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Role of the cytoskeleton in the regulation of Cl^- channels in human embryonic skeletal muscle cells

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Abstract. The effects of volume change and cytoskeleton manipulation on the Cl^- channels in human embryonic skeletal muscle cells were studied. Trypsination, used for production of myoballs, changes the channel properties only a little. When the external osmolarity was reduced from 300 to 270 mosmol/l, the specific Cl^- conductance, g_{Cl}, (at −80 mV) of myoballs increased from 5.1 ± 1.9 to 30.4 ± 12.2 μS/cm² (SD; $n = 6$) within 15 min. Concomitantly, the kinetics of Cl^- currents, elicited by clamping the membrane potential from a negative to positive values, changed from activation and subsequent slow inactivation to instantaneous activation with fast inactivation. G protein activation, protein kinase action or $[Ca^{2+}]_i$ elevation seemed not to be involved in these effects. Similar changes were produced in the absence of a transmembrane osmotic gradient by 500 nM intracellular cytochalasin D (g_{Cl} = 34.3 ± 10.3 μS/cm²; $n = 6$) or 12.5 μM colchicine (g_{Cl} = 15.4 ± 1.4 μS/cm²; $n = 5$). When the external osmolarity was increased to 418 mosmol/l, 1 μM cytochalasin D did not affect g_{Cl}. In four of six cell-attached patches the open probability of the intermediate Cl^- channel was increased after reduction of the bath osmolarity. In inside-out patches, the drugs increased the open probability of the channels. It is concluded that the Cl^- channels are under control of the cytoskeleton.

Key words: Chloride channels – Myoballs – Cytoskeleton – Skeletal muscle

Introduction

Whole-cell Cl^- currents in human myoballs (myotubes that have been transformed to spherical shape by a brief exposure to trypsin) were shown to be conducted predominantly by a Cl^- channel, that was dubbed "intermediate" because of the size of its conductance [8, 27]. This channel is characterized by two gating modes, 1 and 2, that differ in their open probability in the negative potential range [8, 9]. Cells with the majority of their intermediate Cl^- channels in gating mode 2 have a very large Cl^- conductance, g_{Cl}, at the resting potential (> 100 S/F [9, 27]) that is even bigger than in adult human muscle fibres [18].

A major aim of the present work was to test how this conductance is influenced by the cell volume, as this could play a role in processes like cell division and migration during cell differentiation [15]. Volume activation of channels has been demonstrated in several types of cells [1, 4–6, 11, 16, 19, 25, 26].

Myotubes are not suited for such an investigation, as application of drugs affecting the cytoskeleton drastically changes their shape, turning them into myoballs. Therefore we started with myoballs that had their cytoskeleton rearranged for 4–5 h. In order to assess the effect of this rearrangement on the intermediate Cl^- channels, we performed a series of experiments on myotubes that were comparable to our earlier myoball studies [8, 27]. Having established the similarity of the results, we continued to use myoballs. Some of the results of this paper were presented to the German Physiological Society [21].

Materials and methods

Human myotubes and myoballs were cultured as described [2]. Whole-cell currents [13] were recorded through borosilicate glass pipettes (resistance 1–3 MΩ) connected to an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt, Germany). Capacity currents and series resistance were compensated by means of an analog circuit, leakage current was not subtracted. The current records were filtered (3 kHz) and stored on an AT computer equipped with a home-made A/D converter. Series resistance, cell capacitance, and seal resistance were calculated from responses to 20-mV steps imposed on the membrane potential. The mean values for cell capacitance and series resistance were 255 ± 155 pF and 1.0 ± 0.9 MΩ, respectively, resulting in a mean charging time constant of 0.25 ± 0.15 ms (SD, $n = 8$). Recordings with a voltage error at the series resistance of more than 5 mV after compensation

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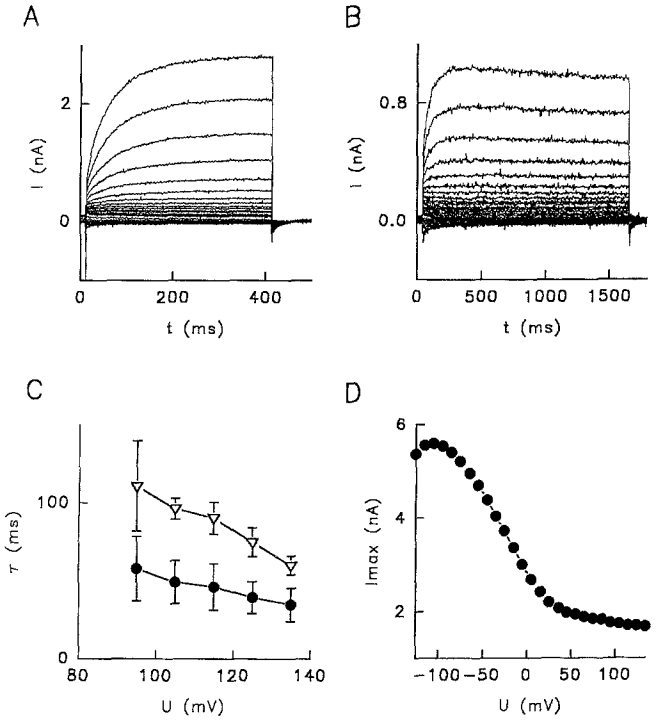

Fig. 1A–D. Properties of Cl^- currents recorded from human myotubes. **A** Responses to test pulses going from a holding potential of −50 mV to the range from −125 mV to + 135 mV in 10-mV steps. Note voltage-dependent activation positive to + 75 mV. **B** Responses of another myotube, same pulse program but longer test pulses. Note very slow inactivation at positive potentials. **C** Time constants gained from mono-exponential fits to the activation of Cl^- currents (●, myotubes; ▽, myoballs; (means ± SD, $n = 6$ each). Activation is faster in myotubes. **D** Voltage dependence of steady-state inactivation. Plot of the maximum current (I_{max}) flowing at the test potential (U) of + 110 mV against the variable potential of prepulses lasting 2 s

were rejected. The seal resistance was usually greater than 5GΩ. Cl^- currents were elicited by test pulses of variable size (from -125 to + 135 mV) and duration (200–1500 ms). Between test pulses the membrane potential was usually held at −50 mV for 5 s.

Single-channel recordings in the cell-attached or inside-out mode [13] were performed with the same set-up. Records were taken at constant clamp potentials, passed through an analogue filter (3 kHz) and stored on a digital tape at a sampling rate of 44 kHz. For off-line evaluation, such a record was divided in periods lasting 110 s and sampled at a rate of 10 kHz. Recordings in the cell-attached mode were made at pipette potentials of + 20, + 40, and −60 mV. Subtraction of these values from the unknown resting potential would yield absolute potential values.

For inside-out recordings, the cells were bathed in normal internal solution. After seal formation, patches were excised by a quick movement through the air-water interface. Usually, first the controls and then the tests were performed at −40, −85 and + 60 mV clamp potential.

Solutions. (All concentrations in mM unless otherwise stated.) For the whole-cell experiments, the standard internal solution contained: 145, CsCl; 1, $MgCl_2$; 3, 4-(2-hydroxyethyl)-1-piperazine ethane sulphonic acid (HEPES); and 0.5, ethylene bis(oxonitrilo)-tetraacetate (EGTA). To determine the selectivity, CsCl was substituted by equimolar CsI. The standard external solution contained: 145, CsCl; 1, $CaCl_2$; 1, $MgCl_2$; and 3, HEPES. Hypotonic extracellular solution was produced by the addition of 1, $CaCl_2$; 1, $MgCl_2$; and 3, HEPES in the ratio 1:0.1. For the investigation of G protein action on the Cl^- currents, 0.1 guanosine 5′-*O*-(2-thiodiphosphate) (GDP[βS], Sigma, Deisenhofen, Germany) was added to the pipette solution. Cytochalasin D or colchicine (Sigma) were added to the internal solution in concentrations as given in the text. In some experiments, the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, was buffered to 10^{-7} M using an internal solution containing: 145, CsCl; 1, $MgCl_2$; 1, $CaCl_2$; 3, HEPES; and 40, *N*-(2-hydroxyethyl)-ethylene diamine-*N,N′,N′*-triacetic acid (HEDTA) [7].

For the single-channel experiments, the patch pipettes were filled with standard external solution. For measurements in the cell-attached mode, the bathing solution contained: 140, NaCl; 3.5, KCl; 1.5, $CaCl_2$; 1, $MgCl_2$; and 3, HEPES. The external osmolarity was reduced by adding: 3.5, KCl; 1.5, $CaCl_2$; 1, $MgCl_2$; and 3, HEPES in the ratio 1:0.1. Inside-out patches were bathed in the standard internal solution. Cytochalasin D and colchicine were applied by adding 10 µl of a stock solution to the 2-ml-containing bath. The stock solutions contained colchicine and cytochalasin D at a concentration of 2 mM and 0.2 mM, respectively, dissolved in standard internal solution.

All solutions were adjusted to pH 7.4 with HCl or CsOH. All experiments were performed at room temperature (21°C).

Data analysis. Whole-cell measurements were analysed using in-house software. For the determination of time constants of activation and inactivation, Marquardt's algorithm was used to fit sums of exponentials to the time course of the currents. When current/voltage relationships were plotted, "instantaneous" current amplitudes were determined 10 ms after the depolarizing steps, to avoid interference with capacitive currents. Open probabilities were calculated by fitting Gaussian distributions to the amplitude distribution of single-channel currents. Statistical significance was tested using the unpaired *t*-test for equal or unequal variance. The level of significance was $P < 0.05$ unless stated otherwise. All results are given as means ± SD.

Results

Cl^- currents recorded from myotubes in the whole-cell mode

A total of eight myotubes were investigated. Their mean cell capacitance was 178 ± 48 pF and their mean series resistance was 4.0 ± 1.5 MΩ, resulting in a mean membrane charging time constant of 0.73 ± 0.36 ms. Whole-cell recorded Cl^- currents in standard external solution were small in the negative membrane potential range. Positive to + 75 mV, current activation appeared, which was more pronounced, the more positive the test potential (Fig. 1A). The time course of activation was monoexponential. With longer lasting test pulses, inactivation became visible (Fig. 1B). The time constants of activation were smaller than in myoballs (Fig. 1C), although the charging time constants were greater. The dependence of the current maximum at a constant test potential on the preceding prepulse potential showed a steady-state inactivation curve with an inflexion point at about −25 mV (Fig. 1D), which is similar to results obtained with myoballs [27]. The g_{Cl} in the negative membrane potential range was 8.9 ± 3.2 µS/cm² ($n = 6$). We concluded that in the myotubes the Cl^- current is conducted by intermediate Cl^- channels in gating mode 1, as in myoballs.

Because of the similarity of the currents in the two preparations, we performed the main part of the study with the more convenient myoballs.

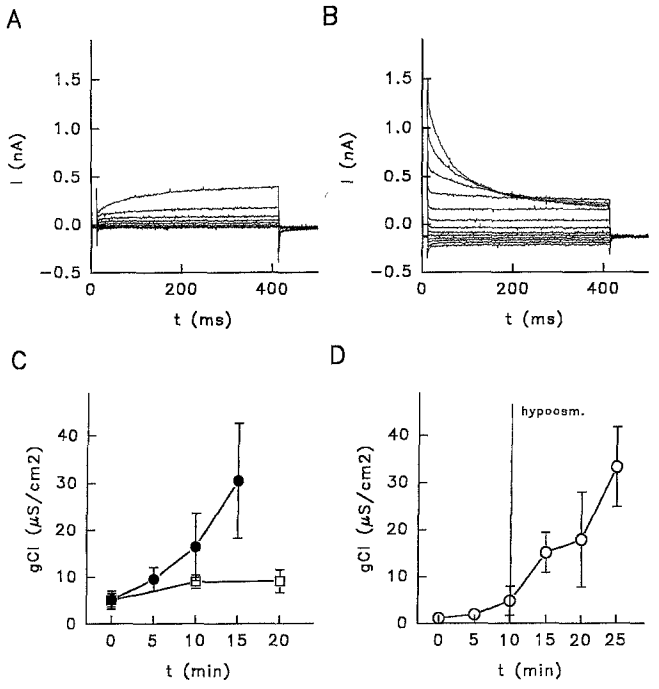

Fig. 2A–D. Effect of lowering the external osmolarity from 300 to 270 mosmol/l on whole-cell Cl^- currents in human myoballs. **A** Controls at normal extracellular osmolarity. Holding potential −50 mV, currents elicited by test pulses going to the range from −125 mV to + 115 mV in 20-mV steps. **B** Responses to same set of pulses 15 min after reduction of the extracellular osmolarity. **C** Time dependence of Cl^- conductance (g_{Cl}, slope of current/voltage relation in the negative potential range) under hypo-osmotic (●, $t = 0$ denoting time of changing to hypo-osmolar perfusion), and under iso-osmotic conditions (□, $t = 0$ denoting time of achieving the whole-cell mode, means ± SD, $n = 6$). **D** Effect of hypo-osmolarity on g_{Cl} of GDP[βS]-perfused myoballs, where GDP[βS] is guanosine 5′-*O*-(2-thiodiphosphate). *Abscissa*, Time after establishment of whole-cell mode ($n = 7$)

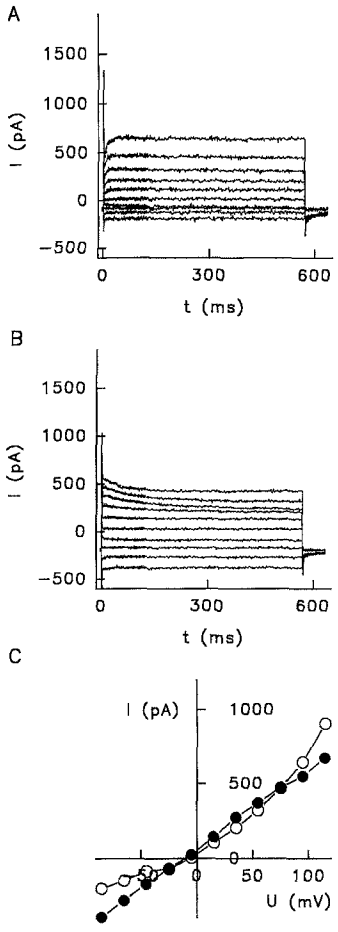

Fig. 3A–C. Effects of lowering the external osmolarity from 300 to 270 mosmol/l on whole-cell Cl^- currents in a human myoball perfused with (in mM): 145, CsI; 1, $MgCl_2$; 0.5, EGTA; 3, HEPES; pH 7.4. **A** Controls at normal extracellular osmolarity. Holding potential −50 mV, currents elicited by test pulses going to the range from −85 mV to + 95 mV in 20-mV steps. **B** Responses to same set of pulses 20 min after reduction of the extracellular osmolarity. **C** Plot of the current maxima from **A** (○) and **B** (●) vs test pulse potential, U

Effect of a reduction of the external osmolarity on whole-cell Cl^- currents of myoballs

After achieving the whole-cell mode, we usually analysed the Cl^- currents first under standard conditions (Fig. 2A). The mean control g_{Cl}, i.e. the slope of the current/voltage relation in the negative potential range, was 5.1 ± 1.9 μS/cm² ($n = 6$). This value increased only slightly (to 9.1 ± 2.5 μS/cm²; $n = 3$) when the cells were just kept under this condition for 20 min (Fig. 2C). After reduction of the external osmolarity from 300 to 270 mosmol/l, the seal remained usually intact for about 15 min. During this time, g_{Cl} slowly increased in all tested myoballs ($n = 6$) to a mean value of 30.4 ± 12.2 μS/cm² (Fig. 2C). A plateau was never reached so that we are concerned to have not recorded the maximum possible value for this condition. Cell swelling was not very obvious, some of cells produced blebs. A further increase of the osmotic gradient seemed to increase also the maximum value of g_{Cl}. Upon halving the external osmolarity, the mean g_{Cl} after 15 min was as much as 102 μS/cm² ($n = 2$). This was not further examined.

Concomitantly with the increase of the conductance, the time course of the Cl^- currents changed from a slow rise to a plateau, to an instantaneous rise with subsequent fast inactivation. The latter was most prominent at test potentials positive to + 75 mV (Fig. 2B). At positive potentials the current amplitude at the end of a 400-ms test pulse became smaller than it was before the change of the osmolarity.

The halide selectivity of the channels conducting these currents was determined from the reversal potential of the anion current with an internal solution having I^- substituted for Cl^-. Before reduction of the external osmolarity, we observed a small anion conductance in the negative potential range and current activation upon membrane depolarization (Fig. 3A). The above-described change of the current kinetics had taken place 20 min after perfusing with a hypo-osmolar solution (Fig. 3B). Plots of the current maxima versus the test pulse potential showed unchanged reversal potentials for both conditions (−7 ± 2 mV; $n = 3$; Fig. 3C). Using the Goldman-Hodgkin-Katz equation [14] we calculated a P_{Cl}/P_I of 1.3, where P denotes permeability.

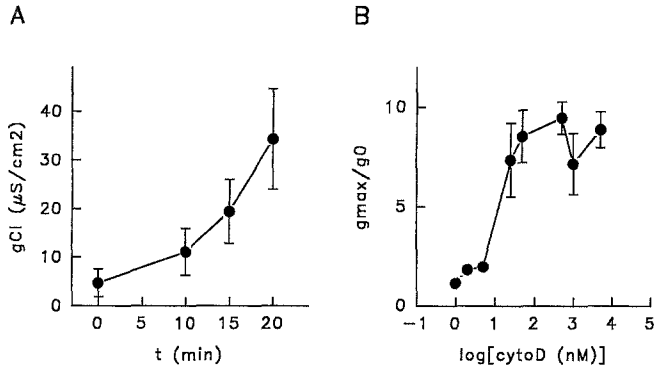

Fig. 4A, B. Effect of intracellular cytochalasin D on g_{Cl} of human myoballs. **A** Time course of the specific g_{Cl} at −80 mV membrane potential after achieving the whole-cell mode, i.e. after access of pipette solution containing 500 nM cytochalasin (mean ± SD, n = 6). **B** Plot of the quotient of maximum g_{Cl} and g_{Cl} immediately after establishment of whole-cell mode against cytochalasin D concentration. Each data point mean value from at least 3 cells

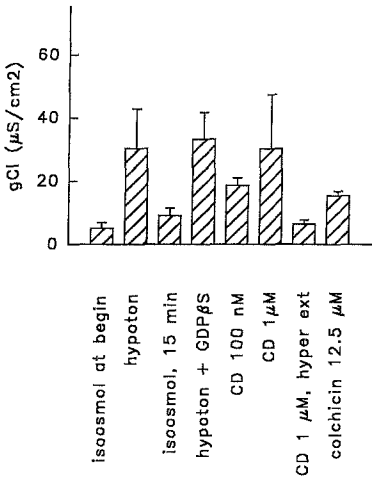

Fig. 5. g_{Cl} values measured under the conditions ascribed to the *columns*. In the two cases without osmotic gradient (*iso-osmol*) values were recorded at times given below the *columns*, in all other cases values correspond to the maxima attained during the recording time of about 15 min (means ± SD). *CD*, Cytochalasin D

In conclusion, the decreased steady-state current at positive potentials obtained with a reduction of the external osmolarity and the result of the selectivity test suggest that the channels conducting the Cl^- current change their kinetics in a way that has already been described for the intermediate Cl^- channel [8, 9], and make it unlikely that another, under control conditions, inactive channel is induced.

Influences of internal GDP[βS] and of $[Ca^{2+}]_i$ on whole-cell Cl^- currents in myoballs

The above-described effects occurred when no ATP or GTP was in the pipette solution, suggesting that a protein kinase was not involved. To test the involvement of a G protein, we added 0.1 mM GDP[βS] to the pipette solution. This had no influence on the time course or maximum value of g_{Cl} attained during our usual osmotic stress (Fig. 2D). Within 20 min, g_{Cl} reached 33.3 ± 8.4 μS/cm² (n = 7). For a test of the involvement of $[Ca^{2+}]_i$, an increase of the internal osmolarity was produced by the addition of high amounts of a Ca^{2+} buffer (40 mM HEDTA, [7]). This did not prevent the increase of g_{Cl} and the described kinetic changes after achieving the whole-cell mode. Within 20 min the maximum g_{Cl} was 86.1 μS/cm² (n = 2).

Effects of drugs affecting the cytoskeleton on whole-cell currents

To investigate how changes of the osmotic gradient might act on the Cl^- channels, we applied two drugs that are known to interact with parts of the cytoskeleton.

Perfusion of myoballs with cytochalasin D [3] under iso-osmotic conditions produced similar effects as lowering the osmotic pressure in the bath, since g_{Cl} increased considerably with time after achieving the whole-cell mode (Fig. 4A). The current kinetics changed likewise. The increase of maximum g_{Cl} was the greater the higher the concentration of the drug (34.3 ± 10.3 μS/cm² at 500 nM, n = 6; Figs. 4B and 5). We also investigated the effect of 1 μM cytochalasin D on myotubes; 10 min after application, g_{Cl} at −80 mV was increased to 28.2 ± 9.3 μS/cm² (n = 3) and the Cl^- current kinetics were changed as in myoballs.

Thus, cytochalasin D mimicked the effects of a volume increase in the absence of a transmembrane osmotic difference. To study this further, we investigated the effect of the drug in the presence of an inverted osmotic gradient (418 mosmol/l outside). Change to an external solution containing (in mM): 200, CsCl; 1, $CaCl_2$; 1, $MgCl_2$; 3, HEPES/CsOH; with simultaneous intracellular application of 1 μM cytochalasin D did not produce the typical changes of g_{Cl} and Cl^- current kinetics (maximum g_{Cl} was 6.4 ± 1.3 μS/cm² 20 min after achieving the whole-cell mode, i.e. after starting perfusion, n = 4).

Colchicine is known to depolymerize microtubules in a variety of cells [20]. In the myoballs, the presence of 12.5 μM colchicine in the pipette produced an increase of g_{Cl} to 15.4 ± 1.4 μS/cm² (n = 5) within 20 min (Fig. 5). Likewise, the kinetics of the Cl^- currents in the negative potential range were changed as shown for reduced external osmolarity (Fig. 2B). The specificity of the colchicine action was shown by the finding that lumicolchicin [23] at the same concentration had no such effect (n = 2).

Effect of a reduction of the external osmolarity on the kinetics of the whole-cell Cl^- currents

The inactivation process seen upon reduction of the external osmolarity and in the presence of the drugs was best described by a sum of two exponentials. The volt-

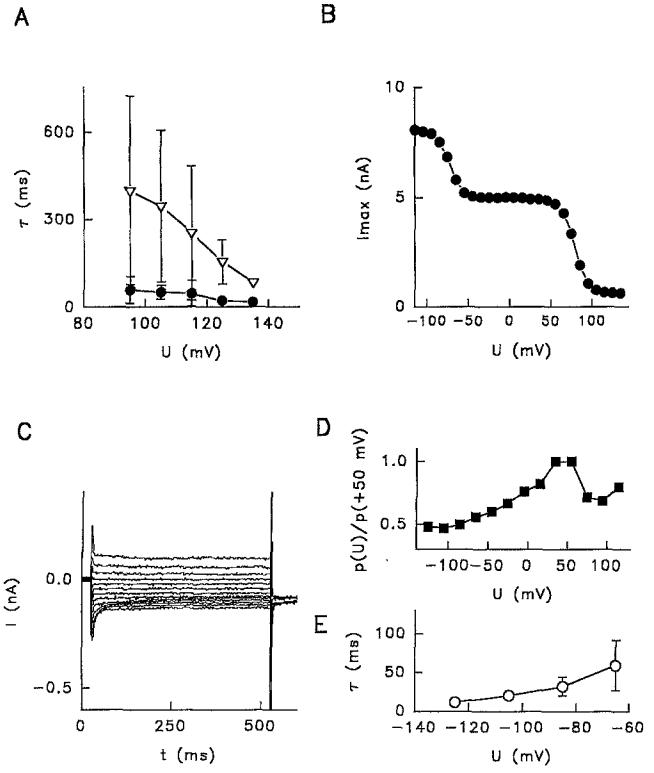

Fig. 6A–E. Kinetics of the volume-affected currents. **A** Potential dependence of the two time constants of inactivation (means ± SD, $n = 4$) obtained by fitting sums of two exponentials to current traces obtained at test potentials positive to + 90 mV. **B** Voltage dependence of steady-state inactivation. Plot of the maximum current flowing at a test potential of + 110 mV against the variable potential of prepulses lasting 2 s. **C** Deactivation of Cl^- currents flowing when, after a 2-s prepulse to + 50 mV, test potentials were varied between 125 mV and + 115 mV. **D** Potential dependence of the relative open probability as calculated by dividing the current amplitudes at the end and the beginning of the traces shown in **C**. **E** Time constants of deactivation (means ± SD; $n = 4$) gained from fits of single exponentials to currents obtained at test potentials negative to −60 mV

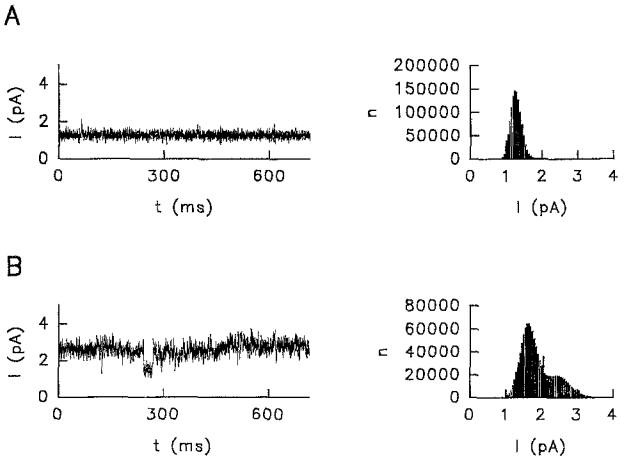

Fig. 7A, B. Change of currents through a myoball Cl^- channel upon reduction of the osmolarity of the bath. Cell-attached patch-clamp recordings. **A** Standard conditions; recording at 40 mV negative to the holding potential. The amplitude histogram (*right panel*) shows that the open probability is less than 1%. **B** The open probability was greatly increased 10 min after lowering the osmolarity of the bathing fluid

age dependence of the fast and the slow time constants are given in Fig. 6A.

The dependence of the instantaneous current I/I_{max} on the prepulse potential U was fitted with a sum of two Boltzmann distributions according to:

$$I/I_{max} = A_1[1 + \exp(U - U_1/V_1)]^{-1} + A_2[1 + \exp(U - U_2)/V_2]^{-1} \quad (1)$$

where A_1 and A_2 are constants ($A_2 = 1 - A_1$), U_1 and U_2 are the positions of the two points of inflexion, and $1/V_1$ and $1/V_2$ are the slopes at these positions (Fig. 6B). The mean values (± SD; $n = 5$) of these parameters were $A_1 = 0.5 \pm 0.15$, $U_1 = -80.3 \pm 4.2$ mV, $V_1 = 12.0 \pm 2.6$ mV, $U_2 = +61 \pm 4.3$ mV, $V_2 = 5.8 \pm 0$ mV.

A pulse program with a positive prepulse potential (+ 50 mV) followed by various test pulse potentials was used to determine the voltage dependence of the steady-state open probability of the underlying Cl^- channels (Fig. 6C). The relative open probability, calculated by dividing the current amplitudes at the end and the beginning of the test pulses [7], revealed a maximum at + 50 mV (Fig. 6D). Because of the pronounced difference of the open probabilities in the negative voltage range, a contribution to the registered current from Cl^- channels that remained unaffected by the osmotic stress should be small.

Test pulses to very negative potentials elicited rapidly deactivating currents. The time constants for this deactivation are given in Fig. 6E.

Effect of a reduction of the external osmolarity on single-channel currents: measurements in the cell-attached mode

For these measurements, myoballs were bathed in a K^+-containing solution so that the resting potential was normal. Three different pipette potentials, + 40 and + 20 mV (hyperpolarizing the patch) and −60 mV were used. Intermediate Cl^- channels were identified by the direction of current flow when the patch was hyperpolarized, and by the channel kinetics. After the controls were recorded, a bathing solution with 270 mosmol/l was applied in which $[K^+]$, $[Ca^{2+}]$ and $[Mg^{2+}]$ were normal, to minimize changes of the resting potential. Under this condition, the gating mode of the intermediate Cl^- channels was changed in the direction expected from macroscopic recordings, however this was not always the case. In four of six tested patches, the open probability of the intermediate Cl^- channel increased, although a halving of the single-channel current amplitude at the negative potentials – characteristic for the transition from mode 1 to mode 2 [9] – was not seen within 20 min (Fig. 7A, B). The increase of the open probability started normally 5–10 min after application of the hypo-osmolar medium. In one patch, the open probability started to increase before the external osmolarity was lowered. In

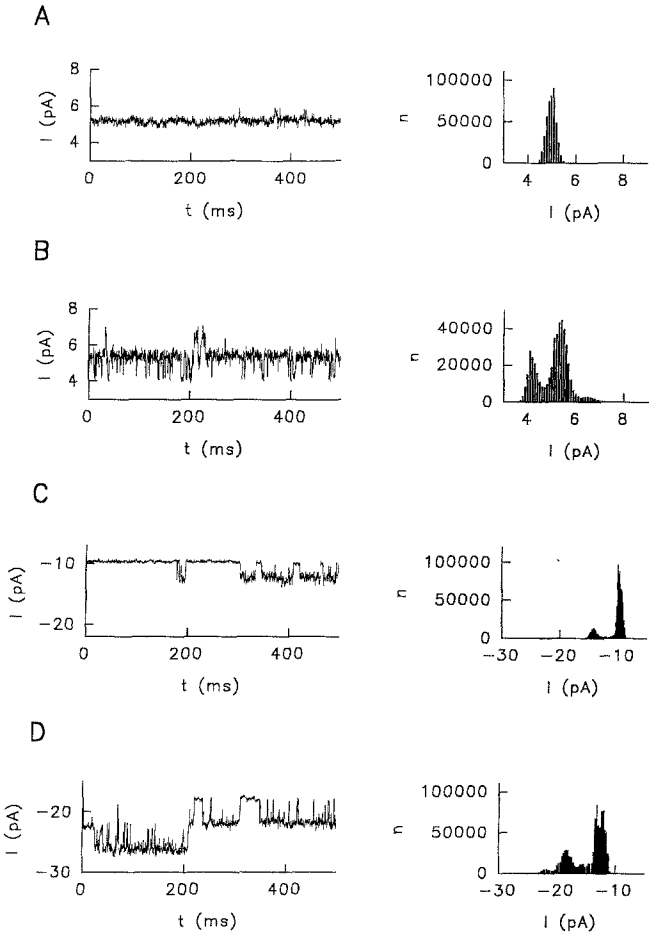

Fig. 8A–D. Effect of 1 μM cytochalasin D on Cl^- currents through an inside-out patch containing at least 2 intermediate Cl^- channels. **A** Original record (*left*) and amplitude histogram (*right*) before addition of cytochalasin D. Holding potential, −85 mV. **B** Same patch, same conditions, 10 min after addition of cytochalasin D. **C, D** Same as **A** and **B**, but with + 60 mV holding potential

prolonged recordings (more than 20 min after application of the hypo-osmolar solution) the open probability decreased to the level at the beginning of the experiment.

Effects of cytochalasin D or colchicine on inside-out patches

Patches were excised from myoballs and single-channel recordings made at + 85, + 40 and −60 mV pipette potential (corresponding to −85, −40 and + 60 mV membrane potential) for a total recording time of 6 min. Then, cytochalasin D or colchicine was added to the bath in concentrations of 1 μM and 10 μM, respectively. The patches remained stable for at least 20 min. Currents were recorded at the same three potentials at various times after drug application. In all these recordings there were no transitions from gating mode 1 to 2 (Fig. 8A–D) although the open probability of the Cl^- channels increased (Figs. 8, 9). The maximum of the open probability was reached at positive membrane potentials, in contrast to the maximum in the negative membrane domain seen during G protein activation [9].

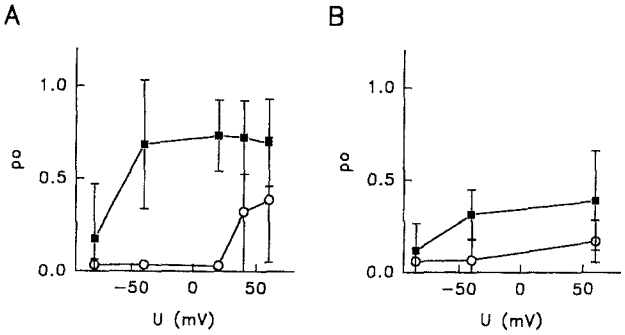

Fig. 9A, B. Open probability measured in excised inside-out patches at various membrane potentials. **A** Open probability before (○) and after (■) addition of 1 μM cytochalasin D. **B** Open probability before (○) and after (■) addition of 10 μM colchicine

The fact that the drugs were able to increase the open probability in excised patches corroborates our earlier conclusion that second messenger systems like protein kinases, G proteins and $[Ca^{2+}]_i$ are not necessarily involved in this kind of channel regulation.

Discussion

Our experiments demonstrate a volume-sensitive g_{Cl} in human embryonic muscle cells that is most likely connected with the "intermediate" Cl^- channel [8]. Following a decrease of the external osmolarity, the instantaneous Cl^- current was much increased over the whole membrane potential range, and the steady-state current was decreased in the positive potential range. This change has been shown to occur when the open probability of intermediate Cl^- channels changes in the negative voltage range [8, 9]. As pointed out, the decrease of the steady-state current cannot easily be explained by the induction of another channel type. The change of the current kinetics results in an increase of the macroscopic g_{Cl} but does not affect the anion selectivity.

Comparison with other volume-sensitive Cl^- channels

In epithelial cells, an outward rectifying channel is activated by hypo-osmolarity [19, 26]. The relationship of these electrophysiologically well-defined channels to recently cloned proteins [22, 25] is unclear. Expression cloning using MDCK cells [22] yielded a cytosolic actin-binding protein which does not conduct the observed currents [1, 17].

Transfection of a fibroblast or a lung epithelial cell line with the MDR-1 gene product leads to volume-activated Cl^- currents in these cells [25]. There are now several indications that the MDR-1 gene product, a P-glycoprotein, is not identical with the volume-activated channel in these cells [6, 16]. The anion selectivity of these channels is I > Br > Cl, they are outward rectifiers, and when the membrane potential is varied starting from a holding value of 0 mV, the recorded currents activate upon hyperpolarization and inactivate upon depolarization [4, 25].

The properties of all these channels are different from those reported here for the embryonic skeletal muscle anion channel. The volume-activated currents in myoballs have a greater selectivity for Cl^- than for I^- and the outward rectification in symmetrical Cl^- is much more pronounced. The steady-state inactivation curve is best fitted with two exponentials whereas in epithelial channels one exponential suffices [19]. When the membrane potential is clamped from a positive to a negative value, Cl^- currents of epithelial cells show a simple recovery from inactivation [25, 26], whereas in myoballs there is a rapid deactivation with a very slow recovery from inactivation.

Clc-2, a member of a family of voltage-gated Cl^- channels present in almost every mammalian cell, is also volume activated [11]. This channel has a greater selectivity for Cl^- than for I^-, however, it differs from the muscle anion channel in that it is activated by hyperpolarization.

The intermediate Cl^- channel is likely to be under the control of the cytoskeleton

Several findings indicate a regulatory action of the cytoskeleton on the intermediate Cl^- channels. The formation of myoballs from myotubes leads to a decrease of the activation time constants. This result cannot be an artefact because the time constants for charging the membrane are much greater for myotubes than for myoballs. When cytochalasin D or colchicine were applied to excised patches kept in osmotically balanced solutions, the open probability increased, although the typical half-conductance state was not observed. An interaction of the cytoskeleton with the channels could also explain the slight increase in g_{Cl} in myoballs kept in osmotically balanced solutions (Fig. 1C) and the incidental increase of the open probability in excised patches [8]. Microfilaments and microtubules are unstable polymers, the polymerization of which depends on the concentrations of ATP and GTP [20]. Perfusion of myoballs with a solution devoid of NTP would lead to a depolymerization of the cytoskeleton and therefore increase g_{Cl}.

Interaction between channels and cytoskeleton is also suggested by the result that in cell-attached recordings the intermediate Cl^- channels were altered when the osmolarity of the bath was decreased, because in this experimental configuration no osmotic pressure ensues across the patch. The increase of the open probability of channels seen in these experiments suggests a transduction mechanism between the swelling of a whole cell and channels in a patch of its membrane that is covered by a pipette.

How is the cytoskeleton related to the volume sensitivity of the Cl^- currents?

Destruction of the cytoskeleton by cytochalasin D or colchicine was followed by changes of the kinetics of the Cl^- currents. These changes did not occur when the cell interior was hypotonic with respect to outside.

Investigating a human colonic cell line, Worrell et al. [26] observed a volume effect on Cl^- currents that was even present when the pipette and bath solutions were osmotically balanced. They explained this observation as being due to intracellular oncotic pressure which, under this experimental condition, leads to cell swelling. This view is corroborated by the differences that we observed for the actions of cytochalasin D under the conditions of whole-cell recording and of inside-out patches lacking oncotic pressure. It should be mentioned that effects of the cytoskeleton on the channel properties are difficult to deduce from single-channel recordings because the production of the seal might influence channel/cytoskeleton interactions. For example, the incidental dissociation of the membrane patch from the cytoskeleton was described for cell-attached patches in *Xenopus* oocytes [12]. Moreover, excised patches are not free from cytoskeletal components [24]. Thus, we are afraid that also, in our patch experiments, an undefined remaining influence of cytoskeleton fractions must be considered.

The results obtained with whole cells agree with the notion of a stabilization of the cell membrane by the cytoskeleton [10]. Myoballs seem to have a rather rigid skeleton which prevents oncotic-pressure-induced swelling and allows the cell volume to swell only very slowly when a hypotonic external medium is applied. When parts of the cytoskeletal structure were removed, the cells seemed to swell even under the small oncotic pressure.

This hypothesis cannot fully account for the results obtained with cell-free patches. Cytochalasin D or colchicine application to inside-out patches lead to increases of the open probability over the whole voltage range investigated. The cytochalasin D and colchicine experiments show that a disruption of the cytoskeleton affects these channels without an osmotic gradient, thus pointing to a more direct interaction between these structures.

Our results are best explained by the assumption of an interaction between the intermediate Cl^- channels and the cytoskeletal network which is modulated by the tension in the membrane/cytoskeleton system. Disruption of the cytoskeleton leads to minor changes of the channel properties when transmembrane osmolarity is balanced, but increases the sensitivity of the channel in the case of an osmotic gradient.

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