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Ion Channels Activated by Swelling of Madin Darby Canine Kidney (MDCK) Cells

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Summary. According to previous studies hyposmotic swelling of Madin Darby Canine Kidney (MDCK) cells leads to a marked decrease of cell membrane resistance. The present study has been performed to identify the underlying ion channels using the patch-clamp technique: reduction of extracellular osmolarity to 230 mmol/liter leads to a transient activation of K^+ channels and a sustained activation of anion channels. The K^+ channels are inwardly rectifying with a single-channel slope conductance of 56 ± 3 pS at −50 mV (cell negative) and of 29 ± 2 pS at 0 mV PD across the patch (150 mmol/liter K^+ in pipette). The same channels are activated by an increase of intracellular calcium activity, as shown previously. The anion channels display a single-channel slope conductance of 41 ± 4 pS at −50 mV (cell negative) and of 25 ± 3 pS at 0 mV PD across the patch (150 mmol/liter Cl^- in pipette). The channel is anion selective and conducts both bicarbonate and chloride with a preference for bicarbonate. Its open probability is not affected by changing intracellular calcium from 0.1–10 μmol/liter. The channels observed explain the effects of cell swelling on PD, ion selectivity and resistance of the cell membrane in MDCK cells.

Key Words MDCK cells · cell volume regulation · K^+ channels · Cl^- channels · patch clamp

Introduction

Cells from a great variety of tissues regulate their volume following sudden exposure to hyposmotic extracellular fluid, i.e., they swell initially but then reduce their volume towards the value in isotonic extracellular fluid. Part of this volume regulatory decrease (VRD) is achieved by extrusion of KCl [1, 2, 9, 14, 16, 19, 22] or possibly $KHCO_3$ [15, 22, 23]. Swelling of MDCK cells by sudden exposure to hypotonic extracellular fluid leads to a variable, transient hyperpolarization followed by a sustained depolarization of the cell membrane [12, 18, 21]. The resistance of the cell membrane decreases to 25% of its original value [10, 20]. While the transient hyperpolarization may be the result of enhanced K^+ conductance, the depolarization is apparently due to activation of an anion-conductive pathway, since it is paralleled by an increase of the cell membrane chloride selectivity.

The present study has been performed to define the ion channels accounting for the observed alterations of macroscopic ion conductances in swollen MDCK cells. The results confirm the observations with microelectrodes and show that hyposmotic cell swelling leads to a transient activation of calcium-sensitive K^+ channels and a sustained activation of unspecific anion channels.

Materials and Methods

MDCK cells from the American Type Culture Collection [5, 17] were used from passage 90 to 110. Serial cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin equilibrated with 95% humidified air and 5% carbon dioxide at 37°C. After growing to confluency monolayers were dispersed by incubation in a calcium- and magnesium-free trypsin-EDTA containing balanced salt solution (pH 7.4), plated on sterile cover glasses and incubated again in the same medium as above for at least 48 hr. Cover glasses with incompletely confluent cell layers were mounted into a perfusion chamber (volume: 0.1 ml, perfusion rate 4 ml/min).

Patch-clamp experiments were carried out according to the method of Hamill et al. [6]. Pipettes were pulled from soft glass (hematocrit tubes, Brand, 749321, Wertheim, Germany). Single-channel current events were measured by means of a L/M-EPC-7 amplifier (LIST-Electronics, Darmstadt, FRG) stored on a VHS videotape recorder (ELIN-6101, Vienna, Austria) via pulse code modulation (SONY PCM-501ES). The experiments were performed under cell-attached and excised-patch configuration. Outward current from the cytoplasm to the pipette is given as positive. The potential of the pipette (V_p) is given in reference to the bath. In the cell-attached configuration the actual PD across the patch is the difference between V_p and V_m, the PD across the unpatched cell membrane (V_m). In cells exposed to hypotonic extracellular perfusate, V_m amounts to −30 mV [18, 20]. For analysis, 4-sec segments of current records were played back through an 8-pole Bessel filter (model 902 LPF, Frequency Devices, Haverhill MA) set at 0.85 kHz and digitized into an Olivetti M28 computer at a sampling rate of 0.5 msec (2 kHz) using a 12-

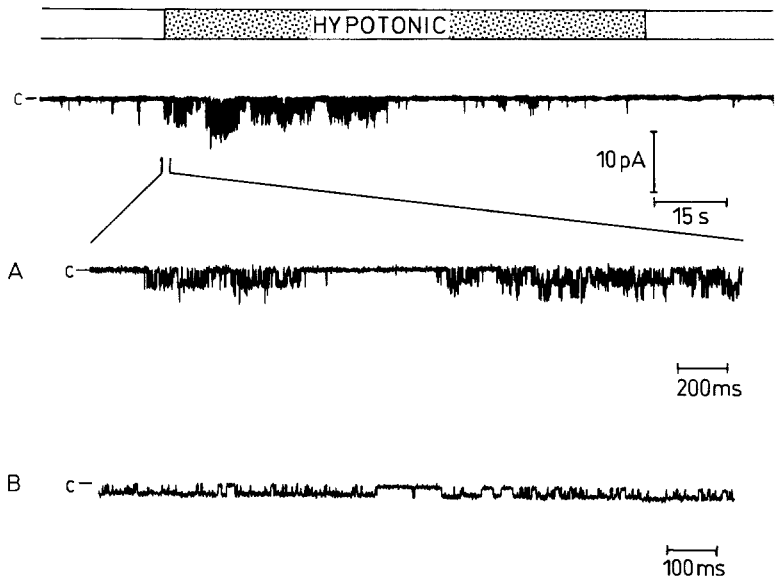

Fig. 1. Effect of decreasing extracellular osmolarity on K^+ channel activity: original tracing of currents recorded in the cell-attached configuration before, during and after reduction of extracellular osmolarity (by removal of 68 mmol/liter mannitol, *c* = closed). Pipette solution contained 145 mmol/liter KCl; zero PD between bath and pipette. Trace *A* is an amplification of the early part of the upper trace, i.e., the early effect of cell swelling. Trace *B* shows currents during prolonged exposure to hypotonic solution. Please note the difference in single-channel current between *A* and *B*. Cell swelling leads to a transient hyperpolarization of the cell membrane, which increases the driving force for K^+ movements across the patch, and thus, leads to a transient enhancement of single-channel current (*A*).

bit A/D-converter (DASH16, Metrabyte, Taunton, MA), analyzed and stored on a 40-MByte hard disk.

Using software written by F. Friedrich [3, 4], the open probability was calculated from amplitude histograms according to the equation:

$$P_o = \left(\sum_{n=1}^{N} (n \cdot tn)\right) \Big/ N \qquad (1)$$

where *tn* are the fractions of the observed time interval, when *n* channels are open, and *N* is the maximal number of channels observed under maximal stimulation.

A second estimate of P_o was made in excised patches from the following equation:

$$P'_o = 1 - tc^{1/N} \qquad (2)$$

where *tc* is the fraction of the observed time interval when all channels are closed.

If the channels open independently and if *N* is indeed the number of all channels in the patch, $P_o \approx P'_o$.

For current records in the cell-attached configuration, the bath perfusate was composed of (in mmol/liter): 80 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 1.2 $CaCl_2$, 0.8 Na_2HPO_4, 0.2 NaH_2PO_4, 20 $NaHCO_3$, 5.5 glucose and 68 mannitol. The solution was equilibrated with 5% CO_2, 95% air (pH 7.4) and kept at 37°C. In some experiments NaCl was reduced to 40 mmol/liter and mannitol increased to 148 mmol/liter. Osmolarity was reduced by omission of mannitol from the bath solution (hypotonic).

The pipette was filled with a solution composed of (in mmol/liter): 145 KCl, 1.2 $CaCl_2$, 10 HEPES-KOH (pH 7.4) and 0.04 phenol red. For the analysis of K^+ channel activity, the pipette tip has been filled with K^+ gluconate instead of KCl. For anion channel records the pipette solution was composed of (in mmol/liter): 145 choline chloride, 1 $BaCl_2$, 10 TEA and 5 Tris-HCl (pH 7.4). Barium and TEA were added to block K^+ channels. It has been shown previously [18], that 1 mmol/liter barium completely eliminates the K^+ transference number of MDCK cells.

The selectivity of the channels for potassium has been estimated from the shift of the reversal potential (V_o) when part of KCl was replaced by NaCl in the pipette. The anion channel selectivity has been estimated in excised patches from the reversal potential (V_o) by using a bath solution composed of (in mmol/liter): 5 choline chloride, 40 choline bicarbonate, 200 mannitol (equilibrated with 10% CO_2, 90% air, pH 7.4) and 45 KCl, 200 mannitol, 5 Tris-HCl (pH 7.4), respectively. The equations used have been described previously [4].

As a reference electrode, a 150 mmol/liter KCl-Agar bridge was used throughout and placed at the solution exit of the chamber. Where appropriate, correction was made for liquid junction potentials. Applicable data are expressed as arithmetic means ± SEM.

Results

In isotonic extracellular perfusate little channel activity is observed in cell-attached membrane patches. However, following reduction of extracellular osmolarity from 288 to 230 mmol/liter a transient activation of K^+ channels is observed in most patches (Fig. 1). As shown in Fig. 2, the open probability of these channels increases from 0.04 ± 0.01 up to 0.26 ± 0.04. The current-voltage relation reveals the inwardly rectifying property of these channels (Fig. 3). The single-channel slope conductance amounts to 29 ± 2 pS (n = 7) at 0 mV PD across the patch and to 56 ± 3 pS (n = 7) at −50 mV, cell negative (Fig. 3). With 150 mmol/liter K^+ in the patch pipette, the reversal potential amounts to −45 ± 3 mV (n = 7, Fig. 3) (pipette negative *versus* bath). Thus, the reversal potential is close to the cell membrane potential, as expected from K^+ channels but not for anion channels (*see below*). Using pipettes filled with 50 mmol/liter KCl and 100 mmol/

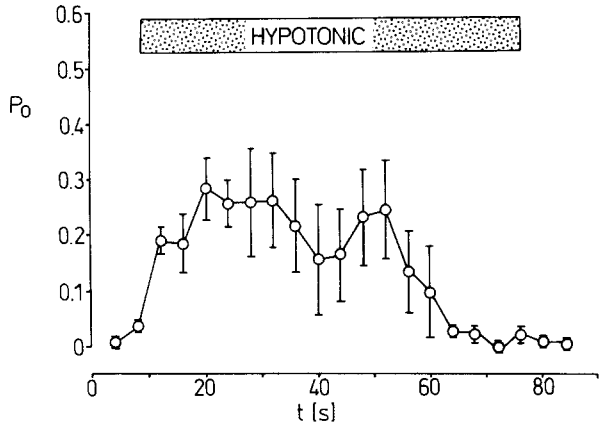

Fig. 2. Open probability (P_o) of the K^+ channels in the cell-attached configuration as a function of time following reduction of extracellular osmolarity by omission of 68 mmol/liter mannitol (n = 6 patches). Given are arithmetic means ± SEM (V_p = 0).

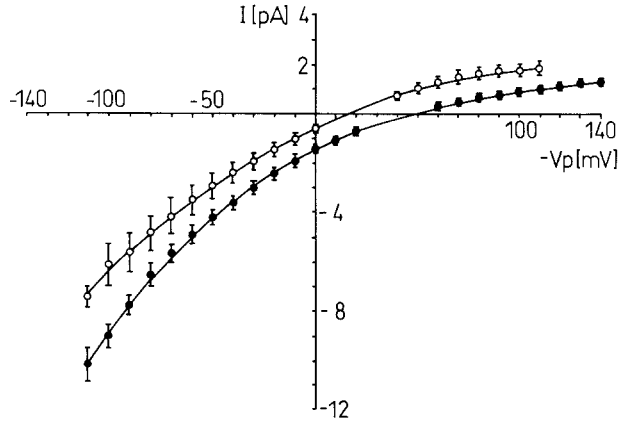

Fig. 3. Single-channel current (I) through K^+ channels in the cell-attached configuration as a function of the PD (V_p) between bath and pipette. Given are arithmetic means ± SEM. Filled symbols stand for pipette solution containing 145 mmol/liter KCl (n = 7 patches); open symbols stand for pipette solution containing 50 mmol/liter KCl and 100 mmol/liter NaCl (n = 5 patches). I = currents from cell to pipette; V_p = PD between pipette and bath. Given a cell membrane potential of −30 mV in hypotonic extracellular fluid, the PD across the patch is 30 mV more positive than V_p.

liter NaCl, the reversal potential is −18 ± 4 mV (n = 5), pipette negative. The change of reversal potential upon partial replacement of KCl with NaCl in the pipette discloses the K^+ selectivity of the channels (0.93 ± 0.17); the potassium permeability is more than 20-fold the sodium permeability.

In 28% of the patches the sustained activation of anion channels is observed following reduction of extracellular osmolarity from 288 to 230 mmol/liter (Fig. 4). The open probability of these channels increases from 0.01 ± 0.01 up to 0.30 ± 0.05 (Fig. 5). A change of V_p from 20 to 0 mV does not significantly modify the open probability (0.30 ± 0.05 at 20 mV *versus* 0.30 ± 0.04 at 0 mV). The single-channel slope conductance amounts to 41 ± 4 pS (n = 5) at −50 mV (cell negative) PD across the patch and to 25 ± 3 pS (n = 5) at 0 mV (Fig. 6). In excised patches with 145 choline chloride in the pipette and the extracellular perfusate in the bath (89.4 mmol/liter chloride, 20 mmol/liter bicarbonate), the reversal potential amounts to −2.3 ± 3.4 mV (n = 4), which is significantly lower than the equilibrium potential for chloride (−13 mV). With 145 mmol/liter choline chloride in the patch pipette and 45 mmol/liter KCl in the bath, however, the reversal potential amounts to 31.4 ± 2.8 mV (n = 6), pipette positive, a value not significantly different from the chloride equilibrium potential (31.5 mV) (Fig. 7). In those experiments the replacement of bath KCl by choline chloride at constant holding potential (100 mV) does not significantly alter the single-channel current (−2 ± 3%, n = 9), a finding consistent with the anion selectivity of the channel. Replacement of 45 mmol/liter KCl by 40 mmol/liter choline bicarbonate and 5 mmol/liter choline chloride results in a reversal potential of −0.7 ± 11.0 mV (n = 6), pipette negative. Accordingly, the channel is conductive to both bicarbonate and chloride with a preference for bicarbonate (P_{Cl}/P_{HCO_3} = 0.3 ± 0.1). At symmetrical bicarbonate concentrations (bath (in mmol/liter): 40 $KHCO_3$, 5 KCl and 200 mannitol; pipette (in mmol/liter): 110 choline chloride and 40 choline bicarbonate), the reversal potential (15 mV, pipette positive) is again clearly lower than the reversal potential for chloride (82 mV) and again consistent with a preference of the channel for bicarbonate.

To test for calcium sensitivity of the anion channel, excised patches have been exposed subsequently to 100 nmol/liter, 1 μmol/liter and 10 μmol/liter calcium in the bath. The open probabilities were 0.13 ± 0.02, 0.14 ± 0.03, and 0.13 ± 0.02, respectively (n = 5).

Discussion

The present study fully confirms the conclusions derived from observations using conventional electrophysiology [10, 18, 20, 21], i.e., that swelling of MDCK cells leads to an initial transient activation of K^+ channels and a sustained stimulation of chloride-conducting anion channels.

The K^+ channel activated has been characterized in previous papers [3, 4, 11] and has been shown to be activated by an increase of intracellular calcium activity. The activation of the K^+ channels

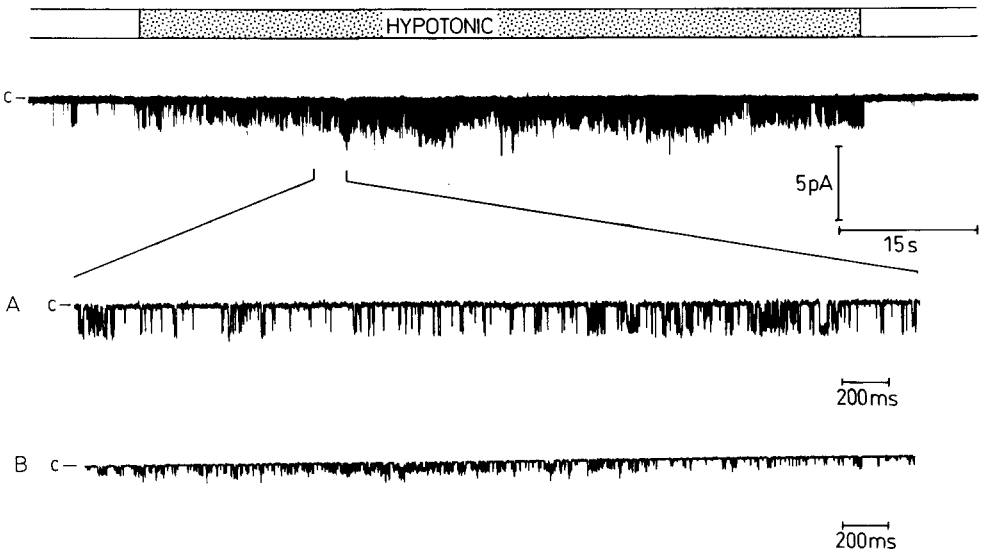

Fig. 4. Effect of decreasing extracellular osmolarity on anion channel activity in cell-attached patches: original tracing of currents recorded in the cell-attached configuration before, during and after reduction of extracellular osmolarity (by removal of 68 mmol/liter mannitol, *c* = closed). Pipette solution contained (in mmol/liter): 145 choline chloride, 1 $BaCl_2$ and 10 TEA, +20 mV PD, pipette positive *versus* bath. Trace *A* is an amplification of the early part of the upper trace, i.e., the early effect of cell swelling. Trace *B* shows currents during prolonged exposure to hypotonic solution. Please note the difference in single-channel current between *A* and *B*. Cell swelling leads to a transient hyperpolarization of the cell membrane, which increases the driving force for Cl^- movements across the patch, and thus, leads to a transient enhancement of single-channel current (*A*).

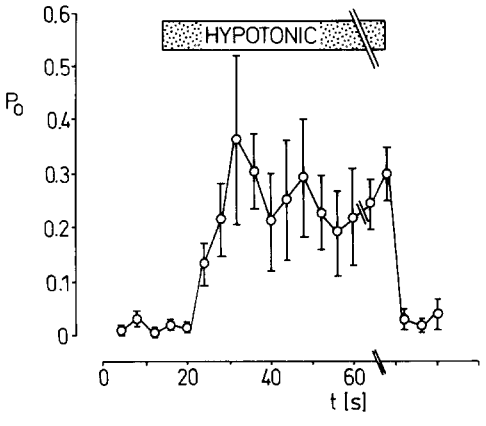

Fig. 5. Open probability (P_o) of the anion channels in the cell-attached configuration as a function of time following reduction of extracellular osmolarity by omission of 68 mmol/liter mannitol. Given are arithmetic means ± SEM (n = 6 patches, +20 mV PD, pipette positive *versus* bath).

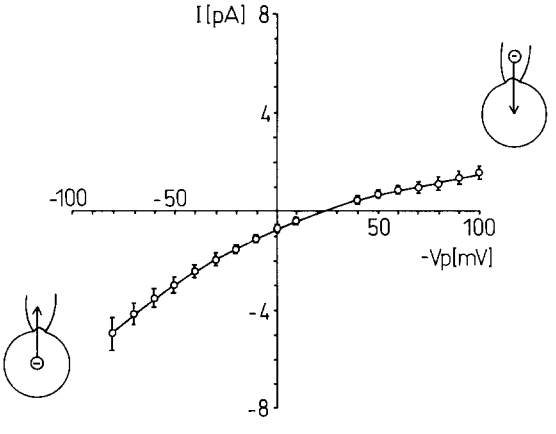

Fig. 6. Single-channel current (I) through anion channels in the cell-attached configuration as a function of the PD (V_p) between bath and pipette. Given are arithmetic means ± SEM, n = 5 patches. (Pipette solution contained (in mmol/liter): 145 choline chloride, 1 $BaCl_2$ and 10 TEA). I = currents from cell to pipette; V_p = PD between pipette and bath.

accelerates the parallel conductive loss of K^+ and Cl^-. However, as suggested from conventional electrophysiology, the K^+ channels are activated only transiently and they inactivate before completion of cell VRD [10, 18]. Thus, given the high K^+ conductance of MDCK cells in the absence of cell swelling, the activation of calcium-sensitive K^+ channels may be supportive but not crucial for regulatory cell volume decrease.

The anion channel activated by cell swelling would allow exit of both chloride and bicarbonate. In a previous study, we have indeed shown that swelling of MDCK cells leads to intracellular acido-

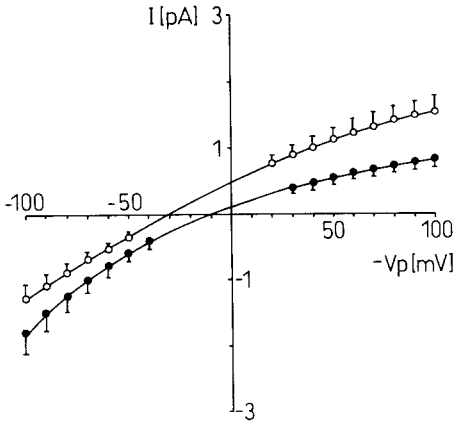

Fig. 7. Single-channel current (I) through anion channels in the excised-patch configuration as a function of the PD (V_p) between bath and pipette. Given are arithmetic means ± SEM. Filled circles stand for bath solution containing (in mmol/liter): 5 choline chloride, 40 choline bicarbonate and 200 mannitol equilibrated with 10% CO_2, 90% air (pH 7.4) (n = 6 patches); open circles stand for bath solution containing (in mmol/liter): 45 KCl, 200 mannitol and 5 Tris-HCl (pH 7.4) (n = 6 patches); pipette solution for both was (in mmol/liter): 145 choline chloride, 1 $BaCl_2$, 10 TEA and 5 Tris-HCl (pH 7.4). I = currents from bath to pipette; V_p = PD between pipette and bath.

sis from 7.23 ± 0.06 to 6.93 ± 0.04 pH units [20], which is—according to this study—at least partially the result of cellular bicarbonate loss through the anion channels. An alternative mechanism allowing loss of cellular bicarbonate is the Cl^-/HCO_3^- exchanger driven by the decreasing intracellular chloride activity. Roy and Sauvé [21] observed that loss of potassium from swollen MDCK cells is twice that of chloride. This anion gap could at least partially be accounted for by bicarbonate. Bicarbonate lost by the cells could be replenished from CO_2, and thus, considerable amounts of bicarbonate could be released by the cells, even if intracellular bicarbonate concentration is low. The intracellular bicarbonate formation leads to the generation of hydrogen ions, which are bound to intracellular buffers due to the acidosis or are consumed by alteration of intracellular metabolism. In liver cells, the metabolic formation of several organic acids is reduced during cell swelling [7, 8, 13]. Accordingly, the bicarbonate loss through the unspecific anion channel may indeed contribute to regulatory cell volume decrease. In straight proximal tubules of the mouse kidney [23] and in the *Necturus* proximal tubule [15], regulatory cell volume decrease is even dependent on the presence of bicarbonate. This is not true for MDCK cells, which still exhibit regulatory cell volume decrease in the absence of exogenous bicarbonate [18].

In conclusion, swelling of MDCK cells leads to a transient activation of calcium-sensitive K^+ channels and sustained activation of an anion channel allowing passage of chloride and bicarbonate. These channels allow the cellular loss of the respective ions leading to regulatory cell volume decrease.

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