Hearing Research, 21 (1986) 205–211
Elsevier

HRR 00691

The temperature dependence of electrical coupling in the organ of Corti

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(Received 27 August 1985; accepted 8 December 1985)

Electrical coupling in an in vitro preparation of the organ of Corti was evaluated during changes in temperature of the bathing media. The effect of cooling the organ from $35 \pm 2°C$ to $17 \pm 3°C$ is to reduce membrane potentials, increase input resistance and decrease coupling ratios. Decreases in coupling ratios ranging from 15 to 75% have been observed. The effects are reversible upon warming. Membrane potentials are very susceptible to depolarization caused by cooling. The reduction in coupling is not due to depolarization nor is it dependent upon extracellular Ca^{2+}. It is conceivable, however, that intracellular stores of Ca^{2+} are released or that intracellular pH is altered.

electrical coupling, gap junctions, organ of Corti, temperature effects, DNP, membrane potentials

Introduction

Gap junctions are thought to mediate intercellular communication between a variety of cell types (Bennett and Goodenough, 1978). These channels permit the direct cellular exchange of materials (ions, dyes and metabolites) up to molecular masses of approximately 1 000 daltons in mammalian cell types (Flagg-Newton et al., 1979). Over the past 20 years a variety of treatments has been shown to influence the conductance of these junctions (Spray and Bennett, 1985). In the supporting cells of the organ of Corti, electrical coupling via gap junctions is present (Santos-Sacchi and Dallos, 1982, 1983; Santos-Sacchi, 1984a) and can be modified by treatments which alter the intracellular activities of Ca^{2+} and H^+ (Santos-Sacchi, 1984b, 1985). Gap junctional communication in the organ of Corti may be important for normal cochlear function (Santos-Sacchi, 1985), and may (1) underlie metabolic cooperation among the supporting cells, (2) provide a means to buffer K^+ in the extracellular spaces of the organ of Corti, as occurs in the CNS by ionically coupled astrocytes, and (3) influence cochlear micromechanics by permitting the spread of ionically mediated tonus alterations throughout the supporting cells.

Many studies have shown that cochlear electrophysiology is quite sensitive to cochlear temperature (Butler et al., 1960; Coats, 1965; Manley and Johnstone, 1974; Brown et al., 1983). Temperature is also known to influence gap junctional communication (Politoff et al., 1967; Payton et al., 1969). The present study evaluates the effects of cooling upon electrical coupling between supporting cells in vitro.

Methods

Guinea pigs were anesthetized with pentobarbitol and killed by decapitation. The whole temporal bone was placed in a perfusion chamber on a Zeiss ACM microscope, and the bony capsule around the two most apical turns was chipped away. The stria vascularis and spiral ligament were removed. Medium 199 (with Hanks salts [1.26 mM $CaCl_2$, 1.7 μM $Fe(NO_3)_5$, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4$, 137 mM NaCl, 4.16 mM $NaHCO_3$, 0.33 mM Na_2HPO_4; for high K^+ media, NaCl was adjusted to maintain tonicityl, pH 7.2–7.4, Gibco, NY) was perfused at a rate of 0.8–1.5 ml/min under an air atmosphere. High K^+ solutions (140 mM) were perfused through the cell chamber in order to evaluate the effects of depolarization on coupling.

2,4-Dinitrophenol (2,4-DNP) (1 mM) was used to evaluate the effect of this metabolic inhibitor on membrane potential. Ca^{2+}-free medium was used to evaluate the effects of extracellular Ca^{2+} upon changes which occur with temperature manipulations. The temperature of the preparation was controlled by Peltier devices utilizing a Bailey Instruments (NJ) controller unit. Electrodes were pulled on a Narishige puller.

Coupling measurements were made with high input impedance devices (WPI KS-700, Dagan 8100-1) capable of constant current injection. Coupling was assessed by injecting negative current pulses of varying magnitudes into one cell and noting the voltage drop in the same and an adjacent cell. Under visual control, Hensen's cells were impaled with electrodes; double-barreled theta glass electrodes were used to inject separately current (I_1) and record voltage drops (V_1) in one cell, while a neighboring cell was impaled with a single-barreled voltage recording electrode (V_2).

Coupling ratio is defined as the voltage drop in cell 2 divided by the voltage drop in cell 1 in response to current injection in cell 1 (V_2/V_1) (Bennett, 1966). Current pulses were generated by a D/A converter controlled by an IBM PC/XT. Coupling ratios were determined online using a Data 6000 waveform analyzer (Data Precision, MA) in conjunction with the IBM. Coupling responses, membrane potentials, coupling ratios and temperature were recorded on a Gould four-channel recorder. Individual coupling responses were digitally stored within the Data 6000 and saved to disk.

Results

The supporting cells of the organ of Corti are very susceptible to cold-induced depolarization. Fig. 1 illustrates the effects of a reduction in temperature from 35 to 15°C upon membrane potential. The Hensen's cell depolarizes coincident with the temperature drop, and repolarizes as the temperature rises. A lack of precise temporal correspondence between the measured temperature change and membrane potential change is possibly due to the inability to place the temperature probe directly on the specimen. In 7 preparations (35 cells), a 10°C drop in temperature caused an average drop in membrane potential of 13.98 ± 4.44 mV, with the original membrane potentials being 40 ± 10 mV. The magnitude of the depolarization associated with the temperature decrease

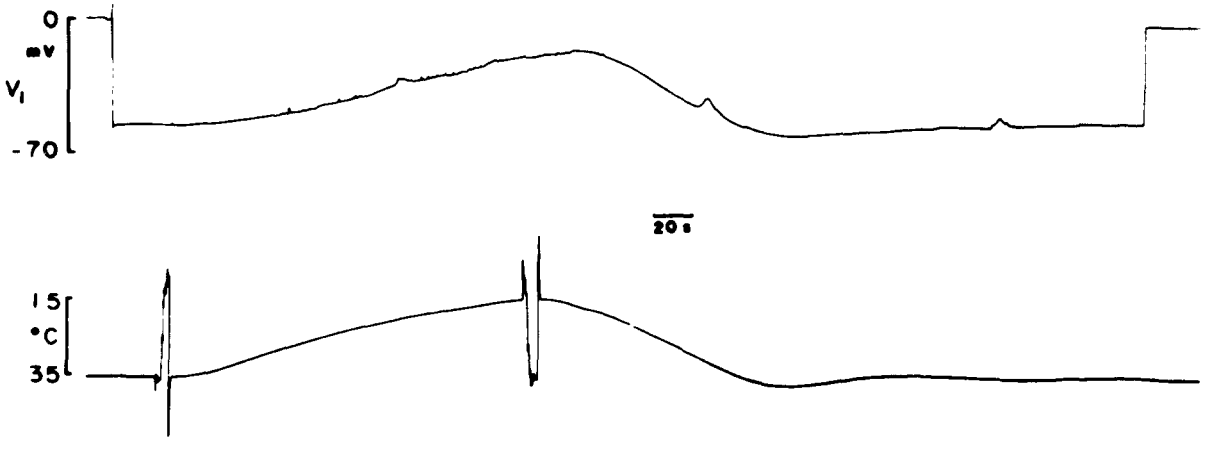


Fig. 1. The effect of cooling upon supporting cell membrane potential. The top trace is a strip chart recording of the membrane potential of a Hensen's cell. The bottom trace indicates the temperature of the perfused media which bathes the cell. As the temperature is reduced from about 36 to 15°C there is a corresponding drop in the membrane potential of the Hensen's cells. Return of the temperature to pretreatment levels causes a repolarization of the membrane potential. Note that the temperature overshoots the initial level during rewarming, and the membrane potential follows this overshoot.

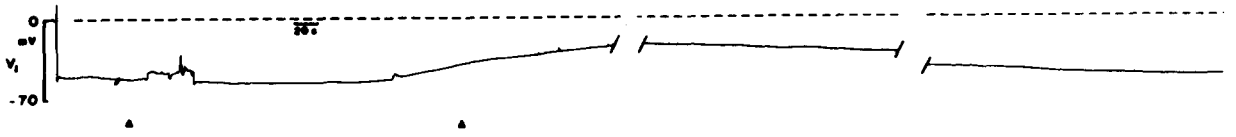


Fig. 2. The effect of 2,4-DNP on membrane potential. A Hensen's cell was recorded from during perfusion of the bath with 1 mM DNP. The first arrow indicates the start of the DNP perfusion and the second arrow indicates the return to normal medium. Membrane potential undergoes a gradual depolarization which is reversible upon washout of the poison. Portions of the trace were removed and accounted for 150 and 340 s, respectively.

was positively correlated with the initial membrane potential of cell ($r = +0.573$, $p < 0.01$, two-tailed). Since a reduction in temperature is known to affect metabolic rate, it was of interest to determine if a metabolic poison would also produce depolarization of Hensen's cells. This might help differentiate between a direct effect upon the cell membrane and an intermediate effect upon metabolism. As is demonstrated in Fig. 2, the metabolic inhibitor 2,4-DNP also depolarizes cells in a reversible manner although the time course is longer because of the time required to wash out the poison.

Electrical coupling between Hensen's cells is dependent upon temperature as well. However, the uncoupling due to cold is variable in extent

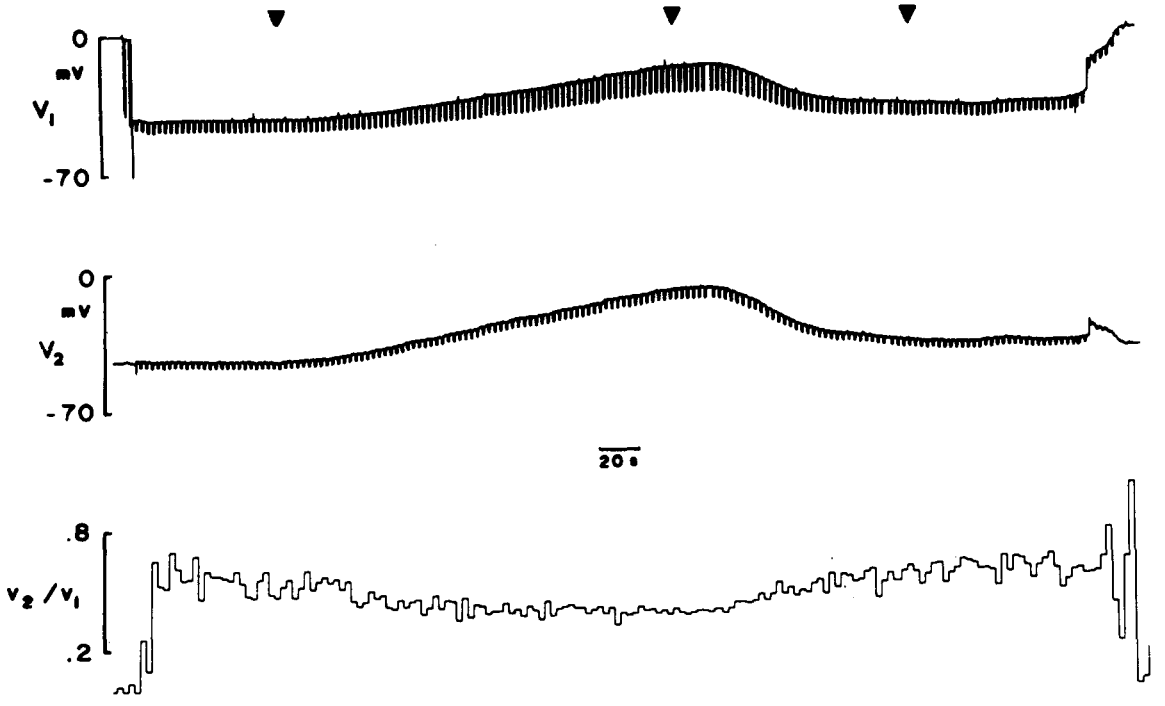


Fig. 3. The effect of cooling upon electrical coupling between supporting cells. Two neighboring Hensen's cells were penetrated with 3 M KCl electrodes. Cell 1 received current pulses of -15 nA through one barrel of a theta glass electrode and the resulting voltage drop was recorded with the other barrel. The coupling response in the other cell was measured with a single barreled electrode. Coupling ratio (bottom trace) was computed on-line and plotted out simultaneously. Temperature of the bath was 37°C initially, and cooling began at the first arrow. A temperature of 15°C was reached at the second arrow, whereupon rewarming began. 37°C was reached at the third arrow. The result of cooling is to reduce membrane potentials, increase input resistance, and decrease coupling ratio. These measures return to pretreatment levels upon rewarming.

despite the fact that depolarization is always substantial. Fig. 3 depicts the effects of lowering the temperature from 37 to 15°C upon electrically coupled supporting cells. In response to cooling, membrane potentials drop, input resistance increases and coupling ratios decrease; upon warming, the measures recover. Reductions in initial coupling ratios ranged from 15 to 75% for temperature changes from 37 ± 2 to 17 ± 3°C. Mean coupling ratios for these temperature extremes are 0.58 ± 0.17 and 0.34 ± 0.11, respectively ($n = 17$, 9 preparations).

Note in Fig. 3 that near the end of the trace there is a sudden drop in the membrane potentials of the cells. This apparently indicates some sort of injury to cell 1, since its membrane potential continues to decline while cell 2's membrane potential recovers. That is, because the cells are electrically coupled, the membrane potential of cell 2 initially follows that of cell 1. The subsequent recovery of cell 2 indicates that uncoupling of the cells is occurring. It is interesting to note that the ratio of initial membrane potential drop in cells 1 and 2 in response to cell 1 injury is, in fact, equivalent to the coupling ratio.

Since uncoupling due to reduced temperature and other treatments (e.g., lowered pH (Santos-Sacchi, 1985)) is accompanied by depolarization, it is of interest to determine if depolarization, per se, is causal. It is known that supporting cells initially have low membrane potentials following removal of the stria vascularis and spiral ligament which recover over time (Santos-Sacchi, 1984a, 1985). Yet, even prior to the recovery of the supporting cell membrane potentials, good coupling is demonstrable (Fig. 4). Furthermore, treatment of Hensen's cells with high potassium solutions, while causing depolarization, neither results in an increase in input resistance nor a concomitant reduction of coupling ratio (Fig. 5). Fig 6 presents a scatter plot of the magnitude of decrease in coupling ratio versus the magnitude (cell

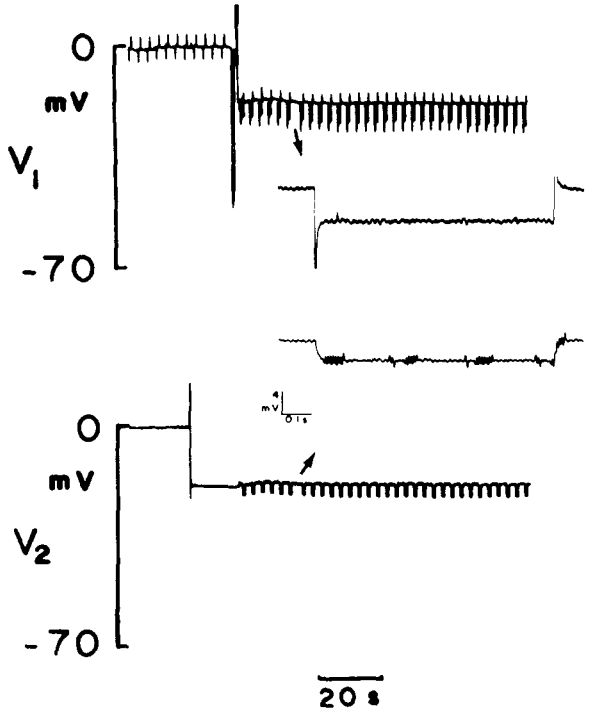


Fig. 4. Good coupling is demonstrable despite low membrane potentials of Hensen's cells. Immediately after removal of the lateral wall of the cochlear duct, membrane potentials drop, but recover over time. This example illustrates that coupling between supporting cells is substantial during periods of reduced membrane potential. Current pulses of −10 nA into cell 1 produces voltage drops in both cells which calculate to a coupling ratio of about 0.7. Inserts are plots of the digitally saved voltage drops in each cell.

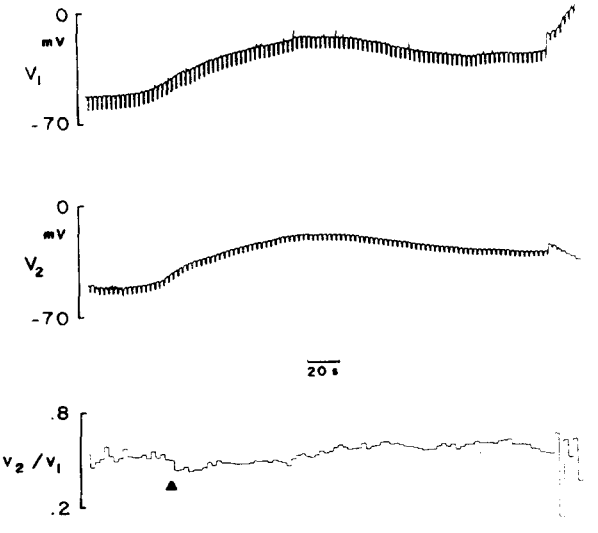


Fig. 5. Effect of depolarization upon coupling in supporting cells. High K^+ media (140 mM) was perfused into the organ chamber for 130 s, and normal medium perfusion was reinstated at the arrow. In response to the high extracellular K^+, a rapid fall in membrane potential is seen. Input resistance decreases slightly, and no uncoupling trend is correlated with the pronounced depolarization. Upon washout of the K^+, membrane potentials repolarize.

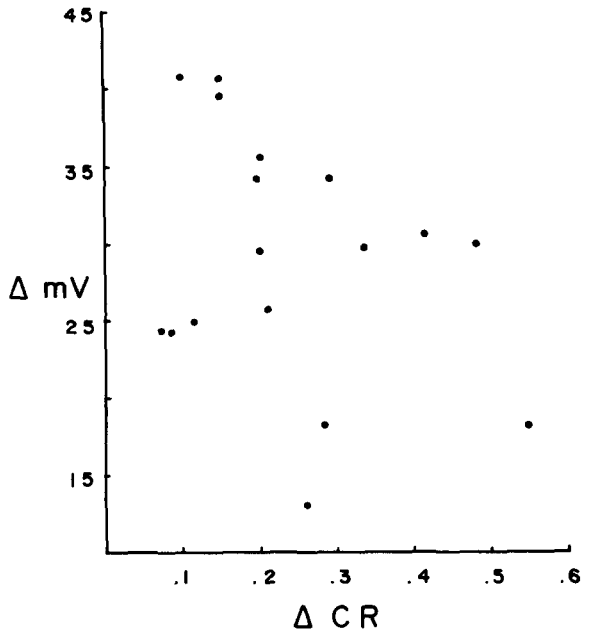
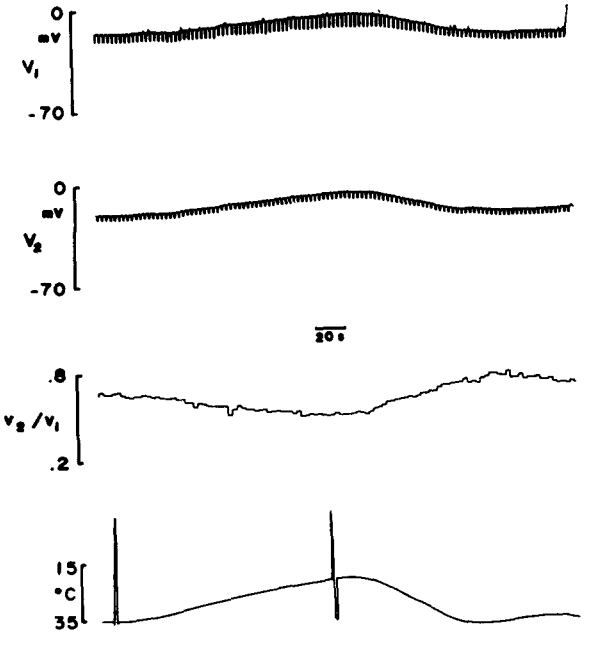


Fig. 6. Scatter plot of the magnitude of depolarization (Δ mV) vs. magnitude of coupling ratio change (Δ CR) in response to cooling the preparation from 37±2°C to 17±3°C. Correlation coefficient is −0.29, but is not significant at the 0.1 level.



pair average) of concomitant membrane depolarization in response to cooling. Correlation between the two is not significant ($r = -0.29$, $p > 0.1$, two-tailed). Thus, depolarization of the cell membrane is probably not related to the uncoupling action of reduced temperature.

The recovery of supporting cell membrane potentials following removal of the lateral well is probably similar to the 'healing over' process observed following injury to cardiac purkinje fibers (DeMello, 1983). Extracellular Ca^{2+} is known to be required for this 'healing over'. This is also the case for the supporting cells, since incubation of the organ of Corti in calcium-free media prevents the recovery of membrane potentials. Fig. 7 demonstrates that coupling during this type of incubation is good, and that reduced temperature still promotes uncoupling. Influx of extracellular calcium is therefore not required for the uncoupling effect.

Discussion

The present results indicate that temperature is an important factor which influences both membrane potentials and coupling ratios of supporting cells of the organ of Corti.

Temperature and metabolic inhibitors have been shown to influence membrane potentials of some cells in other systems, but not of others. For example, Creed and McDonald (1975) found no temperature effect (20–40°C) upon resting potentials of submandibular acinar cells but did find a 50% drop in membrane potentials with prolonged exposure to DNP, in vivo. Similarly, Payton et al. (1969) found only small depolarizations in crayfish giant axons upon cooling to 5°C, which they attributed to alteration of the electrode tip potential. In the present study, only very small (< 5 mV) changes in electrode tip potential were associated with temperature reductions capable of near total depolarization of the supporting cells. Al-

Fig. 7. Effect of cooling on coupling in Ca^{2+}-free media. In this example, it is demonstrated that coupling ratios are decreased upon cooling despite the absence of Ca^{2+} in the external medium, thus indicating that Ca^{2+} influx is not required for uncoupling. Current pulses into cell 1 are −20 nA. Note the close correspondence between coupling ratio and temperature.

though no alterations in membrane potential were noted by Payton et al. (1969), cooling had a profound effect upon junctional communication between crayfish axons. Junctional conductance was found to follow rapidly alterations in temperature, similar to the effects reported here on supporting cell coupling ratios. It is interesting that slow cooling of the crayfish axon apparently does not alter junctional conductance (Ramon and Zampighi, 1980).

Politoff et al. (1967) did find a reduction of membrane potential associated with cooling of salivary glands of *Chironomus thummi* larvae from 20 to 5°C. However, uncoupling of cells from this tissue, which was observed, initially required prolonged cooling. These authors speculated that some change in a metabolically dependent concentration of a cellular constituent (e.g., Ca^{2+}) occurs which is responsible for the uncoupling. Subsequently, they showed that depolarization of these cells by outward currents caused uncoupling, and again they speculated that it was possibly due to an increase in intracellular Ca^{2+}. It is currently known that Ca^{2+} can uncouple cells although H^+ is apparently a more potent uncoupler (Spray et al., 1982). Supporting cells of the organ of Corti can be uncoupled by treatments which increase the intracellular concentrations of either ion (Santos-Sacchi, 1984b, 1985). Whereas membrane potential (both inside-out and transjunctional) may be important factors in junctional conductance of non-mammalian cells, it appears not to be for mammalian cells (Spray and Bennett, 1985). It is demonstrated here that depolarization, per se, of Hensen's cells does not interfere with cellular communication. In addition, an influx of extracellular Ca^{2+} is not responsible for the uncoupling observed; however, the release of an intracellular store of Ca^{2+} or a decrease in intracellular pH is a possibility. Intracellular pH and Ca^{2+} measurements are required to evaluate this issue. Finally, a direct effect of cooling upon membrane structure cannot be ruled out (Payton et al., 1969).

The fact that 2,4-DNP produces a drop in membrane potential may indicate that temperature reductions produce depolarizations via metabolic interference. Nuttall and Lawrence (1978) found minimal changes in the in vivo recorded membrane potentials of supporting cells during anoxia. In fact, membrane potentials remain stable for some time after death of the animal (J.S.S., unpublished observations). These apparent differences between in vivo and in vitro susceptibility to metabolic insult warrants investigation, especially since there is currently widespread use of in vitro preparations to study organ of Corti function.

As indicated in the introduction, cell coupling in the organ of Corti may be very important for the normal functioning of the inner ear. Interference with cell coupling by temperature reductions or metabolic insults may possibly contribute to the altered functioning of the ear which occurs during such manipulations. For example, if the supporting cells act as a buffer to maintain low potassium levels in the spaces of Nuel, uncoupling these cells might compromise this function and ultimately interfere with hair cell and neuronal electrical activity. Experiments which measure extracellular potassium in the organ of Corti during temperature manipulations may shed light on this issue.

Acknowledgements

This work was supported by an RCDA from NINCDS and grants from the Deafness Research Foundation, the Foundation of the UMDNJ, and the NIH (NS 21380-01). I thank Barbara Fate for her technical assistance.

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