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Ion Channels Activated by Osmotic and Mechanical Stress in Membranes of Opossum Kidney Cells

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Summary. For patch-clamp measurements cultured kidney (OK) cells were exposed to osmotic and mechanical stress. Superfusion of a cell in whole cell configuration with hypotonic media (190 mOsm) evokes strong depolarization, which is reversible by returning to the isotonic bath medium. In the cell-attached configuration the exposure to hypotonic media evokes up to six ion channels of homogeneous single-channel properties in the membrane patch. Subsequently, the channels became activated after a time lag of a few seconds. At an applied membrane potential of 0 mV, the corresponding membrane current is directed inward and shows a transient behavior in the time range of minutes. In the same membrane patch these ion channels can be activated by application of negative hydrostatic pressure. The channel has a single-channel conductance of about 22 pS and is permeable to Na^+ and K^+ as well as to Cl^-. It is suggested that volume regulation involves mechanoreceptor-operated ion channels.

Key Words volume regulation · osmotic stress · stretch-activation · ion channels · patch-clamp

Introduction

Proximal tubular epithelial cells have the capacity of regulatory volume decrease (RVD) (Dellasega & Grantham, 1973; Lohr & Grantham, 1986; Kirk et al., 1987a, 1987b). The RVD response in proximal tubular epithelial cells is due to the loss of cytosolic potassium and chloride (e.g. Kirk, DiBona & Schafer, 1987*a*; Kirk, Schafer & DiBona, 1987*b*). An important function of the RVD response in cells performing transcellular transport (e.g. proximal tubule), is to minimize cell swelling associated with acute imbalances in the rates of cell solute entry and exit (Schultz & Hudson, 1986); since osmolarity changes in the extracellular (intratubular, intestinal) fluid of proximal tubules are small (Schafer, 1987), there is only a minimal need for a RVD response to compensate extracellular osmolarity changes.

The RVD response has been characterized in many epithelial and nonepithelial cell types, and it has been shown that conductive and nonconductive ion transport mechanisms can mediate a loss of cellular potassium and chloride (for review *see* Kregenow, 1981; Spring & Ericson, 1982; Hoffmann, 1986; Eveloff & Warnock, 1987). Furthermore, it is known that differences in the osmolyte concentration in the millimolar range across the plasma membrane can result in a membrane stretch phenomenon, equivalent to that occurring by applying a pressure of a few millibar (Anderson, 1972). Membrane stretch can lead to activation of ion channels, and it has been proposed that membrane stretch represents a trigger for the activation of channels that are involved in cell volume homeostasis (for review *see* Sachs, 1987).

In this paper we present results obtained with the patch-clamp technique for an ion channel, whose gating in the cell-attached configuration of the patch-pipette is affected by applying a negative pressure on the membrane patch (mechanical stress) or by exposing the cell to hypotonic medium (osmotic stress). The experiments were performed on an epithelial cell line derived from opossum kidney (OK). This cell line contains a RVD response as analyzed by cell-size determinations (Knoblauch, Montrose & Murer, 1987), as well as many proximal tubular transport functions, such as sodium-coupled transport of inorganic phosphate and hexoses, parathyroid hormone-controlled sodium/proton exchange and sodium phosphate cotransport activity (*see* Malmström & Murer, 1986; Malmström, Stange & Murer, 1987). From the presented data, we suppose that the characterized ion channel which becomes activated in response to mechanical and osmotic stress is identical and might be involved in proximal tubular cell volume homeosta-

sis. Our results appeared in abstract form (Ubl, Murer & Kolb, 1988*a*).

Materials and Methods

Cell Culture

OK cells (Koyama et al., 1978) were originally obtained from Dr. D. G. Warnock (San Francisco, CA) at serial passage 79, and experiments were performed using cells between passage 86 and 100. Cells were maintained in culture in a humidified 5% CO_2 (95% air) atmosphere in DMEM/Hams F12 (1 : 1) culture medium (Gibco) containing supplements of 2 mM glutamine, 50 IU/ml penicillin, 50 μg/ml streptomycin, 20 mM HEPES, and 10% (vol/vol) fetal calf serum. Cells were subcultured as previously described (Malmström & Murer, 1986). For experimental purposes, 3×10^6 cells were plated on sterile coverslips and grown for 2–3 days. At this time the culture was in a subconfluent state. About 1 hr prior to the experiments, the culture medium was replaced by DMEM without supplements and buffered by 20 mM HEPES to pH 7.4. For the patch-clamp recordings the coverslips were broken into pieces and placed into a perfusion chamber. Cells within or at the rim of a monolayer of connected cells were selected at random for the experiments.

Patch-Clamp Experiments

Patch-clamp recordings were carried out according to the method of Neher and Sakmann (Hamill et al., 1981) using a List EPC-7 amplifier. Typically, pipettes of a resistance between 7-12 MΩ were used. The current was recorded either in the cell-attached configuration (on-cell mode), on inside-out membrane patches (cell-free mode), or in the whole-cell configuration. Only in the case of whole-cell current records are the corresponding potentials given as membrane potentials. In the cell-attached mode the membrane potential is experimentally undetermined. Furthermore, it turned out (Results) that the membrane potential showed transient changes in response to varied osmolarity of the bath. Therefore, for records in the cell-attached and also in the cell-free configuration, the given potentials are the applied pipette potentials (U_p) and referred to a potential of 0 mV for the bath. The notation, depolarization or hyperpolarization, refers to corresponding changes of the membrane potential. Positive currents from the pipette into the cytoplasm are drawn as upward currents and denoted as *inward currents*. The currents were stored on a FM tape recorder (Racal Store 4) with a frequency response of DC to 20 kHz. The records were actively low-pass filtered (Krohn-Hite Mod. 3342) at a cut-off frequency of usually 2 kHz.

Electrolyte Solution

For experiments requiring a physiological bath solution, the medium (denoted as *310 mOsm NaCl medium* or *control solution*) contained (mM): 142 Na^+, 4 K^+, 1 Ca^{2+}, 1 Mg^{2+}, 136 Cl^-, 1 SO_4^{2-}, 1 PO_4^{2-}, 20 HEPES, 18 glucose, pH 7.4 at 22–24°C. The osmolarity of this solution was 309 ± 2 mOsm (n = 10), as measured by freezing point depression (digital microosmometer, Roebling, Berlin). The hypotonic medium (denoted as *190 mOsm NaCl medium*), with 189 ± 2 mOsm (n = 10), contained 67 instead of 142 mM NaCl in the medium described above. The time and amplitude course of the membrane potential was measured in the whole-cell configuration at different osmolarity. For these experiments the pipette solution contained (in mM): 146 KCl, 1.2 $MgCl_2$, 20 mM (HEPES at pH 7.4. For adjustment of a free Ca^{2+}-concentration of 10^{-7} M, 1.1 mM EGTA and 0.55 mM $CaCl_2$ were added (Fabiato & Fabiato, 1979). The channel selectivity to different ions was investigated in the cell-attached mode by replacing NaCl by various concentrations of KCl, Na-gluconate or choline-chloride as stated in the text. The perfusion chamber contained a bath solution of 200 μl, which could be replaced by another solution within 15 sec. As reference electrode, a micropipette filled with 150 mM KCl was used and placed at the solution exit of the chamber.

Data Analysis

For determination of single-channel current amplitudes as well as the estimation of the percentage of time during which a channel is open, amplitude histograms of current fluctuations were analyzed in real time at a sampling rate of 9.8 μsec, with a Hewlett Packard (HP) 5420 A Digital Signal Analyzer. The area of Gaussian-like distributed amplitudes of the leak current and the unitary channel currents were calculated on line by a HP 9825A. The ratio of the corresponding areas was taken as an estimate of the time during which one channel was open, and denoted as *channel open probability*. The total number of conducting channels in the membrane patch was derived from the amplitude histogram. The data are presented as mean ± SEM, and n denotes the number of experiments.

Results

Activation of Ion Channels by Hypotonic Bath

In the cell-attached configuration, an osmotic gradient (extracellular hypotonicity) causes cell swelling and thereby a mechanical stress on the cell membrane, whereas in the cell-free configuration, the membrane faces two open systems and osmotic imbalances which do not provoke a mechanical stress. Therefore, the whole-cell and on-cell mode of the pipette configuration was used to measure the effect of osmotic stress on the ion transport across OK-cell membranes.

To investigate the influence of osmotic gradients on membrane potential, whole-cell experiments were performed. Using the control solution as bath medium and a pipette solution containing 146 mM KCl and 10^{-7}M Ca^{2+}, we found a resting membrane potential of −72 ± 4 mV (n = 6). Exchange of the 310 mOsm NaCl bath medium by the 190 mOsm NaCl electrolyte solution caused depolarization of the cell. The potential change started within the time range of 15 sec which was necessary for a complete replacement of the bath volume (Ma-

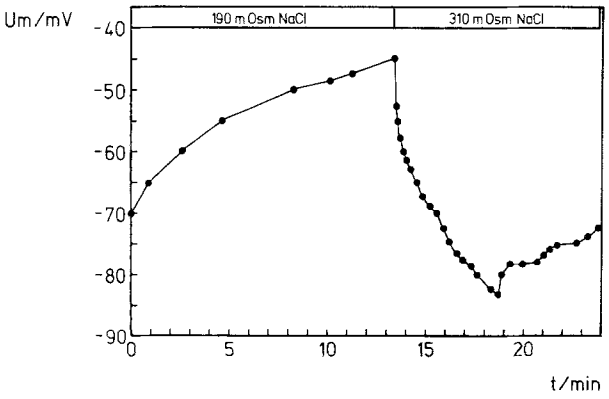

Fig. 1. Membrane potential U_m of an OK cell as function of time for various osmolarities of the bath as indicated. U_m was recorded at current clamp in the whole-cell patch-clamp configuration. Prior to $t = 0$, the cell was exposed to the 310 mOsm NaCl medium and a membrane potential of −70.4 mV was measured. At time zero, the bath medium was replaced by the 190 mOsm NaCl medium and after 14 min exchanged again by the 310 mOsm NaCl medium. The pipette solution contained (mM): 146 KCl, 1.2 $MgCl_2$, 10^{-4} Ca^{2+}, 20 HEPES at pH 7.4

terials and Methods). The time and amplitude course of the membrane potential U_m is given in Fig. 1. Within about 15 min the membrane potential depolarizes to about −45 mV (−41 ± 5 mV ($n = 6$)). Replacement of the hypotonic medium by the control solution caused a repolarization of the membrane potential within 5 min. Typically, after an overshoot in hyperpolarizing direction, the membrane potential returns to values close to the original resting membrane potential.

On-cell current records were performed in parallel to analyze the ion transport systems which might be responsible for the observed change of the membrane potential. The following experimental protocol was used. The membrane patch was sealed and the applied potential was set to 0 mV. In a first series of experiments the 310 mOsm NaCl medium was used as pipette solution and as bath medium. Generally, prior to a change of the osmolarity of the bath, the current record showed only occasionally single upward-directed current pulses within silent periods of up to several minutes. A subsequent exposure of the cells to the hypotonic bath caused, after a variable time lag of about 15 to 60 sec, a stepwise increase of an inward directed membrane current as shown in Fig. 2*a*. This type of current pattern could be observed in 22 experiments out of 41, which were performed at these incubation conditions. In four experiments the channels became activated during the exchange of the bath medium. Figure 2*a* shows that the evoked membrane current is dominated by a successive opening of up to six channels of similar current amplitude. It is noteworthy that the application of osmotic pressure yields to either the simultaneous opening of more than two channels of the type presented in Fig. 2*a*, or no channel activation at all.

In order to rule out the possibility that the osmotically evoked channel activation be mediated by the influence of decreased ionic strength, we exchanged the 310 mOsm NaCl bath medium with a medium from the same osmolarity (190 mOsm NaCl plus 134 mM saccharose) of different osmotic strengths in a series of experiments. No significant change of the current was observed across the membrane patch ($n = 4$); but removal of the saccharose resulted in a subsequent activation of ion channels as described above.

The osmotically evoked membrane current shows a voltage dependency for both the outward and inward component (Fig. 2*b*). For this type of experiment, the applied potential was continuously varied between −37 to +37 mV before and after replacement of the isotonic bath by the hypotonic one. Before the osmolarity was reduced no channel fluctuations could be detected in this membrane patch. The corresponding leak current showed an ohmic behavior, and a conductance of 13.5 pS was derived. After exchange of the isotonic bath medium by the hypotonic solution, the conductance increased. In the vicinity of the zero current potential about a 20-fold increase to 280 pS was estimated. The current (I)-voltage (U_p) relation given in Fig. 2*b* could sufficiently be described by a single exponential function of the type

$$I(U_p) = I(U_p^1) + (I(U_p^1) - I(U_p^2))(1 - \exp(\alpha(U_p - U_p^1))) \quad (1)$$

where $U_p^1(U_p^2)$ denotes the lower(higher) limit of the applied voltage ramp, and α the corresponding voltage sensitivity. For a potential change of 11.5 mV in negative direction, an e-fold increase of the current was obtained. The osmotically evoked membrane current was not found to be significantly dependent on external calcium. The shape of the current-voltage relation in Fig. 2*b* was observed to be independent from Ca^{2+} in the hypotonic bath medium, in the range of 1 mM Ca^{2+} to 10^{-7} M Ca^{2+}. The voltage dependency of the outward directed current is dominated by a potassium current (Ubl, Murer & Kolb, 1988*b; see also* Discussion), and will not be considered here. The following analysis is focussed on the ion channel which determines the osmotically evoked inward current as shown in Fig. 2*a*.

In experiments that showed only two simultaneously fluctuating ion channels, the current volt-

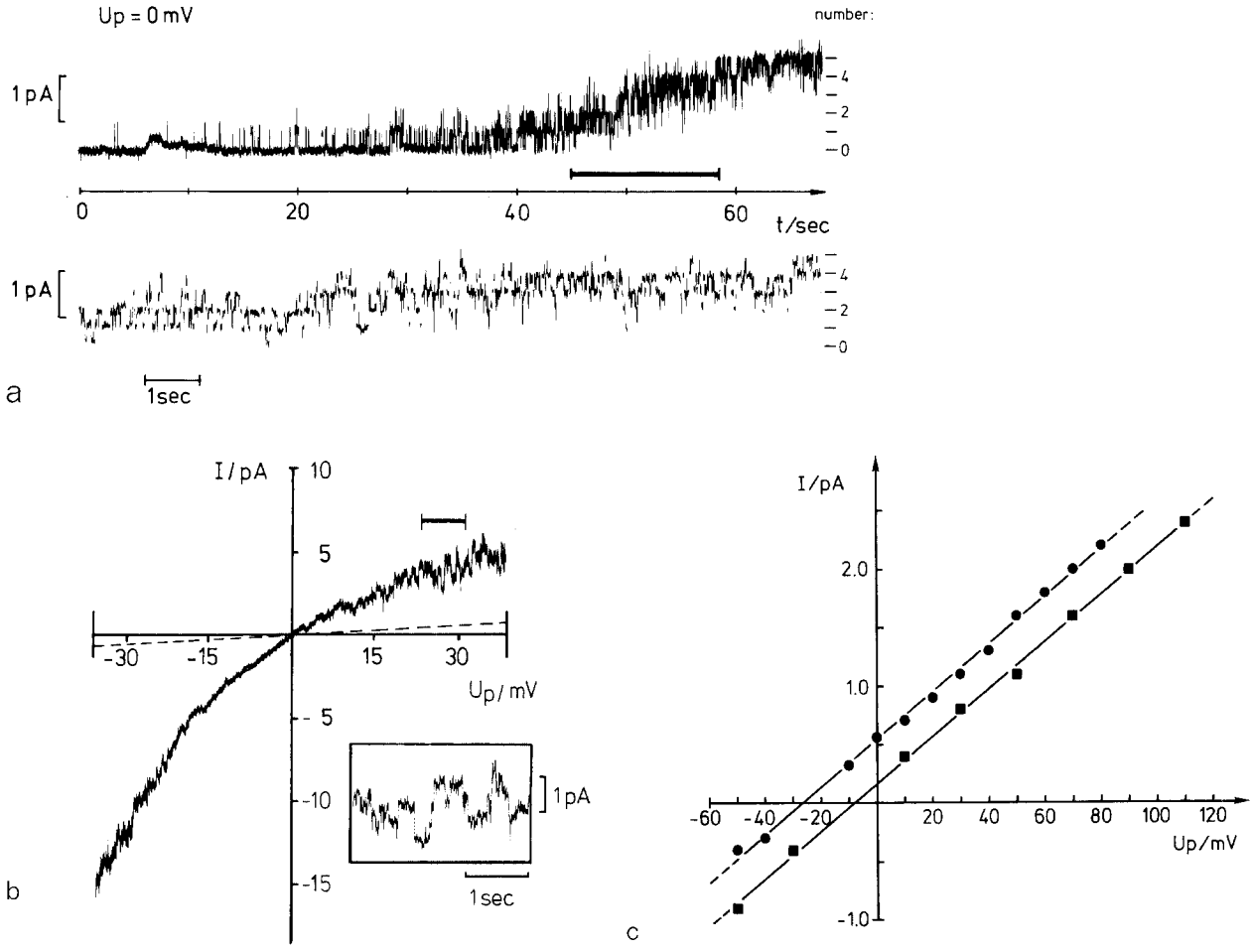

Fig. 2. Osmotically-evoked membrane current in on-cell records. (*a*) Induction of osmotically dependent membrane current. The recording was started about 35 sec after the 310 mOsm NaCl (control) bath had been exchanged for the 190 mOsm NaCl medium. The lower trace shows the current record denoted by a solid bar on an expanded time scale. The pipette potential U_p was held at 0 mV. On the right-hand side the number of simultaneously open channels is indicated. (*b*) Potential dependence of the total current I across the membrane patch before replacement of the control bath medium (dashed line), and 5 min after exposure of the cell to 190 mOsm NaCl medium. The pipette potential was continuously varied within 25 sec between −37 and +37 mV. The inset shows a time-expanded trace of the current as indicated by the solid bar. (*c*) Single-channel current I *vs.* pipette potential U_p, at 190 mOsm NaCl bath. ■ denotes the 310 mOsm NaCl medium and ● denotes the 190 mOsm medium as pipette solution. Corresponding single-channel conductances of 20.3 pS (20.4 pS) and zero current potentials of −8.1 mV (−26.4 mV) were derived, respectively

age-relation of the single-channel amplitude was determined. We obtained a linear relation for the single-channel current in the potential range of about −60 to 80 mV. For the corresponding slope conductance (*see also* Fig. 2*c*), a single-channel conductance of $g = 22.1 \pm 3.1$ pS ($n = 11$) and a zero current potential of -8.0 ± 6.9 mV ($n = 11$) was derived. It should be noted that these values were obtained after cell exposure to the hypotonic bath for about 10 min. A single-channel analysis of osmotically activated channels immediately after their first appearance yielded a value of $g = 35.1 \pm 4.7$ pS as well as a corresponding zero current potential of -30.2 ± 11.2 mV ($n = 5$) (*see* Table). Ion channels of larger single-channel conductance ($g >$ 40 pS) were not observed independently from the used osmolarity of the bath in more than 150 experiments. Aside from the channel type shown in Fig. 2, ion channels with a single-channel conductance of about 8 pS were also observed (in 4 cases of a total of 22). These will not be considered here.

The ion selectivity was analyzed in the cell-attached mode of the micropipette, since in the cell-free configuration, a mechanical stress to the membrane cannot be generated by an osmotic gradient. Furthermore, osmotically evoked ion channels were only transiently activated (*see below* and Fig. 4). Therefore, a time interval of 6 to 10 min was selected after an exchange of the bath, for an estimation of the zero current potential and the single-

Table. Single-channel conductance and zero current pipette potential for ion channels evoked in the on-cell configuration by either osmotic stress using a hypotonic bath (190 mOsm NaCl), or by application of a negative pressure of 25 mbar to the pipette interior[a]

Pipette solution	Channel conductance (pS)	Zero current potential (mV)	n
	Osmotic stress		
Isoton NaCl	35.1 ± 4.7*	−30.2 ± 11.2*	5
	21.1 ± 3.1	− 8.0 ± 6.9	11
Hypoton NaCl	22.8 ± 4.7	−27.8 ± 7.7	11
Hypoton Na-gluconate	25.7 ± 8.1	−28.9 ± 7.3	2
Hypoton choline-Cl	9.9 ± 2.1	−40.4 ± 12.5	7
	Mechanical stress		
Pipette/bath			
Isoton NaCl/hypoton NaCl	19.5 ± 4.2	−26.0 ± 6.1	3
Isoton NaCl/isoton NaCl	27.4 ± 2.9	−26.9 ± 4.2	7
Isoton KCl/isoton NaCl	22.2 ± 5.1	−18.2 ± 7.7	2
Isoton Choline-Cl/hypoton NaCl	9.9 ± 1.6	−25.8 ± 10.5	2

[a] The pipette solution was varied for the experiments as indicated. All experiments were started with 310 mOsm NaCl medium as bath, which was exchanged by the given medium after the pipette had been sealed to the membrane. For experiments with hypotonic pipette solutions and hypotonic bath media, the pipette was sealed to the cell membrane directly following the replacement of the isotonic bath. For further explanations, *see* text.

* The data were obtained following the superfusion of the cells with the hypotonic bath. In all other cases the results were derived within 6 to 10 min after exchange of the bath.

channel conductance. For different experiments the composition of the pipette solution was varied. The results are summarized in the Table. The Table shows that for hypotonic NaCl as a bath, neither a pipette solution with isotonic NaCl, nor hypotonic NaCl, nor hypotonic Na-gluconate had a significant effect on the single-channel conductance. Replacement of sodium in the pipette solution by choline reduced the single-channel conductance to about 10 pS.

In a second series of experiments hypotonic pipette solutions of different compositions were used. A stable seal could not be achieved with a hypotonic pipette solution and isotonic bath medium independent of the electrolyte composition of the pipette. Therefore, the following experimental procedure was used in these cases. The pipette was placed close to the membrane surface, then the control medium was exchanged with a hypotonic one and the giga-seal was established. The patched membrane showed fluctuations of the membrane current (Fig. 3) of the same channel type (*see* the Table and Fig. 2*c*), as observed for isotonic pipette solution. The figure also indicates an increase of the channel open probability at depolarizing membrane potentials. The fluctuation pattern of the channel shows remarkable characteristics, which can be identified by visual inspection of the current pattern. Open-close events in the time range of milliseconds can be well discriminated from occasionally occurring long channel openings with increased electrical noise (Fig. 3). This fluctuation pattern appears to be a characteristic of this channel in the presence of a hypotonic bath and of either a isotonic pipette or a hypotonic pipette solution. Single-channel currents with a conductance of $g = 22.8 \pm 4.7$ pS ($n = 11$) were measured for the 190 mOsm NaCl bath (Fig. 2*c*).

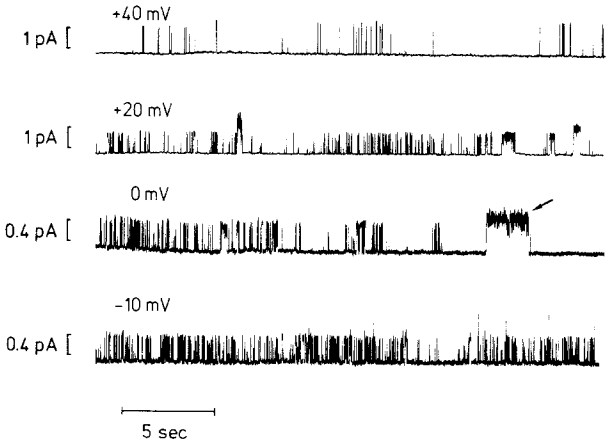

Fig. 3. Potential dependence of an on-cell record for hypotonic bath (190 mOsm NaCl) and pipette solution. The pipette potential was varied as indicated. At zero pipette potential a longer channel opening is marked. For further explanations, *see* text

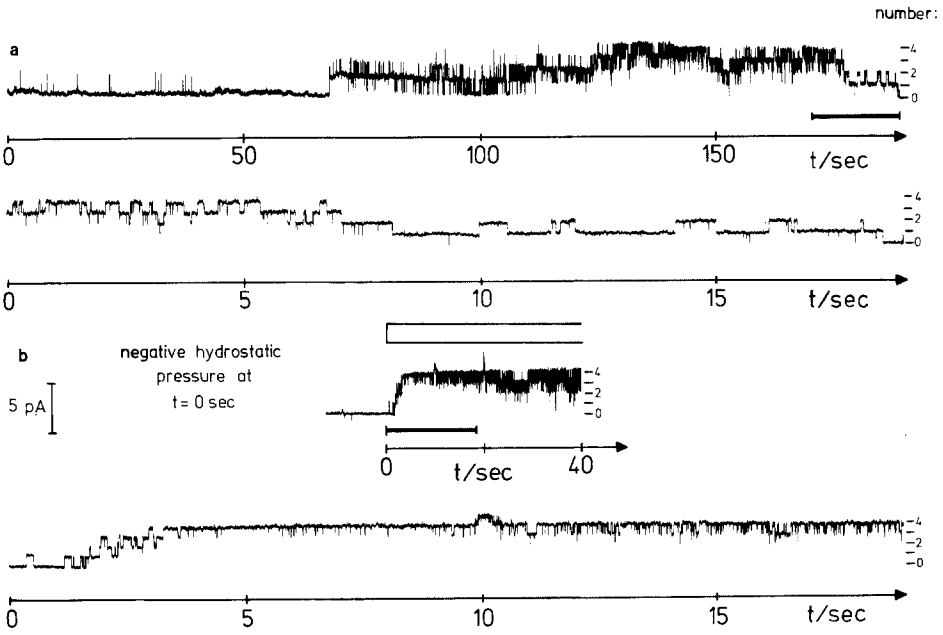

Fig. 4. Activation of a channel population in one on-cell membrane patch by two different activation mechanisms. (*a*) 25 sec prior to time zero, the 310 mOsm NaCl bath medium was replaced by 190 mOsm NaCl. A current trace marked by a solid bar is shown on an expanded time scale below indicating a transient closure of the channels. (*b*) After the transient closure of the osmotically activated channel a negative pressure of 25 mbar is applied to the silent membrane patch, as indicated. The pipette solution was 310 mOsm NaCl and the pipette potential 0 mV. The number of simultaneously open channels is given on the right-hand scale

Activation of Ion Channels by Negative Pressure

The following experiments were performed to obtain evidence that those channels, which are evoked by osmotic stress, can also be activated by mechanical stress. Figure 4*a* shows, for an isotonic pipette solution, the subsequent activation of four channels by a decrease in the osmolarity of the bath. In this typical experiment the induced channel fluctuations showed a transient behavior. Prior to the exposure of the cell to hypotonic medium, no channel fluctuations were observed. About 20 sec after the start of superfusion of the cell by the 190 mOsm NaCl solution, upward directed current fluctuations appeared. Successively, up to four current steps of similar amplitude become evident, and about 200 sec after replacement of the original bath medium, the fluctuations diminished and only the leak current was observed. Closer inspection of the current pattern shows that the size of the current steps decreased by about 30% during this time period at a constantly applied potential of 0 mV. Thereafter, while the corresponding ion channels stayed in a closed state, a negative pressure of 25 mbar was constantly applied to the pipette interior. As Fig. 4*b* shows, a brisk and sustained increase in the inward current was induced. The current consisted again of up to four channels of the same amplitude which subsequently became activated. The channels remained active as long as the negative pressure was applied. Release of the pressure caused a closure of the channels within the same time course as the pressure change. Channel activation of the type shown in Fig. 2*a* and 4, induced by a reduction of the bath osmolarity, could only be observed in experiments which also exhibited current fluctuations due to suction after obtaining the giga-seal. Figure 5 shows pressure-induced channel fluctuations for different electrolytes used as pipette solutions (Fig. 5*a*, high KCl; Fig. 5*b*, control solution; Fig. 5*c*, choline-chloride). Figure 5*b* shows again characteristic channel openings in a range of seconds, as was observed for the osmotically activated ion channel (*compare* Fig. 3).

Current-voltage relations for the corresponding single channel were derived by measuring the current during continuous variation of the applied potential (−10 to 60 mV; *see* for comparison Fig. 2*b*), while a negative pressure of 25 mbar was applied. With the control solution as bath medium and pipette solution, a single-channel conductance of 27.4 ± 2.9 pS and a zero current potential of −26.9 ± 4.2 mV ($n = 7$) was found. The ion selectivity was ana-

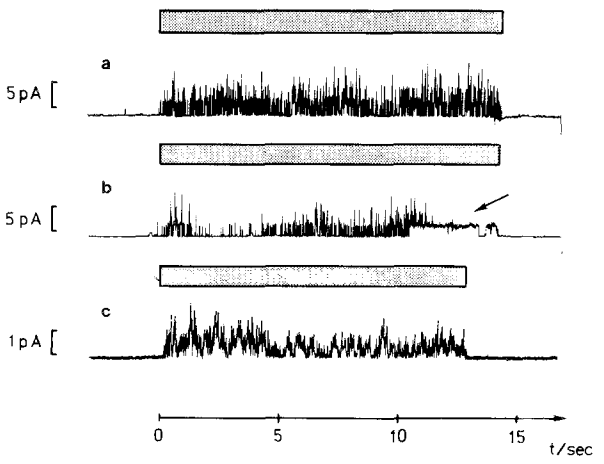

Fig. 5. Endogenous stretch-activated channels of OK-cell membranes in on-cell records for different pipette and bath compositions. The applied pipette potential was 0 mV. The application of a negative pressure (25 mbar) to the pipette interior is indicated above each current trace by a hatched bar, respectively. (*a*) NaCl in the 310 mOsm NaCl medium is replaced by KCl for the pipette solution and the 310 mOsm NaCl medium is used as bath. (*b*) 310 mOsm NaCl for the bath and pipette solution. A channel opening in the range of seconds is marked by an arrow. (*c*) NaCl in the 310 mOsm NaCl is replaced by choline-chloride for the pipette solution and 190 mOsm NaCl medium as bath. The pressure was applied after the osmotically evoked current fluctuations had disappeared (*compare* Fig. 4)

lyzed using different pipette solutions as well as different bath solutions (Fig. 5). The results are summarized in the Table.

Discussion

Cultured OK cells have been used previously as a model epithelial cell for the renal proximal tubular epithelial cell (Miller & Pollock, 1987). Recently, it could be shown, by using electronic cell sizing of suspensions of OK cells, that the cells show regulatory volume decrease (Knoblauch et al., 1988) as response to hypotonic bath media. In the present paper, we have evaluated the electrical response of OK cell membranes in two different experimental maneuvers, those being osmotic stress and mechanical stress. For the application of an osmotic stress, the cultured cell monolayer was exposed to hypotonic media. For the generation of a mechanical stress, a negative hydrostatic pressure was applied to the patch pipette. Our goal was to give evidence that mechanical stress activates ion channels, which might be involved in the mechanism of volume regulation as a response to hypotonic media.

Superfusion with hypotonic media of the cell, which was current-clamped in the whole cell configuration, caused pronounced depolarization (Fig. 1). This appeared to be fully reversible after replacement of the hypotonic bath by a physiological electrolyte solution. It was shown (Knoblauch et al., 1988) that exposure of a suspension of OK-cells to hypotonic medium (190 mOsm), evokes cell swelling followed by a regulatory volume decrease (RVD), resulting in the original volume within 30 to 60 min. The cells swelled to approximately 50% of the predicted osmometric response. The maximal cell volume was reached after about 3 to 5 min. It was concluded from the previous study that during RVD the cells showed membrane depolarization, which is in accordance with our observations. The time course for both mechanisms, cell swelling (Knoblauch et al., 1988) and the cell depolarization is slow (Fig. 1), and occurs in the range of minutes. The slow time course could be caused by an interaction of two mechanisms, fast alterations in the second range of the transmembrane ion gradients upon dilution of the cellular contents and simultaneously an activation of ion transport systems which are responsible for the volume decrease (*see below*). Furthermore, the kinetics of the measured time course of the cell depolarization may be influenced by the pipette solution, since osmotically induced alterations of the composition of the cytosol are suppressed by a rapid equilibration with the pipette electrolyte.

By use of the cell-attached pipette configuration, it was observed that hypotonic challenge evoked in the membrane patch ion channels, with a single-channel conductance of about 22 pS. A decrease of osmolarity (310 to 190 mOsm) evoked up to six ion channels of this conductance, which caused an inward current at an applied potential of 0 mV (Figs. 2*a* and 4). The time scale for the activation ranged from a few seconds (< 15 sec) to about 1 min, which is within the time scale observed for maximal cell swelling (Knoblauch et al.,1988). Since the membrane potential depolarizes (Fig. 1) and the cell content is diluted, the electrochemical gradients should change. This was confirmed by the observation that the osmotically evoked single-channel current amplitude declined in on-cell records by about 30% within about 3 min (Figs. 2*a* and 4). In addition, the corresponding zero current potential shifted (Table). Within a variable period of time (3 to about 10 min), the evoked patch current returned to the resting state. Since the cells were used as cultured monolayers and have epithelial properties, it cannot be ruled out the further ion transport mechanisms are activated at the basal cell membrane. Due to the transient behavior of the current pattern, chemical reaction kinetics based on thermodynamic equilibrium is not applicable for ki-

netic analysis. The presented analysis of the osmotically produced membrane current was focussed on the inward current. In addition, we observed the activation of a further channel population, which dominated the outward current at depolarizing membrane potentials (*see* Fig. 2*b*). The latter was identified as an outward-directed potassium current, depending on cytoplasmic free calcium (Ubl, Murer & Kolb, 1988*b*) and will not be considered here. It is noteworthy that this potassium current could not be activated in the membrane patch by the application of a negative hydrostatic pressure to the pipette. Recently, it was shown that osmotic stress causes an increase of cytoplasmic calcium which activates a maxi-K channel (Christensen, 1987).

In the following we summarize the evidence supporting the suggestion that the same type of ion channel dominating the inward current can be activated by osmotic and mechanical stress.

1. Within the same membrane patch the identical number of ion channels can simultaneously be activated either by a hypotonic medium or by a negative hydrostatic pressure (*see* Fig. 4).

2. A one-to-one correspondence in the appearance of osmotically and mechanically activated channels was found. Either no channel could be evoked in the membrane patch by application of one of these gradients, or both mechanisms initiated the same number and size of current steps. Exposure of the membrane patch to a negative pressure pulse, and thereafter to an osmotic gradient, yielded the identical result as in the foregoing case. In both cases the evoked single-channel fluctuations were characterized by the occasional appearance of long lasting openings (*compare* Figs. 3 and 5).

3. The Table shows the single-channel conductance of the osmotically evoked channel, measured as close as possible to the mechanically activated ion channels during the successive channel closure. The data was acquired using different pipette solutions and agrees with the results derived from the mechanically activated ion channel within the given limits.

Finally, the ion selectivity of the channel, which had to be estimated from cell-attached experiments, will be discussed. Cell-free measurements could not be used to analyze the membrane response to osmotic stress, since with this pipette configuration the membrane separates two open systems. Furthermore, the number of osmotically-evoked ion channels decreased after changing to the inside-out configuration, and in most experiments no further channel activity could be observed. The data of the Table indicate that the channel can either conduct a cation inward current or a chloride outward current. Replacement of extracellular sodium by gluconate results in an chloride outward current. The data are comparable with those obtained for the stretch-activated ion channel (Table). An ion channel of similar conducting properties and ion selectivity has been reported in basolateral membranes of the late proximal tubule of the rabbit kidney (Gögelein & Greger, 1986). A chloride permeability has not been demonstrated for stretch-activated ion channels up to now. Most of the stretch-activated ion channels have been shown to be cation unselective (for review *see* Sachs, 1987). For skeletal muscle, a corresponding single-channel conductance of 35 pS (Guharay & Sachs, 1984) is reported with a doubling, if potassium is used instead of sodium. Similar data have been observed for stretch-activated channels in frog lens (Cooper et al., 1986).

The question arises whether the osmotic gradient can create a mechanical stress in the membrane and/or reduces the number of infoldings. For a cylindrical cell, which is limited for a longitudinal expansion of about a 50% volume increase, would cause about a 22% surface increase. It was found from the morphology of OK cells, which were analyzed as cell monolayers (Malmström & Murer, 1986), that the microvilli in the apical membrane are infrequent events. This qualitative observation would support the conclusion that hypotonic medium could cause membrane stretch in addition to a decrease in the number of microvilli.

In case of stretch-activated channels it is proposed for muscle cells that the transduction of the tension occurs via the viscoelastic actin meshwork, which is directly fixed to the transport molecule (Sachs, 1987). The application of negative pressure causes an increase in the area of the patch-membrane, and a pull of the actin meshwork is applied to the ion channel; as a result, the channel opens. It might be speculated that osmotic stress of the cell membrane, in general, also yields the same type of activation of the ion channels in the patched membranes. Such an interaction of the cytoskeleton and the channel could not only affect the obvious channel opening, but the ion selectivity as well. The association of the cytoskeleton with the channel protein(s) offers also an explanation toward the observation of a closure of the osmotic stress-activated channels, after transition from the on-cell to the cell-free mode.

The observation of an osmotic activation naturally raised the question of a possible relation of this ion channel to some sort of volume-regulatory mechanism. It has been shown for various epithelial and nonepithelial cell types that cell swelling induces a rapid loss of KCl and a regulatory decrease in cell volume (for review *see* Kregenow, 1981; Spring & Ericson, 1982; Hoffmann, 1986; Eveloff & Warnock, 1987). It has been shown that for epithe-

lia the membrane transport mechanisms (conductive and/or nonconductive) involved in the cellular loss of KCl are located in the basolateral membrane, i.e., co-located with the Na^+,K^+-ATPase (e.g. *see* Ussing, 1982; Lau, Hudson & Schultz, 1984; for review, *see* Spring and Ericson, 1982; Spring, 1985; Schultz & Hudson, 1986; Eveloff & Warnock, 1987). Also for renal proximal tubule, the involvement of basolateral K^+ conductance in RVD is clearly documented (Welling, Linshaw & Sullivan, 1985; Lopes & Guggino, 1987). In addition, Lopes and Guggino presented evidence for the involvement of a basolateral-located bicarbonate conductive pathway in RVD, in proximal tubule of *Necturus*. However, it should be noted that in RVD responses usually a Cl^--conductive pathway parallels K^+-conductive pathways. From membrane fractionation experiments we know that Na^+-K^+-ATPase in OK cells grown on impermeable supports as confluent monolayers is predominately located in the basolateral membrane (Malmstroem et al., 1987). Our patch-clamp measurements on subconfluent OK-cell monolayers do not allow us to locate the osmotically and mechanically sensitive ion transport system in either the apical or basolateral membrane of OK cells. Furthermore, it is also not clear whether the on-cell experiments are necessarily correlated with the conductance change induced by volume-regulatory response. The problem arises from the circumstance that in our patch-clamp experiments the osmolarity of the bath could not be changed in synchrony with the pipette solution.

Despite some ambiguity as to the localization of the measured channel type to the apical and basolateral membrane, and its possible involvement in volume regulatory responses, the following seems clear: that maneuvers which uniformly produce profound swelling of the cell by osmotic stress or mechanical membrane tension led to the activation of ion channels permeable for Na, K and Cl which were not conducting under normal transport conditions.

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