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Anion modulation of calcium current voltage dependence and amplitude in salamander rods

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Abstract

Hofmeister anions were used to investigate the ability of Cl^- replacement to produce inhibition and a hyperpolarizing activation shift in L-type Ca^{2+} currents (I_{Ca}) of rod photoreceptors. Inhibition of I_{Ca} largely followed the Hofmeister sequence: $Cl^- = Br^- < NO_3^- < I^- < ClO_4^-$ (ClO_4^- caused the greatest suppression). Anion-induced hyperpolarizing activation shifts also followed the Hofmeister sequence: $Cl^- < Br^- < NO_3^- < I^- < ClO_4^-$ (ClO_4^- caused the largest shift). Agreement with the Hofmeister sequence suggests that these effects are due to anion interactions at the membrane surface. Hofmeister anions also caused similar hyperpolarizing shifts in the voltage dependence of inwardly rectifying cation currents (I_h) and outward K^+ currents (I_K) consistent with the hypothesis that hyperpolarizing shifts arise from anion effects on membrane surface potential. Sulfate and phosphate inhibited rod I_{Ca} and phosphate caused a significant leftward activation shift suggesting these anions are strongly adsorbed to the membrane. Because of the overlap between the physiological voltage range and the lower part of the I_{Ca} activation curve, anion effects on amplitude and activation may influence synaptic transmission at the first retinal synapse.

Keywords: Retina; Hofmeister anion; Chloride; Photoreceptor; L-Type calcium current

1. Introduction

Release of the neurotransmitter, L-glutamate, from rod photoreceptors is mediated by Ca^{2+} influx through dihydropyridine-sensitive L-type Ca^{2+} channels [1–3]. Substances which modulate rod Ca^{2+} channels will therefore modify the transmission of visual information at the first retinal synapse. Since the physiological voltage range for photoreceptors is confined to the lower region of the I_{Ca} activation curve, both changes in I_{Ca} amplitude and small shifts in the voltage dependence of I_{Ca} can have a significant impact on the transmission of light-evoked responses [4,5].

Reducing extracellular [Cl^-] inhibits photoreceptor I_{Ca} causing inhibition of light-evoked currents in second-order neurons [3,6]. Significant suppression of I_{Ca} and synaptic transmission are observed when [Cl^-] is reduced by only 10 mM raising the possibility that physiological [Cl^-] changes in and around synaptic terminals of photoreceptors may modulate neurotransmission [3]. In addition to inhibiting the peak amplitude of I_{Ca}, reducing extracellular [Cl^-] also causes a leftward shift in I_{Ca} activation. The mechanism(s) by which anions influence amplitude and voltage dependence of photoreceptor I_{Ca} are

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Table 1
Extracellular solutions (concentrations in mM)

Chaotropes/Ca	99 NaA[a]	2.5 KA	10 CaA_2	0.5 MgA_2	5 glucose	10 HEPES	
Chaotropes/Ba	99 NaA	2.5 KA	10 BaA_2	0.5 MgA_2	5 glucose	10 HEPES	
SO_4	55.5 Na_2SO_4	1.25 K_2SO_4	2.7 $CaSO_4$	0.5 $MgSO_4$	5 glucose	10 HEPES	57.3 mannitol
SO_4 control	111 NaCl	2.5 KCl	2.7 $CaCl_2$	0.5 $MgCl_2$	5 glucose	10 HEPES	
H_2PO_4	91 NaCl	2.5 KCl	1.8 $CaCl_2$	0.5 $MgCl_2$	5 glucose	10 HEPES	20 NaH_2PO_4
H_2PO_4 control	111 NaCl	2.5 KCl	1.8 $CaCl_2$	0.5 $MgCl_2$	5 glucose	10 HEPES	
I_K and I_h	111 NaA	2.5 KA	0.1 $CdCl_2$	2.3 MgA_2	5 glucose	10 HEPES	
	55.5 Na_2SO_4	1.25 K_2SO_4	0.1 $CdCl_2$	2.5 $MgSO_4$	5 glucose	10 HEPES	57.3 mannitol

[a] A = Cl^-, Br^-, NO_3^-, I^-, or ClO_4^-.

not yet fully understood although previous experiments have ruled out a number of possibilities which might account for these effects, such as indirect effects of Cl^- on pH [3].

Similar to photoreceptors, anions can also modulate the amplitude of I_{Ca} and/or hormone release at neurosecretory synapses in pituitary and adrenal chromaffin cells [7–9]. This suggests that anion modulation of I_{Ca} amplitude may be a general property of the L-type I_{Ca} subtypes that mediate sustained release from sensory receptors and neurosecretory cells. In contrast to the suppressive effects in neurosecretory cells, Cl^- replacement does not significantly inhibit L-type I_{Ca} in vertebrate skeletal and cardiac muscle [10–13]. However, Cl^- replacement does cause a negative activation shift in skeletal muscle. It has been proposed that this anion-induced activation shift is due to changes in membrane surface potential [10,14]. The observation that anions cause an activation shift in the absence of significant inhibition of skeletal muscle I_{Ca} suggests that different mechanisms may mediate these two effects of anions on photoreceptor I_{Ca}.

The ability of anions to alter surface potential, like many other physicochemical properties of anions, follows the Hofmeister (or lyotropic) anion series. The series was first described by Franz Hofmeister from the abilities of anions to precipitate proteins from whole egg white [15]. A representative Hofmeister series based on surface charge at an air/water interface is: $SO_4^{2-} < Cl^- < Br^- < NO_3^- < I^- < ClO_4^-$ [16–18]. Anions to the right of Cl^- in the Hofmeister series, which have been termed chaotropic anions, possess low charge density, exhibit weak hydrogen bonding interactions with water, and destabilize proteins [19,20]. In principle, the weak ion-water interactions of chaotropic anions facilitate their approach to a lipid–water interface and thus increase negative charge at a membrane surface. In contrast, anions to the left of Cl^- in the Hofmeister series, which have been termed kosmotropic anions (e.g., HPO_4^{2-} and SO_4^{2-}), possess high charge density, exhibit strong hydrogen bonding interactions, and help to stabilize proteins [19,20]. Based on this concept, the strong ion–water interactions of kosmotropes will limit their approach to a water-lipid interface.

In the present study, Hofmeister anions are used to probe the sites of anion effects on I_{Ca} activation and amplitude in rod photoreceptors. The results indicate that chaotropic anions produce a negative shift in rod I_{Ca} as a consequence of changes in membrane surface potential. Chaotropic anions also inhibit I_{Ca} with an efficacy following the Hofmeister sequence suggesting that the inhibitory effects arise from anion interactions at the membrane surface. The physiological anions, sulfate and phosphate, inhibit I_{Ca} and phosphate causes a significant leftward activation shift suggesting these anions are strongly adsorbed to the membrane. Because of the limited overlap between I_{Ca} activation and the voltage range for light-evoked responses of photoreceptors, anion effects on both I_{Ca} amplitude and activation could influence synaptic transmission at the first synapse in the visual pathway. Some of these results have been presented previously in abstract form [21,22].

2. Materials and methods

Conventional and perforated patch, whole cell voltage clamp recordings were obtained from rod photoreceptors in the superfused retinal slice prepa-

ration of the larval tiger salamander (*Ambystoma tigrinum*). The retinal slice preparation used in these experiments was the same as that described elsewhere [3,6].

Extracellular solutions used in the various electrophysiological experiments are summarized in Table 1. The experimental solutions described in this table were used to test: (1) effects of chaotropic anions on I_{Ca} (chaotropes/Ca), (2) effects of chaotropic anions on I_{Ba} (chaotropes/Ba), (3) effects of SO_4 on I_{Ca} (SO_4), (4) control solution for SO_4 experiments (SO_4 control), (5) effects of H_2PO_4 on I_{Ca} (H_2PO_4), (6) control solution for H_2PO_4 experiments (H_2PO_4 control), and (7) experiments on outward and inwardly rectifying currents in rods (I_K and I_h). In addition to the compounds described in the Table 1, 0.1 mM niflumic acid and 0.1 mM picrotoxin were added to all solutions and slices were steadily illuminated by bright white light in order to suppress the light-sensitive conductance in intact outer segments. All solutions were continuously bubbled with 100% O_2 and the pH was adjusted to 7.8 with NaOH. The chaotropic anions (Br^-, NO_3^-, I^-, and ClO_4^-) and Cl^- were tested with 10 mM Ca^{2+} or 10 mM Ba^{2+} as the charge carrier. The Ca^{2+} and Ba^{2+} salts of these chaotropic anions are freely soluble. In contrast, the highest free [Ca^{2+}] that could be achieved with the SO_4^{2-} and $H_2PO_4^-$ solutions were 2.7 and 1.8 mM respectively. Therefore, in experiments with SO_4 and H_2PO_4, the free [Ca^{2+}] in control and test solutions were matched using a Ca-sensitive electrode (KWIKCAL, World Precision Instruments). TEACl (10 mM) was also added in experiments with H_2PO_4. In experiments on I_h and I_K, Ca^{2+} was replaced with Mg^{2+} and 0.1 mM Cd^{2+} was added to suppress I_{Ca}.

Whole cell patch electrodes were pulled with a Narashige PB-7 puller from borosilicate pipettes (1.2 mm outer diameter, 0.95 mm inner diameter, omega dot) and had tips of 1–2 μM outer diameter ($R = 10$–15 MΩ). To avoid the rapid rundown that plagues conventional whole cell recordings of I_{Ca}, we used perforated patch recording techniques with the pore-forming antibiotic nystatin. During anion replacement experiments with perforated patch recordings, we were able to maintain stable recordings of I_{Ca} for up to 1 h. The pipette electrolyte solution contained (in mM): 54 CsCl, 61.5 $CsCH_3SO_3$, 3.5 $NaCH_3SO_3$, and 10 HEPES (pH 7.2). Nystatin was mixed in dimethylsulfoxide (120 mg/ml) and then diluted into the electrolyte solution to achieve a final concentration of 480 μg/ml. Fresh nystatin solutions were prepared every 3 h.

Osmolarities of all intra- and extracellular solutions were measured using a vapor pressure osmometer (Wescor 5100C) and adjusted, if necessary, with distilled water or Na anion to 242 ± 2 mOsm. Such care was necessary because removal of Cl^- perturbs the Donnan equilibrium making cells extremely sensitive to small osmotic imbalances.

The input resistance (R_{in}) of rods averaged 853 ± 88.5 MΩ ($n = 53$), cell capacitance averaged 38.9 ± 3.53 pF, and pipette access resistance (R_{ser}) during perforated patch recording averaged 33.8 ± 1.22 MΩ. Photoreceptors were voltage clamped at −70 mV. I_{Ca} was typically tested with voltage ramps (1 mV/ms) from −90 to +60 mV acquired every 10–60 s. Ramps allowed rapid and frequent acquisition of data, more reproducible estimates of V_{50} values, and caused less current inactivation than step series. The amplitude of I_{Ca} evoked by ramps averaged 97.7% ($n = 10$) of the amplitude of I_{Ca} evoked by voltage steps (150 ms step duration). The voltage at which currents were half-maximal (V_{50}) also did not differ significantly for ramps and steps ($P = 0.17$, paired t-test; ramp V_{50} − step $V_{50} = -3.2 \pm 2.11$ mV; $n = 10$).

Conventional, ruptured patch, whole cell recording techniques were used for experiments on I_h and I_K. For these experiments, the intracellular solution contained: 98 mM KCH_3SO_4, 3.5 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 5 mM HEPES, 2 mM D-glucose, 1 mM reduced glutathione, 1 mM ATP-Mg, 1 mM GTP. I_h was evaluated with voltage steps (10 mV, 150 ms) negative to −60 mV; I_K was evaluated at potentials positive to −70 mV.

KCl (3 M)/agar was used as a bridge to the Ag/AgCl reference electrode. The agar bridge was downstream from the retinal slices in the perfusion chamber so Cl^- leached from the agar could not reach the slices. With this arrangement, the change in junction potential measured after switching to various Cl^- substitutes was ±0.2 mV.

Currents were acquired and analyzed using PClamp 6.0 software. Holding potentials were corrected for a junction potential of 10.5 mV calculated

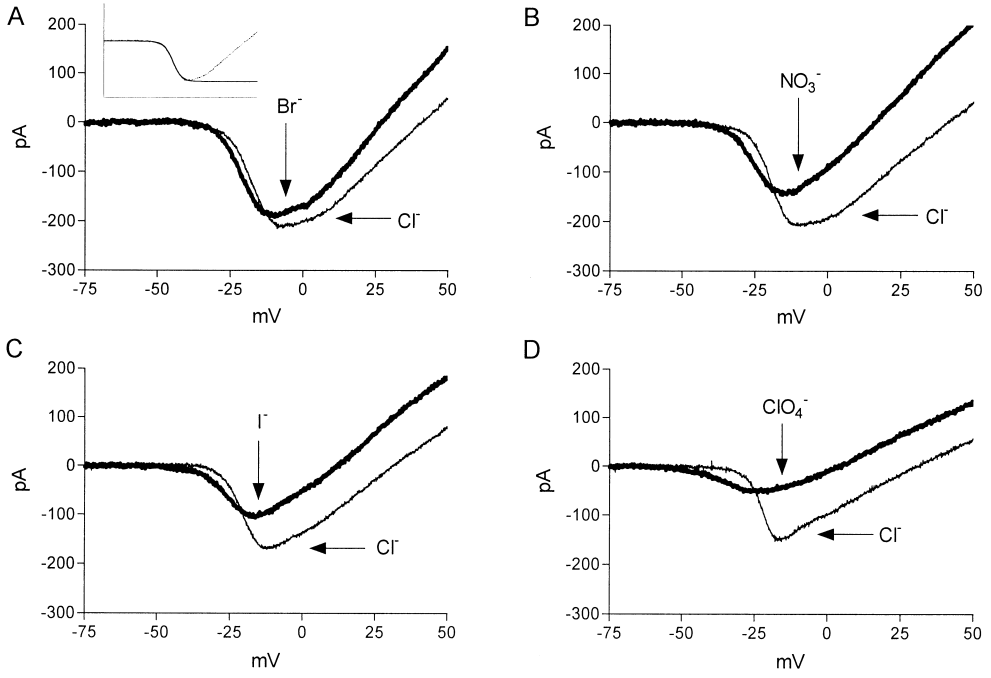

Fig. 1. Leak-subtracted current-voltage relationships obtained in a rod photoreceptor in solutions containing Cl^- (thin trace, all panels), Br^- (A, thick trace), NO_3^- (B, thick trace), I^- (C, thick trace), and ClO_4^- (D, thick trace). Cl^- traces were obtained prior to replacement with the test anion. In this cell, the experimental order was ClO_4^-, I^-, NO_3^-, and Br^-. (Inset) Sigmoidal fit to control data (thin trace, panel A) with Eq. 1.

using PClamp 7.0. The leak conductance was assumed to be ohmic and equal to the minimum conductance between −75 and −55 mV. Leak subtraction of I_{Ca} by block with 0.1 mM Cd^{2+} yielded almost identical voltage profiles ($n=12$). After leak subtraction, the amplitude of I_{Ca} was measured and the voltage at which the current was half-maximal (V_{50}) was determined from the best fit to a sigmoidal function of the form [1]:

$$I = I_{max}(1+\exp(V_{50}-V)/B)^{-1} + C \quad (1)$$

where I = current at voltage V, I_{max} = maximum current, C = baseline constant (near 0 after leak subtraction), and B = slope factor. The inset in Fig. 1A shows an example of the best fit curve fit to a control record in Cl-containing media. The region chosen for fitting extended from base to peak of the inward current. V_{50} values determined from the best-fit curves were consistently within 0.2 mV of the V_{50} values measured at the half-maximal current of the leak-subtracted raw data. Statistical significance was chosen as $P<0.05$ and evaluated using GraphPad Prism. Errors are reported as ± S.E.M.

3. Results

Fig. 1 illustrates effects of the chaotropic anions Br^-, NO_3^-, I^-, or ClO_4^- on I_{Ca}. The figure shows leak-subtracted currents evoked by voltage ramps recorded from a rod with 10 mM Ca^{2+} as the primary charge carrier. The thin trace in each panel shows the currents obtained in Cl^- solution prior to replacement with the indicated test anion (thick trace). The inset in the figure shows the best fit sigmoidal curve from Eq. 1 to the control record in Fig. 1A. Although the figure presents the data in an order which accords with the Hofmeister sequence, the test order in this particular experiment was actually ClO_4^-, then I^-, then NO_3^-, and finally Br^-. Anion test orders were shuffled between experiments to avoid possible order effects. The figure shows progressively greater suppression of I_{Ca} as one increases the chaotropic nature of the anion ($Cl^- < Br^- < NO_3^- < I^- < ClO_4^-$). Fig. 1 also shows that chaotropic anions caused a hyperpolarizing shift in the threshold and peak of I_{Ca}. The hyperpolarizing shifts in outward currents may reflect, at least in

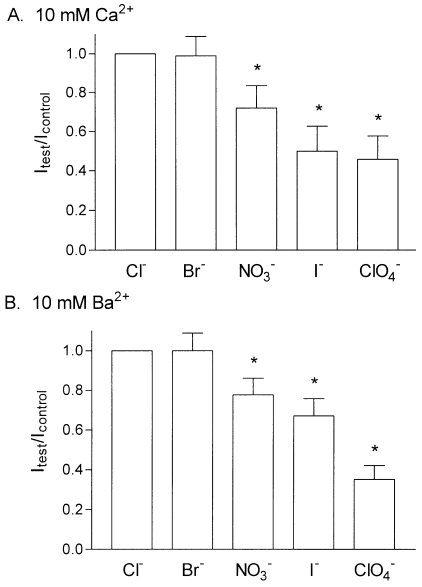

Fig. 2. Suppression of I_{Ca} (A) and I_{Ba} (B) in rods by replacing Cl^- with Br^-, NO_3^-, I^-, or ClO_4^-. Amplitudes of currents obtained in test solutions were normalized to currents obtained in control Cl^- solution ($I_{test}/I_{control}$). Test ramps were acquired after 0.5–2 min applications of test solutions containing Br^-, NO_3^-, I^-, and ClO_4^-. Samples sizes were as follows. Rods, 10 mM Ca^{2+}: Br^-, $n=11$; NO_3^-, $n=9$; I^-, $n=8$; ClO_4^-, $n=7$. Rod I_{Ca} amplitude and V_{50} averaged -193 ± 31.0 pA ($n=13$) and -10.0 ± 1.72 mV, respectively. Rods, 10 mM Ba^{2+}: Br^-, $n=15$; NO_3^-, $n=14$; I^-, $n=13$; ClO_4^-, $n=21$. Rod I_{Ba} amplitude and V_{50} averaged -251 ± 35.1 pA ($n=33$) and -19.7 ± 1.50 mV, respectively. *Significant suppression with respect to control currents in Cl media using Student's t-test ($P<0.05$). Current amplitudes in the four test anions were also significantly different from one another as assessed by ANOVA (Ca^{2+}, $P=0.007$; Ba^{2+}, $P<0.001$).

part, shifts in the activation of incompletely blocked delayed rectifier K^+ currents (see Fig. 7).

The bar graphs in Fig. 2 show the mean fractional amplitudes of rod I_{Ca} and I_{Ba} normalized to the control currents in Cl-containing solution ($I_{test}/I_{control}$). With both Ca^{2+} and Ba^{2+} as charge carriers, the most chaotropic anion, ClO_4^-, produced the greatest suppression (rod I_{Ca}, 0.46 ± 0.120; rod I_{Ba}, 0.35 ± 0.067). In both cases, inhibition of I_{Ca} amplitude largely followed the chaotropic order: $Cl^- = Br^- < NO_3^- < I^- < ClO_4^-$.

As illustrated in Fig. 3, during superfusion with anion test solutions, I_{Ca} consistently exhibited a maximum negative shift prior to the maximum inhibitory effect of anions on I_{Ca} amplitude. The more rapid effect of anions on voltage dependence does not ap-

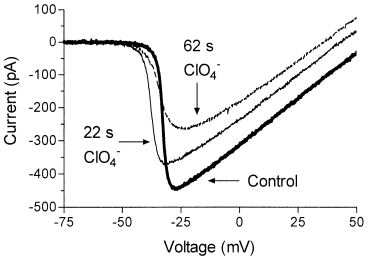

Fig. 3. Anion-induced shift in I_{Ba} voltage dependence preceded maximal suppression of I_{Ba}. Cl^- was replaced with ClO_4^-. Leak-subtracted currents evoked by voltage ramps. Thick line, control current obtained just prior to application of Cl-free medium; thin solid line, current after 22 s perfusion in Cl-free medium; thin dashed line, current after 62 s perfusion in Cl-free medium.

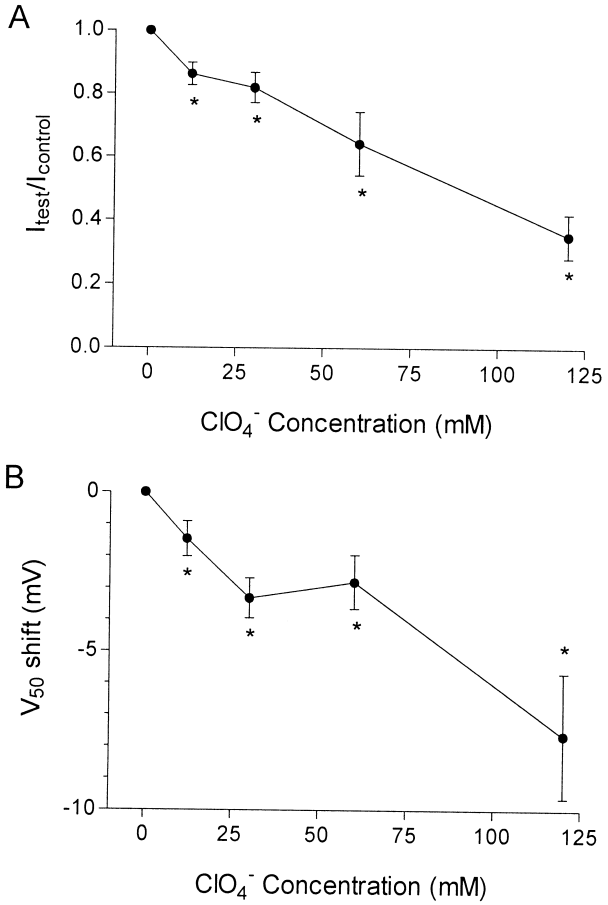

Fig. 4. Concentration-response relationships between ClO_4^- concentration and I_{Ba} amplitude (A) or shift in voltage dependence (B). Amplitude data were determined as described for Fig. 2. V_{50} was determined from the best fit of Eq. 1 to the leak-subtracted I_{Ca} or I_{Ba}. The activation shift was calculated by subtracting the V_{50} obtained in the test solution from the V_{50} in the control, Cl^- solution. Charge carrier: 10 mM Ba^{2+}. *Significant change with respect to control using Student's t-test ($P<0.05$).

pear to reflect a greater sensitivity to low Cl^- concentrations since, as shown in Fig. 4, the concentration dependence of the two effects was similar. Using the most chaotropic (and consequently most efficacious) anion, significant effects on both amplitude and voltage dependence were obtained with ClO_4^- concentrations of 12.5 mM and both effects increased with higher concentrations of ClO_4^-. The slow development of inhibition is instead consistent with experiments suggesting that inhibitory effects of anions are exerted at the intracellular membrane surface [23]. For the purposes of the present study, the rapid effect of anions on voltage dependence was fortuitous because it permitted measurements of anion effects on voltage dependence before currents were substantially inhibited thereby minimizing artefactual voltage shifts arising from series resistance.

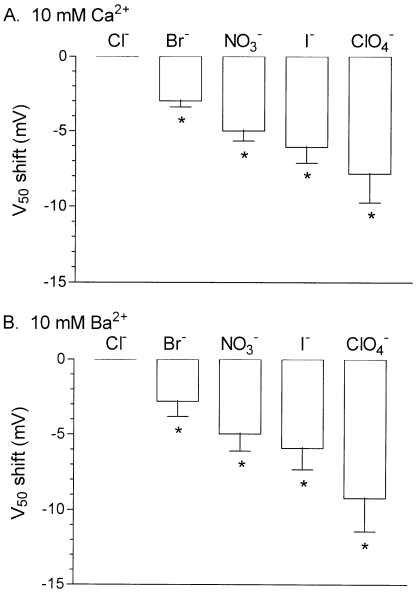

Fig. 5. Shifts in the midpoints (V_{50}) of I_{Ca} (A) and I_{Ba} (B) caused by replacing Cl^- with Br^-, NO_3^-, I^-, or ClO_4^-. V_{50} was determined from the best fit of Eq. 1 to the leak-subtracted I_{Ca} or I_{Ba}. The activation shift was calculated by subtracting the V_{50} obtained in the test solution from the V_{50} measured in the control, Cl^- solution. To minimize artefactual shifts associated with series resistance, test V_{50} values were determined from the first ramp obtained following change to the anion solution. Records were excluded from consideration if the current at the first ramp in the anion test solution showed suppression of >100 pA. Data are from the same cells as in Fig. 2. *Significant shift in voltage dependence with respect to control currents in Cl media evaluated with Student's t-test ($P<0.05$). The shifts induced by each anion also differed significantly from one another as assessed by ANOVA (Ca^{2+}, $P=0.013$; Ba^{2+}, $P=0.023$).

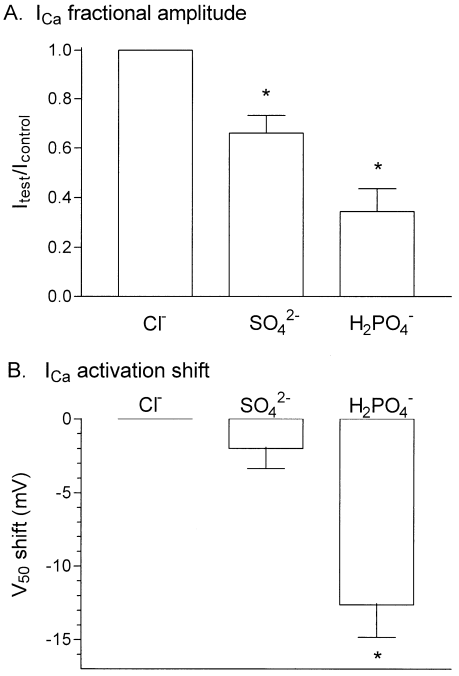

Fig. 6. Effects of kosmotropic anions, SO_4^{2-} (55 mM, $n=24$) and $H_2PO_4^-$ (20 mM, $n=8$), on rod I_{Ca} amplitude (A) and V_{50} (B). In A, the amplitude of currents obtained in test solutions were normalized to currents obtained in control Cl^- solution before application and after washout of test solutions ($I_{test}/I_{control}$). In B, V_{50} values were determined from the best fit of Eq. 1 to leak-subtracted I_{Ca}. The shift in V_{50} was then calculated by subtracting V_{50} in the test solution from V_{50} in the control, Cl^- solution. In SO_4^{2-} trials, control I_{Ca} amplitude and V_{50} averaged 93.2 ± 13.71 pA and -21.7 ± 1.15 mV. In $H_2PO_4^-$ trials, control I_{Ca} amplitude and V_{50} averaged 101.5 ± 17.41 pA and -14.4 ± 1.79 mV. *Significant change with respect to control currents using Student's t-test ($P<0.05$).

Fig. 5 shows the mean anion-induced shift in V_{50} with respect to the V_{50} measured in Cl^-. As with chaotropic effects on amplitude, the largest shift was induced by the most chaotropic anion, ClO_4^- (Ba^{2+}, -9.2 ± 2.19 mV; Ca^{2+}, -7.8 ± 1.92 mV) and the shift in I_{Ca} voltage dependence followed the chaotropic order: $Cl^- < Br^- < NO_3^- < I^- < ClO_4^-$.

SO_4^{2-} and $H_2PO_4^-$ were tested because of their positions in the Hofmeister sequence and because they are, unlike the chaotropic anions tested above, physiologically occurring anions. As shown in Fig. 6, $H_2PO_4^-$ (20 mM) caused significant inhibition and a negative shift in the activation midpoint of I_{Ca} akin to higher concentrations of the most chaotropic

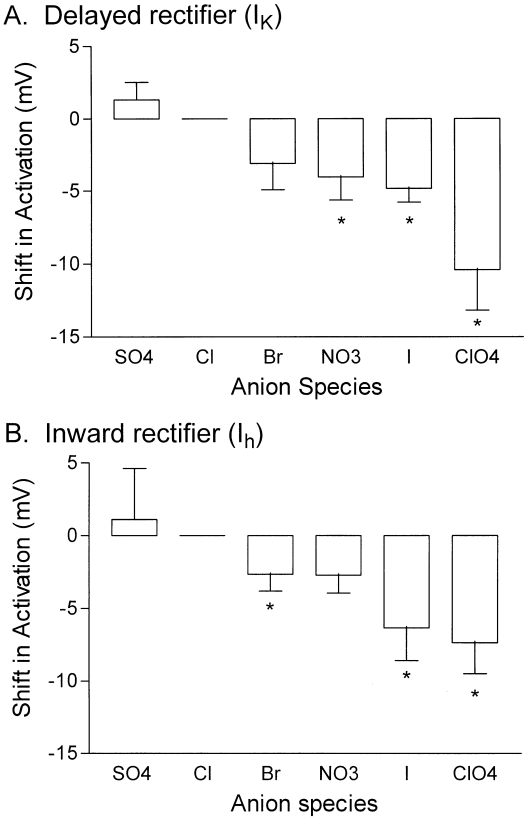

Fig. 7. Effects of Hofmeister anions on I_K and I_h in rods. (A) Anion-induced shifts in the activation midpoints (V_{50}) of I_K. (SO_4^{2-}, $n=7$; Br^-, $n=8$; NO_3^-, $n=9$; I^-, $n=10$; ClO_4^-, $n=7$.) (B) Anion-induced shifts in V_{50} for I_h. (SO_4^{2-}, $n=9$; Br^-, $n=10$; NO_3^-, $n=10$; I^-, $n=10$; ClO_4^-, $n=11$.) V_{50} was determined from the best fit of Eq. 1 to the leak-subtracted, steady-state, current–voltage relationship for I_K or I_h. The activation shift was calculated by subtracting the V_{50} obtained in the test solution from the V_{50} in the control, Cl^- solution. *Significant shift in voltage dependence with respect to control currents in Cl media using Student's t-test ($P<0.05$). Activation shifts induced by the five test anions also differed significantly from one another by ANOVA (I_K, $P<0.001$; I_h, $P=0.034$).

anion, ClO_4^-. SO_4^{2-} (55 mM) caused significant inhibition of I_{Ca} but no significant activation shift.

Previous studies with Hofmeister anions in skeletal muscle suggest that the shift in voltage dependence of I_{Ca} is due to anion-induced changes in membrane surface potential [10,14]. If changes in surface potential account for the anion-induced shift in the voltage dependence of I_{Ca}, then anions should cause similar shifts in other voltage-dependent currents of rods. We therefore tested the effects of Hofmeister anions on the voltage dependence of two additional currents in rods: cation currents activated by hyperpolarization (I_h) and outward K^+ currents activated by depolarization (I_K) [24–26]. I_h and I_K were evoked by a series of 150 ms voltage steps in the presence of 0 mM Ca^{2+} and 0.1 mM Cd^{2+} to block I_{Ca}. In control Cl^- solution, the V_{50} for I_h was -93.2 ± 3.43 mV ($n=29$) and the V_{50} for I_K was $+37.6\pm4.70$ mV ($n=28$). As with I_{Ca}, replacing Cl^- with Br^-, NO_3^-, I^-, or ClO_4^- caused a negative shift in V_{50} for both currents roughly consistent with the chaotropic order (Fig. 7). ClO_4^- produced the largest negative shift (I_h, $-7.32+2.136$ mV; I_K, -10.40 ± 2.762 mV). SO_4^{2-} produced a small positive shift. Consistent with a relatively non-specific effect on surface charge, anion-induced shifts in V_{50} for I_{Ca}, I_h, and I_K were well correlated with one another (I_h vs. I_K, $r^2=0.84$; I_{Ca} vs. I_K, $r^2=0.88$; I_{Ca} vs. I_h, $r^2=0.89$).

4. Discussion

Hofmeister anions suppress rod I_{Ca} and shift activation of voltage-dependent currents to more negative potentials. The abilities of chaotropic anions to shift activation and reduce the amplitude of I_{Ca} were correlated with their order in the Hofmeister series: $Cl^- < Br^- < NO_3^- < I^- < ClO_4^-$. This order differs from the orders of solubility products (K_{sp}) of the anions with Na^+, Ca^{2+}, Ba^{2+}, or H^+. Therefore, it is unlikely that chaotropic anions exert their effects indirectly by non-covalent associations which deplete cations from the membrane. Correlation with the Hofmeister sequence suggests that anion effects on both amplitude and voltage dependence are due to interactions at the membrane surface.

The finding that the size of the anion-induced shift in V_{50} for I_{Ca} was correlated with the chaotropic strength of the anion supports conclusions of earlier studies which suggested that anion-induced activation shifts are due to changes in membrane surface potential [10,14]. (For a discussion of the features and consequences of membrane surface charge, the reader is referred to Hille [27]). Two other results of the present study strengthen this conclusion. First, although there may be small disparities due to local differences in charge distribution, changes in membrane surface potential should have similar effects on all voltage-dependent currents in a cell. Consistent with this prediction, hyperpolarizing shifts in the ac-

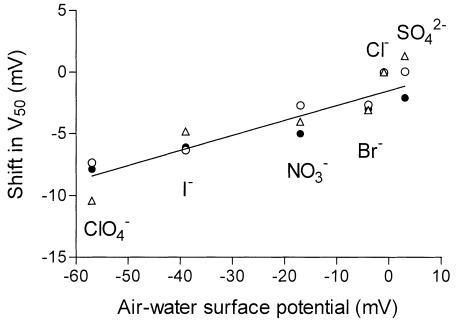

Fig. 8. Anion-induced shifts in the activation midpoints (V_{50}) of I_K (open triangles), I_h (open circles), and I_{Ca} (filled circles) in rods were linearly correlated with anion-induced changes in surface potential at an air-water interface (straight line; $r^2 = 0.836$). Surface potential measurements were made by Frumkin [16] and Randles [18] in solutions containing 2 M Na anion. Data on I_K and I_h were taken from Fig. 7 and data on I_{Ca} were taken from Figs. 5 and 6.

tivation midpoints of I_{Ca}, I_h, and I_K were well correlated with one another. Second, as shown in Fig. 8, anion-induced shifts in the voltage dependence of I_{Ca}, I_h, and I_K are linearly correlated with anion-induced shifts in surface potential measured directly at an air–water interface [16,18] (I_h, $r^2 = 0.92$; I_K, $r^2 = 0.87$; rod I_{Ca}, $r^2 = 0.84$). The surface potential shifts plotted along the abscissa of Fig. 8 were measured with 2 M solutions of Na anion which produce larger surface potential changes than the concentrations used in the present study. At a more physiological concentration of 0.1 M, $NaClO_4$ changes the surface potential by −10 mV at both air–water and water–membrane surfaces [28]. This is comparable to the hyperpolarizing shifts in the activation midpoints of I_{Ca}, I_{Ba}, I_K, and I_h induced by ClO_4^- which ranged from −7.3 to −10.4 mV. We therefore conclude that the activation shifts of I_{Ca} induced by chaotropic anions are due to increases in membrane surface potential.

The large negative shift in I_{Ca} induced by phosphate cannot be accounted for by its generally agreed upon position in the Hofmeister sequence. The ability of phosphate to produce a negative shift in I_{Ca} may result from the fact that phosphate is a physiological anion for which proteins possess many moderate to high affinity binding sites [29]. Plentiful phosphate binding sites will promote its binding to the membrane surface causing an increase in negative surface charge and thus inducing a negative activation shift. It should be noted that the ability of 20 mM $H_2PO_4^-$ to significantly inhibit I_{Ca} and shift its activation indicates that physiological anions can replicate effects seen experimentally with non-physiological Cl^- substitutes in this and other studies (e.g., [3,30]).

Like the effects on voltage dependence, the ability of chaotropic anions to suppress I_{Ca} correlated with their position in the Hofmeister series suggesting that the suppressive effects arise from interactions at the membrane surface. However, the inhibitory effect of Hofmeister anions on I_{Ca} amplitude is probably not a consequence of the shift in activation voltage since this would not be expected to influence the amplitude of the fully activated current. The mechanism of inhibitory effects of anions on photoreceptor I_{Ca} is not yet fully explained. In pituitary melanotrophs, anions appear to exert an indirect effect on Ca^{2+} channels by interacting with pertussis toxin-sensitive G proteins [31,32]. However, we have found that pertussis toxin pretreatment does not alter the sensitivity of photoreceptor I_{Ca} to replacement of Cl^- with ClO_4^- (data not shown). Another possibility is direct interaction of anions with photoreceptor Ca^{2+} channels. This possibility is supported by single channel experiments showing that anions act at the intracellular membrane surface to reduce channel open probability [23].

One interesting consequence of the hyperpolarizing activation shift is that, despite suppression of the peak current, I_{Ca} in the physiological voltage range (below −35 mV) is generally enhanced by Cl^- replacement (e.g., Figs. 1 and 3). This situation is analogous to the increase of I_{Ca} in the physiological voltage range produced by reductions in divalent cation concentration which, like anion substitution, suppresses the peak current and produces a hyperpolarizing activation shift [5]. However, despite a hyperpolarizing activation shift, Cl^- replacement inhibits synaptic transmission from photoreceptors [6]. The inhibition of photoreceptor glutamate release by low Cl^- solutions must therefore involve additional sites of action besides the effects on I_{Ca} described in this paper. For example, increasing I_{Ca} at the resting potential will increase $[Ca^{2+}]_i$ which will in turn enhance Ca-dependent inactivation of I_{Ca} [1]. Changes in photoreceptor membrane potential induced by low Cl^- solutions will further influence both the steady

state activation level of I_{Ca} and the consequent degree of Ca-dependent inactivation. Finally, changes in [Cl^-] can influence other aspects of neurotransmission (e.g., vesicular glutamate uptake) [33].

Results of the present study on the effects of anions complement previous studies on effects of protons and divalent cations [4,5] and further demonstrate the importance of small ions in regulating the voltage dependence and amplitude of photoreceptor I_{Ca}. The presence of Ca^{2+}-activated Cl^- channels and Cl^- channels coupled to glutamate transporters in rod photoreceptors [25,34], as well as GABA-activated and voltage-dependent Cl^- channels in cones [35,36], raise the possibility that Cl^- flux in the tight confines of invaginating photoreceptor terminals may cause sufficient changes in anion concentration near the membrane to alter surface charge distribution and thus alter I_{Ca} activation.

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