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Ca^{2+}-activated K^+ Channels Are Involved in Regulatory Volume Decrease in Acinar Cells Isolated from the Rat Lacrimal Gland

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Abstract. The volumes of acinar cells isolated from rat lacrimal gland were measured on computer by video-imaging. Cells were found to swell on exposure to hypotonic solutions; they subsequently exhibited a regulatory volume decrease (RVD). RVD was inhibited in the absence of extracellular Ca^{2+}, and by the K^+ channel blocker tetraethylammonium chloride (2 mM TEA^+). The possible involvement of K^+ channels in RVD was further investigated in cell-attached patches. Exposing the cells to a hypotonic solution activated channels with a conductance of 141 ± 6 pS (n = 11). These channels were partially blocked by 0.5 mM TEA^+, and channel activation was not observed in the absence of extracellular Ca^{2+}. Experiments in the inside-out patch configuration demonstrated that the channels activated by hypotonic stress were "maxi" Ca^{2+}-activated K^+ channels. It is concluded that the opening of these channels plays an important role in RVD, by facilitating K^+ loss from the cell.

Key words: Ca^{2+}-activated K^+ channels — Cell volume — Regulatory volume decrease — Tetraethylammonium — Lacrimal acinar cells

Introduction

When exposed to hypotonic media, cells behave as osmometers and swell. Many types of cells can subsequently regulate their volume so that they shrink back towards their original size. This phenomenon is commonly referred to as regulatory volume decrease (RVD), and often involves the net loss of K^+, Cl^- and water from the cytoplasm (*reviewed by* Hoffman & Simonsen, 1989). In many cell types, this loss of K^+ and Cl^- is achieved by the parallel activation of K^+ and Cl^- channels (*see* Sarkardi & Parker, 1991).

Recent patch clamp experiments in this laboratory have shown that exposing lacrimal gland acinar cells to hypotonic solutions activates whole-cell K^+ and Cl^- currents (Kotera & Brown, 1993). The Cl^- current activated by hypotonic stress was found to be carried by Ca^{2+}-activated Cl^- channels (Kotera & Brown, 1993). The nature of the K^+ channels involved, however, has not yet been studied. The activation of K^+ and Cl^- channels in these cells by hypotonic conditions may well be linked to RVD. However, little is known about volume regulation in lacrimal and other exocrine acinar cells. Salivary gland acinar cells have been shown to shrink when stimulated to secrete with acetylcholine (Foskett & Melvin, 1989; Nakahari et al., 1990; Larcombe-McDouall et al., 1991; Moran & Turner, 1993). Foskett et al. (1994) have also reported that acinar cells isolated from rat parotid salivary glands undergo RVD when bathed in hypotonic solutions. Steward and Larcombe-McDouall (1989), however, found that acinar cells in the intact perfused rat mandibular salivary gland did not regulate their volume when exposed to hypotonic or hypertonic media.

In the present study, we have measured cell volume changes in isolated rat lacrimal acinar cells by video-imaging. We found that these cells exhibit RVD when exposed to hypotonic solutions. We have also used cell-attached patch experiments to show that Ca^{2+}-activated K^+ channels are activated by hypotonic stress and appear to be involved in the RVD. A preliminary account of some of these data has been presented to the Physiological Society (Park & Brown, 1994).

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Materials and Methods

Cell Preparation

Single isolated lacrimal gland acinar cells were prepared using the procedure described in our previous paper (Kotera & Brown, 1993). The method involved incubating small pieces of gland with trypsin and collagenase. The tissue was then dissociated by repeated pipetting. The resulting single acinar cells were resuspended in Medium 199 (Sigma), and stored in an incubator at 37°C.

Cell Volume Measurements

Clean pieces of glass coverslip (2 × 4 mm; washed in acetone and ethanol), were placed on the bottom of a 35 mm culture dish and covered with Medium 199. Six drops of the freshly isolated cell suspension were then added to the culture dish directly above each coverslip. The dishes were then placed in an incubator at 37°C with 5% CO_2 for 1 hr to allow the cells to adhere to the coverslips. A single coverslip was then placed in the experimental chamber (volume = 50 μl) on the stage of an inverted microscope (World Precision Instruments). The bath was perfused at a flow rate of 3 ml/min. The control (isotonic) bath solution contained (mM): NaCl, 140; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; glucose, 5 and HEPES, 5 (pH adjusted to 7.3 with HCl; osmolality = 280.3 ± 4.5 mOsm, n = 5). The hypotonic solution (osmolality = 191.4 ± 1.2 mOsm, n = 5), was similar to the control solution except that it contained only 90 mM NaCl. In a single series of experiments 100 mM mannitol replaced 50 mM NaCl in the isotonic solution (279 ± 8 mOsm, n = 3). In some experiments 90 mM NaCl was replaced by 90 mM Na glucuronate in the hypotonic solution (196 mOsm; osmolality was adjusted to this value by the addition of 15–20 mM mannitol). The osmolality of the solutions was measured by the freezing-point depression method using a Roebling micro-osmometer (Camlab, Cambridge, UK). All experiments were performed at room temperature (19–23°C).

Cells were observed through a bright-field 25× objective lens, and video images of cells recorded using an EDC-1000 computer camera (Electrim, Princeton, NJ). The images were saved directly on a hard disk at intervals of 0.5, 1, 2 or 5 min. The pixel resolution of each image was 192 × 165 which corresponds to an area of 47 × 40 μm. In subsequent analysis, the area of the image was estimated using AVS computer software (Hewlett Packard), i.e., the number of pixels bounded by the perimeter of each cell was determined. Cell volume was calculated assuming that each cell was spherical (mean volume = 5.93 ± 0.83 pl, n = 10). In all experiments the volume was normalized to the initial volume observed during control superfusion with isotonic solution.

Patch Clamp Recording

Cells were allowed to settle at the bottom of a recording chamber (volume 400 μl), and viewed with an Olympus CK2 inverted microscope. The cells were superfused (3 ml · min^{-1}) with a NaCl-rich, control solution containing (mM): 90 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, 100 mannitol, pH 7.3 (pH adjusted with NaOH). Electrodes were manufactured from hematocrit capillaries (Blue Tip, Monoject Scientific, Eire). These were filled with a K^+-rich solution containing (mM): 140 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 HEPES, pH 7.3 (pH adjusted with KOH), and had tip resistances of between 6 to 8 MΩ. High resistance seals (20 to 40 GΩ) were obtained with about 40% of the electrodes. Single channel activity, in cell-attached or inside-out patches, was monitored with an Axopatch-1B amplifier (Axon Instruments, Foster City, CA). The patches were clamped at a series of potentials and the resultant current records were stored as a digital signal on video tape (40 kHz) using a modified digital audio processor (Sony PCM701).

The control level of activity in cell-attached patches was established by recording single channel currents from cells bathed in the control solution for 1 min. The bath solution was then changed to one of several test solutions for 1 or 2 min. The hypotonic solution was produced by omitting mannitol from the control bath solution. Ca^{2+}-free solutions were made by omitting $CaCl_2$ and adding 0.5 mM EGTA. In the inside-out patch experiments KCl-rich and NaCl-rich bath solutions were used. These were similar to the control solution except that 140 mM KCl or 140 mM NaCl replaced the 90 mM NaCl and 100 mM mannitol. The Ca^{2+} activity in these bath solutions was buffered with EGTA either at 100 nM (0.596 mM $CaCl_2$ and 1 mM EGTA), or 5 nM (0.072 $CaCl_2$ and 1 mM EGTA; *see* Brown, Loo & Wright, 1988). All experiments were performed at room temperature (19–23°C).

Data Analysis

Single channel current amplitudes were measured using either the cursors on a digital storage oscilloscope (Gould 1425), or by computer using the software package pCLAMP 5.5.1 (Axon Instruments). The usual conventions of current flow were observed throughout, i.e., positive charge moving out of the cell (into the electrode) is a positive current. The potential applied to the electrode in cell-attached patches ($-V_p$) does not include the contribution of the membrane potential of the cell. V_m quoted for inside-out patches is the reverse of the applied electrode potential (i.e., $-V_p = V_m$). Lines through the current-voltage (I–V) relationships were fitted by linear regression analysis. The Constant Field equation was used to fit a line through data from an inside-out patch.

Open probabilities (P_o) were determined for sequential 20 sec periods of data using the pCLAMP program. Data were not included for analysis if the control P_o was greater than 0.02, or if patches contained more than two channels. The number of channels in each patch was counted at the end of a recording following excision into the inside-out patch configuration (bath Ca^{2+} at 1 mM). The P_o for a patch containing two channels was estimated, assuming that the channels opened independently of each other, using Eq. (1).

$$P_o = \frac{\text{Time at level 1} + \text{Time at level 2}}{\text{Total time} \times 2} \quad (1)$$

Results

Volume Changes Caused by Hypotonic Shock

The effects of hypotonic bathing solutions on the relative volumes of lacrimal acinar cells are shown in Fig. 1*A*. The volume of eight cells was first measured in the control solution (140 mM NaCl) over a 1 min period. When the cells were superfused with the hypotonic solution (90 mM NaCl; shown by the bar), the mean cell volume increased to a maximum size of 1.32 ± 0.01. This volume was reached after a 120 sec exposure to the hypotonic solution. Cell volume then slowly decreased, so that after 20 min in hypotonic conditions the volume was only 1.18 ± 0.05 (significantly different from max-

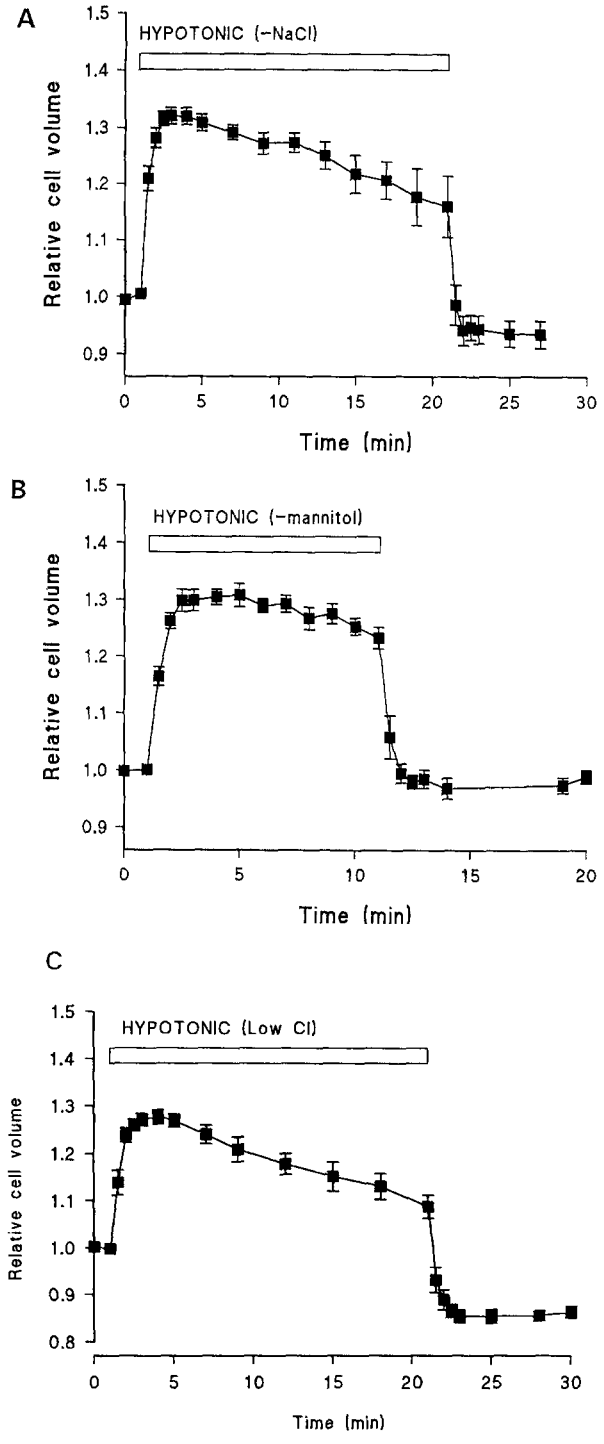

Fig. 1. Volume changes in lacrimal gland acinar cells exposed to hypotonic solutions. Cells were exposed to hypotonic solution (*A* and *B*, 90 mM NaCl; 191 mOsm) for the period indicated by the bar. The isotonic control solution was similar to the hypotonic solution except it contained an additional 50 mM NaCl (*A* and *C*, 280 mOsm), or 100 mM mannitol (*B*, 279 mOsm). (*C*) 90 mM Cl^- in the hypotonic solution was replaced by 90 mM glucuronate (196 mOsm) to increase the gradient for Cl^- efflux. Cell volume was monitored by measuring the area of a video image of the cell. Volumes were then calculated assuming the cell to be spherical, and expressed as a fraction of the control volume (measured over the first minute of the experiment in isotonic conditions). Data are mean ± SEM of eight experiments in *A*, and six experiments in *B* and *C*.

imum value, $P < 0.01$). When the cells were returned to the isotonic solution, they shrank to a relative volume of 0.95 ± 0.03. Each of the eight cells examined responded in this way. Similar results were also obtained when an isotonic solution containing 100 mM mannitol and 90 mM NaCl was replaced by the hypotonic solution (Fig. 1*B*). The maximum cell volume (1.31 ± 0.02, $n = 5$), was attained after 180 sec. Mean cell volume then decreased over the next 8 min to 1.23 ± 0.02 (significantly different from maximum value; $P < 0.01$). On returning to the isotonic medium, the cells shrank to a volume of 0.97 ± 0.02, before recovering over the next 8 min to a volume of 0.99 ± 0.01.

Figure 1*C* shows the effect of removing 140 mM Cl^- from the hypotonic solution (replaced with glucuronate). This maneuver was designed to increase the gradient for Cl^- exit from the cells, and volume regulation appeared to be enhanced under these conditions. In six experiments, the cell swelled to a maximum volume of 1.28 ± 0.01 (3 min), and then shrank to 1.09 ± 0.03 during the following 17 min. On returning to isotonic solutions, the cells quickly shrank to a volume of 0.85 ± 0.01). Replacing Cl^- with glucuronate under isotonic conditions caused only a slight decrease in cell volume to 0.98 in 20 min ($n = 4$).

Cell Volume Regulation Is Inhibited by Tetraethylammonium and Ca^{2+}-free Conditions

Tetraethylammonium (TEA^+) has been reported to inhibit Ca^{2+}-activated K^+ channels in lacrimal gland acinar cells (Trautmann & Marty, 1984; Lechleiter, Dartt & Brehm, 1988). Experiments were performed in the presence of TEA^+ to investigate the possibility that these channels are involved in the regulation of cell volume. Figure 2*A* shows that exposing cells to the hypotonic solution in the presence of 2 mM TEA^+, caused the cells to swell to a relative volume of 1.27 ± 0.02 ($n = 8$; maximum volume attained after 240 sec). Under these conditions, however, the recovery of the cell volume appeared to be inhibited (relative volume after 20 min was 1.27 ± 0.02, not different from the maximum volume, $P > 0.1$). In three control experiments in which cells were exposed to 2 mM TEA^+ in isotonic conditions for 10 min, no significant changes in cell volumes were observed (*data not shown*).

Experiments were performed in Ca^{2+}-free solutions to investigate the involvement of extracellular Ca^{2+} in cell volume regulation. Figure 2*B* shows results from nine experiments in which the cells were exposed to a Ca^{2+}-free, hypotonic solution. The cells swelled to a volume of 1.28 ± 0.02 within 120 sec of exposure to this solution (Fig. 2B). The subsequent recovery of cell volume, however, was greatly inhibited (volume after 20 min = 1.24 ± 0.02, not significantly different from maximum volume, $P > 0.1$). In control experiments in

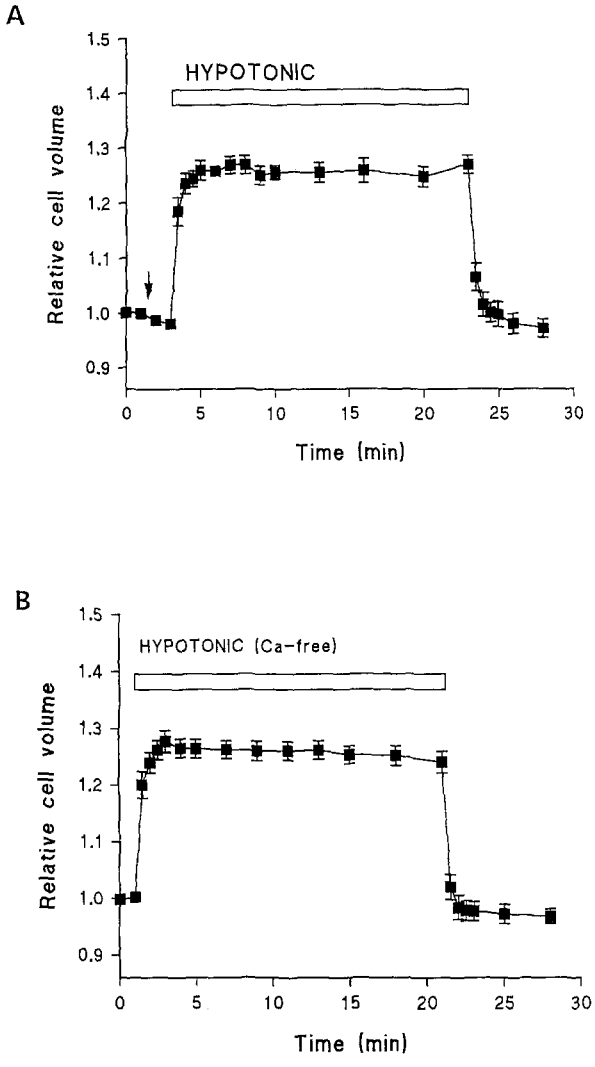

Fig. 2. Inhibition of lacrimal gland cell volume regulation by TEA^+ or Ca^{2+}-free media. (*A*) 2 mM TEA^+ was added to the isotonic solution as indicated by the arrow, and was present throughout the superfusion with hypotonic solution (bar). (*B*) Cells were exposed to Ca^{2+}-free, hypotonic solution (0.5 mM EGTA and no added $CaCl_2$). Cell volume was measured as in Fig. 1, and the isotonic solution contained 140 mM NaCl. Data are mean ± SEM of (*A*) eight and (*B*) nine experiments.

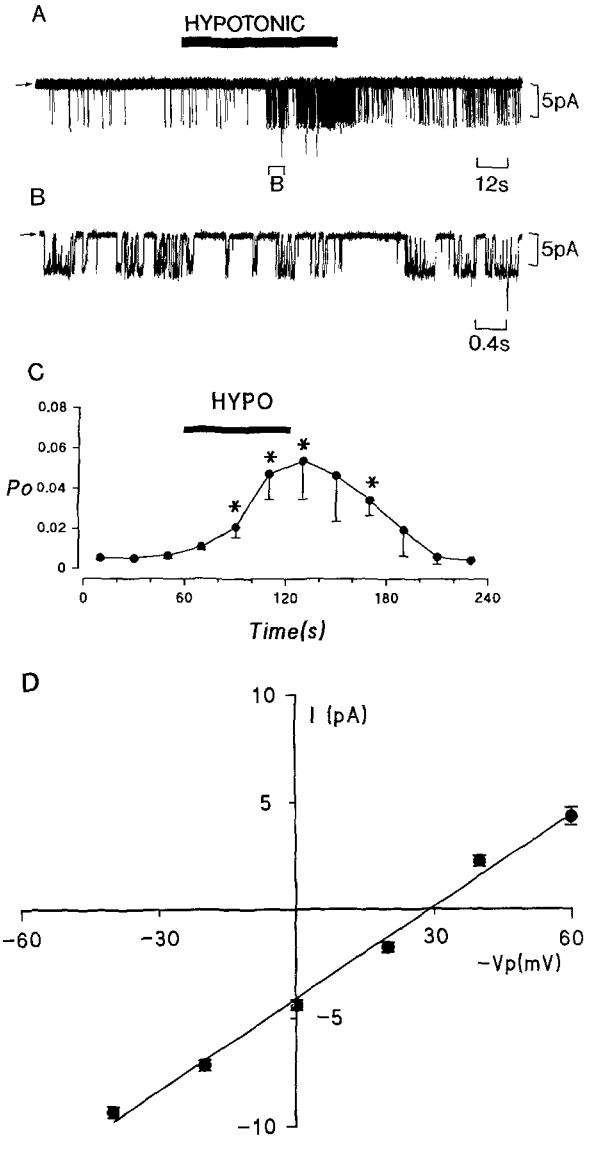

Fig. 3. K^+ channel activation under hypotonic conditions. (*A*) Single channel records from a cell-attached patch containing two K^+ channels. The patch pipette contained a K^+-rich solution, and no potential was applied ($-V_p = 0$ mV). The downward current deflections (inward current) represent channel openings. The cell was superfused with hypotonic solution for the period indicated by the filled bar. (*B*) A 40 sec period of single channel data from Fig. 3*A* displayed on a faster time scale. (*C*) Channel open probability (P_o) plotted as a function of time, for the experiment shown in Fig. 3*A* and four other similar experiments. P_o was calculated for 20 sec periods of channel activity, and the results are expressed as mean ± SEM. (*D*) Current-voltage relationship for the channel in Fig. 3*A* and in 10 other patches. The line through the data was fitted by linear regression. The mean conductance was 141 ± 6 pS and the reversal potential is at $-V_p = 30.1$ ± 1.9 mV.

which the cells were exposed to an isotonic Ca^{2+}-free solution for 5 min (0 $CaCl_2$, 0.5 mM EGTA), cell volume did not change ($n = 4$, *data not shown*).

Activation of K^+ Channels by Hypotonic Stress

Figure 3*A* shows single channel currents from a cell-attached patch on a lacrimal acinar cell which contained two identical channels. The trace shows continuous activity from the patch before, during and after superfusion of the cell with a hypotonic solution. The patch was held with no applied pipette potential throughout the experiment ($-V_p = 0$), and channel openings were observed as downward current steps (mean current amplitude = −4.4 ± 0.2 pA). These inward currents could be carried either by K^+ moving from the electrode

into the cell, or by Cl^- moving in the opposite direction. Channel openings were very infrequent in the control solution. A sudden increase in channel activity was observed within 40 sec of exposing the cell to the hypotonic solution. Figure 3*B* shows a 6.8 sec period of channel activity on an expanded time scale. This period of activity is marked *B* in Fig. 3*A*, and shows channel activity after the introduction of the hypotonic solution. Individual channel openings can be seen more clearly in this trace, and the simultaneous opening of the two channels in the patch can also be observed. Open probability (P_o) for the channels in this experiment increased from 0.003 in the control solution to a maximum value of 0.193 after a 90 sec exposure to the hypotonic solution. Channel activity declined towards the resting value when the cell was returned to the control solution.

An increase in channel activity was caused by hypotonic solutions in five experiments. The mean values of P_o from these experiments are plotted in Fig. 3*C*. A significant increase in P_o was observed within 40 sec of switching to the hypotonic solution ($P < 0.05$). The *I–V* relationship for the channels activated in these experiments, and in six other patches, is shown in Fig. 3*D*. The line through the data was fitted by linear regression analysis and gives a conductance of 141 ± 6 pS, (current reversal at $-V_p$ = 30.1 ± 1.9 mV, n = 11). The conductance of the channel is consistent with that previously measured for Ca^{2+}-activated K^+ channels in these cells (Findlay, 1984; Trautmann & Marty, 1984; Lechleiter et al., 1988). In three experiments, an increase in K^+ channel activity was observed in less hypotonic conditions (110 mM NaCl, *data not shown*).

The Effects of Hypotonic Solutions on K^+ Channel Activity in Ca^{2+}-free Conditions

Figure 4*A* shows single channel records from a cell which was exposed to hypotonic conditions in the absence of extracellular (bath) Ca^{2+}. In this experiment removing Ca^{2+} from the bath solution under isotonic conditions did not cause any change in channel activity. When the cell was superfused with the Ca^{2+}-free, hypotonic solution, channel activity did not appear to increase. Averaged data for P_o from this and three similar experiments are shown in Fig. 4*B* (data from one other experiment in which the Ca^{2+}-free, isotonic solution caused a decrease in channel activity are not included). In each of the four cells examined, superfusion with the hypotonic, Ca^{2+}-free solution failed to cause an increase in P_o (Control = 0.007 ± 0.001; Ca^{2+}-free = 0.008 ± 0.001; Hypotonic, Ca^{2+}-free = 0.005 ± 0.001). Figure 4*C* shows data from a series of six experiments in which the role of Ca^{2+} in K^+ channel activation was further examined. In these experiments a 2 min exposure of the cell to a Ca^{2+}-free, hypotonic solution failed to increase P_o. However, the addition of Ca^{2+} to the hypotonic bath solution did cause a significant increase in P_o, from 0.003 ± 0.001 in control conditions (with Ca^{2+}), to a peak value of 0.036 ± 0.001 ($P < 0.05$).

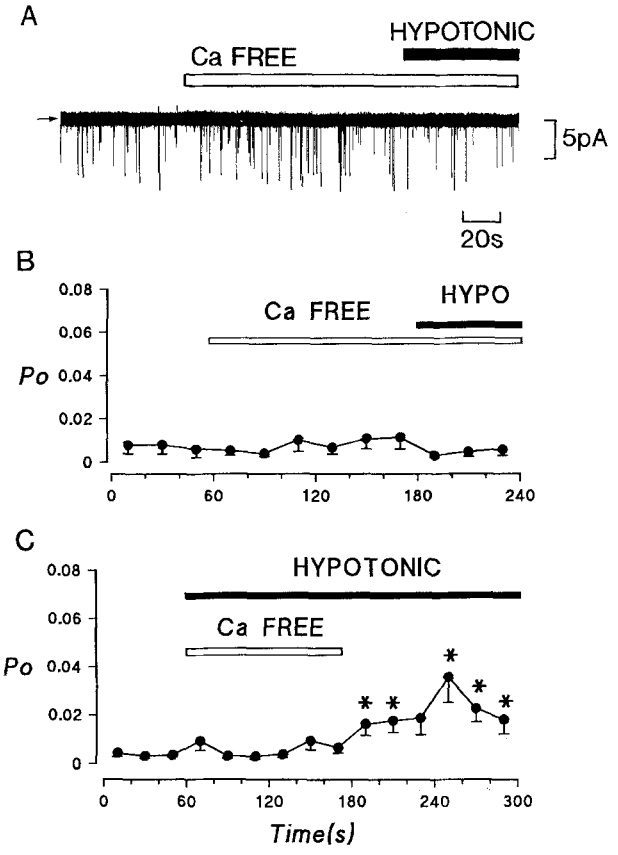

Fig. 4. K^+ channel activation by hypotonic stress is inhibited by Ca^{2+}-free solutions. (*A*) Single channel records from a cell-attached patch (K-rich electrode solution and $-V_p$ = 0 mV). The cell was exposed to: a Ca^{2+}-free isotonic solution and subsequently to Ca^{2+}-free, hypotonic solution. The Ca^{2+}-free solutions contained 0.5 mM EGTA and 0 $CaCl_2$. (*B*) Averaged data on P_o plotted as a function of time, from Fig. 4*A* and three other experiments. (*C*) P_o for K^+ channels in six cell-attached patches during superfusion with hypotonic, Ca^{2+}-free solution and subsequently with hypotonic solution containing Ca^{2+} (1 mM $CaCl_2$).

K^+ Channel Block by TEA^+

Figure 5A shows single channel records from a cell-attached patch containing two K^+ channels, held at $-V_p$ = 0 mV. In this experiment 0.5 mM TEA^+ was included in the pipette solution. TEA^+ is known to be a "fast blocker" of Ca^{2+}-activated K^+ channels, and in this experiment the current amplitude was reduced to about −1.9 pA (Fig. 5*A*). This is less than half of the K^+ current normally observed (−4.4 pA, *see* Fig. 3*A*). The trace in Fig. 5*A* is a continuous record from a cell which was superfused with control and subsequently with hy-

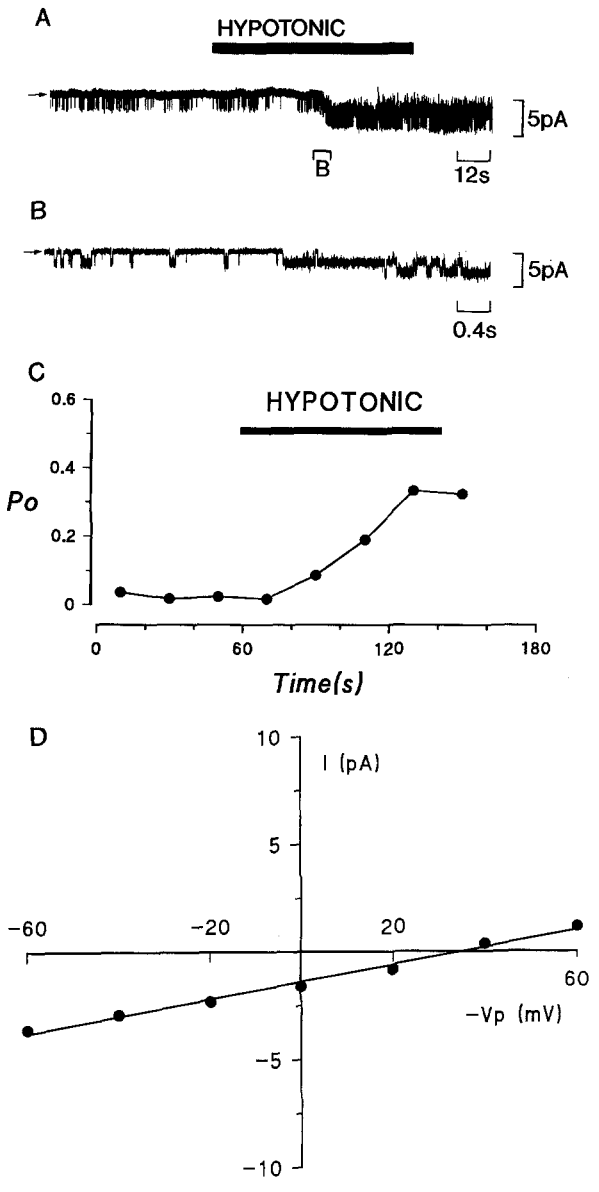

Fig. 5. The K^+ channel activated by hypotonic stress is blocked by TEA^+. (*A*) Single channel activity from a cell-attached patch containing two K^+ channels, recorded with K^+-rich pipette solution which also contained 0.5 mM TEA^+. The cell was bathed in hypotonic solution for the period indicated by the bar. (*B*) A 40 sec period of single channel data from Fig. 5*A* displayed on a faster time scale. (*C*) Plot of P_o as a function of time for the channel in Fig. 5*A*. (*D*) *I–V* relationship for K^+ channel activity recorded with 0.5 mM TEA^+ in the electrode solution. The apparent conductance of the channel was 42.8 ± 2.1 pS and the reversal potential was $-V_p$ = 32.7 ± 3.0 mV (n = 4).

potonic solutions. An increase in channel activity was observed despite the presence of TEA^+. Figure 5*B* shows the initial period of channel activation on an expanded time scale. An increase in both the frequency and the duration of channel openings could clearly be seen when the cell was exposed to hypotonic solution. At the peak of channel activation, channel openings to a second current level were observed due to the simultaneous opening of the two channels. A plot of P_o against time for the two channels in this experiment is shown in Fig. 5*C*. P_o increased from 0.026 in control conditions to 0.389 in the hypotonic solution. Similar results to those in Fig. 5*C* were obtained in two other cells.

The addition of 0.5 mM TEA^+ to the pipette solution was found to reduce the current amplitude in a total of four cell-attached patches. The *I–V* relationship from these channels is shown in Fig. 5*D*. The average conductance was significantly reduced by TEA^+ compared to that measured in control conditions to 42.8 ± 2.1 pS (n = 4; $P < 0.01$). The reversal potential, however, was not significantly changed under these conditions ($-V_p$ = 32.7 ± 3.0 mV).

Properties of the K^+ Channel in an Excised Patch

To characterize the channels activated by hypotonic stress in more detail, experiments were performed in the inside-out patch configuration. Figure 6*A* shows single channel records, at three hyperpolarizing potentials, from an inside-out patch bathed in the KCl-rich solution containing 5 nM Ca^{2+}. Inward currents were observed at each potential; the current amplitude increased with hyperpolarization, while P_o decreased. When the Ca^{2+} activity in the bath was increased to 100 nM, an increase in P_o at all holding potentials was observed (Fig. 6*B*). *I–V* relationships for the K^+ channel in this inside-out patch are shown in Fig. 6*C*. Data are currents recorded with either the 140 mM KCl bath solution (●), or in the 140 mM NaCl solution (■). The line through the data recorded with KCl in the bath gave a conductance of 180 pS with current reversal at V_m = 4.4 mV (i.e., close to the expected equilibrium potential for K^+, V_m = 0). With the NaCl-rich solution, the *I–V* relationship deviated from linearity. The curve through these data was fitted using the Constant Field equation, assuming that the channel is permeable only to K^+.

Discussion

In an earlier study we showed that hypotonic solutions cause an increase in whole-cell Cl^- and K^+ currents in lacrimal gland acinar cells (Kotera & Brown, 1993). We also showed that the Cl^- current was carried by Ca^{2+}-activated Cl^- channels. In the present study the nature of the K^+ channels activated by hypotonic stress was examined, and the involvement of these channels in RVD assessed.

Activation of K^+ Channels

Exposure of lacrimal acinar cells to hypotonic solutions was found to activate channels in cell-attached

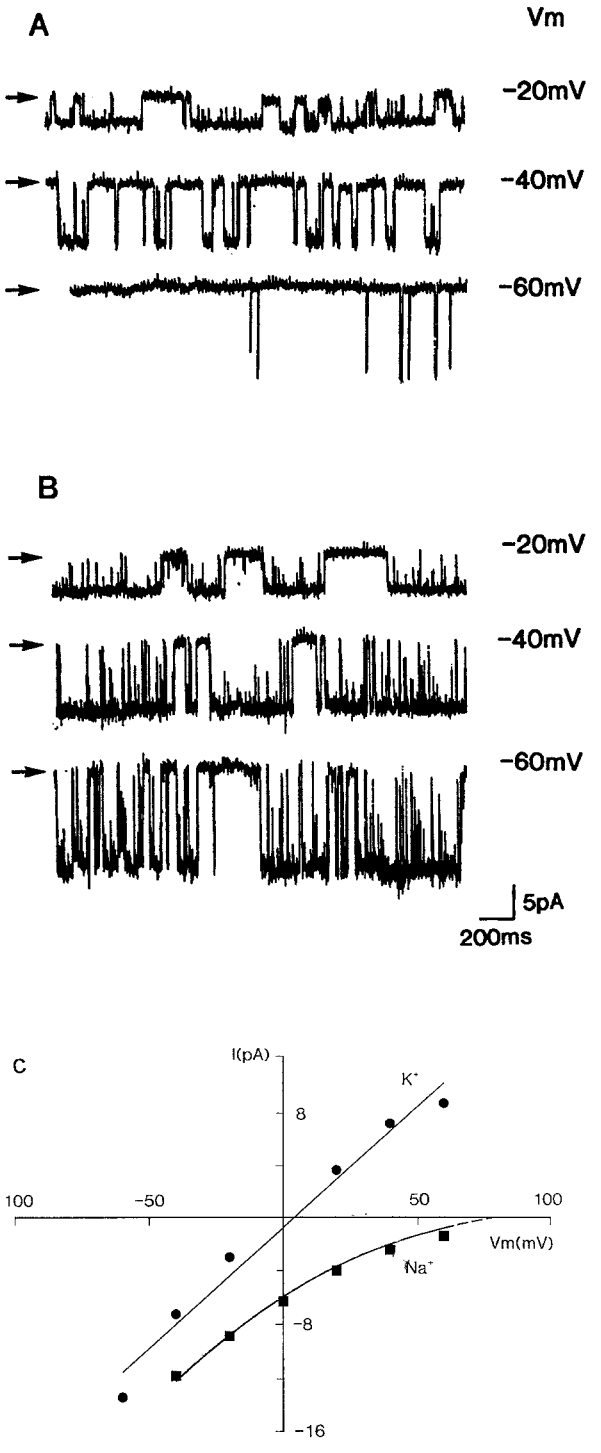

Fig. 6. Properties of the K^+ channel in an inside-out patch. (*A* and *B*). Single channel records for a channel in a patch bathed with K^+-rich solutions (140 mM KCl). Channel openings are shown as downward current steps (arrow indicates closed state). The Ca^{2+} activity in the bath solutions was 5 nM in *A*, and 100 nM in *B*. (*C*) *I–V* relationship for the same channel as in Fig. 6*A* and *B*. The bath solution contained: 140 mM KCl (●), or 140 mM NaCl (■). The straight line was fitted through the K^+-rich data by linear regression (conductance = 180 pS; reversal potential at V_m = 4.4 mV). The curve through the NaCl bath data was fitted using the Constant Field equation assuming that the channel was permeable only to K^+.

patches on the basolateral membrane (*see* Fig. 3). The activated channels carried inward currents (with a KCl electrode solution). The full current-voltage relationship for the channel gave a conductance of 141 pS, and the current reversed at an applied pipette potential ($-V_p$) of 30.1 mV (*see* Fig. 3*C*). These values are similar to those for "maxi" Ca^{2+}-activated K^+ channels which have been previously described in lacrimal gland cells (Findlay, 1984; Trautmann & Marty, 1984; Lechleiter et al., 1988). The channel activated by hypotonic conditions was also partially blocked by 0.5 mM TEA^+ (Fig. 5). The apparent reduction of channel conductance by TEA^+ is another property which is characteristic of "maxi" Ca^{2+}-activated K^+ channels in these cells (Trautmann & Marty, 1984; Lechleiter et al., 1988). The properties of the channels were further investigated in excised inside-out patches (Fig. 6). These experiments demonstrated that the channels were regulated by Ca^{2+} (Fig. 6*A* and *B*), and that they were selective to K^+ against Na^+ and Cl^- (Fig. 6*C*).

The data from these single channel experiments strongly suggest that hypotonic conditions increase the activity of "maxi" Ca^{2+}-activated K^+ channels in lacrimal gland acinar cells. The activation of these channels by hypotonic stress has not been previously observed in acinar cells from exocrine glands. However, there are a number of reports of similar phenomena in other epithelia, e.g., choroid plexus (Christensen, 1987), kidney proximal tubule (Dubé, Parent & Sauvé, 1990), and kidney thick ascending limb cells (Taniguchi & Guggino, 1989). The activation of the channels in the choroid plexus and kidney proximal tubule was thought to be caused by an increase in intracellular Ca^{2+}, and could be inhibited by removing extracellular Ca^{2+}. However, it has also been suggested that the actual physical distortion of the membrane caused by cell swelling could directly activate the K^+ channels in cells from the kidney thick ascending limb (Taniguchi & Guggino, 1989), and also in osteoblast cells (Davidson, 1993). This possibility has not been assessed directly in the present study, but the fact that the removal of external Ca^{2+} inhibited channel activation (Fig. 4), suggests that an increase in intracellular Ca^{2+} may be involved in the lacrimal gland. The same conclusion was also reached for Cl^- current activation by hypotonic shock in these cells (Kotera & Brown, 1993).

Cell Volume Regulation

We have now shown that in lacrimal gland acinar cells, hypotonic stress activates both K^+ channels (this study) and Cl^- channels (*see* Kotera & Brown, 1993). The activation of these channels is thought to be a consequence of cell swelling, and may lead to cell shrinkage (i.e., RVD). In the present study this hypothesis was tested by measuring the volume of cells exposed to hy-

potonic media. Reducing the osmolality of the bathing solution from 280 to 191 mOsm, by reducing the NaCl concentration, caused the cells to swell rapidly. They reached a maximum volume, within 120 sec of exposure to the hypotonic media, which was 32% greater than the control volume. This value is close to that expected if the cell behaved as a perfect osmometer (i.e., 31% increase; estimated assuming an intracellular osmolality of 280 mOsm and that approximately 34% of the total volume is osmotically inactive in acinar cells; *see* Nakahari et al., 1992).

Volume regulatory mechanisms appeared to be activated with a lag-time of about 2 min, and caused a reduction of cell volume to 18% above control over a period of 20 min (Fig. 1*A*). This regulation of cell volume appeared similar to RVD described in other cells (Hoffman & Simonsen, 1989; Sarkardi & Parker, 1991). It is almost certainly the result of the loss of osmotically active substances (probably K^+ and Cl^-) from the cytoplasm, since the cells shrank to below their original control volume when they were returned to the isotonic medium (Fig. 1*A*). The slow rate of cell volume regulation (e.g., 1.32 to 1.18 in 20 min; Fig. 1*A*), may be due to the fact that Cl^- is only slightly above equilibrium in these cells (≈1.4 times equilibrium; Saito et al., 1985), so that there is only a small gradient driving Cl^- efflux. This hypothesis is supported by the data in Fig. 1*C* which show that volume regulation was enhanced when the gradient for Cl^- exit was increased by removing extracellular Cl^-.

Volume regulation in response to hypotonic stress has been demonstrated in many cells, including a variety of epithelia, but it has not previously been reported for lacrimal gland acinar cells. Acinar cells isolated from the rat parotid salivary gland also undergo RVD in hypotonic solutions; however, Steward and Larcombe-McDouall (1989) found that acinar cells in the intact, perfused rat mandibular salivary gland did not volume regulate. One explanation for these differences is that the regulation of ion transport varies in acinar cells from different glands, e.g., we envisage a role for intracellular Ca^{2+} in RVD in lacrimal gland cells, whereas Foskett et al. (1994) suggested that an increase in intracellular Ca^{2+} is not directly involved in RVD in parotid cells. Another possible explanation we considered was that different experimental protocols have been used, e.g., Steward and Larcombe-McDouall (1989) used an isotonic solution containing sucrose, with sucrose omitted to give the hypotonic solution. However, cell volume regulation was observed in similar experiments using mannitol replacement in the present study (Fig. 1*B*). Another methodological difference is that Steward and Larcombe-McDouall (1989) studied cells in the intact perfused gland. Ion transport in cells from the perfused gland might not be the same as in single isolated cells; e.g., regulation of intracellular pH appears to be different (*see* Steward, Seo & Case, 1989; Elliott, Lau & Brown, 1991). It will be interesting to see if cells isolated from the submandibular salivary gland behave more like lacrimal gland and parotid cells in hypotonic conditions (i.e., show RVD).

The Mechanism of Cell Volume Regulation

In many types of cells, volume regulation in response to hypotonic stress occurs by K^+ and Cl^- loss through channels which are activated as a result of cell swelling (Hoffman & Simonsen, 1989). The involvement of the "maxi" Ca^{2+}-activated K^+ channels in lacrimal gland cells was therefore tested by measuring cell volume in conditions which either block these channels or inhibit channel activation. First, K^+ channels were blocked using 2 mM TEA^+. This concentration should almost completely block the channels, since the apparent conductance was reduced by about 60% in the presence of 0.5 mM TEA^+ (Fig. 5), in agreement with the previously measured dissociation constant of 0.23–0.3 mM (*see* Blatz & Magleby, 1984; Brown et al., 1988). Volume regulation was almost completely inhibited by 2 mM TEA^+ (Fig. 2*A*). Volume regulation was also inhibited when Ca^{2+} was removed from the hypotonic solution bathing the cells (Fig. 2*B*). This same maneuver also inhibited K^+ channel activation in this study (Fig. 4), and Cl^- current activation in a previous study (Kotera & Brown, 1993). These observations suggest that "maxi" Ca^{2+}-activated K^+ channels have an important role in lacrimal cell volume regulation. The simultaneous opening of K^+ and Cl^- channels presumably facilitates KCl loss from the cytosol, leading to RVD regulation.

The activation of the Cl^- currents was found to be dependent on extracellular Ca^{2+}, and it was suggested that an influx of Ca^{2+} is caused by cell swelling, possibly via stretch-activated nonselective cation channels. The resultant increase in intracellular Ca^{2+} concentration then causes Cl^- channel activation (Kotera & Brown, 1993). The same mechanism may be involved in K^+ channel activation, since both channel activity and volume regulation were found to be dependent on extracellular Ca^{2+}. The elevation of intracellular Ca^{2+} by Ca^{2+} entry has previously been proposed to initiate volume regulation in some other epithelial cells, e.g., amphibian choroid plexus (Christensen, 1987) and intestinal epithelial cells (Hazama & Okada, 1988).

In summary, "maxi" Ca^{2+}-activated K^+ channels are activated as a result of cell swelling in lacrimal gland acinar cells exposed to hypotonic solutions. These channels appear to be involved in cell volume regulation, since maneuvers which inhibit channel activity also inhibit RVD, e.g., application of TEA^+ as a channel blocker and removal of extracellular Ca^{2+}. Channel ac-

tivation during RVD is probably the result of an increase in intracellular Ca^{2+}.

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