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The selectivity of the hair cell's mechanoelectrical-transduction channel promotes Ca^{2+} flux at low Ca^{2+} concentrations

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ABSTRACT **The mechanoelectrical-transduction channel of the hair cell is permeable to both monovalent and divalent cations. Because Ca^{2+} entering through the transduction channel serves as a feedback signal in the adaptation process that sets the channel's open probability, an understanding of adaptation requires estimation of the magnitude of Ca^{2+} influx. To determine the Ca^{2+} current through the transduction channel, we measured extracellular receptor currents with transepithelial voltage-clamp recordings while the apical surface of a saccular macula was bathed with solutions containing various concentrations of K^+, Na^+, or Ca^{2+}. For modest concentrations of a single permeant cation, Ca^{2+} carried much more receptor current than did either K^+ or Na^+. For higher cation concentrations, however, the flux of Na^+ or K^+ through the transduction channel exceeded that of Ca^{2+}. For mixtures of Ca^{2+} and monovalent cations, the receptor current displayed an anomalous mole-fraction effect, which indicates that ions interact while traversing the channel's pore. These results demonstrate not only that the hair cell's transduction channel is selective for Ca^{2+} over monovalent cations but also that Ca^{2+} carries substantial current even at low Ca^{2+} concentrations. At physiological cation concentrations, Ca^{2+} flux through transduction channels can change the local Ca^{2+} concentration in stereocilia in a range relevant for the control of adaptation.**

Hair cells are epithelial receptors that mediate mechanoelectrical transduction in the sensory organs of the vertebrate internal ear and lateral-line system (reviewed in ref. 1). Protruding from the apical surface of a hair cell, the mechanically sensitive organelle, or hair bundle, comprises actin-filled stereocilia arranged in rows of increasing height. The stereocilia contain mechanically gated cation channels called transduction channels (reviewed in ref. 2) as well as the cellular machinery that mediates adaptation to sustained stimuli (reviewed in ref. 3). When the hair bundle is deflected toward its tall edge by mechanical stimulation, transduction channels open to initiate membrane depolarization. Along the hair cell's basolateral surface, the depolarization activates Ca^{2+} and K^+ currents, which control the rate of neurotransmitter release at afferent synapses.

In addition to playing distinct roles in the response to mechanical stimulation, the hair cell's apical and basolateral surfaces are exposed to very different ionic environments (4). The basolateral membrane is surrounded by perilymph, which, like most extracellular solutions, contains a high concentration of Na^+, a low concentration of K^+, and ≈2 mM Ca^{2+} (5, 6). In contrast, the hair bundle is bathed in endolymph, which resembles intracellular fluid because it is high in K^+ and low in Na^+. Mammalian perilymph, for example, contains ≈150 mM Na^+ and ≈5 mM K^+, whereas endolymph contains 1–15 mM Na^+ and ≈150 mM K^+ (4). In addition to its high K^+ concentration, endolymph is unusual because of its very low Ca^{2+} content. In hearing organs, the Ca^{2+} concentration has been estimated to be as low as 30 μM in mammals (5–8) and 65 μM in reptiles (9). In vestibular organs, the Ca^{2+} concentration is higher than in the cochlea: it measures ≈100 μM in the guinea pig's sacculus and utriculus (6) and ≈250 μM in the guinea pig's semicircular canal (7, 8) and the bullfrog's sacculus (10). Tight junctions between epithelial cells maintain the distinct ionic compositions of endolymph and perilymph (reviewed in ref. 11).

Adaptation to mechanical stimulation is regulated by Ca^{2+} ions that enter the hair bundle through the transduction channels (9, 12–14). Although adaptation has been demonstrated *in vivo* by recordings of eighth-nerve activity (12), it has been primarily characterized with *in vitro* hair-cell recordings, usually in the presence of millimolar concentrations of extracellular Ca^{2+}. Because transduction channels have a modest open probability even in the absence of stimulation, measurable amounts of Ca^{2+} enter stereocilia (15, 16) and affect the adaptive state (17) of resting hair cells in such preparations. Given that Ca^{2+} represents less than 0.2% of the permeant cations in endolymph, however, it is unclear how the transduction channels can pass enough Ca^{2+} ions *in vivo* to regulate adaptation.

Two lines of evidence suggest that substantial Ca^{2+} influx can occur because the transduction channel has a higher affinity for Ca^{2+} ions than for monovalent cations. First, reversal–potential measurements show that the channel is severalfold to several hundredfold more permeable to Ca^{2+} than to monovalent cations (18, 19), which indicates that the channel is Ca^{2+}-selective (20). Second, increasing the external Ca^{2+} concentration decreases transduction currents (9, 10, 14), suggesting that Ca^{2+} can transiently bind to, and thus block, the pore. Although these results indicate that Ca^{2+} binds in the pore of the transduction channel, they do not demonstrate how much Ca^{2+} actually traverses the channel's pore. To address this question, we have compared the transduction currents borne by Ca^{2+}, Na^+, and K^+ when the hair cells' apical surfaces are exposed to various extracellular cation concentrations.

A preliminary report of this work has appeared (21).

MATERIALS AND METHODS

Tissue Preparation. Experiments were performed at room temperature on saccular maculae of the bullfrog, *Rana catesbeiana.* Sacculi were prepared and transepithelial current recordings were performed essentially as described (10, 22). Internal ears were dissected in standard saline solution containing 110 mM Na^+, 2 mM K^+, 4 mM Ca^{2+}, 118 mM Cl^-, 3 mM D-glucose, and 5 mM Hepes at pH 7.25. After the otoconial mass overlying the epithelium had been removed with fine forceps, each macula was attached to a plastic disk with nontoxic glue (Tissu Glu Soft, Ellman International,

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Abbreviation: $NMDG^+$, *N*-methyl-D-glucamine.

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Hewlett, NY) such that the hair bundles protruded into a 1.2-mm hole in the disk's center. With an eyelash and fine forceps, the otolithic membrane was lifted from most of the sensory epithelium, leaving a patch of otolithic membrane coupled to hair bundles with similar axes of mechanical sensitivity (22).

The plastic disk with its attached saccular macula served as the partition of a two-chamber recording apparatus. The lower chamber, whose contents bathed the basolateral surfaces of hair cells, was continuously perfused with oxygenated high-K^+ artificial perilymph containing 110 mM Na^+, 17 mM K^+, 1.36 mM Ca^{2+}, 0.68 mM Mg^{2+}, 129 mM Cl^-, 3 mM D-glucose, and 5 mM Hepes at pH 7.3. The elevated K^+ concentration was used to depolarize the hair cells so that modulation of voltage-gated Ca^{2+} currents and Ca^{2+}-activated K^+ currents contributed little to the measured responses (22).

Test Saline Solutions. Because solutions whose $CaCl_2$ concentrations exceed 80 mM are hyperosmotic compared with frog saline solutions, we set the total cation concentration to 80 mM for all test solutions. This allowed us to directly compare the currents carried by individual cation species and to vary the mole fractions of two cations oppositely. In test solutions containing only one species of permeant cation, *N*-methyl-D-glucamine ($NMDG^+$) was used to maintain the total cation concentration at 80 mM.

Four stock solutions were mixed in various proportions to create the test solutions for perfusing the macula's apical surface. Each stock solution contained 80 mM of either Ca^{2+}, Na^+, K^+, or $NMDG^+$, as well as 3 mM D-glucose and 5 mM Hepes at pH 7.25–7.30; Cl^- served as the predominant anion. Stock solutions containing monovalent cations were supplemented with D-mannitol so that all solutions had similar osmolalities, which lay in the range 221–239 mmol·kg^{-1}.

Five test-solution series were created from the stock solutions; in any series, the concentration of each individual cation varied between 0 mM and 80 mM. In the first series, the concentrations of Ca^{2+} and $NMDG^+$ were varied oppositely so that Ca^{2+} was the only cation that could permeate transduction channels. In the second and third series, the concentration of either Na^+ or K^+ was altered oppositely to that of $NMDG^+$ so that Na^+ or K^+ served as the sole permeant cation. In the fourth and fifth series, which contained two permeant cations, the concentration of either Na^+ or K^+ was varied oppositely to that of Ca^{2+}. For test solutions containing 0.1 mM or 0.25 mM Ca^{2+}, the $NMDG^+$, Na^+, and K^+ stock solutions were supplemented with 1 M $CaCl_2$ to achieve the desired Ca^{2+} concentration.

All chemicals were >99% pure and were purchased from either Sigma or Aldrich. Ca^{2+} contamination of ostensibly Ca^{2+}-free stock solutions was determined to be <1.6 μM with a Ca^{2+}-selective electrode (Orion, Boston). Because they can destroy hair-cell transduction (23, 24), Ca^{2+} chelators were not used to further lower the Ca^{2+} concentration.

Electrophysiological Recording. To evoke receptor currents, we displaced the otolithic membrane with a solid, glass stimulus probe, ≈100 μm in tip diameter, whose shaft was fixed to a piezoelectric bimorph stimulator. The stimulus probe was positioned to move along the axis of maximal sensitivity for those bundles that remained attached to the otolithic membrane. Driving signals for the bimorph stimulator were supplied by a computer programmed in LABVIEW 3.1 (National Instruments, Austin, TX) and were low-pass filtered with an eight-pole Bessel filter whose half-power frequency was set at 0.25–0.35 kHz.

For the measurement of receptor currents, the transepithelial voltage was held near 0 mV with a voltage-clamp amplifier that was attached to paired Ag-AgCl electrodes in each chamber of the recording apparatus. Receptor-current data were filtered at 1 kHz, then digitized and recorded with the computer system at a sampling frequency of 5 kHz. The capacitive transients due to cross-talk between the stimulator and amplifier (22) were subtracted from all receptor currents.

A solution exchange was effected by aspirating most of the fluid from the upper chamber, then filling it with a new solution 12–20 times. A typical exchange was completed in ≈1 min. Because repeatedly lifting and lowering the stimulus probe could dislodge the otolithic membrane, we left the probe coupled to the otolithic membrane during solution changes. By using a probe with a small tip, however, we minimized the barrier that the probe presented to ionic diffusion.

To determine the amount of current carried by different concentrations of permeant cations, we measured the maximal receptor current that flowed in each test solution. Because changing the extracellular Ca^{2+} concentration shifts the transduction channel's open probability at rest (10), we subjected the otolithic membrane to a 50-ms, 3.5-μm positive displacement followed immediately by a comparable negative displacement. For each run, the electrical responses to five stimuli presented 960 ms apart were averaged on-line, the average current was saved, and the peak-to-peak receptor current was calculated. To ensure that the receptor current was stable, we performed a run about once per minute for each test solution. The maximal receptor currents from three of these runs were then averaged to yield one data point for that solution. Multiple data points for each test solution were collected and are expressed as means ± standard errors of means.

We took two precautions to control for the effects of deterioration, which was observed in some preparations. First, we assayed the test solutions in a random order. Second, we normalized the maximal receptor current measured in each test solution to that measured in standard saline solution immediately before application of the test solution (Fig. 1). By normalizing, we could compare the data from recording sessions lasting up to 8 hr; the normalized receptor current for a given test solution remained stable throughout this period. At the beginnings of experiments, preparations produced peak-to-peak receptor currents of 52–132 nA in the presence of standard saline solution. Over the durations of the recordings, this range dropped to 16–40 nA; however, the receptor-current drop between successive applications of standard saline solution that were separated by the perfusion of a test solution averaged only 3%.

To ensure that $NMDG^+$ carried little current through transduction channels, we measured the receptor current for each macula in the presence of 80 mM $NMDG^+$ stock solution. Immediately upon solution exchange, we observed either no receptor current or a small outward receptor current. Over the next 3 min, however, an inward receptor current of 1–6 nA developed. One possibility is that $NMDG^+$ bore this current but that its diffusion to hair bundles was impeded. An alternative possibility is that the current was carried by Ca^{2+} ions that became available as residual otoconia dissolved in the low-Ca^{2+} environment. Because in any event the current that developed represented ≤10% of the smallest response measured in standard saline solution, $NMDG^+$ carried a negligible current in these experiments.

The dissociation of tight junctions can lower transepithelial resistance in solutions devoid of Ca^{2+} (25). This effect is minimal, however, when Ca^{2+} is removed from only the apical epithelial surface (26). Had the transepithelial resistance fallen greatly in our experiments with solutions containing Na^+, K^+, or $NMDG^+$ as the sole permeant cation, our estimates of receptor current might have been distorted by failure of the voltage clamp. We therefore performed control experiments to test whether these solutions affected the transepithelial resistance. With high-K^+ perilymph bathing the basolateral epithelial surface, the transepithelial resistance with standard saline solution in the apical compartment averaged 10.0 kΩ, corresponding to a resistivity of 12.1 mΩ·m^2 (22). The resistance rose to 11.0 kΩ in the presence of 80 mM K^+

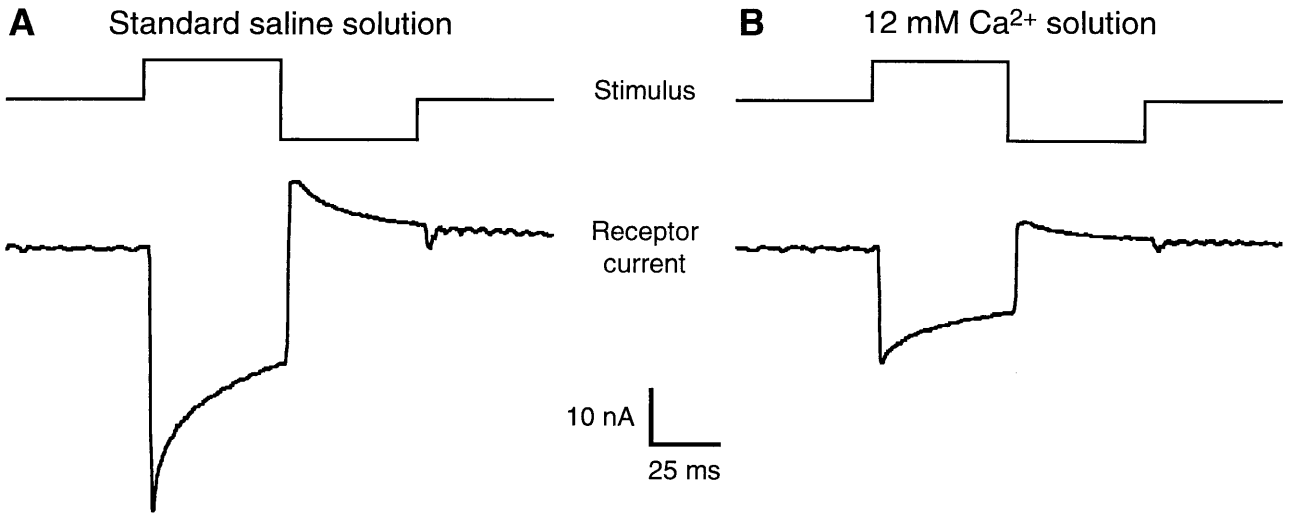

FIG. 1. Transepithelial receptor currents. (*A*) Displacements of the otolithic membrane (top trace) elicited a peak-to-peak receptor current of 60 nA (bottom trace) when the apical surface of the epithelium was bathed in standard saline solution containing 110 mM Na^+, 2 mM K^+, and 4 mM Ca^{2+}. (*B*) With an apical test solution containing 12 mM Ca^{2+} and 68 mM $NMDG^+$, the maximal receptor current measured 4 min after the response in *A* was 26 nA. Each displacement step was ± 3.5 μm. The current traces represent the averaged responses to five presentations of the stimulus train.

and 0.25 mM Ca^{2+}; the value declined slightly, to 9.1 kΩ, in the presence of 80 mM K^+ without added Ca^{2+}. Na^+- and $NMDG^+$-containing solutions likewise had no significant effects on transepithelial resistance. These results confirmed that the accuracy of receptor-current measurements was not affected by the use of low-Ca^{2+} solutions.

RESULTS

To determine the flux of different ions through hair-cell transduction channels, we measured receptor currents carried by various concentrations of Ca^{2+}, Na^+, and K^+. To cover an adequate cation concentration range, we needed to exchange the solution bathing a hair cell's apical surface at least 60 times during a typical experiment. We therefore employed a transepithelial preparation of the bullfrog's saccular macula (10, 12, 19, 22), which offered two distinct advantages for this study. First, because we could record from a single preparation for several hours, we were able to test a complete series of solutions on the same epithelium. Second, by clamping the voltage across the epithelium, we could record the receptor currents flowing simultaneously through several hundred hair cells (Fig. 1). Consequently, receptor currents were readily detectable even for solutions containing low concentrations of permeant cations.

To minimize variability introduced by cellular deterioration and to facilitate comparisons between maculae, we normalized the receptor current supported by each test solution to that measured for standard saline solution immediately prior to solution exchange. Fig. 1*A* shows a typical transepithelial receptor current, measured in the presence of standard saline solution, which was elicited by 3.5-μm positive and negative displacements of the otolithic membrane. After three of these measurements, the solution bathing the apical surface of the epithelium was exchanged for a test solution containing 12 mM Ca^{2+} and 68 mM $NMDG^+$. Three experimental runs were conducted for the test solution; one response is shown in Fig. 1*B*. The test solution was then exchanged for standard saline solution, and the measurement procedure was repeated to determine the normalized receptor current for each test solution.

We first sought to determine the currents carried by single species of permeant cations. Fig. 2 shows the average normalized receptor currents that were measured for solutions containing either Ca^{2+}, Na^+, or K^+. Even at modest concentrations, Ca^{2+} carried substantial receptor currents (Fig. 2*A*): at Ca^{2+} concentrations of 0.25, 1.2, and 4 mM, the normalized currents were respectively 0.03 ± 0.01 ($n = 4$), 0.11 ± 0.01 ($n = 4$), and 0.23 ± 0.01 ($n = 5$). For Ca^{2+} concentrations exceeding 40 mM, the normalized receptor currents appeared to approach saturation.

The most striking feature of these data is that, at concentrations below 40 mM, Ca^{2+} carried considerably more receptor current than did either Na^+ (Fig. 2*B*) or K^+ (Fig. 2*C*). At a permeant-cation concentration of 12 mM, for example, Ca^{2+} carried 20 times as much current as did Na^+. At a concentration of 24 mM, Ca^{2+} still carried 5-fold as much current as did either K^+ or Na^+. For concentrations exceeding 56 mM, however, the currents measured with Ca^{2+} were less than twice those measured with the monovalent-cation solutions, indicating that the flux of Na^+ or K^+ exceeded that of Ca^{2+}.

At equivalent concentrations, Na^+ carried slightly less receptor current than did K^+. Only at a concentration of 76 mM, however, was the normalized receptor current borne by K^+ significantly higher than that carried by Na^+ ($P = 0.02$, Student's one-tailed t test): the normalized receptor current measured for 76 mM K^+ was 0.83 ± 0.03 ($n = 3$) and that measured for 76 mM Na^+ was 0.69 ± 0.04 ($n = 4$). As was the case for K^+-containing test solutions, the small currents carried by relatively low concentrations of Na^+ might have reflected a requirement for multiple occupancy by monovalent cations or a weak blockage of the transduction channel by $NMDG^+$.

To ascertain whether the presence of multiple permeant cation species affected the permeation properties of transduction channels, we measured normalized receptor currents for mixtures of two cations. For a series of solutions containing concentrations of Ca^{2+} and K^+ that varied oppositely, we observed that the relation between the normalized receptor current and the ionic concentration reached a minimum between the concentrations of 1.2 mM Ca^{2+}/78.8 mM K^+ and 12 mM Ca^{2+}/68 mM K^+ (Fig. 3*A*). The normalized receptor current measured for 4 mM Ca^{2+}/76 mM K^+ (0.71 ± 0.01, $n = 5$) was significantly lower than that measured for either 80 mM K^+ (0.82 ± 0.03, $n = 7$; $P = 0.003$) or 80 mM Ca^{2+} (0.84 ± 0.03, $n = 7$; $P = 0.002$).

Although the effect was less dramatic, a receptor-current minimum was also observed for mixtures of Ca^{2+} and Na^+

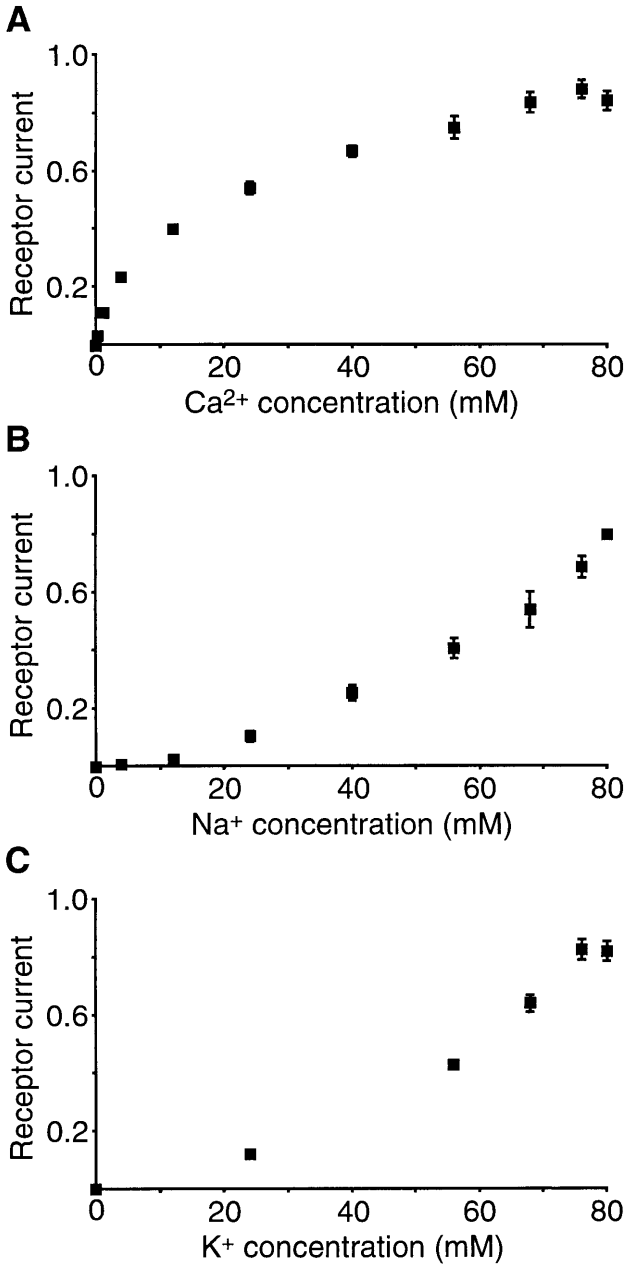

FIG. 2. Normalized receptor currents measured for test solutions containing either Ca^{2+}, Na^+, or K^+. (*A*) At moderate concentrations, Ca^{2+} carried substantial receptor current through transduction channels. Each data point is the average of three to seven measurements from a total of four maculae. (*B*) Na^+ carried little receptor current at concentrations below 24 mM. Each data point is the average of two to five measurements from three preparations. (*C*) K^+ carried receptor currents with magnitudes similar to those measured for Na^+. Each data point is the average of three to seven measurements from three maculae. When it exceeds the size of the symbol, the standard error of the mean is indicated.

(Fig. 3*B*). As for the Ca^{2+}/K^+ mixtures, the normalized receptor current measured for 4 mM Ca^{2+}/76 mM Na^+ ($0.75 \pm 0.02, n = 4$) was significantly lower than that measured for either 80 mM Na^+ ($0.80 \pm 0.01, n = 2; P = 0.04$) or 80 mM Ca^{2+} ($P = 0.02$). For solutions containing Ca^{2+}, the normalized current values measured for identical concentrations of Na^+ and K^+ were not significantly different from one another.

DISCUSSION

Our measurements of receptor currents in the presence of various concentrations of permeant cations extend previous studies that suggest that the hair cell's transduction channel is selective between physiologically relevant cations. In agreement with earlier results (9, 27), we observed that the channel can pass K^+ slightly better than Na^+. Furthermore, our finding that Ca^{2+} carries much more receptor current at moderate concentrations than do monovalent cations indicates that the channel, in addition to having a higher affinity for Ca^{2+} (18, 19), can actually conduct Ca^{2+} more efficiently than monovalent cations.

The receptor-current minimum that we observed for mixtures of Ca^{2+} and monovalent cations suggests an explanation for the transduction channel's high Ca^{2+} affinity and the high Ca^{2+} conductance revealed in this study. If ions permeate the channel's pore independently, we would expect with mixtures of two permeant cations to see a monotonic increase in receptor current as the concentration of the more permeant cation increases. Instead, we observed a drop in current over the Ca^{2+} concentration range of 0–12 mM. The normalized receptor current measured for 4 mM Ca^{2+}/76 mM K^+ was only 67% of that predicted on the assumption of ionic independence, whereas that measured for an equivalent mixture of Ca^{2+} and Na^+ was 82% of the predicted value. The nonmonotonic relation of the current to ionic concentration, called the anomalous mole-fraction effect, has been observed for numerous channels, including voltage-gated Ca^{2+} channels, K^+ channels, and anion channels (reviewed in ref. 20). Such an effect indicates that ions interact with one another as they traverse a channel's pore (see, for example, ref. 28). Due to repulsive ionic interactions within the pore, even ions that bind tightly can achieve high flux rates and thus carry substantial current.

Implications for Hair-Cell Function. When a hair bundle is subjected to excitatory stimulation, each activated transduction channel passes depolarizing current that helps trigger synaptic transmission. The channel simultaneously admits Ca^{2+} into a stereocilium, where the ion regulates adaptation to the stimulus (9, 12–14). Because Ca^{2+} carries substantial current at low concentrations but begins to appreciably block transduction currents in the millimolar range, our data suggest that the transduction channel is optimally suited to fulfill its two functions in cationic environments similar to that of endolymph.

Our results indicate that Ca^{2+} carries more current through transduction channels than suggested by its mole fraction in the bathing solution. Although the interaction of permeant cations within the channel's pore introduces some error, we can use the normalized current measured in the presence of 250 μM Ca^{2+}, which was 0.03, to estimate the rise in intracellular Ca^{2+} concentration in a frog's saccular hair bundle bathed in endolymph. We assume a resting membrane potential of -60 mV (29), a single-channel conductance of 100 pS (9, 15, 30), a resting open probability of 0.2, and a stereociliary volume of 1 fl. In an unstimulated bundle, the Ca^{2+} influx through a single transduction channel is predicted to change a stereocilium's average total Ca^{2+} concentration at a rate of $\approx 0.2\ mM \cdot s^{-1}$ in the absence of buffering and extrusion. When the hair cell receives an excitatory mechanical stimulus, the Ca^{2+} concentration in a stereocilium increases still faster.

That the estimated Ca^{2+} influx *in vivo* is relatively high has several implications for hair-cell physiology. First, even with the hair bundle at rest, Ca^{2+} influx through transduction channels is sufficient to change the stereociliary Ca^{2+} concentration in a biologically relevant range. For example, calmodulin, a Ca^{2+}-dependent protein that regulates adaptation (31), has an affinity for Ca^{2+} in the low micromolar range (32). The second implication is that, because of the high local Ca^{2+} influx, the hair bundle must possess regulatory mechanisms to control the free Ca^{2+} concentration. Indeed, Ca^{2+} pumps are found at a high density in stereocilia and play a role in regulating the hair-bundle Ca^{2+} concentration *in vitro* (33).

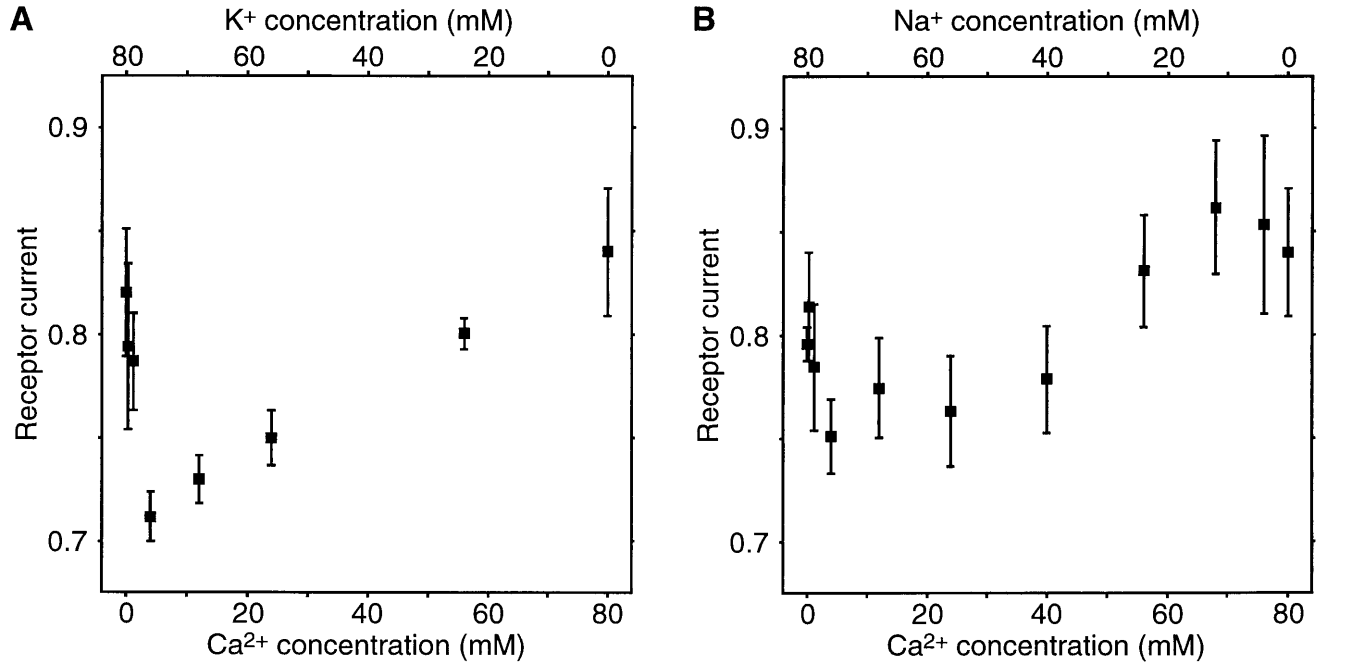

FIG. 3. Normalized receptor currents measured in test solutions containing two permeant cation species. (*A*) With increasing concentrations of Ca^{2+} and decreasing concentrations of K^+, the normalized maximum receptor current first declined and then grew. Each data point is the average of four to seven measurements from two preparations. (*B*) Similar behavior was observed for mixtures of Na^+ and Ca^{2+}. Each data point is the average of two to seven measurements from three maculae. Note the expanded ordinate scales in both panels. The error bars denote standard errors of the means.

In auditory organs, the endolymphatic Ca^{2+} concentration is lower than that of the bullfrog's sacculus (5, 9). The Ca^{2+} flux through transduction channels may therefore be correspondingly lower in auditory than in vestibular hair cells. An intriguing alternative to this possibility is suggested by comparison of our results to those from hair cells isolated from the turtle's basilar papilla (9). For that preparation in the presence of 130 mM Na^+, increasing the extracellular Ca^{2+} concentration from 0.05 to 2.8 mM caused a 50% reduction in transduction current. This reduction may have been a manifestation of the anomalous mole-fraction effect that we observed for Ca^{2+} concentrations in the low millimolar range. The more pronounced reduction measured in auditory hair cells could indicate that the transduction channels of these hair cells have a higher affinity for Ca^{2+} than do vestibular hair cells. Transduction channels from different hair cells thus may be optimized to allow appropriate Ca^{2+} influx in their native cationic environments.

Similarities Between the Hair Cell's Transduction Channel and Other Ion Channels. The anomalous mole-fraction effect exhibited by the transduction channel is less pronounced than those observed for certain other cation channels, such as voltage-gated Ca^{2+} channels (34). The transduction channel's ion-binding site, although selective for Ca^{2+}, therefore has a lower affinity for Ca^{2+} than do those of voltage-gated Ca^{2+} channels.

The transduction channel's selectivity for Ca^{2+} resembles those of cyclic-nucleotide-gated channels, which transduce sensory information in retinal photoreceptors and olfactory neurons (reviewed in refs. 35 and 36). A similar selectivity for Ca^{2+} characterizes ATP-gated ion channels (37, 38) and *N*-methyl-D-aspartate receptors (39). Other permeation properties of the hair cell's transduction channel, however, differ from those of the listed classes of ion channels. For example, the transduction channel's single-channel conductance (9, 15, 30) exceeds that of the other channels above (36, 40, 41).

Although the hair cell's transduction channel may be related to other ion channels at the molecular level, the known biophysical characteristics of the native channel do not warrant its inclusion in a particular class. Furthermore, changing only a few amino acids in the pore region of an ion channel can drastically alter its ion permeability (see, for example, ref. 42), which confounds our ability to classify the transduction channel purely on the basis of its permeability properties. Detailed knowledge of the channel's permeability may, however, facilitate the evaluation of candidate cDNA clones encoding the transduction channel. In conjunction with the ion-selectivity series and permeability ratios, the currents carried by different cations and the anomalous mole-fraction effect represent hallmarks of the native transduction channel to which molecular candidates can be compared.

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