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Calcium-Dependent Chloride Current Activated by Hyposmotic Stress in Rat Lacrimal Acinar Cells

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Abstract. We have identified a whole-cell Cl^- current activated by hyposmotic stress in rat lacrimal acinar cells using the patch-clamp technique. Superfusion of isolated single cells with hyposmotic solution (80% of control osmolarity) caused a gradual increase of the current, which was reversed on return to the control solution. The current-voltage relationship showed outward rectification, and the current showed time and voltage dependence: slowly activated by depolarizing voltages and rapidly inactivated by hyperpolarizing voltages. The increase in current was not observed when intracellular Ca^{2+} was chelated with EGTA. It was also inhibited by the absence of extracellular Ca^{2+}, or the presence of gadolinium ions (20 μM Gd^{3+}). We conclude that in rat lacrimal acinar cells hyposmotic stress activates Ca^{2+}-dependent Cl^- channels as a result of Ca^{2+} influx through a Gd^{3+}-sensitive pathway. The Cl^- channels involved appear to be indistinguishable from those activated by muscarinic stimulation. The inhibitory effect of Gd^{3+} suggests that stretch-activated nonselective cation channels may be responsible for the Ca^{2+} influx.

Key words: Ca^{2+}-dependent Cl^- channels—Cell volume—Gadolinium—Lacrimal acinar cells

Introduction

Most cells, when placed in hyposmotic media, initially swell and then subsequently regulate their volume back towards control values (regulatory volume decrease; RVD). This is usually accomplished through a net loss of KCl and water from the cytosol (*reviewed by* Hoffmann & Simonsen, 1989). Several mechanisms of KCl loss are known to be involved in RVD. However, parallel activation of K^+ and Cl^- channels is probably the most common mechanism in many types of cells, including epithelia (*reviewed by* Sarkadi & Parker, 1991). It has been suggested that intracellular Ca^{2+} plays a fundamental role in controlling KCl efflux during RVD (*reviewed by* Pierce & Politis, 1990). Studies with Ca^{2+}-sensitive fluorescent dyes have demonstrated a significant increase in intracellular Ca^{2+} following hyposmotic stress in a human intestinal epithelial cell line (Hazama & Okada, 1990), in MDCK cells (Rothstein & Mack, 1990), in rabbit proximal tubule (Suzuki et al., 1990; McCarty & O'Neil, 1991) and in medullary thick ascending limb cells (Montrose-Rafizadeh & Guggino, 1991). Recent patch-clamp studies of epithelial cells have shown that K^+ channels are activated by the increase in intracellular Ca^{2+} during RVD (Hazama & Okada, 1988; Weiss & Lang, 1992). It has been suggested that Ca^{2+} influx through stretch-activated nonselective cation channels may be responsible for the increase in intracellular Ca^{2+} and hence for the activation of K^+ channels (Christensen, 1987; Filipovic & Sackin, 1991). In contrast, the Cl^- channels involved in RVD seem to be Ca^{2+} independent in several types of epithelial cells (Hazama & Okada, 1988; Worrell et al., 1989; Solc & Wine, 1991; Banderali & Roy, 1992; Weiss & Lang, 1992).

Cell volume regulation has not been studied in detail in the acinar cells of exocrine glands. It has recently been reported, however, that salivary acinar cells undergo changes in cell volume during muscarinic stimulation (Foskett & Melvin, 1989; Foskett, 1990). The ion transport pathways involved in volume regulation have not yet been identified in these cells. In the present study, we have investigated the effects of hyposmotic stress on the Cl^- conductance in rat lacrimal acinar cells. The results show that Ca^{2+}-dependent Cl^- channels are activated by hyposmotic stress in these cells. A preliminary account of some

of this work has been published in abstract from (Kotera, Brown & Case, 1992).

Materials and Methods

Cell Preparation

Single isolated lacrimal acinar cells were prepared using a modification of the method described by Kanagasuntheram and Randle (1976). Adult Sprague-Dawley rats were killed by an overdose of diethyl ether and the exorbital lacrimal glands were removed. The glands were finely minced and incubated in a Ca^{2+}-free saline solution containing (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 5 glucose, 5 HEPES-NaOH (pH 7.2), supplemented with bovine serum albumin (5 mg/ml)(Sigma), for 10 min at 37°C. The tissue was then incubated in the above solution supplemented with trypsin (0.4 mg/ml)(type XI.Sigma) for 10 min. After washing with Ca^{2+}-free saline, the tissue was incubated in the same solution supplemented with collagenase (0.15 mg/ml)(type II.Sigma) and trypsin inhibitor (2 mg/ml)(type II-S.Sigma) for 15 min. The tissue could then be dissociated mechanically by repeated pipetting through a plastic pipette tip. The cell suspension obtained was filtered through a nylon mesh, and washed with Ca^{2+}-free saline. The resulting single acinar cells and small cell clusters were resuspended in Ca^{2+}-free saline solution. Throughout the dissociation procedure the solutions were gassed with 100% O_2.

Patch-Clamp Recording

Isolated cells were placed in a small chamber (0.4 ml bath volume) on the stage of an Olympus inverted microscope and studied by the standard whole-cell recording method (Hamill et al., 1981). The bath was perfused with solution at a rate of about 2.5 ml/min (2.3–2.9 ml/min). Membrane currents were measured with an Axopatch-1D amplifier (Axon Instruments, Foster City, CA) and stored on a video tape recorder (Akai, Japan) via a digital audio processor (PCM-701ES, Sony, Japan). Voltage pulse protocols were generated by computer using the pCLAMP software (Axon Instruments). Data for current profiles were collected and stored using the same software.

Solutions

The composition of the bath and pipette solutions is shown in the Table. Bath solutions contained 1 mM $CaCl_2$ unless stated otherwise. A range of Ca^{2+} concentrations in pipette solutions was produced by mixing $CaCl_2$ and EGTA as described in the text. Initial experiments were carried out using the KCl (pipette) and NaCl (bath) solutions. However, most experiments were performed using the NMDG-Cl solutions in both bath and pipette in order to study Cl^- current. ATP (2 mM) (Sigma) and GTP (50 or 500 μM) (Sigma) were added to pipette solutions in some experiments.

Hyposmotic bath solutions (215 mOsm) were prepared by dilution with deionized water to 80% of control osmolarity (270 mOsm). A hyperosmotic pipette solution (330 mOsm), which was prepared by adding 50 mM mannitol to the control pipette solution (270 mOsm), was also used to produce cell swelling in some experiments (*see* Doroshenko & Neher, 1992; Valverde et al., 1992). Osmotic differences (pipette–bath) were about 55–60 mOsm in both conditions. The osmolarity of all solutions was measured by a freezing point depression method using a Roebling micro-osmometer (Camlab, Cambridge, UK).

Carbachol (Sigma) was used as a muscarinic agonist in some experiments. Gadolinium, an inhibitor of stretch-activated cation channels (Yang & Sachs, 1989; Filipovic & Sackin, 1991), was added to bath solutions as $GdCl_3$ (Aldrich, UK) in some experiments to give a final concentration of 20 μM. All experiments were performed at room temperature (19–23°C). The data are expressed as the mean ± SEM of n experiments.

Results

Activation of K^+ and Cl^- Currents by Osmotic Stress

Figure 1 shows the effects of hyposmotic stress on the whole-cell current. This experiment was performed using the KCl pipette solution and NaCl bath solution (both 270 mOsm). Outward and inward currents were monitored at 0 and −60 mV, respectively. The current required to clamp the cell at 0 mV, which is close to E_{Cl}, is a measure of the K^+ current. At −60 mV, which is close to E_K, the current is carried by Cl^- (Marty, Tan & Trautmann, 1984). Superfusion with hyposmotic bath solution (215 mOsm) increased both outward and inward currents. This result suggests that both K^+ and Cl^- currents are activated by hyposmotic stress. Similar results were observed in four other cells. Superfusing cells with hyperosmotic solution (330 mOsm) did not cause any change in whole-cell currents (*data not shown*).

To examine the effect of hyposmotic stress on the Cl^- current in more detail, we monitored inward current at −60 mV using NMDG-Cl solutions in the bath and pipette. Under these conditions, replacing the control bath solution (270 mOsm) with hyposmotic bath solution (215 mOsm) caused a large increase in inward Cl^- current (Fig. 2*A*). The current increase was reversed on return to the control bath solution. Current activation began after a delay of about 1 min (0.86 ± 0.13 min, n = 10; range 0.3–1.3 min) on switching to the hyposmotic solution. The peak amplitude of the current was −1.39 ± 0.13 nA (n = 10), which was observed 0.5–2 min after the onset of current increase (1.7 ± 0.17 min, n = 10). Intracellular dialysis with hyperosmotic pipette solution (330 mOsm), while keeping the bath isosmotic (270 mOsm), also increased the Cl^- current (Fig. 2*B*). The increase in Cl^- current in these conditions (peak amplitude was −1.29 ± 0.11 nA, n = 8) was similar to that seen with the hyposmotic bath solution. The current returned to its initial level 5.83 ± 0.31 min (range 5–7 min, n = 6) after the beginning

Table. Composition of pipette and bath solutions

Solutions (mM)	KCl	NaCl	NMDG-Cl	NMDG-Cl[a] (Ca^{2+}-free)	NMDG-Cl (high Ca^{2+})
Pipette[b]					
KCl	140				
$MgCl_2$	2		2	2	2
$CaCl_2$					1.16
NMDG-Cl			145	145	145
HEPES	5		5	5	5
EGTA	0.5		0.5	10	1.34
Bath[c]					
NaCl		140			
KCl		5			
$MgCl_2$		1	1	2	
$CaCl_2$		1	1		
NMDG-Cl			145	145	
HEPES		5	5	5	
EGTA				0.5	

[a] NMDG-Cl: *N*-methyl-D-glucamine chloride.
[b] pH adjusted to 7.2 with KOH or NMDG free base.
[c] pH adjusted to 7.3 with NaOH or NMDG free base.

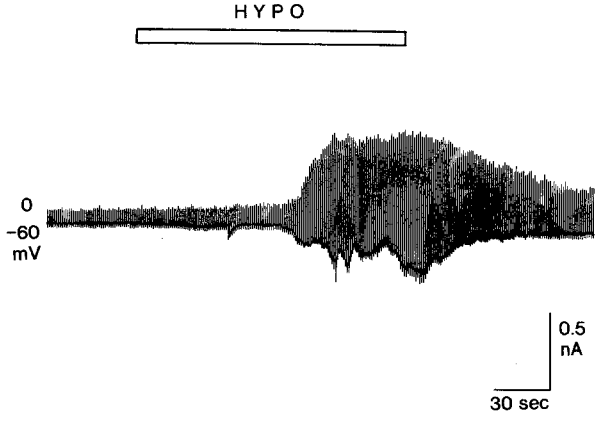

Fig. 1. Hyposmotic stress increases K^+ and Cl^- currents. The holding potential was −60 mV and 200-msec depolarizing pulses to 0 mV were applied every second. The K^+ current appears as upward deflections of the current trace at 0 mV (close to E_{Cl}). The Cl^- current appears as the sustained record at −60 mV (close to E_K). The pipette contained the KCl solution (0.5 mM EGTA, no added Ca^{2+}, 270 mOsm). At the beginning of the experiments, the bath contained NaCl solution (270 mOsm). The bath solution was then changed to hyposmotic solution (0.8 mM Ca^{2+}, 215 mOsm) for the period indicated by the bar. The result is representative of five experiments.

of the current increase. As shown in Fig. 2*C*, when cells were exposed to the hyposmotic bath solution the increase in Cl^- current was also transient. In this experiment, the current decayed in 7.0 ± 1.0 min (range 6–9 min, n = 3).

Properties of the Osmotically Activated Cl^- Current

Figure 3*A* shows the profiles of control and osmotically activated Cl^- currents recorded using a stepped voltage-pulse protocol (1-sec voltage steps at 20 mV increments over the range −100 to 100 mV from a holding potential of 0 mV). Under isosmotic conditions (upper traces), only small currents were observed and they showed little voltage-dependent activation or inactivation. In contrast, much larger currents were observed with the hyposmotic bath solution (lower traces). These currents activated slowly during the depolarizing pulses (between 60 and 100 mV), and inactivated rapidly during the hyperpolarizing pulses (between −60 and −100 mV). The currents measured at the end of each voltage step are plotted against membrane potential in Fig. 3*B*. The currents were much larger in hyposmotic conditions (■) than under control (isosmotic) conditions (●) at all membrane potentials. The current-voltage relationship for the osmotically activated currents showed outward rectification. Current reversal occurred at 3.7 ± 1.3 mV (n = 4), which is much closer to the predicted E_{Cl} (5.6 mV) than to E_{NMDG} (−5.6 mV).

In four experiments 2 mM ATP and 50 or 500 μM GTP were added to the pipette solution. It has been reported that in some cells ATP must be present in

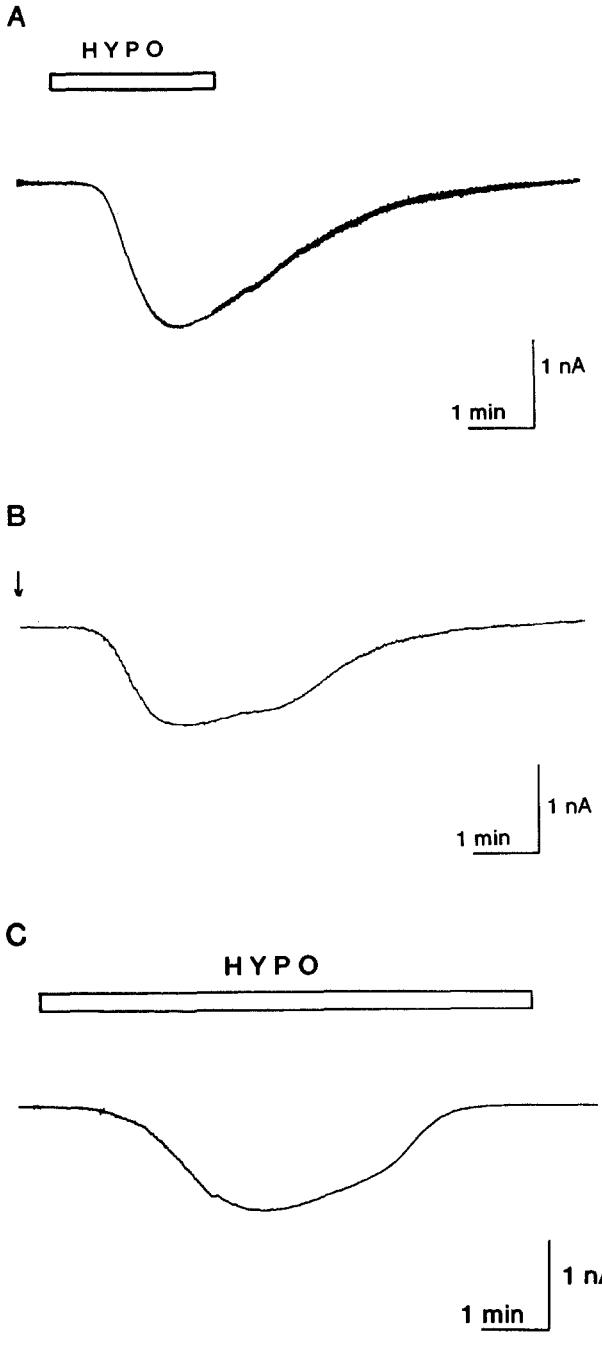

Fig. 2. Changes in Cl^- current in response to osmotic stress. (*A*) The holding potential was −60 mV. The pipette contained NMDG-Cl solution (0.5 mM EGTA, no added Ca^{2+}, 270 mOsm). The bath initially contained control NMDG-Cl solution (270 mOsm), which was changed to hyposmotic NMDG-Cl solution (215 mOsm) for the period indicated by the bar. Similar results were obtained in nine other experiments. (*B*) In this experiment the pipette contained hyperosmotic NMDG-Cl solution (0.5 mM EGTA, 330 mOsm). The bath contained control NMDG-Cl solution (270 mOsm) throughout and the holding potential was −60 mV. The arrow indicates the point at which the whole-cell configuration was established. Similar results were obtained in seven other experiments. (*C*) This experiment was performed under the same conditions as in *A*, except that the bath solution was kept hyposmotic longer (>5 min). Similar results were obtained in two other experiments.

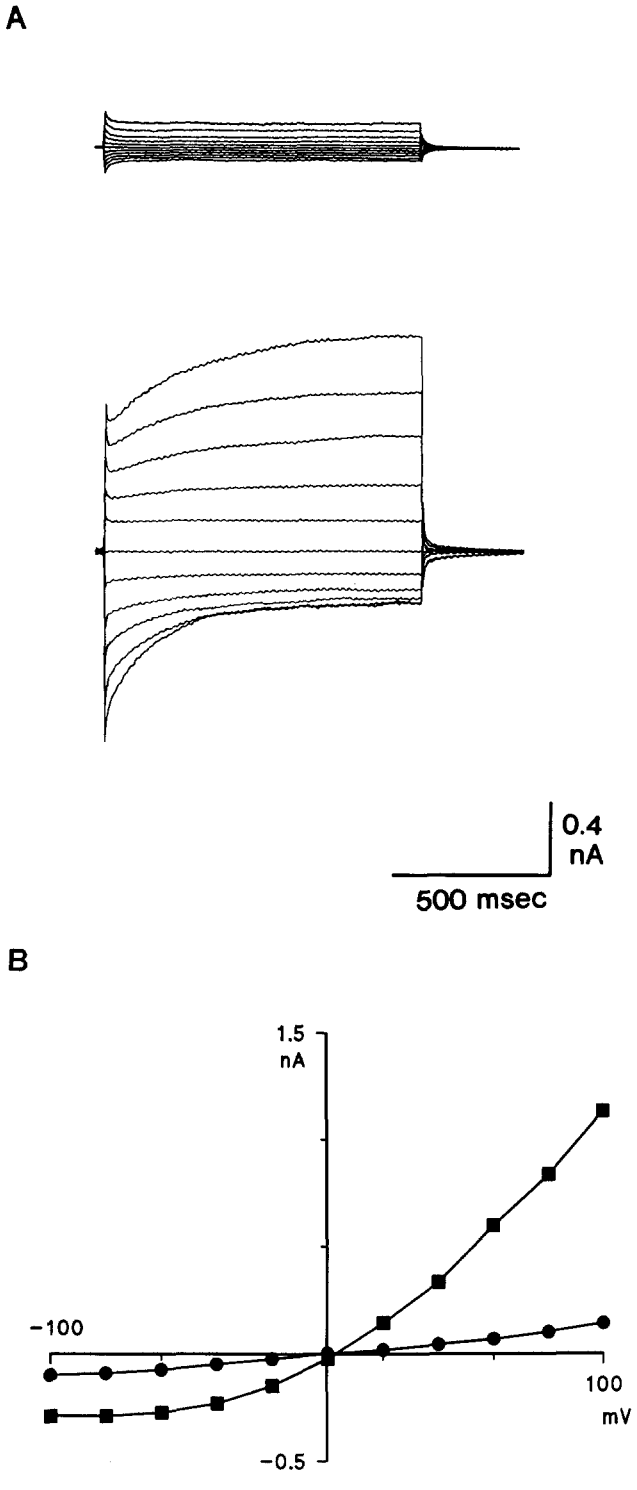

Fig. 3. Current profiles and current-voltage relationships of the Cl^- current. (*A*) Current profiles. The upper traces show Cl^- currents measured under control (isosmotic) condition. The lower traces show Cl^- currents recorded during hyposmotic stress in the same cell. The holding potential was 0 mV and 1-sec voltage steps were applied over the range from −100 to 100 mV at 20-mV increments. The solutions are the same as in Fig. 2*A*. (*B*) Current-voltage relationships for the currents in *A* (control, ●; hyposmotic, ■). The currents were measured at the end of the 1-sec voltage pulse. These results are representative of four experiments.

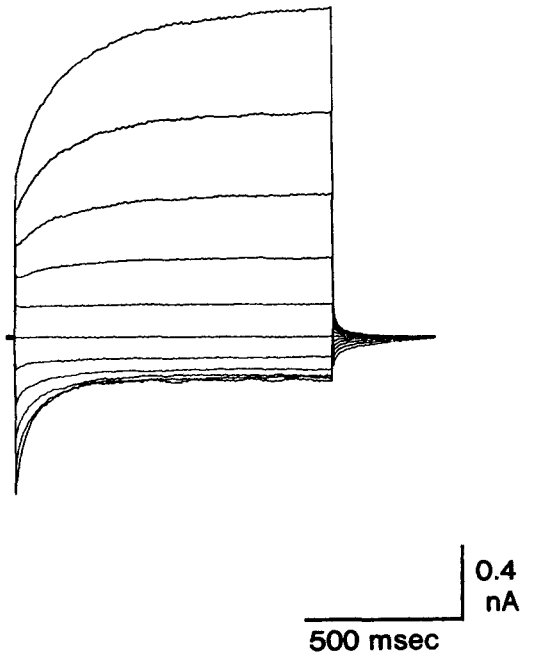

Fig. 4. Current profile for Cl^- currents activated by raised intracellular Ca^{2+}. The voltage pulse protocol was the same as in Fig. 3*A*. The pipette contained high Ca^{2+}-NMDG-Cl solution (intracellular free Ca^{2+}; 500 nM) and the bath contained control NMDG-Cl solution. Similar results were obtained in three other experiments.

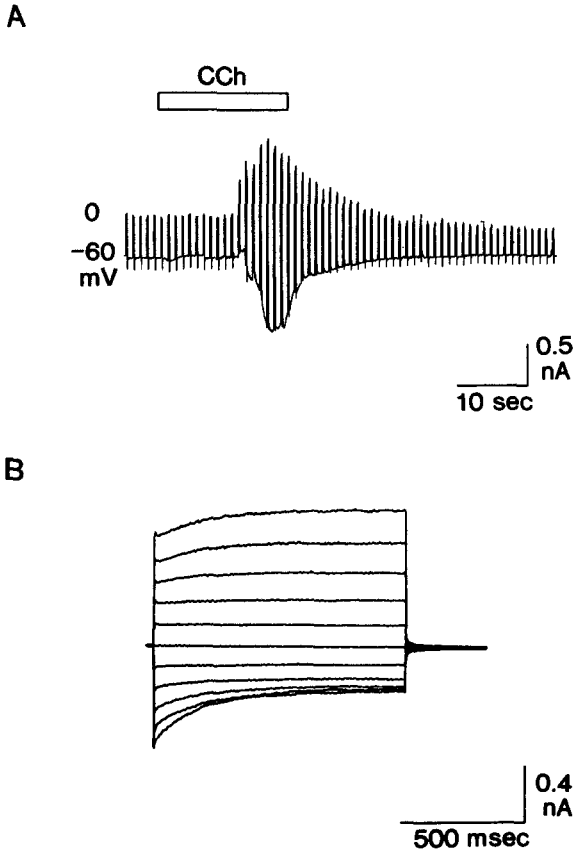

Fig. 5. The current response to muscarinic stimulation. (*A*) K^+ and Cl^- currents activated by CCh. K^+ and Cl^- currents were monitored in the same way as in Fig. 1. The control bath solution was changed to NaCl solution containing 2 μM CCh for the period indicated by the bar. The results are representative of five experiments. (*B*) Current profile for Cl^- currents activated by CCh. The pipette contained NMDG-Cl solution (0.5 mM EGTA, no added Ca^{2+}). The bath solution was NMDG-Cl containing 5 μM CCh. The voltage pulse protocol was the same as in Fig. 3*A*. The results are representative of four experiments.

order to observe volume-regulatory Cl^- currents (Valverde et al., 1992); however, in the lacrimal acinar cells ATP had no significant effect on the Cl^- current observed (*data not shown*).

The voltage dependence of the osmotically activated Cl^- currents in lacrimal acinar cells is different from that of volume-regulatory Cl^- currents in other epithelia, which inactivate at depolarizing potentials (Worrell et al., 1989; Solc & Wine, 1991: Kubo & Okada, 1992; Valverde et al., 1992). They resemble instead the Ca^{2+}-dependent Cl^- currents described previously in rat lacrimal acinar cells (Evans & Marty, 1986). Figure 4 shows Ca^{2+}-dependent Cl^- current measured in our preparation by using a pipette solution in which free Ca^{2+} was buffered at 500 nM. The current profile shown in Fig. 4 is quite similar to that of osmotically activated Cl^- current shown in Fig. 3*A*.

To confirm the similarity between the osmotically activated Cl^- currents and secretory Ca^{2+}-dependent Cl^- currents, we examined the effects of muscarinic stimulation. Figure 5*A* shows the effects of carbachol (*CCh*) on the whole cell current. This experiment was performed using the KCl pipette solution and NaCl bath solution. Superfusion with the bath solution containing 2 μM CCh activated both K^+ and Cl^- currents. The Cl^- currents activated by CCh were then examined using NMDG-Cl solutions in the bath and pipette. Figure 5*B* shows the profile of the activated Cl^- currents recorded using the same voltage pulse protocol as in Fig. 3*A*. This current profile is also quite similar to that of osmotically activated Cl^- current in Fig. 3*A*.

Involvement of Intracellular and Extracellular Ca^{2+} in Cl^- Current Activation

These results suggest that osmotic stress activates Ca^{2+}-dependent Cl^- channels in rat lacrimal acinar cells. We therefore went on to explore the role of

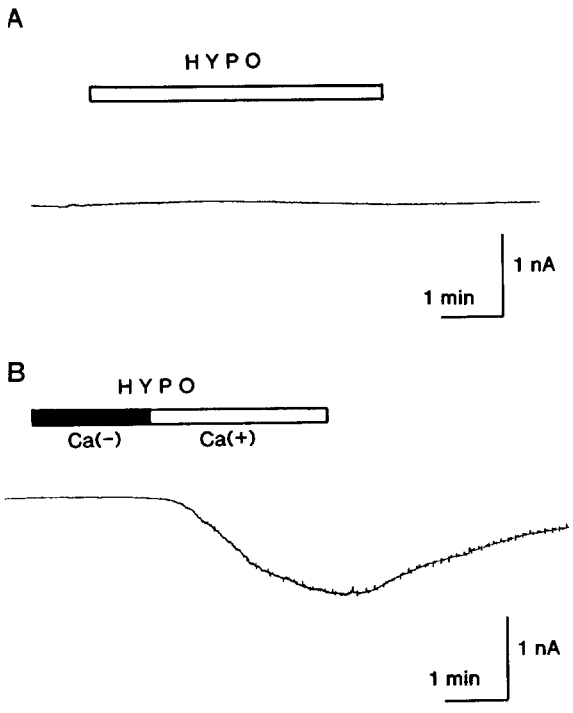

Fig. 6. Ca^{2+} is involved in the activation of Cl^- current during hyposmotic stress. (*A*) Inhibition of the increase in Cl^- current by intracellular EGTA. Cl^- current was recorded at −60 mV with Ca^{2+}-free NMDG-Cl pipette solution (10 mM EGTA, no added Ca^{2+}, 270 mOsm). The bath solution was initially the control NMDG-Cl solution (270 mOsm), which was then changed to hyposmotic solution (215 mOsm) for the period indicated by the bar. The results are representative of five experiments. (*B*) The increase in Cl^- current does not occur in Ca^{2+}-free medium. The control bath NMDG-Cl solution (270 mOsm) was changed first to Ca^{2+}-free hyposmotic solution (0.4 mM EGTA, no added Ca^{2+}, 215 mOsm) and then to control hyposmotic solution (0.8 mM Ca^{2+}, 215 mOsm) for the periods indicated by the bars (*Ca*(−) and *Ca*(+), respectively). The pipette contained NMDG-Cl solution (0.5 mM EGTA, no added Ca^{2+}, 270 mOsm), and the holding potential was −60 mV. Similar results were obtained in seven other experiments.

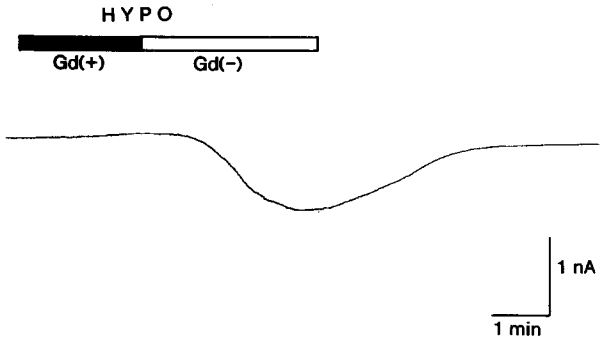

Fig. 7. The effect of extracellular Gd^{3+} on the Cl^- current activated by hyposmotic stress. The control bath NMDG-Cl solution (270 mOsm) was first changed to a hyposmotic solution containing Gd^{3+} (20 μM Gd^{3+}, 0.8 mM Ca^{2+}, 215 mOsm), and then to control hyposmotic solution (0.8 mM Ca^{2+}, 215 mOsm) for the periods indicated by the bars (*Gd*(+) and *Gd*(−), respectively). The holding potential was −60 mV, and the pipette contained NMDG-Cl solution (0.5 mM EGTA, no added Ca^{2+}, 270 mOsm). Similar results were obtained in three other experiments.

Ca^{2+} in current activation. Figure 6*A* shows an experiment in which intracellular Ca^{2+} was strongly chelated by 10 mM EGTA. Under these conditions the hyposmotic bath solution failed to cause any increase in Cl^- current. This result strongly suggests that an increase in intracellular Ca^{2+} is essential for the current activation during hyposmotic stress. To examine whether this increase in intracellular Ca^{2+} was dependent on extracellular Ca^{2+}, we employed a Ca^{2+}-free hyposmotic bath solution (0.4 mM EGTA, no added Ca^{2+}). Figure 6*B* shows that the Cl^- current did not increase during 2 min of superfusion with this Ca^{2+}-free hyposmotic solution. However, the current increased rapidly on changing to a hyposmotic solution containing 0.8 mM Ca^{2+}.

To examine the possible route for Ca^{2+} entry, we performed a series of experiments with Gd^{3+} in the bath solution. Gd^{3+} has been reported to reduce Ca^{2+} entry into the cell by blocking stretch-activated nonselective cation channels (Yang & Sachs, 1989; Filipovic & Sackin, 1991). As shown in Fig. 7, the presence of 20 μM Gd^{3+} in the hyposmotic bath solution inhibited the Cl^- current increase. The effect of Gd^{3+} was reversed by washing the cell with a control hyposmotic solution. The effect of Gd^{3+} was not caused by a direct interaction with the Cl^- channels because Cl^- currents could be observed in the presence of extracellular Gd^{3+} when using a high Ca^{2+} pipette solution (*data not shown*).

Discussion

Osmotically Activated Cl^- Channels

The results presented above demonstrate that Cl^- and K^+ conductances in rat lacrimal acinar cells can be activated by a transmembrane osmotic gradient. The Cl^- current was activated by either extracellular hyposmolarity or intracellular hyperosmolarity (the bath was hyposmotic to cytosol by about 60 mOsm in both conditions), both of which are thought to produce cell swelling (Doroshenko & Neher, 1992; Valverde et al., 1992). Although we did not measure the cell volume changes during the hyposmotic stress in these experiments, the observed transient increase in Cl^- current is likely to be a volume regulatory response induced by cell swelling.

Activation of K^+ and Cl^- currents by osmotic stress has been reported for a number of epithelial cells (Hazama & Okada, 1988; Rothstein & Mack, 1990). An increase in intracellular Ca^{2+} is probably responsible for the activation of K^+ channels during osmotic stress in many epithelia. The mechanism of activation of the Cl^- channels involved in RVD is not well understood (*see reviews* by Hoffmann & Simonsen, 1989; Sarkadi & Parker, 1991). However, recent patch-clamp experiments have identified Cl^- channels which are activated by cell swelling and are independent of intracellular Ca^{2+} in a number of epithelia, e.g., human intestinal epithelial cells (Hazama & Okada, 1988), T84 cells (Worrell et al., 1989), human sweat gland cells (Solc & Wine, 1991), and MDCK cells (Banderali & Roy, 1992; Weiss & Lang, 1992). The Cl^- current activated by osmotic stress in lacrimal gland acinar cells has two distinctive properties which are quite different from those of the volume-sensitive Cl^- currents reported in these epithelia. First, the Cl^- current increase was dependent on an increase in intracellular Ca^{2+} (Fig. 6*A*). Second, the current in lacrimal gland is voltage dependent, with outward currents being activated by depolarization and inward currents inactivated at hyperpolarizing potentials (Fig. 3*A*). In contrast, the volume-sensitive Cl^- currents in the other epithelia show time-dependent inactivation at depolarizing potentials (Worrell et al., 1989; Solc & Wine, 1991; Kubo & Okada, 1992; Valverde et al., 1992). This type of Cl^- current, which has also been shown to be ATP dependent (Valverde et al., 1992), could not be observed in our preparation even in the presence of ATP (and GTP) in the pipette solution. Thus, from these obvious differences it can be concluded that the osmotically activated Cl^- current in lacrimal acinar cells is not the same as the volume-sensitive Cl^- currents in these other epithelial cells.

Comparison with Secretory Cl^- Channels

Ca^{2+}-dependent Cl^- channels play an important role in secretory epithelia. In lacrimal acinar cells muscarinic stimulation activates basolateral K^+ and apical Cl^- channels by increasing intracellular Ca^{2+} (Findlay, 1984; Marty et al., 1984; Trautmann & Marty, 1984; Findlay & Petersen, 1985) and the properties of Ca^{2+}-dependent Cl^- current have been studied in some detail (Evans & Marty, 1986). When we recorded Ca^{2+}-dependent Cl^- currents (Figs. 4 and 5*B*), we found that they resembled the osmotically activated Cl^- currents in several ways: (i) they are activated by Ca^{2+}, (ii) they exhibit outward rectification and (iii) they activate at depolarizing potentials. Thus, it seems likely that the currents activated by muscarinic stimulation and by osmotic stress are carried by the same Ca^{2+}-dependent Cl^- channels.

Ca^{2+} Involvement in Channel Activation

Muscarinic stimulation activates Cl^- current by releasing Ca^{2+} from internal stores with a rapid time course (about 2 sec) and is independent of extracellular Ca^{2+} (Marty et al., 1984). With osmotic stress, channel activation has a much longer delay (about 1 min, Fig. 2*B*), probably reflecting the time to produce osmotic swelling and possibly because Ca^{2+} must enter the cell from the extracellular fluid, although the time to change solution cannot be ignored. The experiments in Fig. 6*B* show that the activation of the Cl^- currents by osmotic stress is dependent on extracellular Ca^{2+}. The increase in intracellular Ca^{2+} which occurs during RVD in other types of cells is dependent on extracellular Ca^{2+} (Bear, 1990; Rothstein & Mack, 1990; Suzuki et al., 1990; McCarty & O'Neil, 1991). Thus, it appears that the activation of the Cl^- currents by osmotic stress is due to the entry of extracellular Ca^{2+} to the cell.

One way by which Ca^{2+} may enter the cell during cell swelling is via stretch-activated channels. It has previously been reported that in some cell types hyposmotic stress can open stretch-activated nonselective cation channels and hence allow Ca^{2+} entry (Christensen, 1987; Bear, 1990). These Ca^{2+}-permeable channels are known to be reversibly inhibited by extracellular Gd^{3+} in micromolar quantities (Yang & Sachs, 1989; Filipovic & Sackin, 1991). In lacrimal gland acinar cells, activation of the Cl^- current by hyposmotic stress was found to be sensitive to Gd^{3+} (Fig. 7). Therefore, we suggest that stretch-activated nonselective cation channels may be opened by hyposmotic stress and work as a Ca^{2+} entry pathway in these cells.

Physiological Role in Lacrimal Acinar Cells

Most cells, apart perhaps from those of the intestine and kidney, are not exposed to large changes in osmolality under normal circumstances. However, in other absorbing and secreting epithelia, e.g., exocrine gland cells and gall bladder, large transcellular ion fluxes can undoubtedly perturb cellular volume homeostasis (Hoffmann & Simonsen, 1989). Accumulation or loss of electrolytes by cells may produce transmembrane osmotic gradients and subsequent changes in cell volume, e.g., in salivary acinar cells muscarinic stimulation of Cl^- efflux is associated

with a large decrease in cell volume (Foskett & Melvin, 1989; Foskett, 1990). To understand epithelial transport fully, it may, therefore, be necessary to study volume regulation in these cells. In this study, we show that K^+ and Cl^- channels are activated by hyposmotic stress in lacrimal acinar cells. It is probable that the activation of these channels is part of RVD response. Further studies must be performed to determine whether the activation of these channels by cell volume change is important during secretion in these cells.

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