J. Physiol. (Lond.)*. 240:505–515.

Barrett, J. N., K. L. Magleby, and B. S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. *J. Physiol. (Lond.)*. 331:211–230.

Bevan, S., P. T. A. Gray, and J. M. Ritchie. 1984. A high conductance anion-selective channel in rat Schwann cells. *J. Physiol. (Lond.)*. 348:18P.

Blatz, A. L., and K. L. Magleby. 1983. Single voltage-dependent Cl^- selective channels of large conductance in cultured rat muscle. *Biophys. J.* 43:237–241.

Blatz, A. L., and K. L. Magleby. 1984. Ion conductance and selectivity of single calcium-activated potassium channels in cultured rat muscle. *J. Gen. Physiol.* 84:1–23.

Colquhoun, D., and A. G. Hawkes. 1982. On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 300:1–59.

Coronado, R., and R. Latorre. 1982. Detection of K^+ and Cl^- channels from calf cardiac sarcolemma in planar lipid bilayer membranes. *Nature (Lond.)*. 298:849–852.

Fukuda, J., G. D. Fishbach, and T. G. Smith. 1976. A voltage clamp study of the sodium, calcium and chloride spikes of chick skeletal muscle cells grown in tissue culture. *Dev. Biol.* 49:412–424.

Goldman, D. 1943. Potential, impedance, and rectification in membrane. *J. Gen. Physiol.* 27:37–60.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch clamp techiques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.

Hodgkin, A. L., and P. Horowicz. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.).* 148:127–160.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.).* 108:37–77.

Hutter, O. F., and D. Noble. 1960. The chloride conductance of frog skeletal muscle. *J. Physiol. (Lond.).* 151:89–102.

Hutter, O. F., and A. E. Warner. 1972. The voltage dependence of the chloride conductance of frog muscle. *J. Physiol. (Lond.).* 227:275–290.

Latorre, R., and C. Miller. 1983. Conduction and selectivity in potassium channels. *J. Membr. Biol.* 71:11–30.

Miller, C. 1982. Open-state substructure of single chloride channels from *Torpedo* electroplax. *Phil. Trans. R. Soc. Lond. B. Biol. Sci.* 299:401–411.

Miller, C., and M. M. White. 1980. A voltage-dependent chloride conductance channel from *Torpedo* electroplax membrane. *Ann. NY Acad. Sci.* 341:534–551.

Palade, P. T., and R. L. Barchi. 1977. Characteristics of the chloride conductance in muscle fibers of rat diaphragm. *J. Gen. Physiol.* 69:325–342.

Sarkadi, B., E. Mack, and A. Rothstein. 1984. Ionic events during the volume response of human peripheral blood lymphocytes to hypotonic media. *J. Gen. Physiol.* 83:497–512.

Schein, S. J., M. Colombini, and A. Finkelstein. 1976. Reconstitution in planar lipid bilayers of a voltage-dependent anion-selective channel obtained from paramecium mitochondria. *J. Membr. Biol.* 30:121–134.

Woodbury, J. W., and P. R. Miles. 1973. Anion conductance of frog muscle membranes: one channel, two kinds of pH dependence. *J. Gen. Physiol.* 62:324–353.