IN VIVO EFFECTS OF HYPEROSMOTIC PERILYMPH PERFUSION ON HAIR CELL AND NEURAL POTENTIALS

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Panel 1/3:

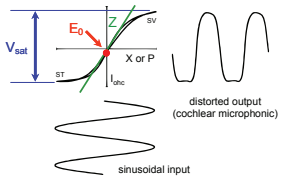
INTRODUCTION

The effect of osmotic bias on cochlear potentials was investigated by perfusion of scala tympani with a modified artificial perilymph. By the addition of sucrose, the mean osmolality of the artificial perilymph was increased by around 15% from 303 ± 6 mOsm/kg H_2O to 349 ± 1 mOsm/kg H_2O. Outer hair cell (OHC) function was assessed using Boltzmann analysis of the low-frequency cochlear microphonic (CM) waveform recorded at the round window. Compound action potential (CAP) thresholds were monitored at multiple frequencies, and the amplitude of the spectrum of the neural noise (SNN) in silence was measured as an indicator of spontaneous neural activity.

METHODS

Animal preparation: Adult pigmented guinea pigs (Cavia porcellus) with normal hearing thresholds were used. All protocols were approved by the Animal Ethics Committee of the University of Western Australia (Approval No. 02/100/184). **Data acquisition:** Near-simultaneous round window recordings were made of i) CAP thresholds at seven different tone-burst frequencies; ii) the SNN recorded in silence (in particular, the mean amplitude of the spectrum calculated between 700 and 1100 Hz); and iii) Boltzmann parameters extracted from the low-frequency CM waveform (Fig. 1; see also Patuzzi & O'Beirne, 1999).

Figure 1: Boltzmann analysis of the low-frequency CM uses an intense, non-traumatic, low-frequency tone (e.g. around 200 Hz) to drive the basal-turn OHCs into partial saturation. The nonlinear transfer curve is then analysed using a curve-fitting process, and yields the following parameters: **Vsat** – proportional to the maximal OHC receptor current for maximal excursions of the hair bundle; **Z** – a sensitivity parameter (in units of meV/Pa) giving the slope of the mechanoelectrical transduction (MET) curve; and **Eo** – an offset parameter (in units of meV) that gives the operating point of the MET channels on the transfer curve, and is used to indicate the quiescent angle of the OHC stereocilia.



Perilymphatic perfusions: Artificial perilymph was pumped at 3 µL/min into scala tympani through a custom-made perfusion pipette placed in a hand-drilled hole in the otic capsule at the first turn. Another hole was made at the apex to allow this fluid to drain into the middle-ear cavity, where it was absorbed by tissue-wicks. The perfusion pipette was removed, rinsed, and refilled between applications of different solutions. The artificial perilymph was based on a formulation by Jenison et al. (1985), shown below. The solutions were warmed to 38° C and adjusted to a pH of 7.4 using HCl or NaOH. The mean osmolality of the hyperosmotic artificial perilymphs (measured by freezing-point depression) was 349 ± 1 mOsm/kg H_2O, compared to the mean osmolality of 303 ± 6 mOsm/kg H_2O for control artificial perilymphs used in this study, and 292.9 ± 5.7 mOsm/kg H_2O for endogenous guinea pig perilymph (Konishi et al., 1984). **Control perilymph composition:** 137 mM NaCl, 5 mM KCl, 12 mM $NaHCO_3$, 11 mM glucose, 2 mM $CaCl_2.2H_2O$, 1 mM $MgCl_2.6H_2O$, 1 mM $NaH_2PO_4.H_2O$. **Hyperosmotic perilymph composition:** As for control perilymph, plus 45 mM sucrose.

RESULTS:

BOLTZMANN ANALYSIS OF THE CM DURING HYPEROSMOTIC PERFUSIONS

The 120 s perfusions caused an average 6 ± 4% increase in maximal CM amplitude (the Vsat parameter; Fig. 2) by the end of the perfusion, consistent with an increase in OHC basolateral permeability, and an 8 ± 1% increase in MET sensitivity (the Z parameter), which may reflect a decrease in OHC axial stiffness. The operating-point (Eo) shifts were more variable: in healthy animals, these perfusions caused an initial Eo shift towards scala vestibuli of 1.7 ± 1.4 meV that was either followed by a brief undershoot towards scala tympani, or initiated a longer-lasting scala tympani Eo shift. Nonetheless, the operating point shifts were small compared to those obtained with other perturbations.

Overlayed data from four 120 s hyperosmotic perfusions (350 mOsm)

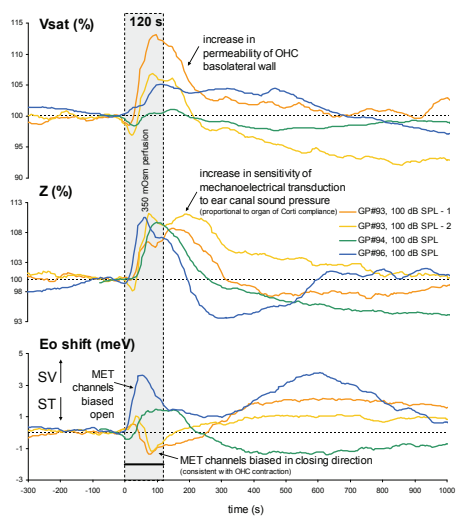


Figure 2: Boltzmann parameters recorded during four two-minute perfusions of 45 mM sucrose hyperosmotic artificial perilymph in three animals. The most consistent changes were those of the Z parameter, an indicator of the overall sensitivity of the MET process. Traces from GP#93 are re-plotted in Figure 3 with accompanying neural measures. Legend: SV = scala vestibuli. ST = scala tympani.

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Panel 2/3:

TRACKED CAP THRESHOLDS & SPONTANEOUS NEURAL ACTIVITY DURING HYPEROSMOTIC PERFUSIONS

The hyperosmotic perfusions caused threshold elevations of up to 30 dB in the high frequencies and reduced SNN amplitude by 7.5 – 20%. Fig. 3 shows overlying results from two consecutive perfusions from one animal. The time-courses of the measured CAP threshold shifts were significantly different for basal and apical frequencies, with the first-turn frequencies showing larger and more rapid threshold shifts that were more closely correlated* to the changes in basolateral permeability than the operating-point changes. The observed pattern of threshold shifts was consistent with the predicted time-course of the sucrose concentration (and therefore osmolality) along the cochlea (Fig. 4).

Figure 5 shows data from Marcon and Patuzzi for similar perfusions at higher sucrose concentrations. The almost identical time-courses of the SP and CAP threshold shifts indicate a mechanical (rather than neural) basis for the loss of sensitivity during these perfusions.

MATHEMATICAL MODELLING OF HYPEROSMOTIC PERFUSIONS

The observed relationship between CAP thresholds, the Vsat parameter, and OHC operating point, was not consistent with the output of our mathematical model of OHC regulation (O'Beirne, 2005; O'Beirne and Patuzzi, 2007). Model simulations of small operating point shifts (Fig. 7) did not cause the large changes in receptor current (reflected experimentally in the Vsat measure) or the large threshold shifts observed experimentally. In other experiments, perturbations which caused operating point shifts larger than those seen with hyperosmotic perfusions (such as DC current injection) generally did not affect CAP or SP thresholds to this extent.

*Pearson correlation coefficient between 22 kHz CAP threshold and Vsat was 0.91, compared to 0.48 for 22 kHz CAP and Eo.

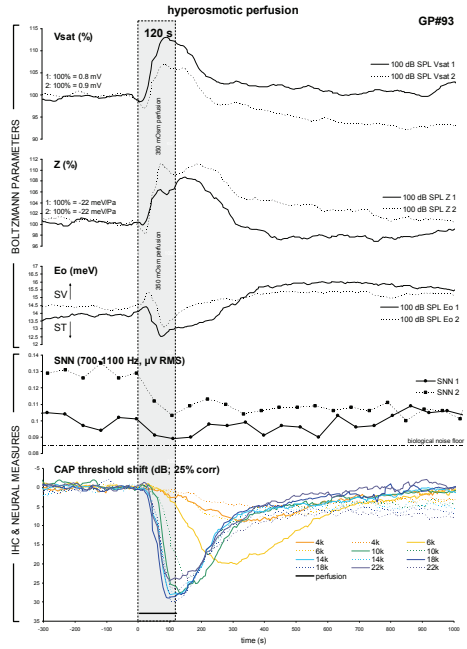

Figure 3: Representative results for two consecutive 2-minute perfusions of 45 mM sucrose hyperosmotic artificial perilymph in one animal, 47 minutes apart. Shown here are the Boltzmann parameters describing mechanoelectrical transduction, the spectrum of the neural noise (SNN) amplitude, and tracked CAP thresholds. The traces are overlaid to illustrate the reversible and reproducible nature of this perturbation.

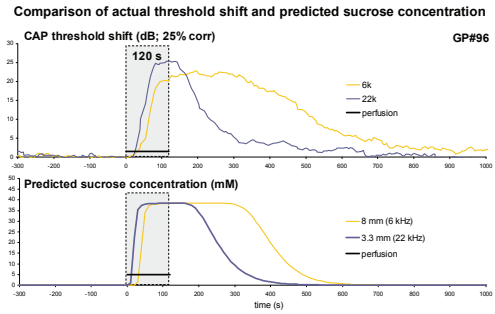

Figure 4: The difference between the CAP threshold time-courses at 6 kHz and 22 kHz was at least partially accounted for by the concentration of the sucrose at the scala tympani predicted by the Washington University Cochlear Fluids Simulator (v1.6h; Salt, 2002) at locations roughly corresponding to the 6 kHz and 22 kHz sites (assuming a 22 mm guinea-pig cochlea and the place-frequency map of Tsuji and Liberman, 1997).

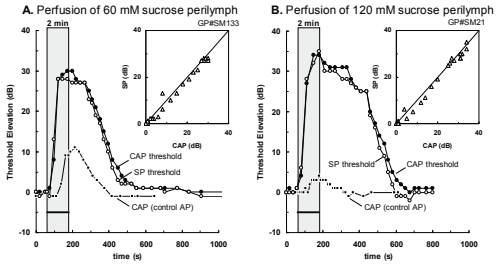

Figure 5: CAP and SP thresholds recorded by Marcon and Patuzzi (2008) during 2-minute perfusions of **A.** 60 mM and **B.** 120 mM sucrose artificial perilymphs. The insets in each figure show that the SP thresholds were proportional to the CAP thresholds, indicating a mechanical (rather than solely neural) basis for the loss of threshold.

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Panel 3/3:

DISCUSSION

The mechanism of the changes seen with hyperosmotic perfusions is not known, but the effects were not consistent with a simple movement of the reticular lamina towards scala vestibuli. The lack of large operating point shifts may indicate an inability of a scala tympani osmotic bias to cause a large static movement of the basilar membrane, due to i) the greater compliance of Reissner's membrane (except in the extreme apex of the cochlea; von Békésy, 1960); and ii) the pressure equalization by the helicotrema.

Despite this, the mechanical (rather than neural) origin of the large CAP threshold shifts observed during hyperosmotic perfusions is supported by the large increases in Vsat and Z, the relatively small decrease in the SNN, and the similar time-courses and magnitudes of the CAP and SP threshold elevations.

In isolated OHCs, Chan and Ulfendahl (1997) noted that a 7.5% increase in the osmolality of the surrounding medium caused a 48% decrease in axial stiffness. The average 8% increase seen in the Z parameter (Fig. 2) is indeed consistent with a decrease in axial stiffness. While the initiation of such a change in stiffness is not within the scope of our model, an increase in cytosolic calcium concentration would i) decrease the axial stiffness of the OHCs (Frolenkov et al., 2003); ii) increase the AC receptor current (as shown by the measured Vsat changes); and iii) decrease the AC receptor potential presumed to drive the active process of the OHCs, thereby causing the mechanical hearing loss typically seen in these experiments.

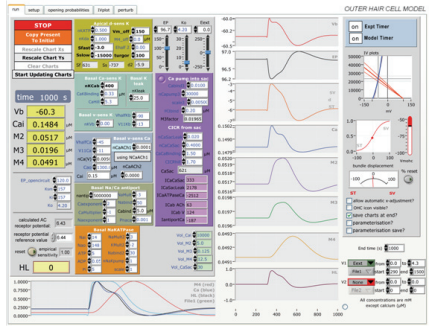

Figure 6: Simulated perfusions of hyperosmotic perilymph were carried out in our mathematical model of OHC regulation by the systematic and timed variation of the parameter representing hair bundle external energy bias, based on estimates of the time-course of perfusate concentration were imported from the Washington University Cochlear Fluids Simulator (v1.6h; Salt, 2002).

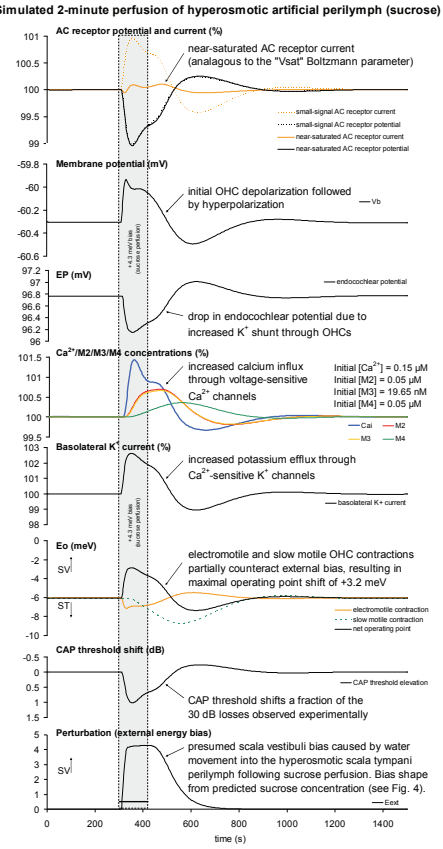

Figure 7: Results from our mathematical model of OHC regulation showing the predicted effects of the perfusion of hyperosmotic artificial perilymph through scala tympani that was sufficient to impart a maximal +4.3 meV hair bundle bias. These model results suggest that the operating point changes seen experimentally were not the major cause of the OHC disruption: Although the time-courses were similar, the simulated changes in Vsat and CAP threshold were a fraction of the magnitude of those observed experimentally.

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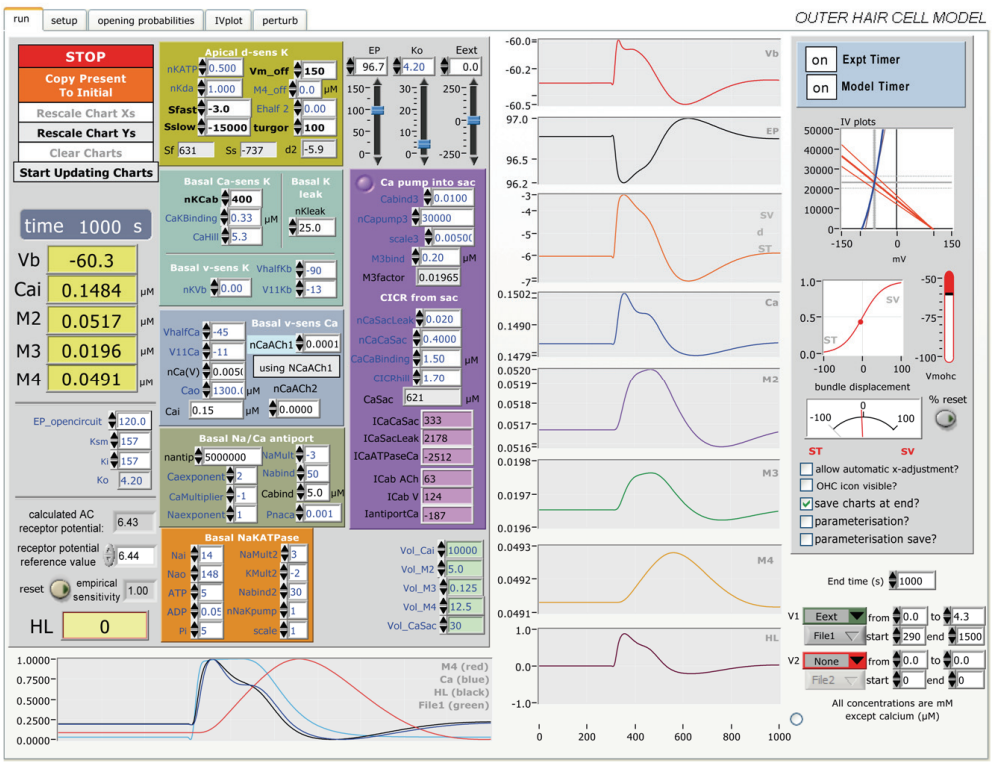
Enlarged version of Figure 6



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