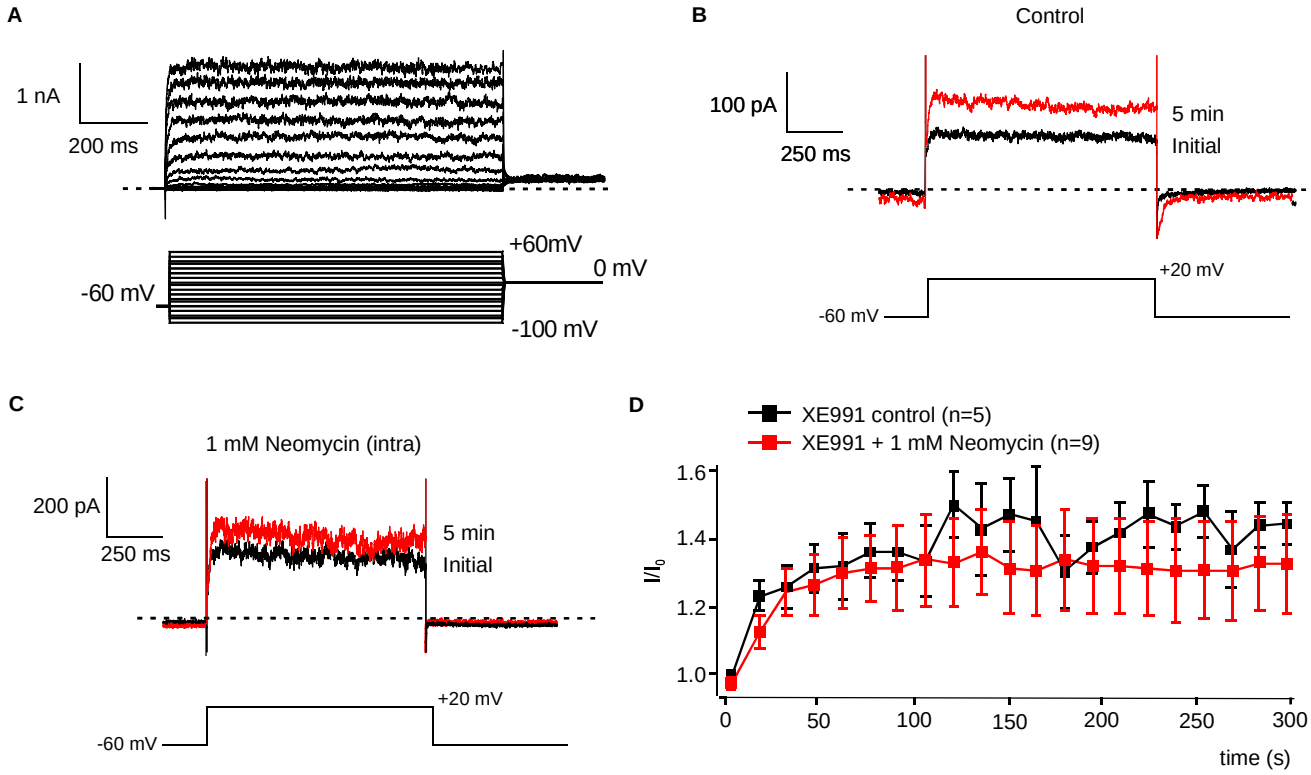
Leitner M.G., Halaszovich C.R. and Oliver D. Aminoglycosides inhibit KCNQ4 channels in cochlear outer hair cells via depletion of $PI(4,5)P_2$

Supplemental Figure 1



Supplemental Figure 1. XE991-insensitive OHC currents are not inhibited by neomycin.

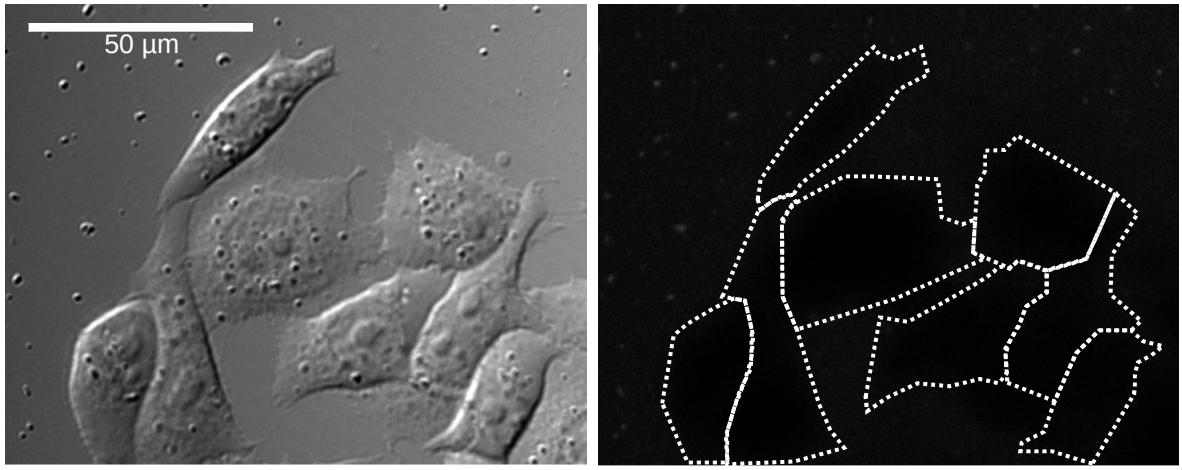
A, Representative family of OHC currents not mediated by KCNQ, isolated by measuring in the presence of the KCNQ channel blocker, XE991 (10 µM).

B, XE991-insensitive whole-cell currents from a rat OHC in response to a depolarizing voltage step (10 µM XE991 in extracellular medium). Traces shown were recorded immediately (*black*) or 5 min after (*red*) establishment of whole-cell configuration. The patch pipette contained standard intracellular medium.

C, Recordings as in (B), but with additional 1 mM neomycin in the intracellular solution.

D, Averaged time course of XE991-insensitive currents from experiments as in (B,C). Note that currents showed the same moderate amplitude run-up under control conditions and with additional neomycin in the intracellular solution.

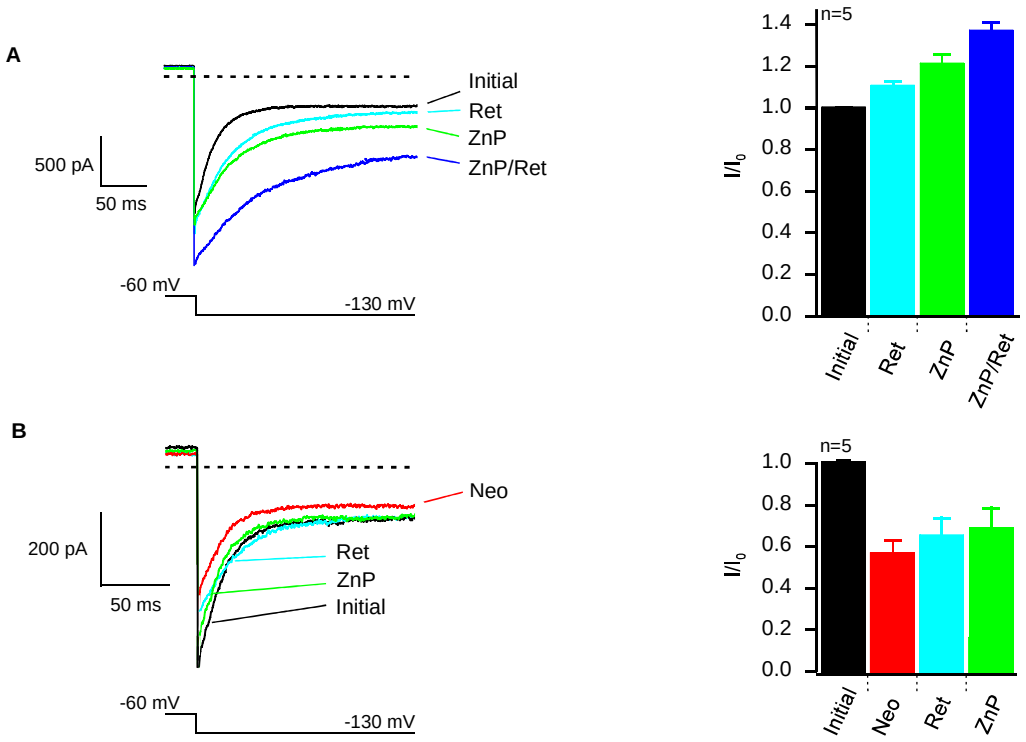
Supplemental Figure 2



Supplemental Figure 2. Aminoglycosides do not enter cultured CHO cells.

Confocal imaging of CHO cells upon application of fluorescently labelled neomycin (NTR, 1mM; 5 min; right panel; left panel, DIC image) shows no detectable intracellular fluorescence, indicating that entry into these cells is absent or very small. Lines in right panel indicate outlines of individual cells. Laser power and detector gain settings were the same as used for imaging of NTR uptake into OHCs (Figure 5).

Supplemental Figure 3

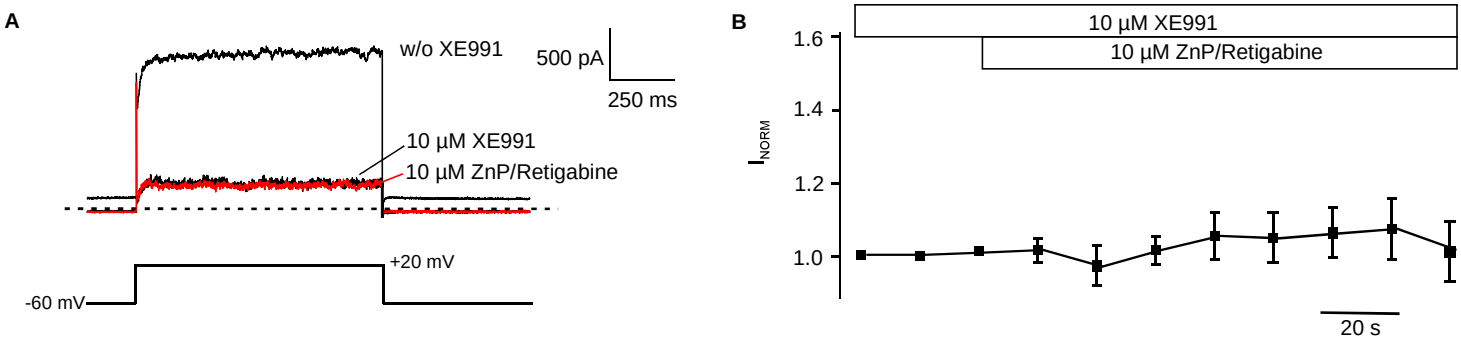


Supplemental Figure 3. Effects of KCNQ channel openers on $I_{K,n}$.

A, Extracellular application of the KCNQ channel openers, retigabine and zinc pyrithione (10 µM each), either separately or in combination augmented $I_{K,n}$ under control conditions.

B, In the presence of intracellular neomycin (500 µM) applied via the patch pipette (10 min), application of either retigabine or zinc pyrithione only slightly increased $I_{K,n}$.

Supplemental Figure 4

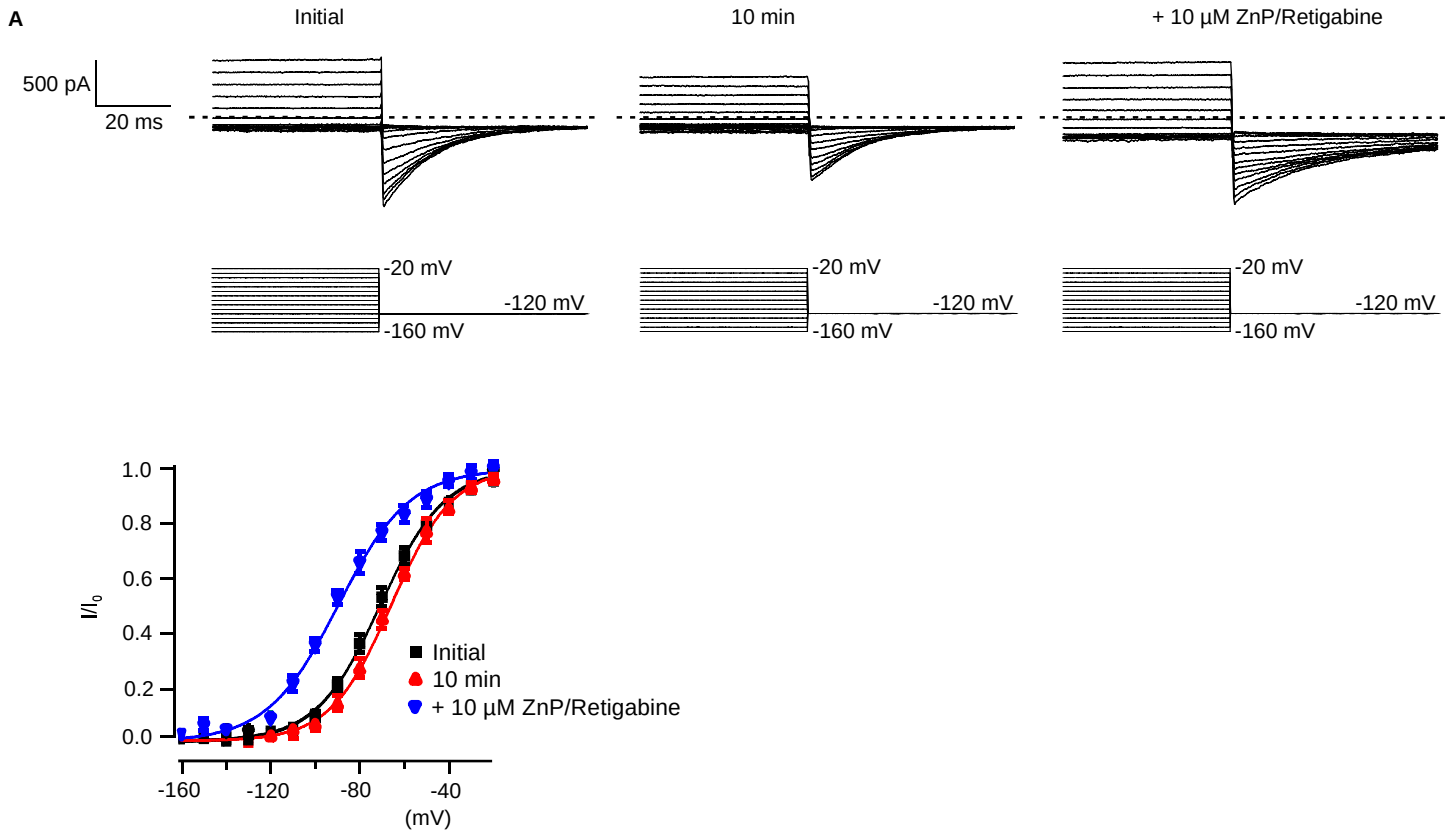


Supplemental Figure 4. XE991-insensitive currents of OHCs are not affected by KCNQ channel openers.

A, Representative whole cell currents from a OHC, elicited by a depolarizing voltage step under control conditions (standard intracellular solution), during application of XE991 (10 µM), and during application of additional extracellular zinc pyrithione plus retigabine (10 µM each).

B, Averaged time course of current amplitudes obtained as in (A) from 6 OHCs demonstrates that combined application of zinc pyrithione and retigabine does not enhance XE991-insensitive currents. Currents are displayed normalized to current amplitude prior to application of KCNQ activators.

Supplemental Figure 5



Supplemental Figure 5. KCNQ channel openers rescue $I_{K,n}$ currents by increasing maximum amplitude and shifting voltage dependence.

A, Representative voltage clamp recordings of $I_{K,n}$ tail currents from an individual OHC, obtained with pipette solution containing 500 µM neomycin. Currents were recorded immediately after patch rupture (*left panel*), after diffusion of neomycin into the cell (*middle panel*), and upon subsequent extracellular application of zinc pyrithione plus retigabine (*right panel*) (10 µM each). Note that overall current amplitudes are decreased by neomycin and potentiated by the KCNQ openers.

B, Normalized I-V curves, obtained from tail current measurements as in (A). Solid lines indicate fits of a two-state Boltzmann function to the data.

Voltage dependence of activation is only slightly altered by neomycin. Voltage at half-maximal activation was V_h = -70.7 ± 2.0 mV (n = 6 OHCs) for initial unblocked currents, and V_h = -65.7 ± 1.5 mV in the presence of neomycin. KCNQ channels openers shifted activation to more negative voltages, yielding V_h = -89.6 ± 3.9 mV.