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Effects of Lipophilic Ions on Outer Hair Cell Membrane Capacitance and Motility

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Abstract. The outer hair cell (OHC) from the mammalian organ of Corti possesses a bell-shaped voltage-dependent capacitance function. The nonlinear capacitance reflects the activity of membrane bound voltage sensors associated with membrane motors that control OHC length. We have studied the effects of the lipophilic ions, tetraphenylborate (TPB^-) and tetraphenylphosphonium (TPP^+), on nonlinear capacitance and motility of isolated guinea-pig OHCs. Effects on supporting cells were also investigated. TPB^- produced an increase in the peak capacitance (Cm_{pk}) and shifted the voltage at peak capacitance (V_{pkCm}) to hyperpolarized levels. Washout reversed the effects. Perfusion of 0.4 μM TPB^- caused an average increase in Cm_{pk} of 16.3 pF and V_{pkCm} shift of 13.6 mV. TPP^+, on the other hand, only shifted V_{pkCm} in the positive direction, with no change in Cm_{pk}. The contributions from native OHC and TPB^--induced capacitance were dissected by a double Boltzmann fitting paradigm, and by blocking native OHC capacitance. While mechanical response studies indicate little effect of TPB^- on the motility of OHCs which were in normal condition or treated with salicylate or gadolinium, the voltage at maximum mechanical gain ($V_{\delta Lmax}$) was shifted in correspondence with native V_{pkCm}, and both changed in a concentration-dependent manner. Both TPB^--induced changes in Cm_{pk} and V_{pkCm} were affected by voltage prepulses and intracellular turgor pressure. TPB^- induced a voltage-dependent capacitance in supporting cells whose characteristics were similar to those of the OHC, but no indication of mechanical responses was noted. Our results indicate that OHC mechanical responses are not simply related to quantity of nonspecific nonlinear charge moved within the membrane, but to the effects of motility voltage-sensor charge movement functionally coupled to a mechanical effector.

Key words: Cochlea — Gating current — Tetraphenylborate — Motility — Capacitance

Introduction

Acoustic signal processing by the mammalian cochlea is accomplished by two classes of sensory hair cells: the inner hair cells (IHCs) and outer hair cells (OHCs). IHCs are solely mechano-electrical transducers, while OHCs function, in addition, as mechanical effectors. It is believed that the unique voltage-dependent mechanical response of the OHC provides feedback into the basilar membrane, thereby sharpening the passive mechanical vibration of the cochlear partition (Brownell et al., 1985; Ashmore, 1987; Santos-Sacchi & Dilger, 1988; Ruggero, 1992). The OHC demonstrates a nonlinear gating charge movement or, correspondingly, a voltage-dependent capacitance that has characteristics similar to those of OHC motility, indicating that membrane-bound voltage sensor/motor elements control OHC length (Santos-Sacchi, 1990, 1991, 1993; Ashmore, 1989, 1992; Dallos et al., 1991; Iwasa, 1994).

The causal relation between OHC nonlinear charge movement and mechanical response is an important feature in theories of OHC motility where voltage-dependent conformational changes in putative lateral membrane molecular motors fluctuate between two areal states (Santos-Sacchi, 1993; Huang & Santos-Sacchi, 1994; Iwasa, 1994). Accordingly, intrinsic gating charge movement represents the reorientation of charged moieties within a membrane-bound, protein motor molecule. Only this charge movement should be coupled to OHC mechanical activity. Membrane bound charge movement associated with extraneous sources, e.g., the cell's

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normal complement of voltage-gated ionic channels or exogenous membrane bound charge, should neither effect nor directly affect OHC motility. Furthermore, blockade of motility-related gating charge should be sufficient to block motility.

To evaluate these predictions, we attempted to induce additional nonlinear charge movement within the OHC plasma membrane with the exogenous hydrophobic ions, tetraphenylborate (TPB^-) and tetraphenylphosphonium (TPP^+). Furthermore, salicylate and Gd^{+3}, known blockers of OHC motility and charge movement (Santos-Sacchi, 1991; Shehata, Brownell & Dieler, 1991; Tunstall, Gale & Ashmore, 1995; Kakehata & Santos-Sacchi, 1996), were evaluated to dissect out contributions of native nonlinear charge movement during hydrophobic ion treatment. Hydrophobic ions such as TPB^- or dipicrylamine have been used to amplify small capacitive signals of secretory vesicle release (Oberhauser & Fernandez, 1995). The ions have also been used as probes of tight junctions (Turin et al., 1991), and membrane structure (Benz & Läuger, 1977; Benz & Nonner, 1981; Dilger & Benz, 1985; Smejtek & Wang, 1991) and function (Reyes & Latorre, 1979; Fernandez, Bezanilla & Tayor, 1982). The TPB^--induced current is similar, in some ways, to that which arises from voltage-dependent ionic channel gating (Benz & Nonner, 1981).

We report here that TPB^- augments native OHC nonlinear capacitance and produces a negative shift of the equivalent voltages at peak capacitance (V_{pkCm}) and maximal mechanical gain (V_{dLmax}), but does not enhance the mechanical response. TPP^+ only causes a shift in the voltage dependencies. Such results might be expected from simple charge screening. These data highlight the specific and causal relationship between the native nonlinear capacitance and the mechanical activity of the OHC.

Materials and Methods

Methods

Guinea pigs were anesthetized with halothane and killed by cervical dislocation. OHCs and supporting cells were isolated enzymatically with dispase I (0.5 mg/ml for 10 min followed by gentle trituration through a polyethylene pipette) in a modified Leibovitz medium which contained (in mM): NaCl 142, KCl 5.37, $MgCl_2$ 1.47, HEPES 5, $CaCl_2$ 2 and dextrose 5; 300 mOsm, pH 7.2. The cells were then transferred to a 700-μl perfusion chamber. All experiments were conducted at room temperature (~23°C). A Nikon Diaphot inverted microscope with Hoffmann optics was used to observe the cells during electrical recording. All experiments were videotaped.

Single OHCs and supporting cells were studied under whole cell voltage-clamp conditions using an Axon 200A amplifier at a holding potential of −80mV. Initial resistances of patch pipettes were 2–3 MΩ, corresponding to tip sizes of 1–2 μm (Hamill et al., 1981). Residual series resistance ranged from 3 to 7 MΩ. All data collection and analysis were performed with an in-house developed Windows-based program, **jClamp** (www.med.yale.edu/surgery/otolar/santos/jclamp.html). Ionic blocking solutions were used to remove voltage-dependent ionic conductances so that capacitive currents could be analyzed in isolation (Santos-Sacchi, 1991; Huang & Santos-Sacchi, 1993). The patch pipette solution contained (in mM): CsCl 140, $MgCl_2$ 2, EGTA 10, HEPES 5, with pH 7.2 and osmolarity 300 mOsm (adjusted with dextrose). The external ionic blocking solution contained (in mM): $BaCl_2$ 10, $CoCl_2$ 2, $MgCl_2$ 1.47, NaCl 100, $CaCl_2$ 2, HEPES 5, with pH 7.2 and osmolarity 300 mOsm (adjusted with dextrose). In this solution $BaCl_2$ replaced the usually employed blockers, CsCl and TEA (Huang & Santos-Sacchi, 1993), because both caused precipitation with sodium tetraphenylboron. The reagents (Na^+ TPB^- and TPP^+ Cl^-) were freshly prepared and applied using the Y-tube method (Murase et al. 1990) at a rate of 0.3 to 0.65 ml/min. These two hydrophobic ions were chosen for their opposite charges, which we presumed might provide for differing effects. We reasoned that the effectiveness in influencing capacitance and motility might depend on the sign of the hydrophobic ion relative to that of the native charge. Whole chamber perfusion was continuous.

A tracking procedure was used to continuously monitor the voltage at peak capacitance (V_{pkCm}) after obtaining whole-cell configuration (Kakehata & Santos-Sacchi, 1995). Membrane potential was corrected for the effects of residual series resistance. Peak capacitance (Cm_{pk}) values were also monitored during the tracking procedure using transient analysis of capacitive currents induced by a −10 mV step. Detailed evaluation of total membrane capacitance was made at different potentials by transient analysis of currents induced by a voltage stair step stimulus, and the capacitance function was fit to the first derivative of a two state Boltzmann function relating nonlinear charge to membrane voltage (dQ/dV; Santos-Sacchi, 1991; Huang & Santos-Sacchi, 1993)

$$C_m = Q_{max}\frac{ze}{kT}\frac{b}{(1+b)^2} + C_{lin}$$

where

$$b = \exp\left(\frac{-ze(V - V_{pkcm})}{kT}\right)$$

Q_{max} is the maximum nonlinear charge moved, V_{pkCm} is voltage at peak capacitance or equivalently, at half-maximal nonlinear charge transfer, V_m is membrane potential, z is valence, C_{lin} is linear membrane capacitance, e is electron charge, k is Boltzmann's constant, and T is absolute temperature.

Pipette pressure was monitored via a T-connector to a pressure monitor (WPI, Sarasota, FL), and modified when required with a syringe connected to the Teflon tubing attached to the patch pipette holder (Kakehata & Santos-Sacchi, 1995). The cylindrical shape of OHCs was maintained during data collection. Measures of voltage-induced (−160 to 40 mV, 20 mV increment) mechanical responses were made off the video monitor during playback with a differential optoresistor technique (Santos-Sacchi, 1989; 1991). Mechanical data were fit to a two state Boltzmann function to determine the voltage at maximum mechanical gain ($V_{\delta Lmax}$).

Dose-response data (charge *vs.* hydrophobic ion concentration) were averaged and are given as mean ± SE. A fit was made according to a modified Michaelis-Menten equation with the use of a least-squares routine (Sigma Plot, CA),

$$Q = Q_{max}\,(C^n/(C^n + K_D^n))$$

where Q is the charge induced by the hydrophobic ion, C is hydropho-

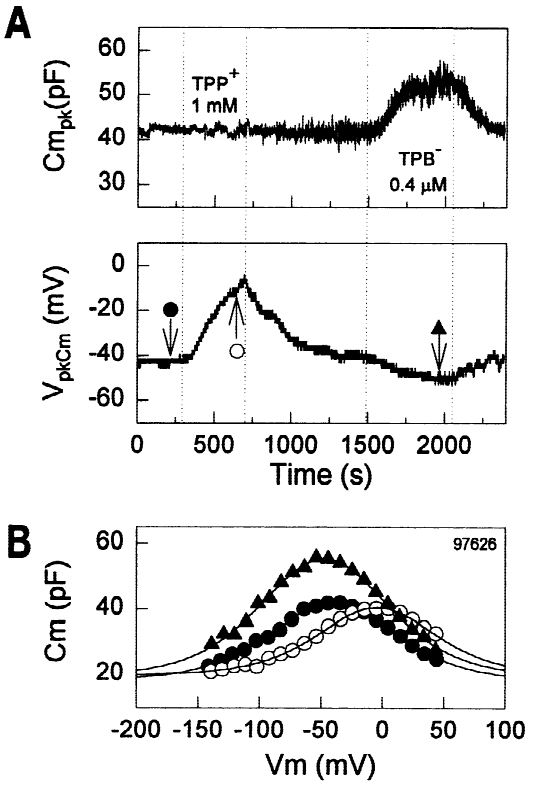

Fig. 1. Effects of TPP^+ and TPB^- on Cm_{pk} and V_{pkCm} of an OHC obtained by the tracking technique (*A*) and the voltage stair step technique (*B*). (*A*) After Cm_{pk} and V_{pkCm} stabilized, TPP^+ (1 mM) was applied extracellularly at the first dotted line. V_{pkCm} shifted to depolarized levels with no change in Cm_{pk}. Washout at the second dotted line caused recovery. TPB^- (0.4 μM) was applied extracellularly at the third dotted line. V_{pkCm} shifted slightly to hyperpolarized levels with a increase in Cm_{pk}. Washout following TPB^- treatment started from the 4th line. Arrows indicate points at which capacitance functions were determined by the voltage stair step technique for the same cell. (*B*) Fits (solid lines) for the capacitance data indicate Cm_{pk}, V_{pkCm}, Q_{max} and z of 42.1 pF, −40.2 mV, 3.26 pC and 0.75 (closed circles, control); 40.6 pF, −4.4 mV, 2.69 pC and 0.79 (opened circles, TPP^+ treatment); 55.7 pF, −47.4 mV, 5.08 pC and 0.73 (closed triangles, TPB^- treatment).

bic ion concentration, K_D is the dissociation constant, and n is the Hill coefficient.

Results

OHCs possess a capacitance that depends upon transmembrane voltage, and which derives from motility-related gating currents (Fig. 1). Membrane-bound, charged molecules from external sources can also contribute to membrane capacitance. This is illustrated in Fig. 1 where the effects of TPB^- and TPP^+ on Cm_{pk} and V_{pkCm} are monitored with the tracking technique described in the methods (Fig. 1*A*); in addition, a more detailed analysis of membrane capacitance is provided by the stair step protocol (Fig. 1*B*). Under our experimental conditions, TPP^+ had no effect on Cm_{pk}, but shifted V_{pkCm} to depolarized levels. In contrast, TPB^- increased Cm_{pk} and shifted V_{pkCm} to hyperpolarized levels. Washout reversed the effects (Fig. 1*A*). The average onset and recovery time constant for 0.4 μM TPB^- was 102.2 ± 25.91 sec ($n = 5$) and 184 ± 60.74 sec ($n = 4$), respectively.

The average steady state shift of V_{pkCm} induced by 1 mM TPP^+ was 33.9 ± 1.7 mV (mean ± SE; $n = 10$; stair analysis). The difference in Cm_{pk} before and during TPP^+ treatment was 0.57 ± 0.3 pF. Higher concentrations of TPP^+, up to 10 mM, were equally ineffective in altering Cm_{pk}. On the other hand, the average elevation of Cm_{pk} and shift of V_{pkCm} induced by 0.4 μM TPB^- were 16.3 ± 2.7 pF and −13.6 ± 3.04 mV ($n = 15$), respectively.

To study the concentration effects of TPB^- on OHC nonlinear capacitance, TPB^- was applied by 'Y-tube' in increasing concentrations ranging from 0.2 μM to 1 μM, without intermittent washing. Tracking commenced immediately after whole cell configuration, and stair step analysis was performed after V_{pkCm} reached steady state at each concentration. Figure 2*A* illustrates the TPB^- concentration dependence of OHC Cm_{pk} and V_{pkCm}. By gradually increasing the concentration of TPB^-, correspondingly larger increases in Cm_{pk} and V_{pkCm} shifts were elicited. Concentrations greater than 1 μM could induce very large capacitances, which compromised the effectiveness of our voltage clamp.

Contributions to the voltage-dependent capacitance observed in Fig. 2*A* are derived from two distinct sources of nonlinear charge movement (native and TPB^--induced), which theoretically should be separable analytically. Unfortunately, it is clear from the data that the characteristics of native and TPB^--induced charge movement are not disparate enough to produce a bimodal capacitance function, which could easily be fit by the sum of two Boltzmann functions. Nevertheless, we have devised a procedure which estimates the TPB^- contribution assuming that in addition to augmenting charge, TPB^- causes a shift in the voltage dependence of OHC native charge movement, as occurred in the presence of TPP^+. Thus, we first obtained fits (single Boltzmann) of OHC native capacitance prior to TPB^- treatment and used these parameters (all fixed except for V_{pkcm} which was permitted to vary) in subsequent fits (sum of two Boltzmanns) of capacitance in the presence of TPB^-. Figure 2*B* illustrates the result, and indicates that, opposite to the effects TPP^+, the native OHC capacitance function is shifted in the negative direction by the negatively charged TPB^-.

This shift in V_{pkcm} was independently confirmed in a group of OHCs where the effects of TPB^- on OHC motility and capacitance were studied in concert. Before and during TPB^- treatment, voltage-induced OHC move-

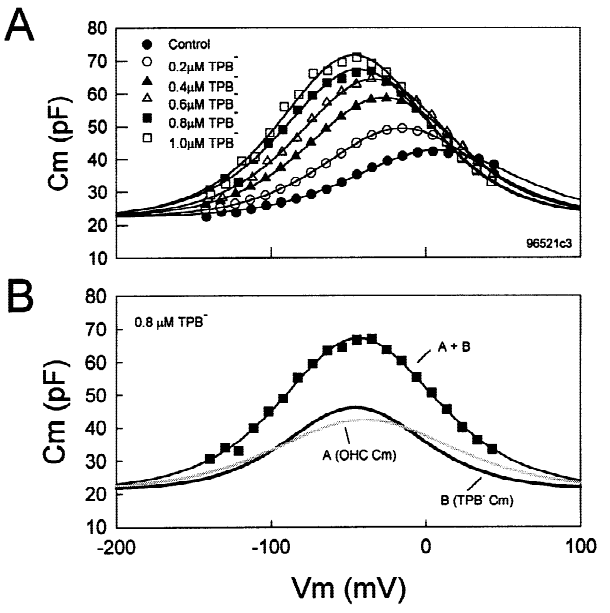

Fig. 2. (*A*) Concentration dependence of TPB^- effects on Cm_{pk} and V_{pkCm} of an OHC. Cm_{pk} increased and V_{pkCm} shifted to hyperpolarized levels in a concentration-dependent manner. Fits (solid lines) for capacitance indicate Cm_{pk}, V_{pkCm}, Q_{max} and z of 42.5 pF, 6.5 mV, 2.89 pC and 0.72 (closed circles, control), 49.4 pF, −15.3 mV, 3.61 pC and 0.77 (open circles, 0.2 μM TPB); 58.8 pF, −26.8 mV, 4.81 pC and 0.78 (closed triangles, 0.4 μM TPB); 64.4 pF, −33.4 mV, 5.48 pC and 0.79 (open triangles, 0.6 μM TPB); 67.2 pF, −43.1 mV, 5.85 pC and 0.79 (closed squares, 0.8 μM TPB) and 71.6 pF, −46.1 mV, 6.26 pC and 0.81 (open squares, 1 μM TPB). (*B*) Example of extraction technique used to assess the individual contributions of TPB^--induced and native OHC capacitance to the overall capacitance function. *A*, native capacitance of OHC; *B*, TPB^--induced capacitance; *A + B*, total capacitance with 0.8 TPB^- treatment. *See* text for details.

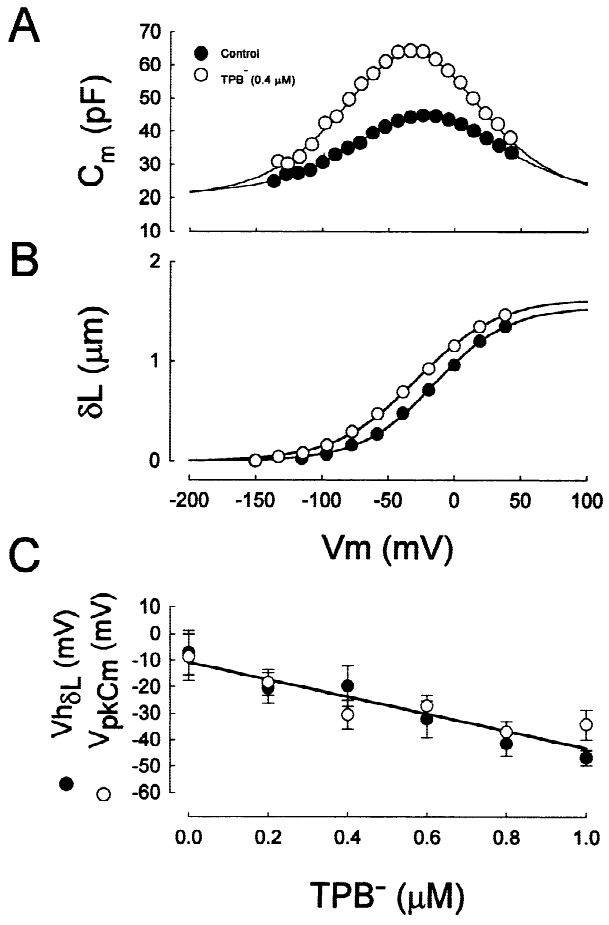

Fig. 3. (*A*) Voltage-dependent capacitance and (*B*) voltage-induced length change of seven OHCs measured before and during TPB^- treatment. Length changes induced by 20 mV steps from −160 to 40 mV, but corrected for series resistance. Fits for average overall capacitance data indicate Cm_{pk}, V_{pkCm}, Q_{max} and z of 44.8 pF, −21.9 mV, 3.69 pC and 0.67 (closed circles, control); 63.9 pF, −32.3 mV, 6.3 pC and 0.72 (open circles, 0.4 μM TPB). Fits for average mechanical data indicate $V_{\delta Lmax}$ and z of −14.9 mV and 0.9 (closed circles, control); −28.5 mV and 0.81 (open circles, 0.4 μM TPB). Cell length was 64.29 ± 1.44 μm (mean ± SE; $n = 7$). (*C*) Correspondence between shifts in extracted OHC V_{pkCm} (determined as in text) and $V_{\delta Lmax}$ of OHCs treated with TPB^- as a function of concentration. Each point is the mean ± SE ($n = 3$).

ment and voltage-dependent capacitance were obtained by voltage steps (−160 to 40 mV, 20 mV increment) and stair step analysis, respectively. As shown in Fig. 3*A* and *B*, TPB^- had a significant effect on the magnitude of Cm_{pk} but little effect on the magnitude of voltage-dependent movement. The ratio of total Q_{max} in the presence of TPB^- to Q_{max} in its absence was 1.71, whereas the corresponding ratio of δL_{max} was 1.06 ($n = 7$). These results indicate that an increase in OHC nonlinear capacitance by an external source does not result in significant enhancement of cell movement. Although TPB^- does not alter the magnitude of OHC motility, its voltage dependence is susceptible. In fact, the effect on $V_{\delta Lmax}$ corresponds to the effect on the extracted value of native OHC V_{pkcm} (Fig. 3*C*); both shift in the hyperpolarizing direction in a concentration-dependent manner. This is expected since the voltage dependencies of OHC motility and nonlinear capacitance are known to coincide (Kakehata & Santos-Sacchi, 1995). Within a limited concentration range of TPB^- (0.2–1.0 μM), the response slope is −32 mV/μM ($n = 3$). The dose-response data indicate a Hill coefficient of 1.85 ± 0.10, Q_{max} of 5.38 ± 0.23 pC, and K_d of 0.50 ± 0.03 μM.

Other ways to dissociate the two components of nonlinear charge movement may include delivery of prepulse voltages (Santos-Sacchi, Kakehata & Takahashi, 1997, 1998; Santos-Sacchi, 1997), alterations of lateral membrane tension (Iwasa, 1993; Gale & Ashmore, 1994; Kakehata & Santos-Sacchi, 1995), and treatments with salicylate (Tunstall et al., 1995; Kakehata & Santos-Sacchi, 1996) or Gd^{+3} (Santos-Sacchi, 1991), since these manipulations may selectively influence native OHC charge movement.

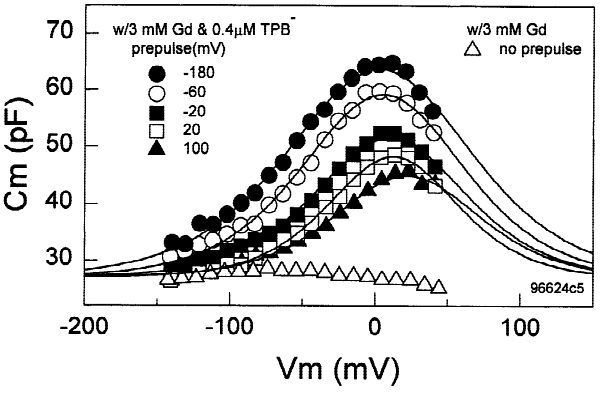

Fig. 4. Effects of 1-sec voltage prepulse on Cm_{pk} and V_{pkCm} of an OHC treated with gadolinium and TPB^-. Once reduction of OHC nonlinear capacitance by Gd^{+3} (3 mM) reached steady state (reduction of Cm_{pk} from 41.2 to 28.2 pF), TPB^- (0.4 μM) was added to the solution. Control capacitance function (open triangles, 3 mM Gd^{+3}, no prepulse) could not be reliably fit. After the appearance of TPB^+-induced capacitance, prepulse effects were studied. Cm_{pk}, V_{pkCm}, Q_{max} and z were 63.7 pF, 2.8 mV, 6.28 pC and 0.60 (closed circles, −180 mV prepulse); 59.2 pF, 5.3 mV, 4.86 pC and 0.68 (open circles, −60 mV prepulse); 51.6 pF, 10.3 mV, 3.46 pC and 0.73 (closed squares, −20 mV prepulse); 48.3 pF, 12.5 mV, 2.57 pC and 0.85 (open square, 20 mV prepulse) and 44.9 pF, 22.1 mV, 2.42 pC and 0.76 (closed triangles, 100 mV prepulse).

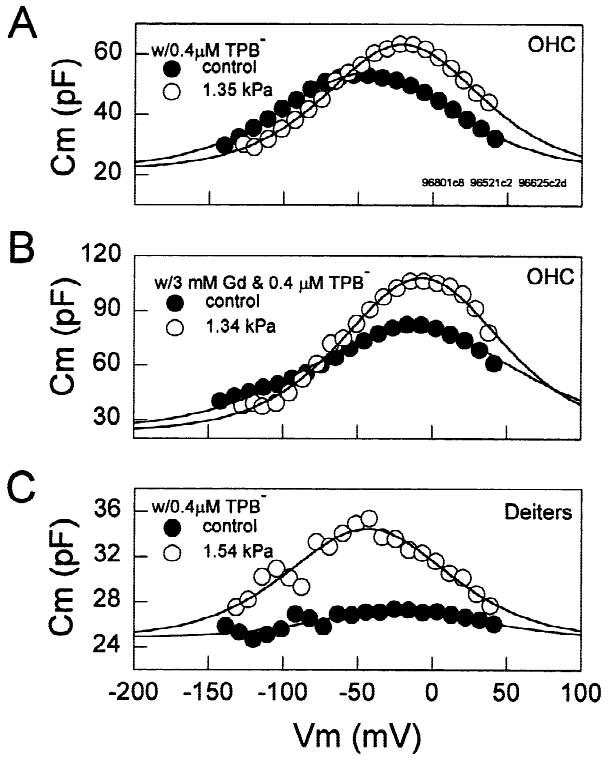

Fig. 5. Effects of positive intracellular pressure on C_m of OHCs (*A*, *B*) and Deiters cells (*C*). (*A*) After OHC C_m was increased by 0.4 μM TPB and reached steady state, positive intracellular pressure caused a positive shift in overall V_{pkCm} and increase in Cm_{pk}. (*B*) OHC nonlinear capacitance was blocked with Gd^{+3}, and followed by treatment with TPB^-. After Cm_{pk} reached steady state, increased intracellular pressure caused a positive shift in V_{pkCm} and an increase in Cm_{pk}. (*C*) TPB^- (0.4 μM) induced a nonlinear capacitance in Deiters cells. In this case, after reaching steady state, positive pressure caused a negative shift in V_{pkCm} and an increase in Cm_{pk}

In the untreated OHC, negative prepulse voltages shift V_{pkCm} in the positive direction and visa versa, with little effect on Cm_{pk} (Santos-Sacchi et al., 1997, 1998). In the TPB^- treated OHC, changes in V_{pkCm} and Cm_{pk} were found (*data not shown*) and could not be accounted for by effects on native charge alone; indeed, these data suggested that TPB^- induced charge movement was susceptible to prepulse effects, as well. To confirm this, gadolinium (3 mM) was first used to block intrinsic OHC nonlinear capacitance, so that TPB^- charge movement could be studied in isolation. Figure 4 illustrates the effects of prepulses on the voltage dependence of the isolated TPB^--induced nonlinear capacitance. As the prepulse potential decreased from 100 to −180 mV, TPB^--induced nonlinear capacitance progressively increased in magnitude and V_{pkCm} became more negative. This is distinctly different from prepulse effects on the intrinsic nonlinear capacitance of the OHC (Santos-Sacchi et al., 1997, 1998).

Positive turgor pressure shifts OHC V_{pkCm} to depolarized levels and reduces Cm_{pk} (Iwasa, 1993; Gale & Ashmore, 1994; Kakehata & Santos-Sacchi, 1995). However, in OHCs treated with TPB^-, whereas positive intracellular pressure also shifts V_{pkCm} to depolarized levels, Cm_{pk} increases (Fig. 5). With 0.4 μM TPB treatment, Cm_{pk} and V_{pkCm} of the cell shown in Fig. 5*A* were 53.3 pF and −27.2 mV at steady state. Positive intracellular pressure (1.35 kPa pipette pressure) increased Cm_{pk} by 6.1 pF and shifted V_{pkCm} in the depolarized direction by 17.6 mV. The average elevation of Cm_{pk} and shift of V_{pkCm} induced by positive pipette pressure (0.95 ± 0.14 kPa) was 6.1 ± 1.35 pF ($n = 4$) and 15.5 ± 3.66 mV ($n = 4$), respectively. After intrinsic OHC nonlinear capacitance was blocked by gadolinium (3 mM), positive intracellular pressure still increased TPB^--induced capacitance and shifted V_{pkCm} in the depolarized direction (Fig. 5*B*). However, there was a greater increase in Cm_{pk} and smaller shift in V_{pkCm}. Under this condition, the average elevation of capacitance and shift of V_{pkCm} caused by positive pressure (1.23 ± 0.16 kPa) was 14.8 ± 3.74 pF and 5.35 ± 1.97 mV ($n = 4$), respectively. Positive intracellular pressure affected supporting cells in a similar way (Fig. 5*C*). The average increase in capacitance was 5.5 ± 0.83 pF ($n = 4$) by positive pressure (1.37 ± 0.26 kPa). Similar results were obtained by changing intracellular pressure with hypotonic solutions (*data not shown*).

Salicylate blocks OHC voltage-dependent capacitance and decreases the cell's mechanical response (Kakehata & Santos-Sacchi, 1996). We found that the pres-

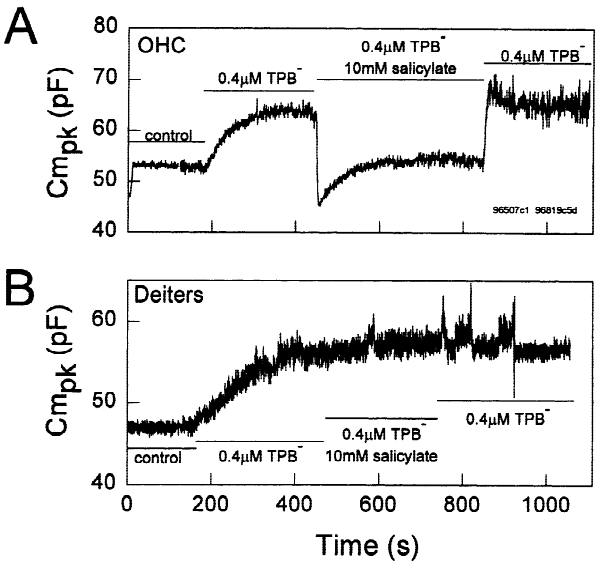

Fig. 6. Effects of salicylate on intrinsic and TBP$^-$ induced nonlinear capacitance. Cm_{pk} of (*A*) an OHC and (*B*) a Deiters cell monitored by the V_{pkCm} tracking technique. Same treatments were used for both cells. Salicylate specifically blocks intrinsic OHC nonlinear capacitance. Salicylate (10 mM) had no effect on Cm_{pk} of the Deiters cell.

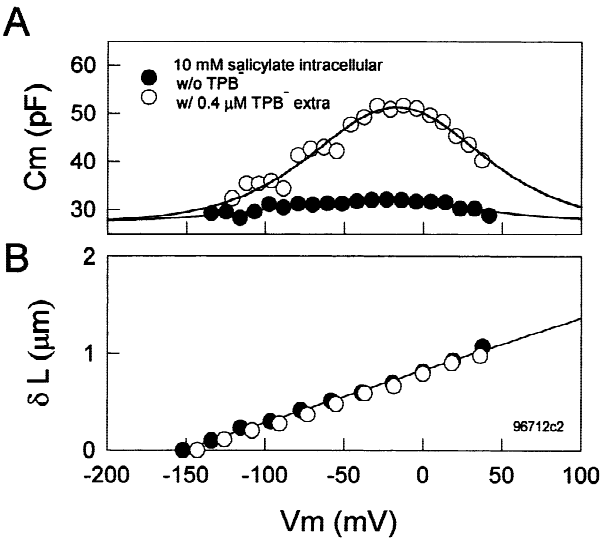

Fig. 7. Effects of TPB$^-$ on (*A*) capacitance and (*B*) movement of OHCs whose nonlinear capacitance was blocked by salicylate. Length changes wree induced by 20-mV steps from −160 to 40 mV and series resistance was corrected. Capacitance was evaluated with the stair step protocol. Fits (solid lines) for capacitance data indicate Cm_{pk}, V_{pkCm}, Q_{max} and *z* of 32.1 pF, −31.8 mV, 0.82 pC and 0.59 (closed circles, w/o TPB); 51.4 pF, −16.9 mV, 2.43 pC and 0.72 (open circles, w/ 0.4 μM TPB). Fits for mechanical data indicate $V_{\delta Lmax}$ and *z* of 119 mV and 0.091 (closed circles, w/o TPB$^-$); −9.1 mV and 0.1213 (open circles, w/ 0.4 μM TPB). Despite the induction of a prominent nonlinear capacitance by TPB$^-$, no recovery of the mechanical response was observed. Cell length: 71 μm.

ence of TPB$^-$ molecules does not affect the reduction of OHC nonlinear capacitance caused by salicylate. In Fig. 6, Cm_{pk} of an OHC and supporting cell were monitored. TPB$^-$ (0.4 μM) increased Cm_{pk} of the OHC (top trace) to a steady-state value of 63.8 pF. Subsequent treatment with salicylate (10 mM), reduced Cm_{pk} to 45.6 pF, with a time constant of 1.69 sec. The average time constant was 3.79 ± 0.94 sec (mean ± SE, $n = 8$). This rate is faster than that observed by Kakehata and Santos-Sacchi (1996), and may be due to the presence of TPB$^-$ or the higher perfusion rate used here. Although Cm_{pk} recovered partially over the course of salicylate perfusion, reaching 52 pF, no recovery of mechanical response was noted. After removal of salicylate, Cm_{pk} increased again due to the reversible effect of salicylate on OHC nonlinear capacitance. The same treatment used for OHCs was applied to supporting cells (Fig. 6, bottom). TPB$^-$ also increased Cm_{pk} of Deiters cells, but to a lesser and more variable degree. The average elevation of Cm_{pk} by 0.4 μM TPB$^-$ was 9.5 ± 1.2 pF for OHCs ($n = 15$) and 5.5 ± 1.0 pF for Deiters cells ($n = 11$). The latter figure does not include another 11 Deiters cells whose capacitance did not show an increase by at least 5 min after onset of TPB$^-$ treatment. No effect of salicylate on Cm_{pk} was found and no mechanical response was detected for TPB$^-$-treated Deiters cells.

The effects of salicylate (or gadolinium) on OHC motility are not alleviated by TPB$^-$ treatment (Fig. 7). The figure illustrates the effects of TPB$^-$ on C_m and movement of an OHC whose nonlinear capacitance was blocked by intracellular salicylate. Simultaneous measures of capacitance and voltage dependent movement were made before and during extracellular application of 0.4 μM TPB$^-$. For this cell, Cm_{pk} was reduced to a steady-state level of 32.1 pF by 10 mM salicylate, which is consistent with the results of Kakehata and Santos-Sacchi (1996). During TPB$^-$ treatment, peak capacitance increased to 51.7 pF. Although TPB$^-$-induced an additional nonlinear capacitance of 19.64 pF and shifted overall V_{pkCm} from −31.83 to −16.28 mV, the difference in maximum length change was only 0.09 μm. The average difference in maximum length change for 3 cells was 0.03 ± 0.02 μm.

Discussion

The present results show that the hydrophobic ion, TPB$^-$, but not TPP$^+$, increases nonlinear charge movement, as evidenced through capacitance measures, in OHCs and supporting cells from the organ of Corti. The enhancement of nonlinear charge movement, which is accompanied by a concentration-dependent shift in the voltage at peak capacitance, is greater for OHCs than for supporting cells. This suggests that membrane structural parameters such as thickness, dielectric constant, dipole poten-

tial, or the degree of the lipid hydrocarbon chain order (Ketterer, Randic & Läuger, 1971; Benz & Läuger, 1977; Reyes & Latorre, 1979; Fernandez et al., 1982) may be different between the two types of cells; another clear difference between the cells is the existence of intrinsic voltage-sensor/motor elements in OHCs. The latter difference is exemplified by the results from salicylate treatments. Salicylate reduced OHC membrane capacitance attributable to intrinsic lateral membrane sensor/motors, which is in agreement with earlier studies (Tunstall et al., 1995; Kakehata & Santos-Sacchi, 1996), but did not reduce the TPB^--induced capacitance of supporting cells or OHCs. This evidence further supports the notion that salicylate directly and specifically acts on the sensor/motor of the OHC (Kakehata & Santos-Sacchi, 1996), and not via some nonselective mechanism to impede all charge movement within the membrane.

The characteristics of TPB^--induced nonlinear capacitance are similar in some ways to those of the OHC intrinsic capacitance, namely, each has a shallow voltage dependence and is affected by voltage prepulse and membrane tension. However, there are obvious differences which are readily revealed when the OHC's intrinsic nonlinear capacitance is blocked. Whereas the OHC nonlinear capacitance shifts its voltage dependence in the negative direction with positive prepulse (Santos-Sacchi, Kakehata & Takahashi, 1998), the voltage dependence of TPB^--induced capacitance does the opposite. Also, whereas positive intracellular pressure decreases OHC peak capacitance (Gale & Ashmore, 1994; Kakehata & Santos-Sacchi, 1995), TPB^--induced peak capacitance increases. The reasons for these differences are not obvious, but may possibly relate to differences in sign of the mobile charged species.

Despite some similarities between TPB^--induced nonlinear charge movement and OHC intrinsic nonlinear charge movement, the former cannot effect mechanical responses, indicating that TPB^- charge movement does not alter lateral membrane motor conformation. Similarly, in frog myelinated nerve, lipophilic dipicrylamine ions did not interact with Na channels (Benz & Nonner, 1981). However, we found that TPB^--induced shifts in OHC intrinsic V_{pkCm} which are mirrored by similar shifts in V_{dLmax}. Since TPB^- does not directly alter the magnitude of OHC motility, it is probable that the ion's effects on motility are via electrostatic interactions with the motility voltage sensor; that is, the voltage dependence of intrinsic OHC capacitance is likely shifted by TPB^-, just as it is by TPP^+.

A variety of evidence indicates that the OHC's native nonlinear charge movement is causally related to the cell's mechanical response. First, blockers of OHC nonlinear capacitance cause reductions in OHC motility (Santos-Sacchi, 1991; Tunstall et al., 1995; Kakehata & Santos-Sacchi, 1996). Second, the kinetics of charge movement and mechanical response coincide (Santos-Sacchi, 1990, 1991; Gale & Ashmore, 1997). Third, treatments which induce shifts in the voltage dependence of nonlinear charge movement or capacitance similarly shift that of the mechanical response (Fig. 3*C*; Kakehata & Santos-Sacchi, 1995). Fourth, mechanical stimulation of the lateral membrane elicits the salicylate blockable motility-related gating charge movement (Gale & Ashmore, 1994; Takahashi & Santos-Sacchi, 1997). Finally, the present data indicate that induced nonspecific charge movement cannot evoke or recover blocked mechanical responses.

The apparent discrepancy between the degree of salicylate-induced reduction in capacitance and mechanical response indicated by the closed symbols in Fig. 7, and noted previously (Kakehata & Santos-Sacchi, 1996) may be readily accounted for. First, the relative decrease in capacitance and motility is not known since the pipette contained the drug and the effect is near immediate (Kakehata & Santos-Sacchi, 1996); no pre-drug measures are available. Second, the native nonlinear charge movement is not completely abolished, and in fact may be extended over a wider voltage range than under normal conditions (reduced z), so that measurement could be limited by our ability to deliver extreme voltages. Futhermore, we have previously shown that when the nonlinear capacitance remaining after salicylate treatment is blocked by Gd^{+3}, the mechanical response is fully abolished (Kakehata & Santos-Sacchi, 1996). Third, the whole-cell mechanical response magnitude may be dependent upon many physical criteria, including turgor pressure (Shehata et al., 1991; Santos-Sacchi, 1991), whole-cell stiffness (Dallos et al., 1997), and possibly membrane visco-elasticity (Santos-Sacchi et al., 1997, 1998). The linearity of the residual mechanical response may be an expression of a reduction in z which would necessarily provide an apparent linearization within a restricted voltage range.

In summary, TPB^- ions increase the magnitude of nonlinear capacitance and modify V_{pkCm} and V_{dLmax} of OHCs. TPB^--induced nonlinear capacitance is independent of OHC intrinsic nonlinear capacitance. We conclude that the OHC mechanical response is not simply related to quantity of nonspecific nonlinear charge moved within the membrane, but to the effects of motility voltage-sensor charge movement functionally coupled to a mechanical effector.

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