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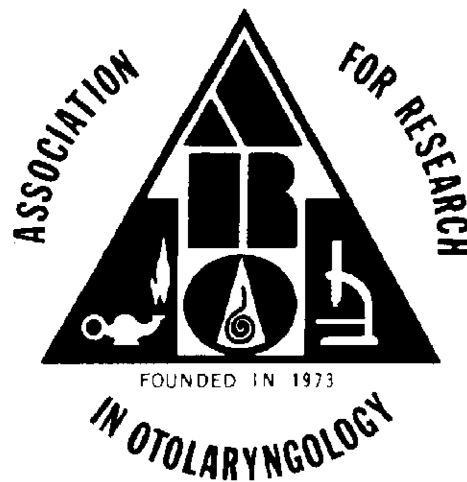
ABSTRACTS

OF THE

SIXTEENTH MIDWINTER RESEARCH MEETING

ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY



FEBRUARY 7-11, 1993

TRADEWINDS

ST. PETERSBURG BEACH, FLORIDA

464 METABOLIC CONTROL OF OHC FUNCTION: PHOSPHORYLATION AND DEPHOSPHORYLATION AGENTS SHIFT THE VOLTAGE DEPENDENCY OF MOTILITY RELATED CAPACITANCE.

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In the latter part of the last decade, it was shown that outer hair cells (OHCs) from the organ of Corti change their length as a function of voltage. Subsequently, a whole cell voltage-dependent capacitance (nonlinear charge movement) was measured in these cells (Ashmore, 1989), and such capacitance changes were shown to be closely co-related to cell length change (Santos-Sacchi, J Neuroscience 11:3096, 1991). Although the molecular basis of OHC length and capacitance changes remains to be determined, we explored the phosphorylation modulation on this OHC response.

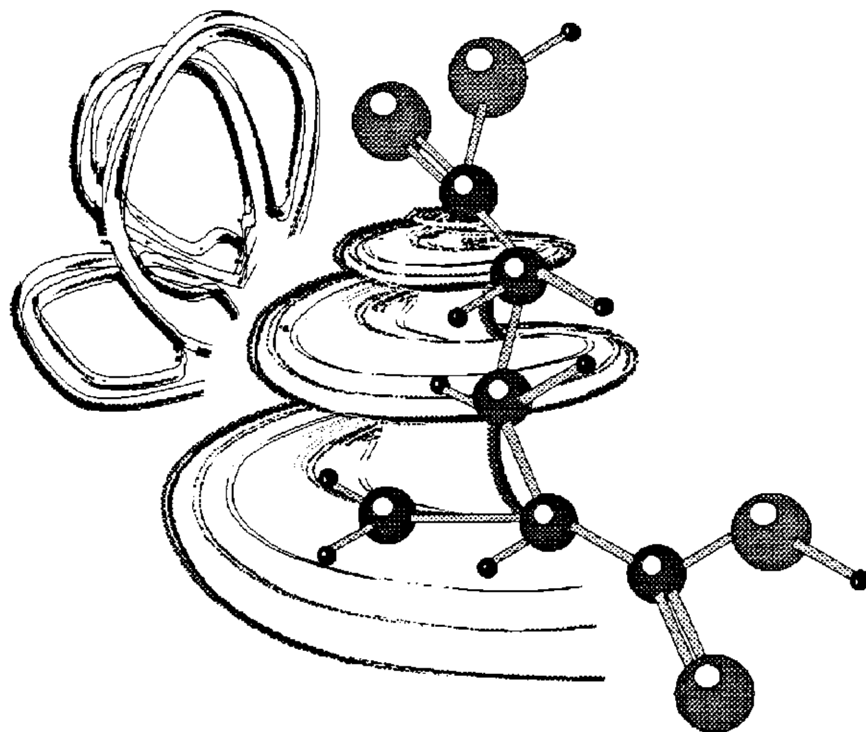
Voltage-clamp whole cell recording was applied to freshly isolated guinea pig OHC cells. Ionic currents in the cells were blocked and the nonlinear capacitance was measured with transient analysis. Cells were held at -80 mV, and staired voltage steps from -160 to 60 mV were applied. The nonlinear capacitance was fit to the first derivative of a Boltzmann function and the voltage at half-maximal charge movement or peak capacitance (V_h) was calculated. Our preliminary data show that in 33 cells the V_h was -45.3 +/- 5.3 mV (mean +/- SE) and the cell resting potential (RP, the zero current potential) was -23.9 +/- 4.4 mV. There was a high correlation between Vh and RP (r=0.838). In our preparation calcium, sodium and potassium channels were blocked; the mechanism responsible for the RP is under further investigation.

Interestingly, when the cells were locally superfused for 3 minutes with 20 mM 2,3-butandione monoxine (BDM), a non-specific phosphatase (Wilson & Ginsburg, Biochem Biophys Acta 18:168, 1955), the Vh was shifted leftward about -43.3 +/- 17.9 mV (N=6, mean +/- SD) without changing RP. When these cells were further exposed to 10 mM 8-bromo-cAMP, a membrane permeable phosphorylation-inducing agent, Vh shifted back to the right about 40.8 +/- 16.9 mV. If OHCs were pre-treated with 20 mM BDM in the bath for 15 minutes, the V_h of those cells (n=3) was around -80 mV, far more negative than non-treated cells; RP was between 0 and - 10 mV. BDM does not alter the resting potential of skeletal muscle (Gage at al, Bri J Pharmacol 100:467, 1990). These data indicate that OHC nonlinear capacitance undergoes phosphorylation and dephosphorylation regulation, and imply that the auditory effects of metabolic insult in vivo could be due to a shift of the voltage-to-movement function along the voltage axis.

(Supported by NINDCD grant # DC00273)

Inner Ear Neuropharmacology

Neuropharmacologie de l'Oreille Interne



First International Symposium

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PROGRAM AND ABSTRACTS

PROGRAMME ET RESUMES

Montpellier, France - September 14-15, 1994

Montpellier, France - 14-15 septembre 1994

EFFECTS OF PUTATIVE EFFERENT TRANSMITTERS ON THE OHC MOTILITY VOLTAGE SENSOR.

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We have been evaluating the possible effects of phosphorylating agents, including efferent transmitters, on the voltage dependency of OHC nonlinear capacitance, an indicator of the motility voltage sensor. Evaluations of V_h (voltage at peak capacitance) under whole-cell voltage clamp with ionic blockers were made with a voltage stair step protocol, which has been previously described (Huang and Santos-Sacchi, 1993; Biophysical J.). Evaluations are limited to those cells which maintained a resting membrane resistance of at least 50 MΩ. Application of drugs was accomplished by gravity feed through a four barreled pipette or Y tube, and altered the media around the whole cell. Changes in V_h are relative to that obtained in the first couple of minutes after establishment of recording. Initial studies indicated that BDM (2,3-butandione monoxin), a chemical phosphatase, produces a shift of V_h to hyperpolarizing levels within 3 minutes of application. This agent (20 mM) produced a shift in V_h of -21 ± 4 mV in 10 out of 13 cells. This type of shift might be expected, since it has been shown that phosphorylation of K channels shifts the channel's voltage dependence in the positive direction (Perozo and Bezanilla, 1990). The shift in OHCs was reversible by wash with 10 mM 8-bromo cAMP; however, simply washing the cells also produced a reversal. 8-bromo cAMP (2 mM) alone produced essentially no change in V_h (n=3). However, in two cells, intracellular perfusion via the patch pipette with the catalytic subunit of PKA evidenced a shift in V_h greater than -20 mV, opposite that expected for electrostatic effects of phosphorylation. In 6 of 11 cells, carbachol (100 µM) produced a shift to depolarizing levels of +13 ± 5 mV; a smaller shift to negative potentials was observed in 4 of the remaining cells (-5 ± 3 mV). ACh (100 µM), on the other hand, produced an initial slight shift to positive potentials (+2.7 mV ± 1.3 mV; n=4) immediately after application, but then reversed direction at 2 minutes (-4.7 ± 0.8 mV) during application. This trend continued during a 3 minute wash with a total shift to negative potentials of -13.2 ± 2.3 mV. The data indicate that dephosphorylation tends to shift the nonlinear capacitance function to hyperpolarizing levels. However, efferent transmitters which presumably enhance phosphorylation via protein kinases produce inconsistent results. It is possible that mechanisms subsequent to protein phosphorylation are involved, i.e., perhaps the overall negative voltage shift observed with ACh is related to a positive charge screening potentially provided by an internal Ca^{+2} release working upon lateral plasma membrane voltage sensors. Further experiments are underway. (Supported by NIH grant NIDCD DC-00273).

outer hair cell, motility, voltage sensor, phosphorylation, efferent