Effects of Ca^{2+} on rabbit translens short-circuit current: evidence for a Ca^{2+} inhibitable K^+ conductance

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Abstract

Purpose. To characterize the effects of medium Ca^{2+} levels on rabbit lens electrical properties. Early studies with wholly submerged lenses had shown that Ca^{2+} removal from the bath resulted in an increased Rb^+ efflux, a consequence of an increased Na^+ permeability and lens depolarization.

Methods. Lenses were bathed within Ussing-type chambers under short-circuited conditions, an arrangement in which the translens short-circuit current (I_{sc}) is carried across the posterior lens surface mainly by an influx of Na^+, and across the anterior face largely by a K^+ efflux.

Results. Under the present conditions in which the effects of Ca^{2+} were characterized unilaterally, the above established effects could only be ascribed to the posterior surface. When Ca^{2+} removal was limited to the anterior face, the I_{sc} increased from 11.87 ± 1.17 to 17.04 ± 1.52 $\mu A/cm^2$ (means ± SE's, n = 18; an accompanying translens resistance (R_t) decrease of 0.23 ± 0.049 $K\Omega \cdot cm^2$ was also recorded). Conversely, increasing the control, anterior-bath $[Ca^{2+}]$ from 1.8 to 3.6 mM reduced the K^+ efflux-dependent I_{sc} from 10.54 ± 1.09 to 8.93 ± 1.02 (n = 10, with an R_t increase of 0.11 ± 0.013). These changes were reversible, Na^+-independent, and fully inhibited by the presence of K^+ channel blockers (quinidine or Ba^{2+}). Inhibitions of the Ca^{2+} effects were also obtained with strontium, a Ca^{2+} surrogate. The I_{sc} was less responsive to changes in the Ca^{2+} content of the posterior bath. Removal of the cation caused a gradual 1.65 ± 0.72 $\mu A/cm^2$ increase (n = 9, with an R_t decrease of 0.090 ± 0.021 $K\Omega \cdot cm^2$). In the absence of posterior Na^+, Ca^{2+} withdrawal resulted in highly variable responses, with some specimens exhibiting salient current increases, suggesting that an outwardly directed, posterior efflux of an anion could also have been affected. During the course of this study it was consistently observed that the removal of Na^+ from the anterior bath led to an I_{sc} decrease of 2.62 ± 0.22 $\mu A/cm^2$ (n = 32, with an R_t increase of 0.35 ± 0.029 $K\Omega \cdot cm^2$). This change occurred in both the presence of ouabain and the absence of Ca^{2+}, suggesting that it did not result from an inhibition of the Na^+-K^+ pump current nor from a reversal in putative Na^+/Ca^{2+} exchange activity. Small I_{sc} increases upon anterior Na^+ withdrawal (1.68 ± 0.17, n = 7), consistent with Na^+ efflux from the lens, could only be observed with K^+ channels inhibited with Ba^{2+}. Also congruent with the observations of a relatively limited anterior Na^+ permeability, was the finding that the induction of nonspecific cation channels with amphotericin B reduced the I_{sc} by allowing Na^+ from the anterior bath to enter the lens. Thus, changes in lens I_{sc} can differentiate changes in K^+ permeability across the native anterior epithelium from changes in Na^+ permeability.

Conclusions. Overall, these results suggest that lens Ca^{2+}-mobilizing agents (e.g. acetylcholine) could trigger the inhibition of epithelial K^+ conductance(s) by the direct action of Ca^{2+} on K^+ channels. Curr. Eye Res. 15: 1198–1207, 1996.

Key words: electrolyte transport; Ussing chamber; acetylcholine; nifedipine; calcium-sensitive potassium conductance; lens; rabbit

Introduction

Measurements of translens short-circuit current (I_{sc}) provide a convenient approach for continuously recording epithelial basolateral K^+ conductance(s) plus the Na^+-K^+ pump current (1, 2). The method is based upon the fact that these electrogenic elements are distributed asymmetrically in toad, rat and rabbit lenses so that their activity represents the major source of the translenticular potential difference (PD_t), anterior side positive, that develops upon isolation of the anterior and posterior lens aspects in a bicameral chamber (2–4). In this arrangement, the I_{sc} is carried across the posterior face mainly by an influx of Na^+ and across the anterior surface largely by a K^+ efflux.

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Heretofore, lens I_{sc} recordings have been used to demonstrate the modulation of K^+ conductance(s) by pH and tonicity changes in the toad lens (2), and by muscarinic-receptor activation in the rabbit lens (4). The latter study suggested that an immediate consequence of applying the lens-Ca^{2+} mobilizing agent acetylcholine (5) was closure of a subset of K^+ channels due to Ca^{2+} activity within the epithelium.

Early studies with wholly submerged rabbit lenses had shown that Ca^{2+} removal from the bath resulted in increased Rb^+ efflux, an indirect aftermath of increased Na^+ permeability and lens depolarization (6). Under the present conditions, in which the effects of Ca^{2+} were examined unilaterally, these established effects may only be ascribed to the posterior face. Anteriorly, altered extracellular Ca^{2+} levels, and presumed changes in intracellular levels, appear to exclusively modulate epithelial K^+ channels directly (i.e. changes in K^+ conductance(s) are not secondary to the regulation of Na^+ permeability), while on the posterior aspect, Ca^{2+} may also regulate an anion channel (presumably Cl^-).

Materials and methods

Translenticular electrical measurements

Lenses removed from adult albino rabbits that had been killed by CO_2 asphyxiation were mounted between Ussing-type hemichambers as described in detail previously (4). Briefly, the polar aspects of the lenses were electrically isolated from each other with each respective polar surface bathed within its own compartment. The cross-sectional areas of the surfaces interfacing with the bathing media was typically $\approx$0.75 cm^2 for lenses that had equatorial diameters of 10.5 ± 1 mm.

Agar-NaCl-filled polyethylene tubing served as a salt bridge connecting each bathing compartment to Ag/AgCl electrodes for translens PD (PD_t) measurements. A second pair of bridges, connected to an automatic voltage-clamp system (7), was used to short-circuit PD_t. The current needed to keep PD_t at O mV (i.e. the I_{sc}) was continuously recorded. For translens resistance (R_t) measurements, the current needed to offset the short-circuited condition by ± 10 mV was measured every 5 min for a few s.

Representative experiments are illustrated below. For these, photocopies of the chart recordings were made, from which the I_{sc} traces were highlighted by close cropping and covering of the chart grids with paint prior to photography. The transitory I_{sc} deflections induced by the automatic pulsing system for R_t determinations were not preserved; the final figures contain remnants of such. Most quantitative data is detailed in the figure legends. Unless stated otherwise, all given changes in the electrical parameters were significant as paired data at the $P < 0.05$ level.

Solutions

The basic physiological medium used to bathe the lenses contained (in mM): 1.8 calcium gluconate, 1.2 $MgCl_2$, 2 K_2HPO_4, 110 NaCl, 5 sodium gluconate, 25 $NaHCO_3$, 10 glucose, and 12 sucrose. For a Ca^{2+}-free medium, the basic solution merely lacked calcium gluconate. For a Na^+-free medium, NaCl, sodium gluconate, and $NaHCO_3$ were replaced by N-methyl-D-glucamine (NMDG)-HCl, NMDG-gluconic acid, and choline bicarbonate.

All solutions measured 285 ± 5 mOsmol per $Kg{\cdot}H_2O$ (adjusted with small amounts of crystalline sucrose as needed) and pH 7.40 ± 0.05, when bubbled with a humidified 5% CO_2-95% air mixture; such gassing also provided a circulation within each hemichamber. Heating coils within each bath were used to maintain a 36°C temperature.

For unilateral electrolyte substitution experiments, the volume on one side was reduced in half by vacuum aspiration, and the remaining solution was rapidly perfused in the presence of circulation and constant aspiration with 12 volumes of the replacement medium, which was then used to restore the original volume. This maneuver was completed within 2 min and occasionally led to a disturbance of the salt bridges, causing a deflection in the trace at the point of the medium exchange (e.g. Fig. 4, bottom).

Chemicals

All chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO). Ouabain octahydrate, quinidine hydrochloride and amphotericin B were stored as aqueous, 100-fold concentrated, stock solutions at 5°C. Also stored at refrigerator temperature, in ethanol, was nifedipine (10^{-2}M).

Results

Rabbit lenses stabilized under short-circuit conditions exhibit an I_{sc} of $\approx$10 $\mu A/cm^2$ with an R_t of $\approx$1.8 $K\Omega{\cdot}cm^2$. The former parameter can be rapidly eliminated (4) by introducing anteriorly 5 mM Ba^{2+}, an inhibitor of epithelial K^+ channels (8, 9) plus 0.1 mM ouabain, inhibitor of the rheogenic Na^+-K^+ pump (10). These agents have no effect on the rabbit lens I_{sc} when added posteriorly.

Effects of anterior medium [Ca^{2+}] on I_{sc} and R_t

When [Ca^{2+}] of the anterior bath was increased from 1.8 to 3.6 mM, the I_{sc} decreased by $\approx$15% with an accompanying 6% R_t increase (n = 10, Fig. 1, top). Although small in magnitude, the R_t increase occurred in all cases and is thus significant. The effect of elevated medium Ca^{2+} was not seen when (a) K^+ channels were blocked by Ba^{2+} and the I_{sc} only reflects the Na^+-K^+ pump current (n = 4, Fig. 1, bottom), nor (b) when the K^+ channel blocker, quinidine (0.2 mM), was present in the anterior bath (n = 4, not shown). The addition of Ba^{2+} under control conditions reduced the I_{sc} by about 2/3 and increased R_t by $\approx$17% (n = 18, Fig. 1, legend); also a relatively meager R_t change given that Ba^{2+} introduction to the lens preparation eliminates the major component of the current flow across the anterior surface. Quinidine (0.2 mM) is a less extensive inhibitor of rabbit lens K^+ conductance(s) than Ba^{2+} (5 mM) as determined by the sequential additions of these agents on the control I_{sc}. Current reductions elicited by the former represented

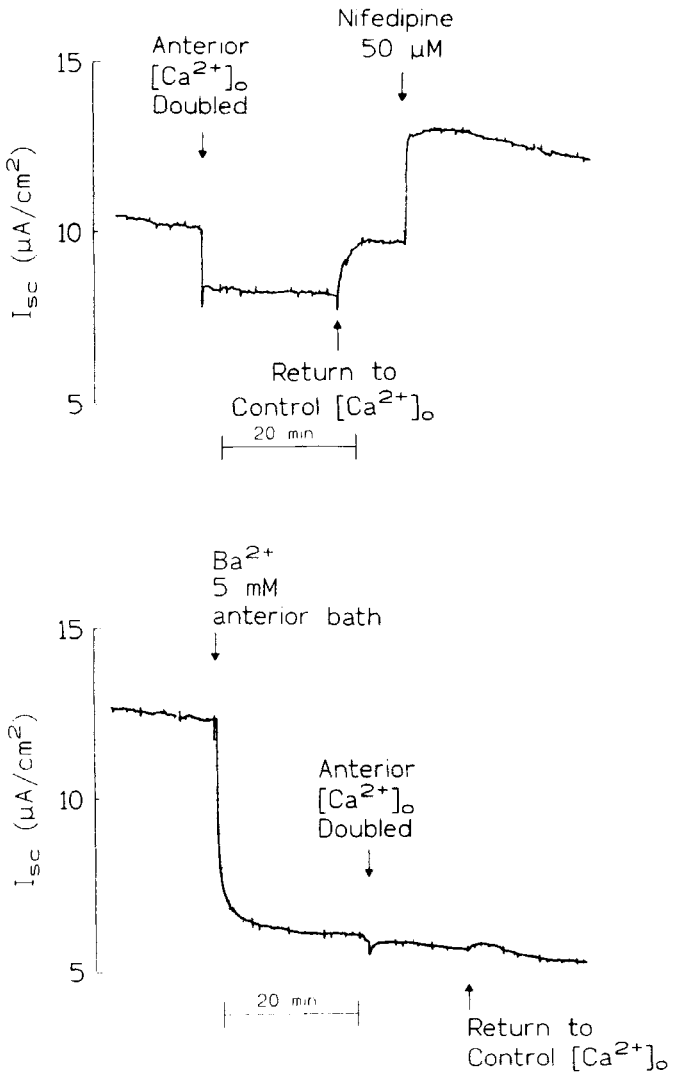

Figure 1. (**Top**) Translens electrical effects from increased $[Ca^{2+}]$ in the anterior hemichamber of rabbit lenses isolated in an Ussing-type chamber. A representative photocopy of a continuous recording of the short-circuit current (I_{sc}) is shown. At the 1st arrow, $[Ca^{2+}]_o$ was increased from 1.8 to 3.6 mM by dilution from an aqueous preparation of calcium gluconate, which was readied in an amount appropriate for 0.1 M by sonication and heating to 50°C. The control I_{sc} was reduced from 10.54 ± 1.09 to 8.93 ± 1.02 μA/cm² (means ± SE's, n = 10 lenses) and R_t increased from 1.80 ± 0.12 to 1.91 ± 0.12 KΩ·cm². At the 2nd arrow, the anterior hemichamber was rapidly perfused with the control medium. At the 3rd arrow (nifedipine), the Ca^{2+} channel blocker was diluted into the anterior solution from a 10^{-2} M stock; this addition increased the I_{sc} from 9.47 ± 1.09 to 12.40 ± 1.48 μA/cm² (n = 10). The effect of nifedipine on R_t (a 0.09 ± 0.04 KΩ·cm² reduction) was marginally significant ($p < 0.08$). (**Bottom**) A photocopy of an I_{sc} recording illustrating the absence of a Ca^{2+} effect on the I_{sc} in the presence of Ba^{2+}. At the 1st arrow, $BaCl_2$ was diluted into the epithelial-side bath from a 1 M stock; the I_{sc} was reduced from 9.56 ± 0.81 to 2.94 ± 0.42 μA/cm² and R_t increased from 1.76 ± 0.09 to 2.06 ± 0.09 KΩ·cm² (n = 18). At the 2nd arrow, 1.8 mM calcium gluconate was added to the Ba^{2+}-containing bath; marked effects on the electrical parameters were not recorded in 4 lenses in which this condition was completed, which was also the case in 4 additional lenses in which quinidine was used in place of Ba^{2+} (not shown). At the final arrow, the excess Ca^{2+} was removed.

64 ± 3.7% (n = 6) of the total I_{sc} inhibited by the combined presence of quinidine and Ba^{2+}, suggesting that those K^+ channels sensitive to elevated $[Ca^{2+}]$ were selectively included among those blocked by quinidine.

Another channel blocker found to affect the rabbit lens I_{sc} was nifedipine, an agent that obstructs Ca^{2+} channels (11). Its introduction to the anterior medium under control conditions increased the I_{sc} variably; on average a 30% increase was obtained with only a marginally significant decrease in R_t ($p < 0.08$; n = 10, Fig. 1). The direction of this I_{sc} change implies that the I_{sc} response to altered medium $[Ca^{2+}]$'s may occur as a consequence of changes in intracellular Ca^{2+} levels after permeation of the cation via a nifedipine-sensitive pathway. Nifedipine did not affect the I_{sc} when added to an anterior bath lacking Ca^{2+} (n = 15, not shown), nor when it was added to one containing Ba^{2+} (n = 2, not shown).

Consistent with the inverse relationship between anterior Ca^{2+} levels and the I_{sc}, exclusion of the cation from the anterior hemichamber increased the current by ≈44% and reduced R_t by ≈13% (n = 18, Fig. 2, top). Such an increase did not occur when Ca^{2+} was removed in the presence of either Ba^{2+} (Fig. 2, bottom) or quinidine; instead small I_{sc} decreases were recorded without significant effects on R_t (Fig. 2, legend). This experiment suggested that Ca^{2+} removal could, at best, only have a limited effect on the Na^+ permeability of the anterior surface. With epithelial K^+ conductances inhibited, had the removal of Ca^{2+} increased the Na^+ inflow across the basolateral face markedly (i.e. a shunt to the pump), a pronounced I_{sc} reduction with an R_t decrease would have been expected.

It was shown previously (4) that the elevated I_{sc} evoked by the absence of anterior Ca^{2+} could be reduced and restored by the sequential reintroduction and removal of the cation at 0.5 mM steps. Further experiments in this regard were completed and resulted in an I_{sc} of 17.79 ± 1.70 μA/cm² (n = 8) with the anterior bath lacking Ca^{2+}; a current of 14.08 ± 1.39 (n = 8) with 0.5 mM Ca^{2+}; and a recovery to 16.87 ± 1.67 (n = 8) upon its removal. When the 0.5 mM Ca^{2+} was then reintroduced in the presence of nifedipine (10 μM), which has no effect on the I_{sc} in the absence of Ca^{2+}, the Ca^{2+}-elicited reduction was limited to 0.94 ± 0.23 (from 16.87 to 15.93). Additional titrations of Ca^{2+} into the nifedipine-containing hemichamber determined that a concentration of 4 mM was needed to obtain a current reduction of 4.22 ± 0.46 (n = 8); indicating that the $[Ca^{2+}]$ must be present at a level ≈8-fold higher than that used in the absence of the Ca^{2+} channel blocker for approximately equivalent reductions in current to occur.

Because the calcium analogue, strontium, inhibits the depolarization of the frog lens that occurs in Ca^{2+}-free media (12), some experiments characterized the effects of Sr^{2+} on the rabbit translens electrical parameters. It was observed that anterior incremental additions of $SrCl_2$ between 1 to 10 mM decreased the I_{sc}, moderately, in a concentration-dependent manner without affecting R_t markedly. Its posterior addition had no effect (n = 8). At the concentration in which Ba^{2+} was used in this study (5 mM), for example, anterior Sr^{2+} addition caused a 2.45 ± 0.44 μA/cm² (n = 15) reduction without a significant effect on R_t. The methodology used, i.e. the continuous record-

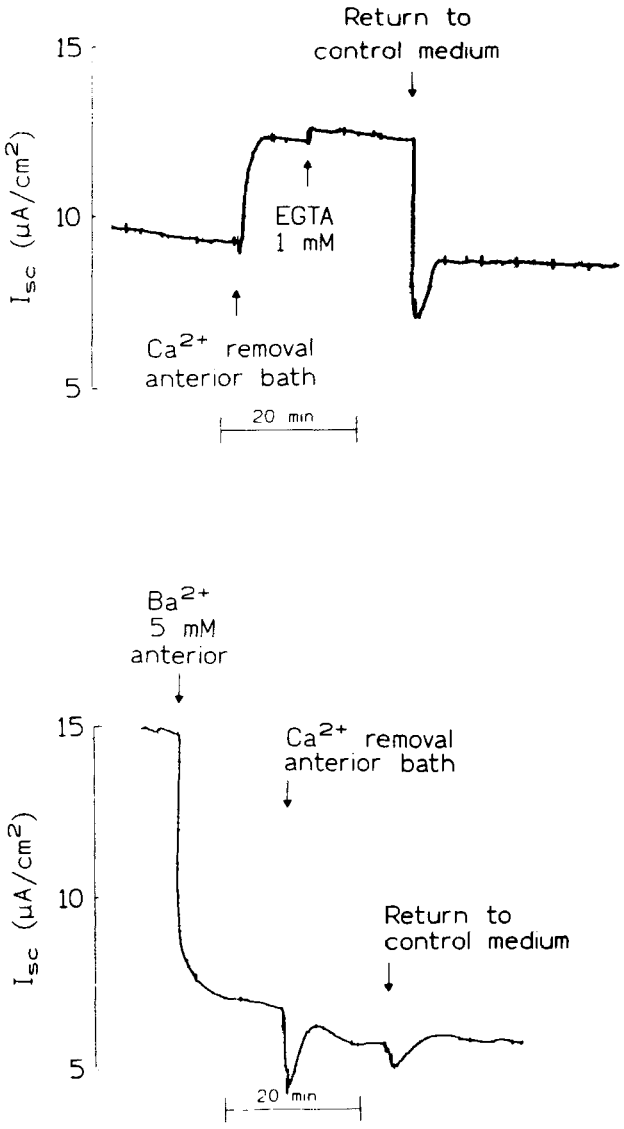

Figure 2. (**Top**) Effect of anterior Ca^{2+} removal and reintroduction on the rabbit lens I_{sc}. At the 1st arrow, the anterior hemichamber was rapidly perfused with a Ca^{2+}-free medium; the I_{sc} increased from 11.87 ± 1.17 to 17.04 ± 1.52 μA/cm² and R_t decreased from 1.84 ± 0.09 to 1.61 ± 0.06 KΩ·cm² (n = 18). With 4 lenses the tetrasodium salt of EGTA was diluted in the Ca^{2+}-free bath from a 0.5 M stock (2nd arrow); the electrical parameters were not markedly affected by this introduction. At the final arrow, the control medium was reintroduced. (**Bottom**) An I_{sc} recording demonstrating that Ca^{2+}-free perfusions did not have a stimulatory effect in the presence of Ba^{2+}. The Ca^{2+}-free medium introduced at the 2nd arrow contained 5 mM Ba^{2+}; following this change the I_{sc} stabilized 0.84 ± 0.29 μA/cm² lower than before the removal of Ca^{2+} (a 25% decrease; n = 7). R_t was not affected (2.04 ± 0.13 vs. 2.02 ± 0.11; n = 7). (When quinidine was used in place of Ba^{2+} (not shown) the I_{sc} was reduced by 0.34 ± 0.16 (n = 4) upon the withdrawal of anterior Ca^{2+}.) At the final arrow, the control, Ba^{2+}-free, Ca^{2+}-rich medium was reintroduced, showing the absence of a prompt recovery to Ba^{2+} inhibition.

ing of the translens I_{sc} as a measure of macroscopic epithelial K^+ conductances, cannot readily discern the mechanism of the Sr^{2+} effect. At least 3 hypothetical possibilities exist: (a) Sr^{2+} entering the lens via Ca^{2+} channels and competing intracellularly with Ca^{2+}; (b) Sr^{2+} entry via K^+ channels and competition with Ca^{2+}; and (c) a partial K^+ channel blockade precluding a significant change in R_t. It was determined, however, that the Sr^{2+}-evoked I_{sc} reduction was completely reversible, repetitively elicited, and inhibited by 86 ± 4.7% (n = 6 as paired data) by Ba^{2+} pretreatment, by 63 ± 7.1% (n = 5, paired data) by quinidine pretreatment and by 15 ± 3.5% (n = 5, paired data) by nifedipine.

To assess if Sr^{2+} affected the Ca^{2+}-elicited I_{sc} changes, experiments analogous to those shown in Figures 1 and 2 were completed, with the difference that 5 mM Sr^{2+} was used instead of Ba^{2+}. The presence of Sr^{2+} prevented the I_{sc} rise upon Ca^{2+} removal. Unlike with Ba^{2+} (Fig. 2), in which Ca^{2+} elimination led to a small I_{sc} decrease in all cases, with Sr^{2+} present, the Ca^{2+} removal either resulted in small increases or decreases (delta I_{sc} = −0.21 ± 0.50, n = 12; i.e. no statistically significant change in current or R_t was measured). A contrast between Sr^{2+} and Ba^{2+} was also evident in experiments in which the [Ca^{2+}] of the anterior bath was increased from 1.8 to 3.6 mM, similar to the experiments in Figure 1. While Ba^{2+} completely inhibited this Ca^{2+} effect, I_{sc} reductions, albeit reduced ones, still occurred (−0.66 ± 0.13, n = 9) in the presence of Sr^{2+}. These results are consistent with the earlier suggestion that Sr^{2+} more adequately serves as a Ca^{2+} substitute than Ba^{2+} (12); the latter blocking K^+ conductance(s) (8, 9) while Sr^{2+} could compete with Ca^{2+} at various binding sites, each with distinct affinities.

Other experiments determined the effects of anterior Ca^{2+} removal in the absence of anterior Na^+ (Figs. 3 and 4). Removal of the latter from the anterior bath under control conditions resulted in a net 28% current reduction with a 20% increase in R_t (n = 32, Fig. 3, legend). This response could be considered anomalous, because the exchange of medium Na^+ with NMDG should have led to an I_{sc} increase due to the diffusion of Na^+ from the lens. Without anterior Na^+, Ca^{2+} removal increased the I_{sc} by 3.72 ± 1.05 μA/cm² (mean ± SE, n = 6; Fig. 3, top), while in the bilateral absence of Na^+, removal of the cation led to an I_{sc} increase of 5.42 ± 0.98 (n = 8, not shown). These changes are not different as unpaired data from the 5.17 ± 0.64 (n = 18) current increase obtained in the presence of Na^+ (Fig. 2, top).

The anomalous I_{sc} reduction obtained upon anterior Na^+ removal also occurred with lenses pre-treated with ouabain, indicating its independence from the activity of the Na^+-K^+ pump (Fig. 3, bottom). Under control conditions, the anterior addition of the glycoside promptly inhibited the I_{sc} by ≈29% (Fig. 3, legend) without affecting R_t (n = 10); its presence did not interfere with the Ca^{2+}-free effect.

An experimental condition demonstrating the freely reversible nature of the Na^+-independent Ca^{2+} effects was also completed (Fig. 4).

Effects of posterior medium [Ca^{2+}] on I_{sc} and R_t

Given that the above experiments indicate that epithelial K^+ channels are acutely susceptible to regulation by Ca^{2+}, and that earlier studies suggested that the stimulation of Rb^+ efflux from

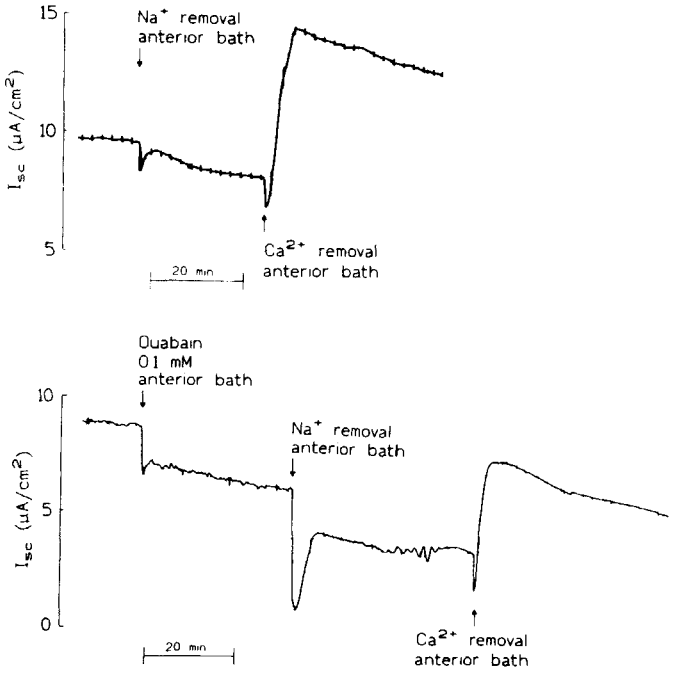

Figure 3. (**Top**) A recording of the I_{sc} across the rabbit lens demonstrating the absence of an anterior Na^+ dependence for the current stimulation due to anterior Ca^{2+} removal. At the 1st arrow, the control medium in the epithelial-side hemichamber was replaced by the homologous NMDG solution. In the absence of anterior Na^+, the I_{sc} decreased from 9.13 ± 0.55 to 6.51 ± 0.45 μA/cm² and R_t increased from 1.78 ± 0.05 to 2.13 ± 0.06 KΩ·cm² (n = 32). In 6 lenses, the anterior surface was then perfused with an NMDG solution lacking Ca^{2+} (2nd arrow); the I_{sc} increased by 3.72 ± 1.05 μA/cm² (a 56% change) and R_t decreased by 0.36 ± 0.11 KΩ·cm² (a 19% change). The trace presented shows the largest Ca^{2+}-free effect that was obtained. (**Bottom**) Independence of anterior Na^+- and Ca^{2+}-free effects from Na^+-K^+ pump activity. Ouabain was present in the anterior hemichamber from the point of the 1st arrow. Introduction of the glycoside reduced the control I_{sc} from 10.74 ± 0.80 to 7.66 ± 0.77 without an effect on R_t (1.86 ± 0.13 vs. 1.89 ± 0.13; n = 10). The subsequent replacement of anterior Na^+ with NMDG (2nd arrow) reduced the I_{sc} by 3.71 ± 0.47 μA/cm² (a 48% change) and increased R_t by 0.34 ± 0.04 KΩ·cm² (an 18% change; n = 10). At the last arrow, the NMDG solution was exchanged by one lacking Ca^{2+}; the I_{sc} increased by 3.23 ± 0.36 (82% change) and R_t decreased by 0.20 ± 0.04 (a 9% change; n = 10).

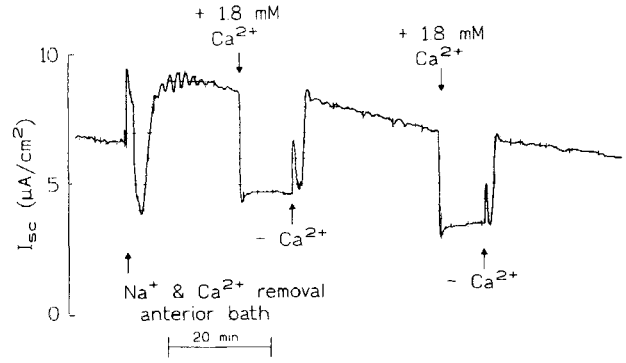

Figure 4. A recording demonstrating the reversibility of the Na^+-independent inverse relationship between anterior-medium Ca^{2+} levels and rabbit lens I_{sc}. At the 1st arrow, the anterior lens surface was perfused with an NMDG solution lacking Ca^{2+}; the I_{sc} increased by 3.86 ± 0.62 μA/cm² (a 45% increase; n = 5) without a statistically significant effect on R_t, which either slightly increased (2 cases) or decreased (3 cases). At the 2nd and 3rd arrows, anterior Ca^{2+} was sequentially reintroduced and removed by dilution from a concentrated calcium gluconate stock followed by perfusion with the Ca^{2+}-free, NMDG solution, a sequence that was repeated at the final two arrows. The I_{sc} inhibitions upon the Ca^{2+} reintroductions were –2.67 ± 0.75 μA/cm² (n = 5, 2nd arrow) and –2.50 ± 0.71 (n = 5, 4th arrow).

the rabbit lens in Ca^{2+}-free media was secondary to an initial increase in Na^+ permeability (6), the effects of Ca^{2+} removal from the posterior bathing medium was also characterized. Overall, however, the absence of posterior Ca^{2+} resulted in less salient and more variable I_{sc} changes than those seen anteriorly (Fig. 5, top). When posterior Ca^{2+} was removed without Na^+ in the posterior bath, more striking I_{sc} rises were obtained (Fig. 5, center and bottom). On average, posterior Ca^{2+} removal under control conditions (Fig. 5, top) increased the I_{sc} by 1.65 ± 0.72 μA/cm² (n = 9); such removal in the absence of Na^+ (Fig. 5, center) increased the I_{sc} by 2.95 ± 1.13 (n = 5), while the simultaneous removal of posterior Na^+ and Ca^{2+} (Fig. 5, bottom) gave an increase of 8.63 ± 2.41 (n = 8). Statistically, only the first and third I_{sc} changes are clearly different from each other as unpaired data. Even with this variability, one may suggest that Ca^{2+} regulates another posterior permeability pathway in addition to its regulation of Na^+ influx. Consistent with this, the two latter I_{sc} changes occurred concomitantly with relatively larger R_t decreases than those recorded in the presence of Na^+ (Fig. 5, legend).

Posterior Na^+ removal, in itself, gave rise to a biphasic response (Fig. 5, center) with the I_{sc} stabilizing at 79% of the control level (n = 11). It is suggested that upon Na^+ removal the PD across the posterior aspect hyperpolarizes so that Cl^- (normally at equilibrium) could then carry current due to its posterior efflux. Enough equivalents of Cl^- exist within the lens to carry current for several h. The immediate ≈50% I_{sc} reduction evoked by posterior Na^+ removal would represent a minimal estimate of the contribution of Na^+ influx to the control translens I_{sc}.

The variability obtained upon posterior Ca^{2+} removal may be explained if one posits that it resides in the extent to which Cl^- is above equilibrium in each individual lens. The rationale for suggesting an asymmetrical efflux of Cl^- is based upon the finding that its permeability across the posterior surface is twice its anterior permeability in frog lenses (13). Indirect support for the position that Ca^{2+} regulates a posterior Cl^- permeability was found by pre-incubating rabbit lenses for 2h without Cl^- prior to the removal of posterior Na^+ and Ca^{2+}. In no case did Ca^{2+} removal affect the I_{sc} when Na^+ and Cl^- were not present (n = 6, not shown). Also observed, but not shown, were the facts that the control I_{sc} was not responsive to posterior [Ca^{2+}] increases from 1.8 to 3.6 mM (n = 4), and that the increased I_{sc} obtained upon the simultaneous removal of posterior Na^+ and Ca^{2+} (Fig. 5, bottom) was not extensively inhib-

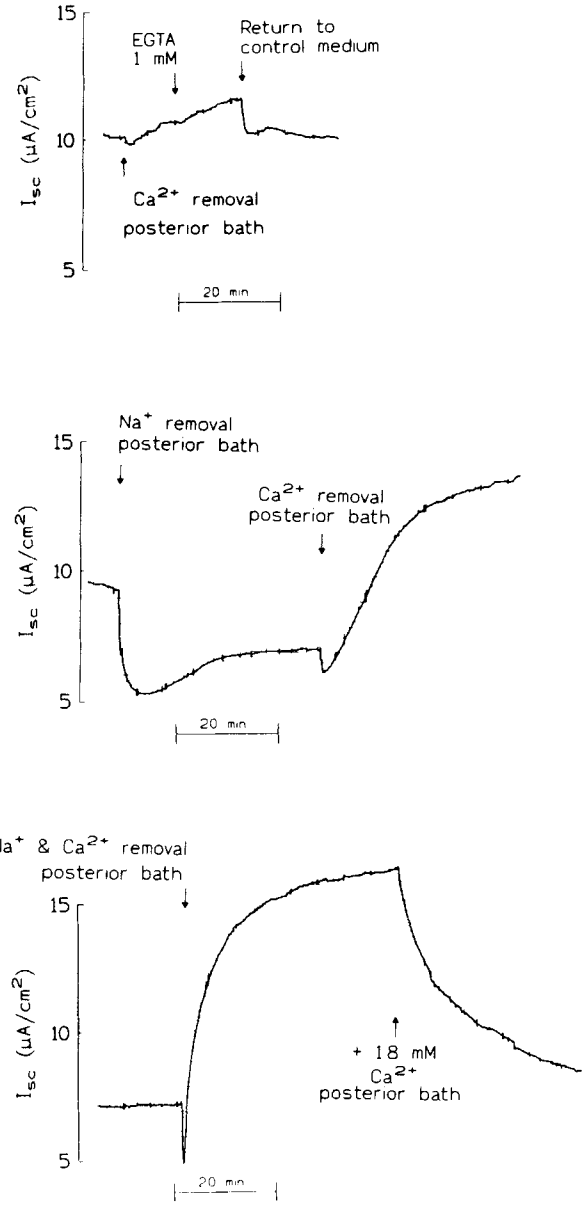

Figure 5. (**Top**) Effect of Ca^{2+} removal from the posterior bathing medium on rabbit lens I_{sc}. Perfusing the posterior lens aspect with a medium lacking Ca^{2+} resulted in a gradual I_{sc} increase from 10.89 ± 1.31 to 12.54 ± 1.29 μA/cm² with an R_t decrease from 2.04 ± 0.10 to 1.95 ± 0.09 KΩ·cm² (n = 9). Overall, the introduction of EGTA to the Ca^{2+}-free medium did not affect the response statistically. (**Center**) Effect of posterior Ca^{2+} removal in the absence of posterior Na^+. Exchanging the posterior control medium with the homologous NMDG solution resulted in an immediate 56 ± 9% I_{sc} inhibition, followed by a gradual recovery and I_{sc} stabilization at 7.61 ± 1.46 μA/cm² (79% of control, n = 11) within 20–30 min. R_t immediately increased 0.32 ± 0.04 KΩ·cm² (20% change) upon Na^+ removal and then decreased by 0.16 ± 0.07 during the partial I_{sc} recovery (n = 11). In 5 cases, Ca^{2+} was then removed from the posterior bath, resulting in a 2.95 ± 1.13 μA/cm² increase (43% change) and 0.18 ± 0.06 KΩ·cm² decrease (10% change). The figure shows the record from the lens that gave the largest response to Ca^{2+} removal. (**Bottom**) Effect of simultaneous Na^+ and Ca^{2+} removal from the posterior bath. Replacement of the control posterior medium with an NMDG solution lacking Ca^{2+} resulted in a direct I_{sc} stimulation of 8.63 ± 2.41 μA/cm² (an 85% average increase) with a 0.25 ± 0.07 KΩ·cm² reduction (a 15% decrease) in R_t (n = 8). The record from a lens in which the I_{sc} more than doubled is given. Ca^{2+} was diluted into the posterior bath at the final arrow.

ited by the subsequent application of 5 mM Ba^{2+} to the posterior bath [I_{sc} reduced from 19.06 ± 3.53 to 15.82 ± 2.62 (n = 4)]; the latter change is suggestive of a limited depolarization across the posterior aspect upon addition of the K^+ channel blocker.

The anomalous Na^+-free effect on I_{sc}

Given the I_{sc} reduction elicited by anterior Na^+ removal (Fig. 3), an experimental condition addressed the possibility that the absence of the cation from the anterior bath led to a reversed Na^+/Ca^{2+} exchange activity which in turn increased intracellular Ca^{2+} levels, leading to the observed inhibition. This notion was excluded by the fact that anterior Na^+ removal in the absence of medium Ca^{2+} (Fig. 6) did not prevent the anomalous Na^+-free effect. Further, the I_{sc} response to the introduction of ouabain was not affected by the absence of anterior Na^+ (ΔI_{sc} = 2.82 ± 0.73, n = 4 in Figure 6, versus 3.08 ± 0.40, n = 10 in Fig. 3), indicating that the Na^+-K^+ pump current had been maintained by the movement of Na^+ into the lens from the posterior bath.

However, when anterior-medium Na^+ was removed under conditions in which K^+ channels were blocked by Ba^{2+}, I_{sc} increases of 1.68 ± 0.17 μA/cm² were recorded (n = 7, Fig. 7). This suggests that K^+ conductance(s) must be inhibited in order to observe the expected increase in I_{sc} produced by the diffusion of Na^+ from the lens to the anterior bath. Under these conditions, the subsequent removal of anterior Ca^{2+} induced an I_{sc} reduction similar to that in Figure 2 (bottom trace). Although an explanation for this latter, Ca^{2+}-free I_{sc} reduction was not determined, an effect on Cl^- diffusion is possible. Overall, these experiments indicate that the anomalous Na^+-free effect is linked to a closing of epithelial K^+ channels, and that the absence of anterior Ca^{2+} does not appear to increase the Na^+ permeability of the epithelial surface.

A final series of experiments was designed to corroborate that the rabbit lens epithelium does not express a significant Na^+ conductance and that changes in I_{sc} can indeed differentiate changes in K^+ permeability across the anterior epithelium from changes in Na^+ permeability. The induction of monovalent cation channels (amphotericin B addition, Fig. 8) to the anterior medium of lenses bathed without anterior Na^+ led, on average (n = 6), to an ≈70% increase in I_{sc} with a 25% R_t reduction. This current was relatively insensitive to Ba^{2+} (Fig. 8, top) and could be reversed by the addition of 60 mM Na^+ to the anterior hemichamber, a result consistent with a limited Na^+ conductive pathway in the native anterior surface. Further, about 0.8 of the current through amphotericin B-induced cation channels was still carried when the posterior bath lacked Na^+ (Fig. 8, bottom), similar to the extent of current flow through native anterior K^+ channels bathed without posterior Na^+.

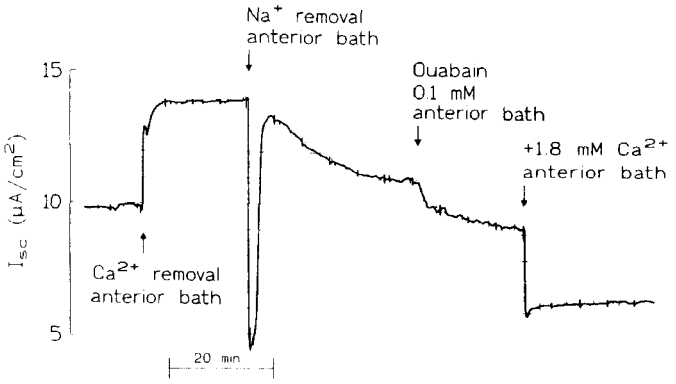

Figure 6. Effects of anterior Na^+ removal and ouabain introduction in the absence of anterior Ca^{2+} on the rabbit lens I_{sc}. The anterior medium lacked Ca^{2+} from the point indicated. At the 2nd arrow, Na^+ was removed anteriorly reducing the I_{sc} by 4.00 ± 0.51 $\mu A/cm^2$ (22% inhibition) and increasing R_t by 0.24 ± 0.08 $K\Omega \cdot cm^2$ (a 14% change, n = 4). The introduction of ouabain (3rd arrow) inhibited the I_{sc} by 2.82 ± 0.73 (a 20% reduction, n = 4). Calcium gluconate was reintroduced at the final arrow.

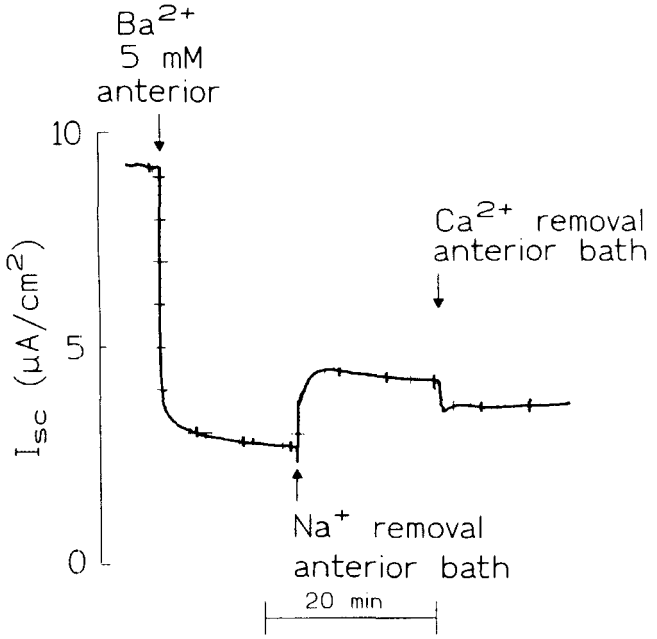

Figure 7. Effect of anterior Na^+ removal in the absence of epithelial K^+ conductance(s). The K^+ channel blocker was present in the anterior bath from the point indicated. At the second arrow ($-Na^+$), the anterior medium was replaced with an NMDG solution containing 5 mM $BaCl_2$; the I_{sc} increased by 1.68 ± 0.17 $\mu A/cm^2$ and R_t decreased by 0.15 ± 0.03 $K\Omega \cdot cm^2$ (n = 7). From the final arrow, the anterior Ba^{2+}-containing NMDG medium lacked Ca^{2+}, eliciting an I_{sc} decrease of 1.21 ± 0.28 $\mu A/cm^2$ without a measurable effect on R_t.

Discussion

Upon mounting the rabbit lens in a bicameral Ussing-type arrangement, whereby the polar surfaces are electrically isolated from each other, a PD_t of about 20 mV, anterior side positive, develops. This PD_t, which represents the difference in the membrane potentials across the anterior and posterior surfaces, lens center as reference (14), arises from the fact that the PD across the anterior face of the rabbit lens (PD_a) is about 55 mV, while across the posterior aspect (PD_p), it is about 35 mV (15). These respective lens surface PD's reflect distinct permeability and transport properties on each side of the lens (16).

Under short-circuit conditions, which enables the characterization of each face in isolation, PD_t is set to 0 mV by sending the appropriate amount of current (the I_{sc}) across the lens needed to do so; in effect PD_p is hyperpolarized and PD_a depolarized until each respective PD attains the same value. The amount of current across the anterior and posterior surfaces, as well as, that measured in the external circuit must be equal. Any transport or permeability changes at either face will, of course, affect the I_{sc} across each face. Because of the large volume and ionic content of the lens, the ionic species carrying the I_{sc} at each surface need not be the same. Experimental maneuvers at either lens face that affect the permeability or transport processes at that surface in turn affect the amount of I_{sc}.

The combined introduction of Ba^{2+} and ouabain to the anterior bathing solutions of toad, rat and rabbit lenses leads to the virtual elimination of the I_{sc} across these specimens (2–4), indicating that the continuous recording of lens I_{sc} can be used to monitor epithelial basolateral K^+ conductance(s) plus the Na^+-K^+ pump current. In some preparations, particularly with toad and rat lenses, a small residual Ba^{2+}- plus ouabain-insensitive I_{sc} remains (<10%), suggesting that Ba^{2+} inhibition of the various K^+ channels known to be present in the lens (9) was incomplete. In the toad lens, this residual current can be reversed by increasing the $[K^+]$ of the anterior bath (unpublished observations). (No inhibitors have yet been found that eliminate the I_{sc} when added posteriorly.)

Overall, the nature of the translens I_{sc} differs from most other transepithelial I_{sc} measurements (e.g. those across absorbing or secreting epithelia) in which the majority of an I_{sc} is a direct measure of a net transmural flux of an electrolyte. The only net flux demonstrated across the lens was that of Na^+ (1), and its magnitude, (in $\mu A/cm^2$) in the toad lens, was comparable to the effect of the anterior addition of ouabain on the I_{sc} of that species (2). Additional radiotracer and ion substitution experiments on toad lenses excluded the presence of net translens K^+, Cl^- and HCO_3^- fluxes (1, 16). Thus, the translens movement of positive charge appears to result from the aforementioned net Na^+ flux generated by the epithelial pump (representing less than 1/3 of the translens current) plus an apparent net gain of Na^+ across the posterior face with a balancing K^+ efflux across the anterior face. This nonsteady-state situation nevertheless supports stable I_{sc}'s for several h due to the quantity of K^+ equivalents in the bulk of the lens.

Most lens physiology studies have used completely submerged lenses (i.e. zero paracellular resistance) in order to determine the effects of Ca^{2+} on lens permeability characteristics (6, 12, 17–19). Hence, it is fundamentally valid to use lenses isolated in Ussing-type chambers under short-circuited conditions in order to examine such effects unilaterally, with particular emphasis on the influence of Ca^{2+} on the epithelial aspect.

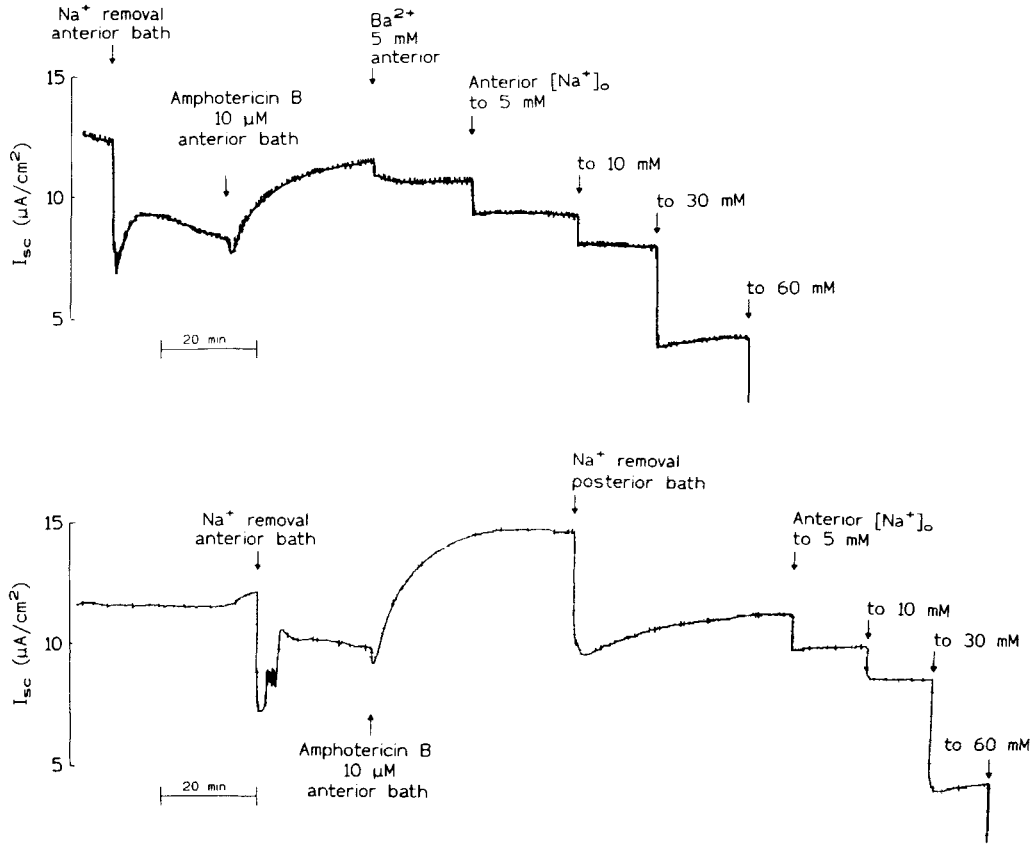

Figure 8. Recordings demonstrating that changes in rabbit lens I_{sc} can distinguish between anterior K^+ permeability changes versus changes in Na^+ permeability. Following the introduction of amphotericin B to lenses bathed in the anterior absence of Na^+, the I_{sc} increased by 5.79 ± 1.91 μA/cm² (69% change) and R_t decreased by 0.54 ± 0.13 KΩ·cm² (25% change, n = 6). Thereafter, either Ba^{2+} was added anteriorly in 3 cases (**Top**) or Na^+ was removed posteriorly (n = 3, **Bottom**). The introduction of Ba^{2+} reduced the I_{sc} by 0.56 ± 0.17 (a 5% inhibition) without affecting R_t measurably. Posterior Na^+ removal inhibited the I_{sc} by 3.14 ± 1.12 μA/cm² (a 20% decrease) and R_t increased by 0.17 ± 0.06 KΩ·cm² (an 8% rise, n = 3). At the 4th arrow of each recording, a fraction of the anterior bath was replaced with an amphotericin B-containing, complete, physiological solution so that the final $[Na^+]$ would be 5 mM; the I_{sc} was reduced by 2.16 ± 0.65 (19% decrease) with an accompanying R_t reduction of 0.11 ± 0.02 (5% decrease; n = 6). At the remaining arrows the anterior $[Na^+]$ was incrementally increased as indicated by removing a fraction of the bath and replacing it with complete medium. The subsequent I_{sc} reductions occurred without measurable R_t changes. At 60 mM Na^+, the I_{sc} reversed; positive current flowed in the anterior-to-posterior direction by 1–2 μA/cm². With a full complement of 140 mM Na^+ (not shown), the I_{sc} was −9.11 ± 1.67 μA/cm² (n = 6).

The present study has demonstrated the following in the rabbit lens. (A) Epithelial K^+ channels are acutely sensitive to the $[Ca^{2+}]$ of the anterior bath, with existent K^+ conductance(s) displaying an inverse relationship to extracellular Ca^{2+} levels. (B) Increases in K^+ conductance(s) under Ca^{2+}-free conditions occur independently of medium $[Na^+]$ indicating that enhanced K^+ efflux is not secondary to an increase in Na^+ permeability. (C) The anterior lens surface expresses a limited Na^+ permeability that is not increased in the absence of Ca^{2+}. (D) Increases in medium $[Ca^{2+}]$ over the control level do not affect the permeability of the posterior aspect. And (E) The absence of Ca^{2+} from the posterior medium may enhance the Na^+ permeability of the posterior surface, but in addition, Ca^{2+} regulates an anionic permeability, apparently a Cl^- channel.

These results describe properties that thus far appear to be unique to the rabbit lens. In more extensively characterized amphibian lenses, it is unequivocal that the absence of Ca^{2+} from the bathing medium results in lens depolarization (17, 20), a consequence of a Na^+ permeability increase (17) perhaps via a cation channel showing a greater selectivity for Na^+ over K^+ (21), or via one with little, Na^+-K^+ selectivity (20).

Indeed, short-circuited toad lenses also exhibit Na^+-dependent I_{sc} decreases upon anterior Ca^{2+} withdrawal (22). In addition, the latter study measured lenses under open-circuit conditions, with a microelectrode impaled into the fibers near the equator. It was shown that the absence of Ca^{2+} depolarized the lens aspect in contact with the medium lacking the cation. The PD across the contralateral lens surface was unaffected.

Since lenses have tight junctions between epithelial cells (23), it can be assumed that the rabbit lens Ba^{2+}-sensitive I_{sc} reflects the activity of K^+ channels at the basolateral membrane. Thus, the inverse relationship between K^+ conductance(s) and bath Ca^{2+} levels requires that rabbit lens PD_a must change in a manner opposite to that described for the toad. Accordingly, preliminary microelectrode experiments [using the mounting arrangement in (22)] have determined under

open-circuit conditions that anterior Ca^{2+} withdrawal hyperpolarizes PD_a by ≈10 mV (n = 4), while increasing anterior $[Ca^{2+}]$ from 1.8 to 3.6 mM depolarizes PD_a by ≈3 mV (n = 4). Changes in rabbit lens PD_p upon the removal of Ca^{2+} from the posterior bath were not detected (n = 4), suggesting that Na^+ and Cl^- permeabilities were affected equivalently.

It seems likely that epithelial Ca^{2+} either blocks rabbit lens K^+ channels directly (or inhibits them somehow) from the cytosolic side of the channel. While the present methodology cannot determine this, some indications consistent with this notion have been obtained. The introduction of either the Ca^{2+} ionophore A23187, or the inhibitor of the Ca^{2+}-ATPase of the intracellular storage compartments, thapsigargin, which leads to a rise in cytosolic Ca^{2+} levels (24, 25), results in I_{sc} inhibitions (4). Further, the fact that nifedipine only increased the I_{sc} when the anterior bath contained Ca^{2+} (Fig. 1), also suggests that Ca^{2+} may be acting from within the cells.

Similarly unexpected was the anomalous effect evoked upon anterior Na^+ removal. Instead of an increase in the I_{sc}, the absence of Na^+ led to a reduced current. Perhaps K^+ channels closed in response to an initially rapid (and unrecorded within the time resolution of the methodology used) lens hyperpolarization across the anterior aspect as Na^+ left the lens. Evidence for this possibility rests upon the facts that the anomalous effect was ouabain-independent, and precluded by K^+ channel blockade. In the course of preparing these experiments, it was also observed that the unilateral removal of Cl^- from the anterior bath increased the I_{sc} by 4.39 ± 1.03 μA/cm² (a 31% rise, n = 7; unpublished data), a change obstructed by Ba^{2+} pretreatment (n = 2). These results would appear to be consistent with the known presence of voltage-gated K^+ channels in the lens (9, 26).

Finally, the native Na^+ channels on the lens epithelium are apparently, relatively impervious elements, as determined from the fact that the presence of artificial cation channels (i.e. amphotericin B) reversed the I_{sc} completely when physiological Na^+ levels were present in the anterior bath. This demonstration supports the view that changes in I_{sc} elicited by anterior ionic replacements reflect overwhelmingly changes in the permeability of K^+ and not of Na^+. The changes in R_t, given for the various protocols depicted above, are also in accordance with this conclusion.

In summary, a unilateral characterization of the intact rabbit lens has demonstrated that the Na^+ permeability of the epithelium is not the major element that is regulated by anterior Ca^{2+}. Rather, elevated Ca^{2+} levels inhibit the K^+ conductance(s) of this species. This property may underlie the temporary closing of K^+ channels and lens depolarization that occurs upon the administration of muscarinic agonists (4, 27). A physiological role for this activity remains to be determined.

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