SINGLE VOLTAGE-DEPENDENT CHLORIDE-SELECTIVE CHANNELS OF LARGE CONDUCTANCE IN CULTURED RAT MUSCLE

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ABSTRACT Single-channel currents of an anion-selective channel in the plasma membrane of cultured rat muscle cells (myotubes) were recorded with the patch-clamp technique (Hamill, O.P., A. Marty, E. Neher, B. Sakmann, and F.J. Sigworth, 1981. *Pfluegers Arch. Eur. J. Physiol.*, 391:85–100). The channel is selective for Cl^- over cations, and has an unusually large single-channel conductance of ~430 pS in symmetrical 143 mM KCl. The channel is often active at 0 mV, opening and closing spontaneously. When active, steps from 0 mV to either negative or positive membrane potentials close the channel to an apparent inactivated state. The mean effective time that a channel is open before it inactivates is ~1.19 s for steps to −30 mV and 0.48 s for steps to +30 mV. Returning the membrane potential to 0 mV results in recovery from inactivation. Calcium ions are not required for channel activity.

INTRODUCTION

Cell membranes contain one or more conductance systems that control their permeability to Cl^- ions (Hodgkin and Horowicz, 1959; Costantin and Podolsky, 1967; Hutter and Warner, 1972; Palade and Barchi, 1977; Fukuda, 1975; Grinstein et al. 1982). The properties of some Cl^--selective channels that underlie or contribute to the Cl^- permeability have been studied by incorporating channels into lipid bilayers. Schein et al. (1976) have reported a voltage-gated Cl^--selective channel from mitochondria outer membrane (Colombini, 1979) with a conductance of ~500 pS (100 mM KCl); White and Miller (1981) have described a voltage-gated Cl^--selective channel from electroplax with a conductance of 16 pS; and Coronado and Latorre (1982) have observed a voltage-gated Cl^--selective channel from heart sarcolemma with a conductance of 55 pS. The newly developed patch-clamp technique (Neher and Sakmann, 1976; Hamill et al., 1981) also provides a means with which to study single-channel properties. An added advantage of the patch-clamp technique is that channels can be studied in their native membrane environment. In this paper we use the patch-clamp technique to describe the single-channel properties of a Cl^- channel in the plasma membrane of embryonic rat skeletal muscle. This channel has a large conductance of 430 pS in 143 mM KCl, is closed by either positive or negative voltage steps from 0 mV, and does not require Ca^{2+} for activity. An abstract of this study has appeared (Blatz and Magleby, 1983).

METHODS

Single-channel recordings (Hamill et al. 1981) were obtained from excised patches of plasma membrane from primary cultures of rat skeletal muscle (myotubes) (Barrett, et al., 1981). Experiments were performed on both inside-out patches (the normal intracellular surface was exposed to the bathing solution and the normal extracellular surface was exposed to the solution in the recording pipette) and on outside-out patches (the normal extracellular surface was exposed to the bathing solution and the normal intracellular surface was exposed to the solution in the pipette). The solution at the normal intracellular membrane surface contained (in millimolar) 143 KCl, 0.5 *N*-methyl-D-glucamine, EGTA, 2 $MgCl_2$, 5 TES (*N*-Tris[hydroxymethyl]-methyl-2-aminoethane sulphonic acid), 0.42 $CaCl_2$ (free [Ca^{2+}], 0.4 μM), pH 7.2. The solution at the normal extracellular membrane surface was usually identical except no $CaCl_2$ was added. In a few specified experiments, the Ca^{2+} was also omitted from the intracellular solution.

The effect of high concentrations of KCl was examined by adding KCl to the appropriate solution. Measurements of diffusion potentials associated with such solution changes were sufficiently small (<1 mV) that it was not necessary to correct the measured membrane potentials. To examine the effects of other ions, KCl was replaced with an isomolar amount of the new salt. Single-channel currents were recorded on an FM tape recorder (bandwidth, 0–5 kHz) and displayed on a chart recorder at reduced speed for analysis. The effective frequency response of the patch-clamp recording system was reduced to 1 kHz before analysis with low-pass filtering. Experiments were performed at room temperature, 21–25 °C.

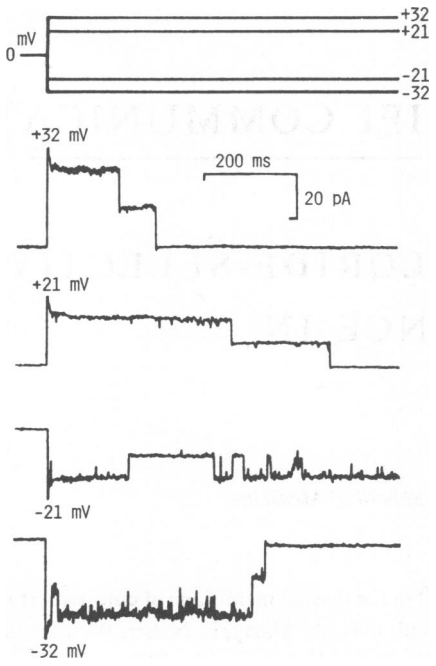

FIGURE 1 Single-channel currents recorded from an excised patch of plasma membrane (inside-out patch) from embryonic rat muscle cell (myotubes). Membrane potentials are expressed as the potential at the normal intracellular side minus the potential at the normal extracellular side. The membrane potential was held at 0 mV and then stepped as indicated in the schematic voltage traces (*upper plot*). The resulting current traces for each voltage step are plotted below. The small decline in current during the first 10 ms after each voltage step is a capacitive artifact. Upward-current deflections correspond to outward currents.

RESULTS AND DISCUSSION

Fig. 1 shows single-channel currents recorded from an inside-out excised patch of membrane. For each record the membrane potential of the patch was held at 0 mV and then stepped to and maintained (for several seconds) at the indicated potentials. Single-channel currents were outward (upward) for positive potentials and inward (downward) for negative potentials (143 mM KCl on both sides of the membrane). At +32 mV, two channels were open immediately after the voltage step; one closed after 150 ms and the other after 230 ms, as indicated by the downward steps in the current record at these times. Two channels were also open immediately after the voltage steps in each of the other records and then closed. Both channels were closed 2.6 s after the step to −21 mV.

Occasionally, channels that were open after a voltage step from 0 mV would close and then reopen, as shown for −21 and −32 mV in Fig. 1, but most channels typically closed within a few seconds after the voltage step and did not reopen, suggesting that the channels were entering an inactivated state. Once the channels became inactivated, returning the membrane potential to 0 mV was found to remove inactivation with a time constant of ~100 ms. A reduction of channel activity with time after a voltage step is often associated with voltage-gated channels (Sigworth and Neher, 1980; Horn et al., 1981). Ca^{2+} was not required at either membrane surface for the observed channel activity.

The current record was considerably noisier when the channels were open than when closed, and the magnitude of the noise appeared greater at negative membrane potentials and increased with the number of open channels. Complete channel closures (or blockade) too brief to be captured by the limited frequency response of the recording system and/or rapid changes in single-channel conductance, perhaps due to partial channel closures, could lead to fluctuations in the open-channel current record. Rapid transitions between open and closed states of the channel (Colquhoun and Sakmann, 1981) could also give rise to flickering of the single-channel currents towards the closed-channel current level observed in the experimental records in Fig. 1. If this is the case, then the effective channel open times observed in these experiments and plotted in Fig. 2 may represent burst duration (Colquhoun and Hawkes, 1981; Colquhoun and Sakmann, 1981). Current steps of less than single-channel unit amplitude were also occasionally observed, suggestive of possible reduced conductance states of the channel. Increased open-channel current noise has been observed previously in Ca-activated K^+ channels (Pallotta et al., 1981) and acetylcholine-activated channels (Sigworth, 1982).

Channels typically stayed open longer before entering the inactivated state after steps to negative membrane potentials rather than to positive potentials. This is shown in Fig. 2, which presents semilogarithmic plots of cumulative distribution of effective channel open times. The effective mean open time after stepping the membrane potential from 0 to +30 mV was 0.48 s, whereas the effective mean open time after steps from 0 to −30 mV

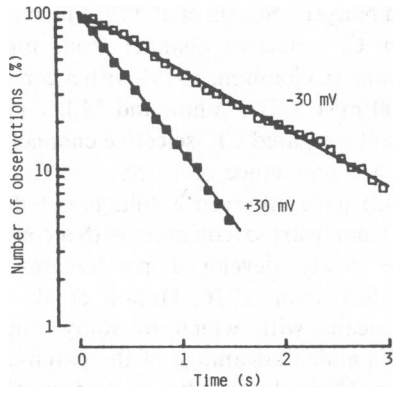

FIGURE 2 Semilogarithmic plots of cumulative effective channel open-time distributions obtained after stepping the membrane potential from 0 to +30 mV or −30 mV. The ordinate is the percentage of openings having a duration of the indicated time or longer. The lines, which are least-squares fits to the data, decay with time constants of 1.19 s at −30 mV and 0.48 s at +30 mV. Results are from 217 openings at −30 mV and 290 openings at +30 mV.

was 1.19 s, almost 2.5 times longer. Effective channel open times were exponentially distributed as indicated by the straight lines in Fig. 2. This observation is consistent with channel closing being a random, memoryless process (Magleby and Stevens, 1972).

The conductance of the channels in Fig. 1 was determined from a plot of single-channel current amplitude against membrane potential. The data are plotted in Fig. 3 *A* as open symbols. The reversal potential is 0 mV, as would be expected for symmetrical solutions. The dashed line indicates a slope of 450 pS. Data from a similar experiment from a different patch are plotted in Fig. 3 *B* where the conductance was 390 pS. The conductance of single channels in eight patches bathed on both sides of the membrane with 143 mM KCl was 430 ± 15 pS (mean ± SEM).

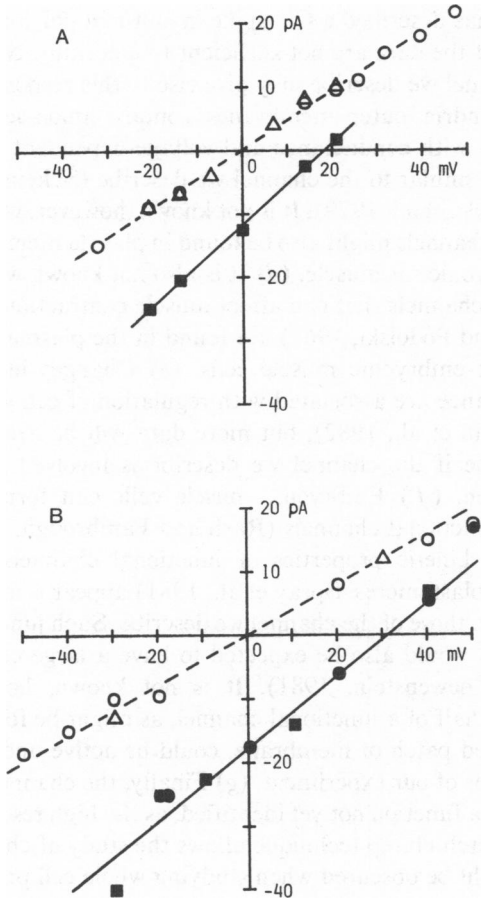

FIGURE 3 Plots of single-channel current amplitude against membrane potential obtained from experiments like those shown in Figs. 1 and 4. The open circles (○) plot current amplitudes with 143 mM KCl on both sides of the membrane for two different membrane patches, *A* and *B*. The filled symbols (●, ■) were obtained after increasing the concentration of KCl at the normal intracellular membrane surface to 400 mM in *A* and 600 mM in *B*. Returning the concentration of KCl at the inner membrane surface to 143 mM reversed the effect, giving the results plotted as open triangles (△). Changing the solution back to 600 mM KCl in *B* gave the results plotted as filled circles (●).

The conductance of this channel is more than twice that of the Ca-activated K channel (Pallotta et al. 1981) and about an order of magnitude greater than the conductance of voltage-sensitive K^+ and Na^+ channels (Conti and Neher, 1980; Sigworth and Neher, 1980), the acetylcholine-activated channel (Hamill et al., 1981), and the Cl^--permeable channels in electroplax (White and Miller, 1981) and heart sarcolemma (Coronado and Latorre, 1982). The conductance is similar to that of a Cl^--selective channel in outer mitochondrial membrane (Schein et al., 1976; Colombini, 1979). The large conductance and characteristic kinetics shown in Fig. 1 (staircase decrease in current following steps to positive or negative potentials) provided a rapid means with which to differentiate this channel from other channels in rat muscle plasma membrane.

While channels of the type shown in Fig. 1 were observed in less than 5–10% of membrane patches examined, patches with this channel typically contained two to three such channels, with the number ranging from 1 to 6. These observations are consistent with two possibilities: the channels typically exist in clusters at low density, or the channels are typically at a mean density of ~3 per membrane patch, but are normally in a nonfunctional state.

Unlike the Ca-activated K^+ channel (Barrett et al., 1982), the number of functional channels of the type shown in Fig. 1 in any particular membrane patch could

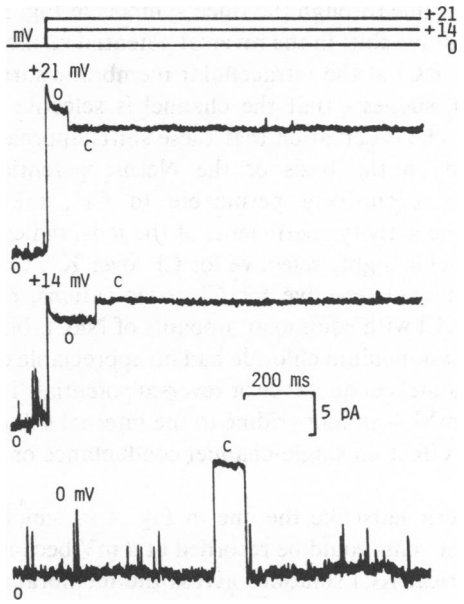

FIGURE 4 Single-channel currents recorded from an excised membrane patch with 143 mM KCl at the normal extracellular surface and 400 mM KCl at the normal intracellular surface. The membrane potential was held at 0 mV and then stepped to +14 or +21 mV as indicated in the voltage diagram (*upper plot*). The resulting current traces for each voltage step are plotted below. The channel was open (O) before and immediately after the steps and closed (*c*) shortly thereafter. The bottom current record was obtained while holding the membrane potential at 0 mV; the channel opened and closed spontaneously at this potential.

change during the course of an experiment. The appearance of such channels was often associated with holding potentials greater than ~+60 mV or with the exposure of the inner membrane surface to 143-mM concentrations of cations such as Na^+, NH_4^+, and *N*-methyl glucamine, although such conditions were not necessary to observe such channels. The observation that the channel sometimes appeared and disappeared during an experiment suggests that the overall functional state may be modulated by some, as yet, unknown factor. Once the channel is in the functional state, its activity would then be controlled by membrane potential.

To determine whether K^+ or Cl^- carried the current through the channel, we increased the concentration of KCl at the intracellular membrane surface from 143 to 400 mM while maintaining the concentration at the extracellular membrane surface at 143 mM. As shown by the current records in Fig. 4, single-channel currents no longer reversed at 0 mV as in Fig. 1, but were clearly inward at this potential and reversed between +14 and +21 mV. The single-channel current amplitudes under these conditions are plotted as filled squares in Fig. 3 *A*. The continuous line through the squares indicates a reversal potential of +18 mV and a single-channel conductance of 630 pS. Increasing the concentration of KCl at the intracellular membrane surface to 600 mM KCl while maintaining the concentration at the extracellular surface at 143 mM gave a reversal potential of +32 mV, as indicated by the continuous line through the filled symbols in Fig. 3 *B*.

The positive shift in the reversal potential as the concentration of KCl at the intracellular membrane surface was increased, suggests that the channel is selective for Cl^- over K^+. The observation that these shifts approach those calculated on the basis of the Nernst potential for a membrane exclusively permeable to Cl^-, taking into account the activity coefficients of the ions, indicates that the channel is highly selective for Cl^- over K^+. Consistent with a channel selective for Cl^- over cations, replacing internal KCl with equimolar amounts of NaCl, NH_4Cl, or tetraethylammonium chloride had no appreciable effect on single-channel conductance or reversal potential. The addition of 1 mM 4-aminopyridine to the internal solution also had little effect on single-channel conductance or reversal potential.

In experiments like the one in Fig. 4 in which single-channel currents could be recorded at 0 mV because of the asymmetrical KCl solutions across the membrane, it was observed that the channel often opened and closed spontaneously at 0 mV. Stepping to positive potentials from 0 mV in this experiment resulted in channel closure and inactivation, similar to Fig. 1. The kinetics of channel opening with voltage steps appeared complex. In a few experiments, it was observed that stepping to negative potentials of ~−60 mV when the channels were closed at 0 mV led to channel opening within less than a few milliseconds in an occasional trial. Closed channels could also be opened in ~1 out of 10 trials by stepping from −60 to +10 mV for a few milliseconds and then back to −60 mV. Once the channels opened in these experiments, they then closed within a few seconds to an apparent inactive state, consistent with the kinetics in Figs. 1 and 4. If these channels have similar kinetics in the intact cell, then they could contribute to the repolarization following an action potential.

The specific cellular function of the Cl^--selective channel described in this report is not yet clear, but there are a number of possibilities to consider. (*a*) Muscle cells of adult frogs (Hodgkin and Horowicz, 1959; Hutter and Warner, 1972) and rats (Palade and Barchi, 1977) possess large Cl^- conductances at steady membrane potentials near the resting potential. The Cl^--permeable channel we describe was typically inactive at such steady membrane potentials, but we cannot exclude that it may contribute to this conductance in intact cells because we used excised membrane patches for our experiments. (*b*) Fukuda (1975) has described a Cl^- spike in cultured chick muscle cells, but the data are not sufficient to determine whether the channel we describe may give rise to this response. (*c*) Mitochondria outer membranes contain anion-selective channels with conductance and voltage-dependent gating kinetics, similar to the channel we describe (Schein et al., 1976; Colombini, 1979). It is not known, however, whether similar channels might also be found in plasma membranes of embryonic rat muscle. (*d*) It is also not known whether the Cl^- channels that can affect muscle contraction (Costantin and Podolski, 1967) are found in the plasma membrane of embryonic muscle cells. (*e*) Changes in anion conductance are associated with regulation of cell volume (Grinstein et al., 1982), but more data will be needed to determine if the channel we describe is involved in this regulation. (*f*) Embryonic muscle cells can form cell-to-cell junctional channels (Rash and Fambrough, 1973), and the kinetic properties of junctional channels (amphibian blastomeres, Spray et al., 1981) appear somewhat similar to those of the channel we describe. Such junctional channels would also be expected to have a large conductance (Loewenstein, 1981). It is not known, however, whether half of a junctional channel, as might be found in an excised patch of membrane, could be active under the conditions of our experiment. (*g*) Finally, the channel may perform a function not yet identified, as the high resolution of the patch-clamp technique allows the study of channels that might be obscured when studying whole cell preparations.

We thank A. L. Zimmerman and B. S. Pallotta for helpful suggestions and J. N. Barrett for contributing myotubes.

Supported by grants NS 07044, NS 10277, NS 12207 from the National Institutes of Health, and a grant from the Muscular Dystrophy Association.

Received for publication 20 January 1983 and in final form 1 April 1983.

REFERENCES

Barrett, J. N., E. F. Barrett, and L. B. Dribin. 1981. Calcium-dependent slow potassium conductance in rat skeletal myotubes. *Dev. Biol.* 82:258–266.

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.

Blatz, A. L., and K. L. Magleby. 1983. Voltage dependent chloride-selective channels of large conductance in cultured rat skeletal muscle. *Biophys. J.* 41 (2, Pt. 2):62*a*. (Abstr.)

Colombini, M. 1979. A candidate for the permeability pathway of the outer mitochondrial membrane. *Nature (Lond.).* 279:643–645.

Colquhoun, D., and A. G. Hawkes. 1981. On the stochastic properties of single ion channels. *Proc. R. Soc. Lond. B. Biol. Sci.* 211:205–235.

Colquhoun, D., and B. Sakmann. 1981. Fluctuations in the microsecond time range of the current through single acetylcholine receptor ion channels. *Nature (Lond.).* 294:464–466.

Conti, F., and E. Neher. 1980. Single channel recordings of K^+ currents in squid axons. *Nature (Lond.).* 285:140–143.

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.

Costantin, L. L., and R. J. Podolsky. 1967. Depolarization of the internal membrane system in the activation of frog skeletal muscle. *J. Gen. Physiol.* 50:1101–1124.

Fukuda, J. 1975. Voltage clamp study on inward chloride currents of spherical muscle cells in tissue culture. *Nature (Lond.).* 257:408–410.

Grinstein, S., C. A. Clarke, A. Dupre, and A. Rothstein. 1982. Volume-induced increase of anion permeability in human lymphocytes. *J. Gen. Physiol.* 80:801–823.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques 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 fibers. *J. Physiol. (Lond.).* 148:127–160.

Horn, R., J. Patlak, and C. F. Stevens. 1981. Sodium channels need not open before they inactivate. *Nature (Lond.).* 291:426–427.

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

Loewenstein, W. R. 1981. Junctional intercellular communication: the cell-to-cell membrane channel. *Physiol. Rev.* 61:829–913.

Magleby, K. L., and C. F. Stevens. 1972. A quantitive description of end-plate currents. *J. Physiol. (Lond.).* 223:173–197.

Neher, E., and B. Sakmann. 1976. Single-channel currents recorded from membrane of denervated frog muscle fibres. *Nature (Lond.).* 260:799–802.

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

Pallota, B. S., K. L. Magleby, and J. N. Barrett. 1981. Single channel recordings of Ca^{2+}-activated K^+ currents in rat muscle cell culture. *Nature (Lond.).* 293:471–474.

Rash, J. E., and D. Fambrough. 1973. Ultrastructural and electrophysiological correlates of cell coupling and cytoplasmic fusion during myogenesis *in vitro*. *Dev. Biol.* 30:166–186.

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. Mem. Biol.* 30:99–120.

Sigworth, F. J. 1982. Fluctuations in the current through open ACh-receptor channels. *Biophys. J.* 37(2, Pt. 2):309*a*. (Abstr.)

Sigworth, F. J., and E. Neher. 1980. Single Na^+ channel currents observed in cultured rat muscle cells. *Nature (Lond.).* 287:447–449.

Spray, D. C., A. L. Harris, and M. V. L. Bennett. 1981. Equilibrium properties of a voltage-dependent junctional conductance. *J. Gen. Physiol.* 77:77–93.

White, M. M., and C. Miller. 1981. Probes of the conduction process of a voltage-gated Cl^- channel from *Torpedo* electroplax. *J. Gen. Physiol.* 78:1–18.