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Ion selectivity of volume regulatory mechanisms present during a hypoosmotic challenge in vestibular dark cells

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Abstract

Volume regulation during a hypoosmotic challenge (RVD) in vestibular dark cells from the gerbilline inner ear has previously been shown to depend on the presence of cytosolic K^+ and Cl^-, suggesting that it involves KCl efflux. The aim of the present study was to characterize hypoosmotically-induced KCl transport under conditions where a hypoosmotic challenge causes KCl influx via the pathways normally used for efflux. Net osmolyte movements were monitored as relative changes in cell volume measured as epithelial cell height (*CH*). A hypoosmotic challenge (298 to 154 mosM) in the presence of 3.6 or 25 mM K^+ and loop-diuretics (piretanide or bumetanide) caused an increase in *CH* by about a factor of 1.2 presumably due to the net effect of primary swelling defined as osmotic dilution of the cytosol and RVD involving KCl efflux. A hypoosmotic challenge in the presence of 79 mM K^+ and loop-diuretics, however, caused *CH* to increase by a factor of over 2.4. Presumably, this large increase in *CH* was due to the sum of primary and secondary swelling. Secondary swelling depended on the presence of extracellular K^+ and Cl^- suggesting that it involved KCl influx followed by water. The ion selectivity of secondary swelling was $K^+ = Rb^+ > Cs^+ \gg Na^+ = NMDG^+$ and $Cl^- = NO_3^- = SCN^- \gg$ gluconate$^-$. Secondary swelling was not inhibited by Ba^{2+}, tetraethylammonium, quinidine, lidocaine, amiloride, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid, 4-acetamido-4′-diisothiocyanatostilbene-2,2′-disulfonic acid, 4,4′-dinitrostilbene-2,2′-disulfonic acid, 5-nitro-2(3-phenylpropylamino)benzoic acid, acetazolamide, or ethoxyzolamide. These data define a profile of the hypoosmotically-induced KCl transport pathways. The ion selectivity and the blocker insensitivity are consistent with the involvement of the apical slowly activating K^+ channel (I_{sK} or minK channel) and the basolateral 360 pS Cl^- channel. The involvement of these channels, however, remains to be demonstrated.

Keywords: Regulatory volume decrease; I_{sK} channel; minK channel; LC chloride ion channel; Vestibular labyrinth

1. Introduction

Vestibular dark cells transport K^+ from perilymph to endolymph in the inner ear [1,2]. Imbalances between K^+ uptake and release during transepithelial transport pose a threat to cell function and necessitate mechanisms for volume regulation. Volume regulation in vestibular dark cells during a hypoosmotic challenge has recently been shown to occur as fast as solution changes could be made and to depend on the presence of cytosolic K^+ and Cl^- [3]. These observations suggested that cytosolic K^+ and Cl^- are released as osmolytes, however, the mechanisms involved in the release of KCl remained unclear. Many cells use K^+ and Cl^- channels, a KCl cotransporter or parallel K^+/H^+ and Cl^-/HCO_3^- exchangers for the release of KCl during a hypoosmotic challenge [4]. It is not known whether vestibular dark cells contain a KCl cotransporter or parallel K^+/H^+ and Cl^-/HCO_3^- exchangers, however, several K^+ and Cl^- channels have been demonstrated. Vestibular dark cells contain in their apical membrane a slowly activating K^+ channel (I_{sK} channel, also called minK channel) [5], a maxi-K^+ channel [6] and a non-selective cation channel [7] and in their basolateral membrane a 95 pS and a 360 pS Cl^- channel [8].

The question arises whether any of these constitutive ion channels support the net KCl efflux during regulatory volume decrease in the presence of a hypoosmotic challenge. One step toward the answer of this question is to compare the ion selectivities and blocker sensitivities of these channels with those of the net KCl flux which occurs

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during a hypoosmotic challenge. Ion selectivities and blocker sensitivities were obtained in the presence of an elevated K^+ concentration and loop-diuretics. A hypoosmotic challenge under these conditions causes cell swelling which is the sum of primary cell swelling due to osmotic dilution of the cytosol and secondary cell swelling which is due to KCl influx followed by water. Hypoosmotically-induced KCl efflux during regulatory volume decrease and hypoosmotically-induced KCl influx during secondary cell swelling may occur via the same pathway if rectifying transport mechanisms for KCl are absent. Hypoosmotically-induced KCl influx was chosen for the present study since the ion selectivity of influx can be obtained with greater certainty than that for efflux which would rely on loading the cells with replacement ions. Secondary cell swelling has also been used by others as a tool for the determination of the ion selectivity of regulatory volume decrease, e.g., [9–12].

Parts of this study have been presented at recent meetings [13–15].

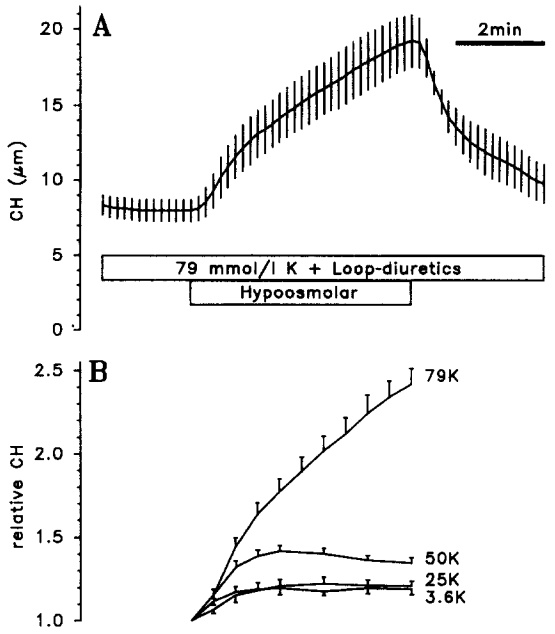

Fig. 1. Effect of a hypoosmotic challenge in the presence of different K^+ concentrations and loop-diuretics on cell height *CH*. (A) The osmolarity was lowered from 298 to 154 mosM (Hypoosmolar) in the presence of 79 mM K^+ and loop-diuretics ($n = 4$). (B) Summary of data normalized to the *CH* at the beginning of the hypoosmotic challenge in the presence of 3.6 mM K^+ (3.6K, $n = 4$), 25 mM K^+ (25K, $n = 6$), 50 mM K^+ (50K, $n = 11$) and 79 mM K^+ (79K, $n = 4$). Loop-diuretics (either 100 µM piretanide or 50 µM bumetanide) were present in all these experiments to prevent K^+-induced cell swelling. The time bar in A applies also to B.

2. Methods

2.1. Preparation

Gerbils (4–10-week-old) were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and decapitated. Experiments were conducted under a protocol approved by the Creighton University Animal Care and Use Committee. The technique of dissection has been described previously [16]. Briefly, dark cell epithelium was dissected at 4°C from an ampulla of a semicircular canal, folded into a loop, and transferred to a bath chamber where experiments were conducted at 37°C.

2.2. Solutions

The composition of solutions is listed in Table 1. All solutions were titrated to pH 7.4 and the osmolarity was measured by freezing point depression (Osmette A, Precision Instruments). Piretanide (a gift from Dr. Greger, Freiburg, Germany), bumetanide, quinidine, lidocaine, amiloride, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, Sigma, St. Louis, MO), 4,4′-dinitrostilbene-2,2′-disulfonic acid (DNDS, Molecular Probes, Eugene, OR) and 5-nitro-2(3-phenylpropylamino)benzoic acid (NPPB, a gift from Dr. H. Englert, Hoechst, Pharmaceutical Research, Frankfurt a.M., Germany) were pre-dissolved in dimethylsulfoxide (DMSO) to a final DMSO concentration of less than 0.1%.

2.3. Data acquisition

Relative changes of cell volume were monitored by measuring cell height. Cell height (*CH*) was measured as previously described [3,16]. The perfusate, which had access to both the apical and the basolateral membrane, was exchanged 3 times/s. The microscope image of the folded tissue was viewed with a black/white video camera (Panasonic WV-1550), mixed with a time signal and displayed on a monitor (PVM-122, Sony, Park Ridge, NJ) as well as recorded on videotape (AG-1960, Panasonic, Secaucus, NJ). Two methods (A and B) were used which yielded nearly identical results when applied to the same experiment. For method (A), a computer generated image of two vertical cursors was mixed on-line with the microscope image (NTSC Recordable Videocard, USVideo, Stamford, CT). The two cursors were adjusted independently to overlay the apical and basal border of the epithelium. The calibrated distance between the two cursors (cell height) was written into an ASCII file at a rate of 0.5 Hz. Data shown in Fig. 1A and 2A were smoothed by averaging 5 data points in a moving window.

For method (B) the microscope image was mixed with a time signal and recorded on videotape. The time signal was used for identification of single frames. For analysis of cell height, single frames were selected and six cell height measurements were averaged from each frame (Java, Jandel Scientific, San Rafael, CA). The location of the individual measurements on each frame remained constant for all frames in a given experiment.

2.4. Data presentation and statistics

Data are given as average ± standard error of the mean (S.E.). The number of observations (n) is equal to the number of epithelial samples. For statistical analysis, averages of original data were compared using Student's t-test for paired and unpaired samples. Averages of normalized values shown in Figs. 1 and 2 were compared with Student's t-test after a logarithmic transformation. A logarithmic transformation has been suggested to restore normal distribution which is required for Student's t-test [17]. One-way analysis of variance (ANOVA-Dunnett) was used for the statistical analysis of the data shown in Figs. 3 and 4 (BMDP Statistical Software, Los Angeles, CA). Differences were assumed to be significant when $P < 0.05$.

3. Results

3.1. Effect of different K^+ concentrations present during a hypoosmotic challenge

In previous studies, it has been shown that elevation of the K^+ concentration leads under isosmotic conditions to K^+-induced cell swelling which can be inhibited by the loop-diuretics bumetanide and piretanide [16,18]. This result was confirmed in the present study. In the presence of loop-diuretics (100 μM piretanide or 50 μM bumetanide) *CH* was not significantly different when K^+ was 3.6 or 79 mM (solution 1, Table 1, 7.8 ± 0.8 μm, $n = 9$ versus solution 7, 7.4 ± 1.0 μm; $n = 19$). A hypoosmotic challenge in the presence of loop-diuretics and 3.6 mM K^+ (solutions 1 and 2), 25 mM K^+ (solutions 3 and 4), 50 mM K^+ (solutions 5 and 6) or 79 mM K^+ (solution 7 and 8) caused *CH* to increase at the end of 5 min by a factor of 1.18 ± 0.03 ($n = 4$), 1.21 ± 0.03 ($n = 6$), 1.34 ± 0.03 ($n = 11$), or 2.4 ± 0.1 ($n = 4$), respectively (Fig. 1). There was no significant difference between the factor of *CH*

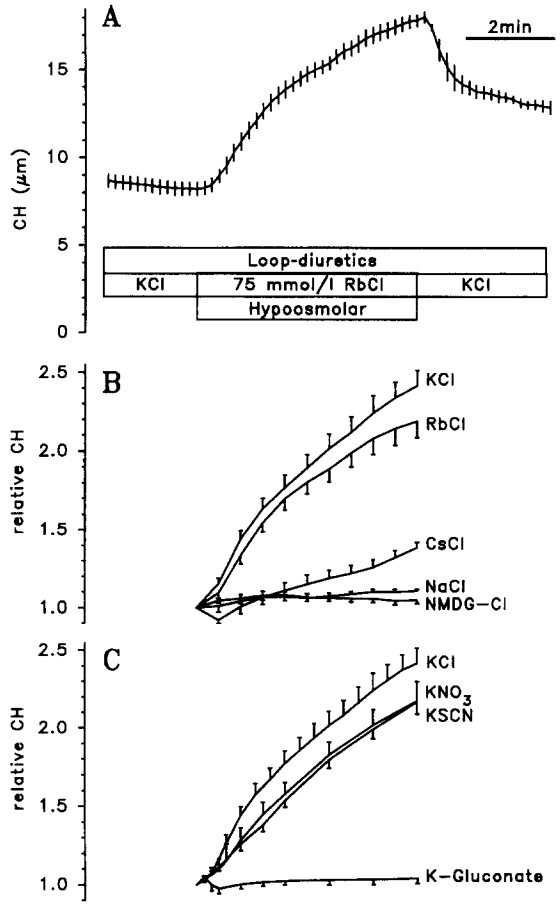

Fig. 2. Effect of a hypoosmotic challenge in the presence of different cations and loop-diuretics on *CH*. (A) The osmolarity was lowered from 298 to 154 mosM (hypoosmolar) and simultaneously 75 mM KCl were replaced with an equimolar amount of RbCl ($n = 4$). (B and C) Summary of data normalized to the *CH* at the beginning of the hypoosmotic challenge. For comparison, data labeled KCl were replotted from Fig. 1. (B) Simultaneous with the hypoosmotic challenge 75 mM KCl were replaced with an equimolar amount of NMDG-Cl, NaCl, CsCl or RbCl (each $n = 4$). (C) Simultaneous with the hypoosmotic challenge 75 mM KCl were replaced with an equimolar amount of potassium gluconate, KNO_3, or KSCN (each $n = 4$). A loop-diuretic (50 μM bumetanide) was present in all these experiments to prevent K^+-induced cell swelling. The time bar in A applies also to B and C.

Table 1
Solutions (in mM)

Solution	1	2	3	4	5 [a]	6 [a]	7	8 [b]
KCl	–	–	21.4	21.4	46.4	46.4	75.0	75.0
NaCl	150.0	75.0	91.1	53.6	66.1	28.6	–	–
NMDG-Cl	–	–	–	–	–	75.0	–	
$MgCl_2$	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$CaCl_2$	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
K_2HPO_4	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
KH_2PO_4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Glucose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mannitol	–	–	75.0	–	75.0	–	–	
mosM	298	154	298	154	298	154	298	154

NMDG-Cl, *N*-methyl-D-glucamine chloride.

[a] For experiments involving 20 mM TEA, NaCl was reduced by 20 mM. Further, for experiments involving 5 mM Ba^{2+}, phosphate buffer was replaced by 5 mM Hepes, KCl was increased by 3.6 mM, and NaCl was reduced by 11 mM.

[b] In some experiments KCl was replaced by either RbCl, CsCl, NaCl, NMDG-Cl, KNO_3, KSCN or potassium gluconate.

increase in the presence of 3.6 and 25 mM K^+, however, the factor of *CH* increase was significantly larger in the presence of 50 and 79 mM K^+. The latter two K^+ concentrations were chosen for the further experiments directed toward the ion dependence and selectivity and towards the inhibitor sensitivity of the mechanisms involved in volume regulation during a hypoosmotic challenge.

3.2. Ion dependence and selectivity of hypoosmotically-induced KCl influx

The ion dependence and selectivity of the mechanisms involved in cell swelling during a hypoosmotic challenge were investigated in the presence of 79 mM K^+ and 50 μM bumetanide. Inducing hypotonicity by removal of NMDG while simultaneously replacing 75 mM KCl with 75 mM RbCl, CsCl, NaCl, or NMDG-Cl (solutions 7 and 8) caused *CH* to increase at the end of 5 min by a factor of 2.2 ± 0.1 ($n = 4$), 1.38 ± 0.03 ($n = 4$), 1.11 ± 0.01 ($n = 4$) or 0.98 ± 0.01 ($n = 5$), respectively (Fig. 2A and B). No significant difference was observed between the factor of *CH* increase in the presence of KCl or RbCl, however, a significantly smaller increase occurred in the presence of NaCl, CsCl or NMDG-Cl. The observation that CsCl initially caused significant cell shrinking remains unexplained.

Inducing hypotonicity by removal of NMDG while simultaneously replacing 75 mM KCl with 75 mM KSCN, KNO_3 or potassium gluconate (solutions 7 and 8) caused *CH* to increase at the end of 5 min by a factor of 2.2 ± 0.1 ($n = 4$), 2.2 ± 0.1 ($n = 4$) or 1.04 ± 0.03 ($n = 4$), respectively (Fig. 2C). No significant difference was observed between the factor of *CH* increase in the presence of KCl, KSCN or KNO_3, however, a significantly smaller increase in *CH* occurred in the presence of potassium gluconate. These observations suggest that net ion transport induced by the hypoosmotic challenge depends on the presence of both, K^+ and Cl^-, and that the ion transport mechanisms involved carry the cations K^+ and Rb^+ and the anions Cl^-, NO_3^- and SCN^-.

3.3. Inhibitor sensitivity of hypoosmotically-induced KCl influx

The inhibitor sensitivities of the ion transport mechanisms involved in volume regulation during a hypoosmotic challenge were investigated in the presence of 50 mM K^+ and loop-diuretics (100 μM piretanide or 50 μM bumetanide). Inhibition of the ion transport mechanisms involved in volume regulation was expected to cause a reduction of cell swelling during hypoosmotic removal of 75 mM NMDG-Cl (solutions 5 and 6). However, the cationic blockers 5 mM Ba^{2+} ($n = 7$), 20 mM tetraethylammonium (TEA, $n = 7$), 1 mM quinidine ($n = 7$), 5 mM lidocaine ($n = 7$) or 1 mM amiloride ($n = 7$) or the anionic blockers, 1 mM DIDS ($n = 7$), 1 mM SITS ($n = 7$), 1 mM DNDS ($n = 7$), 10^{-5} M NPPB ($n = 4$), 10^{-4} M acetazolamide ($n = 8$) or 10^{-4} M ethoxyzolamide ($n = 4$) had no significant effect.

4. Discussion

In a previous study it was shown that a hypoosmotic challenge which was expected to cause an increase of *CH* by about a factor of 2 caused *CH* to increase by a factor of only 1.07 when volume regulatory mechanisms were unimpeded and by a factor of about 1.20 when volume regulatory mechanisms were inhibited, e.g., by depletion of cytosolic K^+ or Cl^- [3]. The latter *CH* increase apparently represents cell swelling by dilution of the cytosol due to osmotic water influx. Significantly larger increases in *CH* were observed in the present study when similar hypoosmotic challenges were applied in the presence of 50 or 79 mM extracellular K^+ and loop-diuretics (Fig. 1). This secondary cell swelling occurred apparently in excess of the primary swelling defined as hypoosmotic dilution of the cytosol [9,10,12]. Reversal of the K^+ and Cl^- gradients in the presence of only 50 mM K^+ and 79 mM Cl^- is conceivable under the assumption that the cytosolic K^+ and Cl^- concentrations were reduced due to inhibition of the $Na^+/Cl^-/K^+$ cotransporter by the loop-diuretics and due to dilution of the cytosol by osmotic water influx during the hypoosmotic challenge.

It can be assumed that both hypoosmotically-induced KCl efflux during regulatory volume decrease and hypoosmotically-induced KCl influx during secondary cell swelling occur via the same pathway as long as strongly rectifying transport mechanisms for K^+ and Cl^- are not involved. Under this assumption it is possible to study hypoosmotically-induced KCl flux as efflux during regulatory volume decrease or as influx during secondary cell swelling.

For the determination of the ion selectivity sequence it is best to measure unidirectional ion flux. Even though the observation of secondary cell swelling is not a unidirectional flux measurement, it is certainly a measurement of a net influx. Consistent with this view is the observation that secondary cell swelling was absent when either extracellular K^+ was replaced with the impermeable ion $NMDG^+$ or when extracellular Cl^- was replaced with the impermeable anion $gluconate^-$ (Fig. 2).

Secondary cell swelling involving hypoosmotically-induced KCl influx was chosen in the present study for the determination of the ion selectivity and blocker sensitivity since the ion selectivity of influx can be obtained with greater certainty than that for efflux which would rely on loading the cells with replacement ions. Secondary cell swelling has also been used by others as a tool for the determination of the ion selectivity of regulatory volume decrease, e.g., [9,10,12].

Mechanisms frequently involved in volume regulation during a hypoosmotic challenge include K^+ and Cl^- channels, a KCl cotransporter or parallel K^+/H^+ and Cl^-/HCO_3^- exchangers [4]. Participation of parallel K^+/H^+ and Cl^-/HCO_3^- exchangers in hypoosmotically-induced KCl influx is unlikely since swelling was neither inhibited by amiloride, a blocker of the K^+/H^+ exchanger [19], nor by DIDS or SITS, blockers of the Cl^-/HCO_3^- exchanger [20], nor by acetazolamide or ethoxzolamide, blockers of the formation of HCO_3^- by carbonic anhydrase [21] (Figs. 3 and 4). Equally unlikely is an involvement of the KCl cotransporter in hypoosmotically-induced KCl efflux or influx. During a hypoosmotically-induced KCl efflux in the presence of 3.6 mM extracellular K^+ and absence of loop-diuretics an increase in the current through and the conductance of the apical I_{sK} channel has been observed [22]. These observations suggest that KCl efflux involved a conductive pathway rather than a KCl cotransporter which is electroneutral. If a KCl cotransporter were activated to carry the main KCl flux during a hypoosmotic challenge, it would be expected that the competing K^+ flux through the I_{sK} channel would either remain unchanged or would decrease. The observation that the current through and the conductance of the I_{sK} channel increased during a hypoosmotic challenge provides evidence against the hypothesis that a KCl cotransporter is involved as main KCl pathway. Further, KCl influx and swelling during a hypoosmotic challenge in the presence of 79 mM extracellular K^+ and loop-diuretics was supported equally by Cl^-, NO_3^- and SCN^-. The KCl cotransporter, however, at least in red cells is impermeable to NO_3^- and SCN^- [23,24].

It is most likely that the KCl flux during a hypoosmotic challenge occurred via the I_{sK} channel and via Cl^- chan-

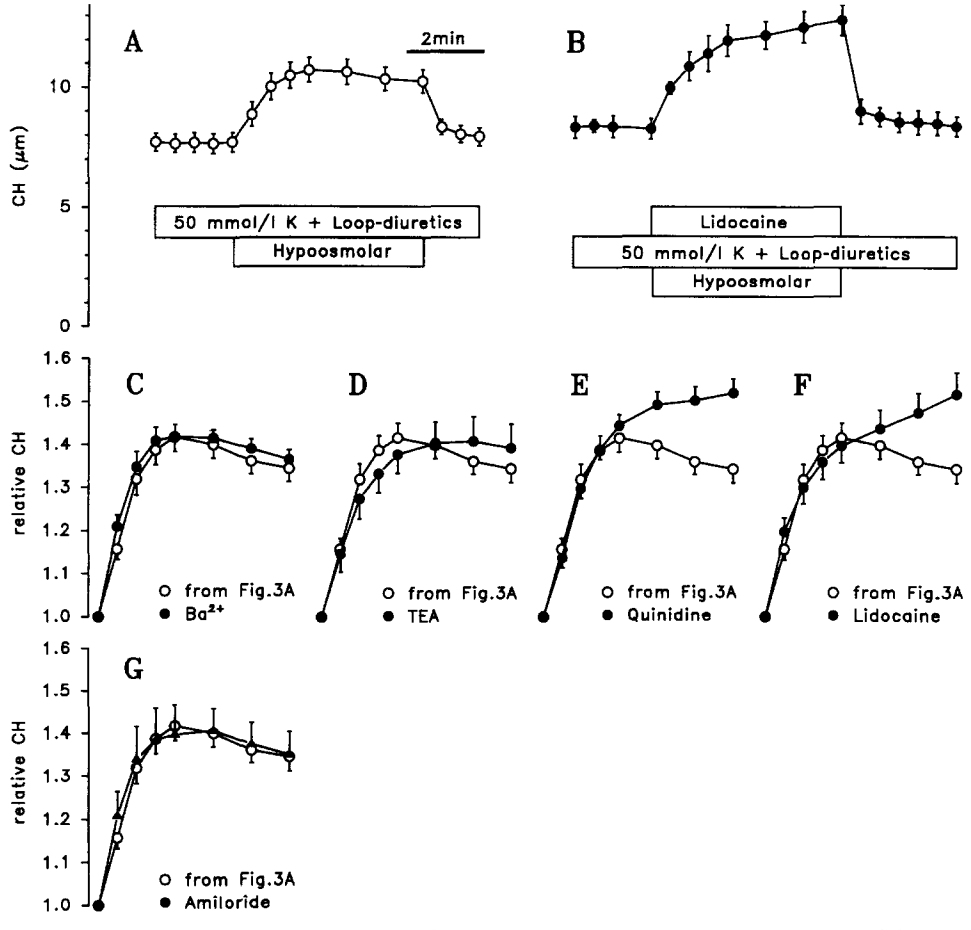

Fig. 3. Effect of a hypoosmotic challenge in the presence of 50 mM K^+ and loop-diuretics and different blockers on *CH*. (A) The osmolarity was lowered from 298 to 154 mosM (Hypoosmolar) in the presence of 50 mM K^+ and loop-diuretics ($n = 11$). These experiments serve as a control and are plotted for comparison in C–G and A–F (open circles). (B) The osmolarity was lowered from 298 to 154 mosM in the presence of 50 mM K^+, loop-diuretics and 5 mM lidocaine ($n = 7$). (C–G) summaries of data normalized to the *CH* at the beginning of the hypoosmotic challenge. The osmolarity was lowered from 298 to 154 mosM in the presence of 50 mM K^+, loop-diuretics and 5 mM Ba^{2+} (C, $n = 7$), 20 mM TEA (D, $n = 7$), 1 mM quinidine (E, $n = 7$), 5 mM lidocaine (F, $n = 7$), or 1 mM amiloride (G, $n = 7$). Inhibitors in C–F were added simultaneously with the hypoosmotic challenge as shown in B, whereas in F the inhibitor was added 40 s prior to the hypoosmotic challenge. Loop-diuretics, either 100 μM piretanide or 50 μM bumetanide, were present in all these experiments to prevent K^+-induced cell swelling. The time bar in A applies to the whole Figure. According to the statistical test (ANOVA-Dunnett) no significant differences were observed between experiments in the absence and presence of inhibitors. Whether apparent differences in E and F are of biological significance remains undetermined.

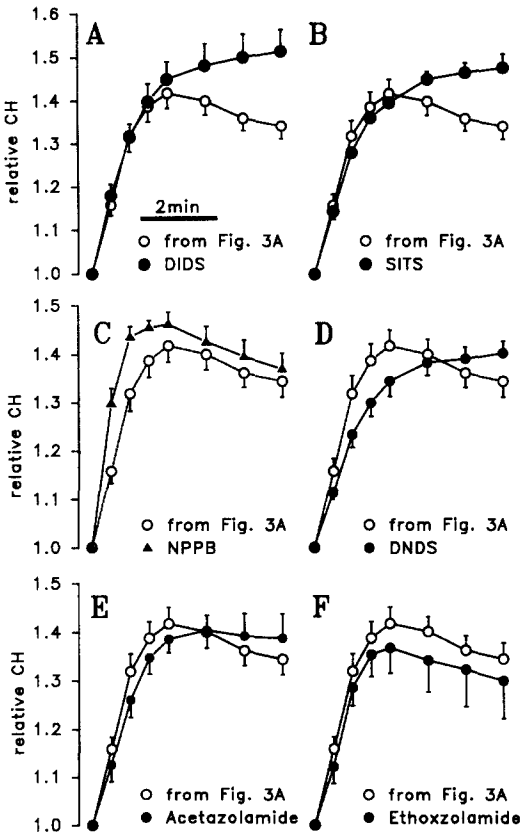

Fig. 4. Effect of a hypoosmotic challenge in the presence of 50 mM K^+ and loop-diuretics and different blockers on *CH*. Experiments were conducted similar as shown in Fig. 3. The osmolarity was lowered from 298 to 154 mosM in the presence of 50 mM K^+, loop-diuretics and 1 mM DIDS (A, $n = 7$), 1 mM SITS (B, $n = 7$), 10^{-5} M NPPB (C, $n = 4$), 1 mM DNDS (D, $n = 7$), 10^{-4} M acetazolamide (E, $n = 8$), or 10^{-4} M ethoxyzolamide (F, $n = 4$). Control experiments (open circles) in the absence of blockers were taken from Fig. 3A. Inhibitors in A, B and D–F were added simultaneously with the hypoosmotic challenge as shown in Fig. 3B, whereas in C the inhibitor was added 40 s prior to the hypoosmotic challenge. Loop-diuretics, either 100 μM piretanide or 50 μM bumetanide, were present in all these experiments to prevent K^+-induced cell swelling. The time bar in A applies to the whole Figure. According to the statistical test (ANOVA-Dunnett) no significant differences were observed between experiments in the absence and presence of inhibitors. Whether apparent differences in A and B are of biological significance remains undetermined.

nels since vestibular dark cells contain an unusually large Cl^- conductance [25]. The membrane resistance decreased from 800 MΩ in the absence of extracellular Cl^- to 17 Ω when Cl^- was added to the perfusate. The observations that the membrane resistance remained low in the presence of the loop-diuretic piretanide [25] suggests that this large Cl^- conductance is available for Cl^- flux in experiments of the present study.

If volume regulation during a hypoosmotic challenge involves K^+ and Cl^- channels, the question arises whether it is likely that any of the previously described ion channels are involved. In order to answer this question, we compared the ion selectivity and the blocker sensitivity of the hypoosmotically-induced KCl influx with those of the described ion channels. The properties of the 360 pS Cl^- channel in the basolateral membrane and the I_{sK} channel in the apical membrane of vestibular dark cells match the present characterization of the volume regulatory mechanisms. Even though both the 360 and the 95 pS Cl^- channels are insensitive to the blockers DIDS, DNDS, NPPB and quinine, and impermeable to gluconate$^-$, only the 360 pS Cl^- channel is equally permeable to Cl^- and NO_3^- [8], similar to hypoosmotically-induced KCl-influx (Fig. 2). The 360 pS Cl^- channel has been found under isotonic conditions with the patch clamp technique with an incidence of only 3% [8] which is probably too low to be a relevant pathway. It remains to be determined whether an increase in the incidence of this channel is part of the volume regulatory response. The involvement of the maxi-K^+ or the non-selective cation channel is unlikely. The maxi-K^+ channel is impermeable to Rb^+ and sensitive to Ba^{2+}, TEA, quinidine and lidocaine [6] and the non-selective cation channel is equally permeable to K^+, Rb^+, Na^+ and Cs^+ [7]. Even though the ion selectivities and blocker sensitivities of the I_{sK} channel have not yet been determined in vestibular dark cells, data from other preparations are available. The I_{sK} channel is activated by a hypoosmotic challenge [26], permeable to K^+ and Rb^+ but impermeable to Cs^+ and relatively insensitive to TEA ($K_i > 45$ mM) and Ba^{2+} ($K_i = 9$ mM in the presence of 50 mM extracellular K^+) [27]. It is conceivable that the I_{sK} channel is involved as major K^+ flux pathway in volume regulation of vestibular dark cells since these properties are compatible with those of the hypoosmotically-induced KCl influx. Indeed, in the presence of 3.6 mM K^+, an increase in the current through and the conductance of the I_{sK} channel was observed during a hypoosmotic challenge [22]. The I_{sK} channel and the 95 pS Cl^- channel are thought to be mainly involved in the transport of K^+ across the apical and Cl^- across the basolateral membrane. Even though these channels are present under isosmotic conditions no cell swelling was observed under isosmotic conditions in the presence of bumetanide or piretanide and 50 or 79 mM K^+ (Fig. 1A and 3A). This observation is consistent with the finding that the apical I_{sK} channel is inactivated when the basolateral $Na^+/Cl^-/K^+$ cotransporter is inhibited by bumetanide [5].

In conclusion, the present data suggest that volume regulation during a hypoosmotic challenge involves an activation of a KCl flux. The ion selectivities and inhibitor sensitivities of KCl influx are consistent with the involvement of the I_{sK} channel previously described in the apical membrane and the 360 pS Cl^- channel previously described in the basolateral membrane of vestibular dark cells. The involvement of these channels in hypoosmotically-induced KCl flux, however, remains to be demonstrated.

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