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# The effects of cytoplasmic acidification upon electrical coupling in the organ of Corti

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The supporting cells of the organ of Corti are joined to one another by gap junctions, and electrical coupling among them is known to be good. It is demonstrated here, using an in vitro preparation, that electrical communication between Hensen's cells can be modified by treatments which are known to cause cytoplasmic acidification. Treatment of the preparation with 100% $CO_2$-saturated medium causes a drop in membrane potential, increase in input resistance, and decrease in steady-state coupling ratio. These measures return to pretreatment levels upon washout of the $CO_2$ medium. Also, direct injection of $H^+$ into a Hensen's cell uncouples that cell from the supporting cell network. An increase in coupling ratio is sometimes observed immediately before and after uncoupling due to $CO_2$ treatment. In fact, in some cases it is possible to solely increase coupling ratios with limited $CO_2$ treatments, although prolonged treatment with $CO_2$ invariably produces uncoupling. This phenomenon may be due to an increase in cell resistance without a change in junctional conductance. A few possible roles for gap junctions in the inner ear are suggested, and the significance of the present results discussed.

carbon dioxide, organ of Corti, cell coupling, gap junction, pH

## Introduction

In the latter part of the last decade various groups demonstrated by electronmicroscopy the presence of gap junctions between all the supporting cells of the mammalian organ of Corti [13,17,18]. These results suggested that the supporting cells form a functional syncytium interconnected via low-resistance pathways. This concept was recently confirmed by studies which demonstrated electrical coupling in the supporting cells both in vivo and in vitro [24,25,26]. The supporting cells of the organ of Corti supply physical support for the receptor cells (inner and outer hair cells); and although current concepts of cochlear function rest heavily upon micromechanical interactions between receptor cells [20,35], it is difficult to ignore a possible influence of supporting cells on cochlear micromechanics. In addition, because of the avascular nature of the organ proper, metabolic cooperation between supporting cells may be important in meeting the metabolic demands of the organ of Corti.

Cell-to-cell communication can be modulated experimentally by treatments (e.g. $CO_2$ treatment) which alter intracellular hydrogen ion activity [31,33]. For example, Turin and Warner [37,38] have shown that cytoplasmic acidification of coupled *Xenopus* blastomeres produces a reversible electrical uncoupling. Subsequently, uncoupling (electrical or dye) due to cytoplasmic acidification has been demonstrated in many cell types [6,7,12,19,22,27].

The pH of the cochlear fluids is near 7.2–7.4 [34], and may be influenced by the very high carbonic anhydrase activity of the inner ear [10]. The pH of the endolymph can be markedly reduced within minutes after the onset of anoxia [4]. Because of the possible importance of hydrogen ion control in the normal and pathologic functioning of the inner ear, the present studies were designed to evaluate the effects of cytoplasmic acidification upon electrical coupling in an in vitro preparation of the organ of Corti.

## Methods

Guinea pigs were anesthetized with pentobarbital and killed by decapitation. The cochleas were quickly removed, and the apical and third turn in one piece was microdissected free after removing the spiral ligament and stria vascularis. The preparation was transferred to a perfusion chamber on a Nikon Diaphot inverted microscope. Alternately, 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 chipped away. The stria vascularis and spiral ligament were removed. In some instances membrane potentials of the Hensen's cells were measured before and after the removal of the stria vascularis and spiral ligament. Both chambers were maintained near 37°C with Peltier devices (Bailey Instruments, NJ). Medium 199 (with Hanks' salts (1.26 mM $CaCl_2$, 1.7 $\mu$M $Fe(NO_3)_3$, 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 HEPES-buffered media (25 mM) and high $K^+$ media, NaCl was adjusted to maintain tonicity), pH 7.2–7.4, Gibco, NY; in some experiments gassed with 100% oxygen) was perfused at a rate of 0.8–1.5 ml/min. 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; four electrode techniques were used to record from Hensen's cells. (1) Single-barreled electrodes were inserted into adjacent or neighboring cells. Using bridge balance techniques, the voltage drops in the two cells ($V_1$, $V_2$) were measured in response to current injection in one ($I_1$). Alternately, a sampling technique using the switched current clamp mode of the Dagan 8100-1 was used to obtain the voltage drop in the current injected cell ($V_1$). Using this sampling technique, measurement of $V_1$ was obtained without contamination from electrode voltage drop. (2) Double-barreled electrodes or theta glass electrodes were used to separately inject 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 ($V_2$). (3) Patch-type electrodes (140 mM KCl, 2 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, pH 7.2) were used to suck into adjacent Hensen's cells. These electrodes permit the rapid exchange of electrode fluids within the cell interior. The switched current clamp was used in these cases. (4) Three separate electrodes were used: one for current injection ($I$) in one cell, one for voltage recording ($V_1$) in a cell nearby, and another for voltage recording in a cell more distal ($V_2$).

Coupling responses, membrane potentials, and current magnitude were recorded on a Gould four channel recorder. In some experiments extracellular pH was measured and recorded as well. Current pulses were generated by an A/D converter controlled by an IBM PC/XT. Coupling ratios were either determined offline, by visual inspection of the records, or online using a Data 6000 waveform analyzer (Data Precision, MA) in conjunction with the IBM. Individual coupling responses were digitally stored within the Data 6000 and saved to disk.

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 ($V_2/V_1$) [2] *. $CO_2$ effects were evaluated by measuring the changes in steady state coupling after introducing various amounts of 100% $CO_2$-saturated medium into the perfusion system, followed by a return to normal medium. Control perfusions of media buffered to low pH levels were performed to evaluate the effects of extracellular acidification. HCl- (0.1M) filled electrodes were used to evaluate the effects of intracellular ionophoretic injection of hydrogen ions. High $K^+$ solutions (70 mM) were perfused through the cell chamber in order to evaluate the effects of depolarization on coupling.

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* In a two cell system, coupling ratio is determined by $R_{m2}/(R_j + R_{m2})$, where $R_{m2}$ is the membrane resistance of cell 2 and $R_j$ is the junctional resistance between cells 1 and 2. Thus, changes in coupling ratio may occur due to changes in membrane and/or junctional resistance. Whereas it is possible to calculate membrane resistance and junctional conductance in a two cell system, from experimentally determined input and transfer resistances, it is not possible to do the same for a cellular syncytium (see [2]).

## Results

Electrical coupling in the supporting cells of the organ of Corti, utilizing Hensen's cells as a model, is very good. Frequently, coupling ratios greater than 0.8 are found between adjacent Hensen's cells (Fig. 1). It was previously reported that the membrane potentials of Hensen's cells in vitro ($-63 \pm 10$ mV; $n = 111$; [26]) are very close to those obtained in vivo when in vitro recordings are made by passing electrodes through Reissner's membrane in the intact cochlear duct. After removal of the stria vascularis and spiral ligament, however, membrane potentials are dramatically reduced, but subsequently recover during the next few hours of incubation. For example, in a series of 9 preparations the membrane potential was $-32 \pm 10.9$ mV ($n = 27$) at the beginning of the experiment, and within a few hours the membrane potential recovered to $-58.96 \pm 8.99$ mV. Despite low membrane potentials at the outset, coupling is present.

Treatment with $CO_2$-saturated medium disrupts electrical communication between Hensen's cells. Fig. 2a demonstrates the effects of a 45 s bolus of $CO_2$-saturated medium injected into the perfusion system. Coupling ratio in this example is initially greater than 0.6. When the $CO_2$ medium enters the cell chamber, indicated by the drop in extracellular pH, several changes in steady-state conditions are noted. Initially, cell membrane potentials depolarize, in this case 25–30 mV, relative to resting potentials. The voltage drop due to $-5$ nA current pulses in cell 1 indicates that cell input resistance increases, and concurrently, electrical communication between the two cells decreases, as indicated by the coupling ratio. As the extracellular $CO_2$ is washed away, and presumably intracellular pH returns to normal, membrane potentials, input resistance and coupling ratio return to pretreatment levels. This uncoupling procedure is repeatable upon further exposures to $CO_2$-saturated medium. Fig. 2b presents individual coupling responses during a similar uncoupling event. The top two traces show the voltage drops in cell 1 and 2 in response to a $-10$ nA pulse in cell 1. The coupling ratio is 0.66. The bottom measures were taken at the maximum point of uncoupling after a 40 s bolus of $CO_2$ medium. The coupling ratio at this point is 0.22. Equivalent perfusions of the organ with low pH-buffered media produced neither depolarizations nor uncoupling, although in one case of prolonged exposure a slight increase in coupling ratio occurred.

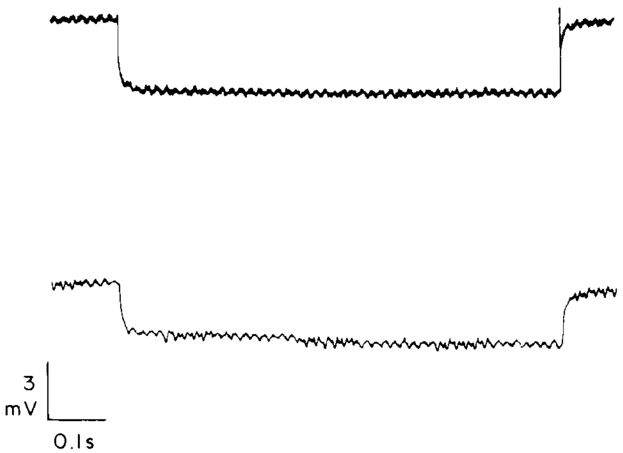

Fig. 1. Neighboring Hensen's cells, separated by one intervening cell, were impaled with electrodes. The traces were digitally stored and plotted. The top trace is of a cell with a membrane potential of $-62$ mV into which a 0.8 s current pulse of $-10$ nA was injected through one barrel of a theta glass electrode. The resulting voltage drop measured through the other barrel indicates an input resistance of 0.7 MΩ. The membrane potential of the neighboring cell depicted in the bottom trace is $-60$ mV and the coupling response measured indicates a coupling ratio of about 0.8.

Direct acidification of Hensen's cell cytoplasm can be accomplished by intracellular ionophoretic injection of $H^+$ ions. Injection into one cell of a neighboring pair uncouples only the injected cell; other neighboring cells in the tissue network remain coupled. Fig. 3 demonstrates the uncoupling effect of $H^+$ injection. It can be seen that in response to injection of $H^+$ the coupling response in the adjacent cell decreases, followed by recovery. Associated with the increase in intracellular hydrogen ion activity is a drop in membrane potential. On many occasions injection of $H^+$ caused complete irreversible uncoupling, associated with lasting depolarization of the injected cell. Suction into Hensen's cells with patch pipets containing acidic solutions also demonstrated a drop in coupling associated with depolarization.

Fig. 4 again shows uncoupling, accompanied by membrane depolarization and increased input resistance following treatment with $CO_2$-saturated

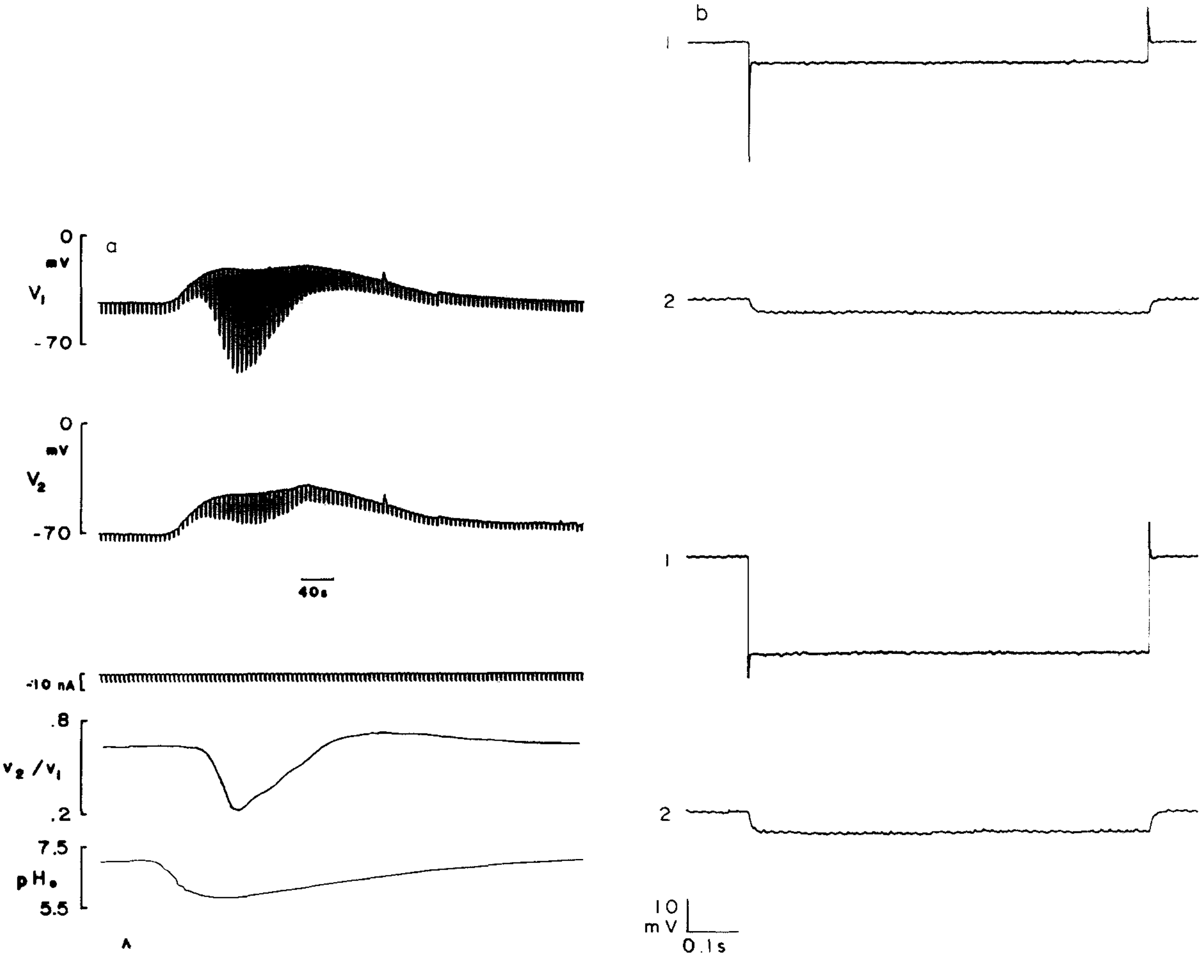

Fig. 2. (a) Impaled Hensen's cells are separated by two intervening cells in this case. A double-barreled electrode was used in cell 1. Steady-state coupling ratio is about 0.64. The arrow indicates the end of a 45 s injection of $CO_2$-saturated medium into the perfusion system, whereupon perfusion of normal medium is reinstated. The $CO_2$ medium reaches the chamber at the time indicated by the drop in extracellular pH (bottom trace). Associated with the $CO_2$ treatment is a drop in the membrane potentials, an increase in input resistance, and a decrease in coupling ratio. Upon washout of the $CO_2$ medium, these measures return to pretreatment levels. (b) Conditions were the same in (a); however, individual coupling responses were digitally stored during steady-state coupling (top two traces, cell 1 and cell 2; coupling ratio 0.66) and during maximum uncoupling (bottom two traces; coupling ratio 0.22) in response to a 40 s treatment with $CO_2$ medium. Capacitive coupling of the double-barreled glass is apparent.

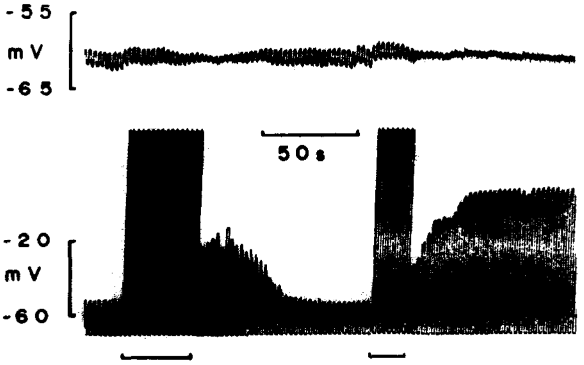

Fig. 3. Neighboring Hensen's cells were impaled with single-barreled electrodes, one containing 0.1 M HCl and the other 3 M KCl. Constant current pulses of −10 nA were injected into one cell through the HCl electrode (bottom trace) and the coupling response in the other cell was recorded (top trace). It was impossible to balance out the voltage drop in the high resistance HCl electrode. At the first bar indicator, a positive steady current of 3.1 nA was superimposed on the pulsed negative current in order to eject $H^+$ from the electrode into the cell. Consequently, the coupling response decreased (top trace) along with the membrane potential of the injected cell (bottom trace). Both measures recovered, whereupon another positive current injection produced irreversible uncoupling and depolarization in the injected cell.

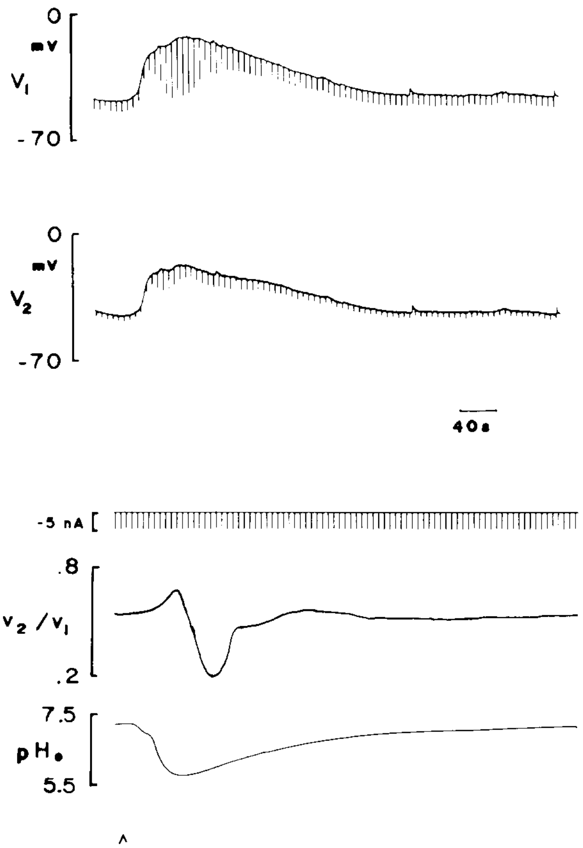

Fig. 4. Adjacent Hensen's cells were impaled with single-barreled electrodes. Current was injected into the cell depicted in the top trace by means of the switched current clamp mode of the Dagan 8100-1. The first arrow indicates the end of a 30 s injection of $CO_2$-saturated medium into the perfusion system. An increase in coupling ratio can be observed prior to the uncoupling effects of intracellular acidification. After washout of the $CO_2$ medium, measures return to steady state.

medium. In this instance, however, there is a transient increase in coupling ratio preceding the drop in coupling ratio. After return to normal medium there is recovery of the cells. Preliminary experiments indicate that high potassium medium causes the membrane potentials to depolarize, and the input resistance to decrease slightly. There is no dramatic uncoupling as occurs with $CO_2$ treatment.

The increase in coupling ratio noted in Fig. 4 is often seen prior to and sometimes following uncoupling due to $CO_2$ treatment. It is possible, however, to observe instances where only an increase in coupling ratio occurs in response to $CO_2$ treatments. Fig. 5 demonstrates this phenomenon. In response to $CO_2$ treatment, the membrane potentials depolarize, and input resistance increases; however, there is a dramatic increase in coupling ratio from a steady state level of just over 0.6 to nearly 1. The electrical measures return to pretreatment levels following washout of the $CO_2$ medium. This phenomenon is further demonstrated in Fig. 6 where three separate electrodes are used to gauge current spread through the organ's supporting cells. The ratio of voltage drops between these two Hensen's cells, one more distal than the other to the current injected cell, increases in response to $CO_2$ treatment. However, it is always possible to inevitably produce uncoupling with prolonged $CO_2$ treatments.

## Discussion

The drop in membrane potentials of the Hensen's cells in response to removal of the lateral wall of the cochlear duct may be accounted for by the presence of gap junctions between the Claudius' cells and the Hensen's cells. The removal of the lateral wall is accomplished by tearing at the interface of these two cell types and consequently the intracellular space of the torn cells is exposed to the extracellular medium. Damage to coupled cardiac muscle fibers also results in a drop in membrane potential of coupled cells [8], and in damaged lens fibers there is a leakage of $K^+$ ions into the extracellular medium [3]. Over time, as the cytoplasmic faces of the gap junctions in the injured cells close off, the membrane potentials of the undamaged cells recover, and the $K^+$ leakage subsides. This process is known as healing over.

The supporting cells of the organ of Corti can be reversibly uncoupled by treatments which acidify their cell cytoplasm. $H^+$ injection directly acidifies the cell interior. $CO_2$ treatment is known to acidify cytoplasm by crossing the cell membrane and becoming hydrated by the action of carbonic anhydrase. It then dissociates to $H^+$ and $HCO_3^-$ intracellularly. The process is reversible upon washout of extracellular $CO_2$. The extent of cytoplasmic acidification is determined by the amount of $H^+$ released or injected intracellularly and the cell's buffering capacity. In the present

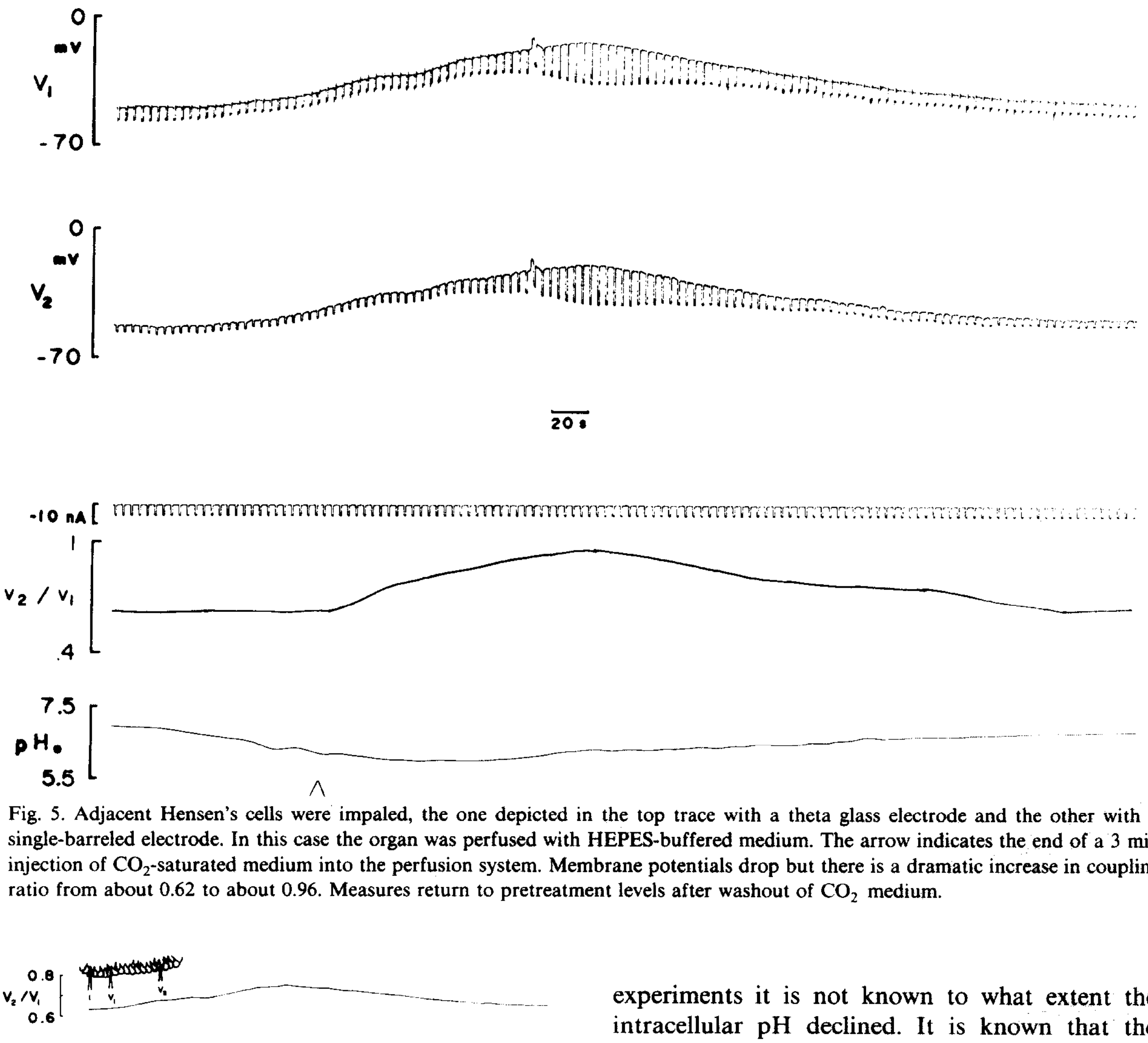

Fig. 5. Adjacent Hensen's cells were impaled, the one depicted in the top trace with a theta glass electrode and the other with a single-barreled electrode. In this case the organ was perfused with HEPES-buffered medium. The arrow indicates the end of a 3 min injection of $CO_2$-saturated medium into the perfusion system. Membrane potentials drop but there is a dramatic increase in coupling ratio from about 0.62 to about 0.96. Measures return to pretreatment levels after washout of $CO_2$ medium.

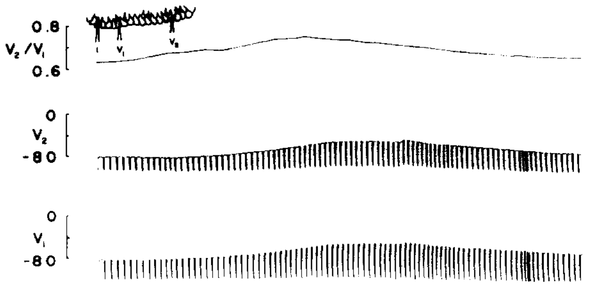

Fig. 6. Three separate Hensen's cells were impaled, one with a current injecting electrode, another with a voltage recording electrode and a more distal one with another voltage recording electrode. The beginning of the traces marks the end of a 25 s injection of $CO_2$ medium into the perfusion system. Current pulses of $-40$ nA were delivered about every 2.5 s. The ratio of voltage drops between the two distal cells increases concurrently with membrane depolarization, indicating an increase in the space constant. Measures return to pretreatment levels as the $CO_2$ medium is washed out.

experiments it is not known to what extent the intracellular pH declined. It is known that the slope of junctional conductance vs. intracellular pH varies among cell types [22,29]. The susceptability to uncoupling depends upon the steepness of this slope, and the position of the normal resting intracellular pH relative to the $pK_H$; thus a similar acidification in two different cell types may have different effects. Intracellular pH measurements are required to determine the precise relationship between coupling and pH in the supporting cells.

Uncoupling of the supporting cells can also be accomplished by intracellular $Ca^{2+}$ injection [23]. There are known to be intracellular interactions between $H^+$ and $Ca^{2+}$, such that increases in one ion's activity can increase the activity of the other. However, Spray et al. [33,30], at least in their model system, have demonstrated a far greater effect of $H^+$ than $Ca^{2+}$ upon junctional communication. This is still a controversial issue and indeed membrane potential (inside–outside and transjunctional) may play an important role in the control of junctional conductance [21,32]. Although preliminary studies indicate that membrane potential appears not to play a critical role in cell coupling in the supporting cells in vitro, further studies, perhaps using isolated supporting cells and voltage clamp techniques, may be required to fully evaluate the issue. Such studies performed in other cell systems have demonstrated uncoupling due to cytoplasmic acidification under voltage clamp conditions; still there appears to be interactions between membrane potential, $H^+$, and $Ca^{2+}$ in the control of junctional conductance [21,32].

The increase in coupling ratio that is occasionally observed upon treatment with $CO_2$ medium was an unexpected result. It is, however, important because it demonstrates that the intracellular spread of current between supporting cells can be increased under certain conditions. This may have important consequences as discussed below. Three explanations of this phenomenon can be presented. First, it may be possible that the effect is actually due to an increase in junctional conductance caused by an *increase* in intracellular pH. Reber and Weingart [22] have found a decrease in the longitudinal resistance of Purkinje fibers in response to an increase in intracellular pH above resting levels. Perhaps the effect of sub-uncoupling levels of $CO_2$ primes the supporting cells to enhance their $H^+$ pumping and buffering capabilities, thus raising intracellular pH. It should be stated, however, that in one preliminary attempt to raise Hensen's cell pH by $NH_4Cl$ treatment, no effects were noted upon supporting cell coupling. This must be investigated further, however. Second, there may be a decrease in the intracellular $Ca^{2+}$ activity due to cell acidification. This has been shown to occur in sheep cardiac Purkinje fibers [15]. A decrease in $Ca^{2+}$ activity might increase junctional conductance. Third, and probably the simplest explanation may be that the membrane resistance of the supporting cells increases without a concomitant decrease in junctional conductance. Such an effect would produce a greater flow of ions through gap junctions relative to the flow to ground (extracellular medium). Thus the space constant would increase. These possibilities are being evaluated.

Previous studies have suggested that all the supporting cells of the organ of Corti are coupled via gap junctions [13,17,18]. However, it is difficult to quantify the exact distribution of gap junctions by electron microscopy. Light microscopic visualization by the fluorescent antibody (against gap junction protein [14]) technique may provide a more comprehensive demonstration of gap junction distribution in the organ of Corti. Still, the demonstration of gap junctions cannot provide information as to the extent of electrical coupling; nor can the demonstration of electrical communication necessarily indicate that larger molecules (dyes, metabolites) are able to pass through gap junctions. The previously reported lack of dye spread between Hensen's cells illustrates this problem [26]. Others have found this to be the case in other electrically coupled cells [1,36]. Recent efforts to reinvestigate this issue, have revealed that injection of Lucifer Yellow into Hensen's cells causes depolarization, which raises the possibility that the dye is toxic to these cells and may uncouple them. In any case, the existence of electrical communication and the possibility of metabolic communication in the supporting cells may indicate roles in the inner ear similar to those proposed in other tissues. They are discussed below.

(1) The supporting cells may provide a $K^+$ buffering system whereby increases in local extracellular $K^+$ in response to hair cell and neuronal activity are reduced by active uptake of $K^+$ by supporting cells, as is thought to occur in the CNS by astrocytes [28,11]. The syncytial nature of the supporting cells might then allow shunting of $K^+$ from the tunnel and spaces of Nuel through the supporting cells to areas of lower $K^+$ concentration (nonactive regions of the duct) or perhaps through metabolic means into the scala media. Supporting cells are known to have microvilli on the endolymphatic surface which are ATPase positive [16]. Thus high $K^+$ concentrations would be reduced in regions where the ion might interfere with hair cell and neural activity.

(2) Metabolically, the supporting cells may provide for the rapid dissemination of energy sub-

strates in areas of the cochlea duct requiring these molecules. Perhaps the external sulcus cells whose projections are intertwined with spiral ligament capillaries [9] initiate a transfer of metabolites through the Claudius cells to the Hensen's cells and then on to the other supporting cells. The fact that there is a drop in supporting cell membrane potentials, with a subsequent healing over, when the spiral ligament and stria vascularis are removed by means of a tear in the Claudius cell–Hensen's cell interface, indicates that electrical communication occurs between this interface. Thus the possibility of a metabolic route also exists.

(3) Finally, the syncytial nature of the supporting cells may permit pervasive modifications of supporting cell tonus which could affect organ of Corti micromechanics. If ionic conditions arose in a localized area due to local receptor-neuronal activity, and these in some manner effected alterations in local supporting cell structural–contractile proteins, gap junctional communication might permit the spread of these ionically mediated alterations to areas beyond the area of local activity. It is interesting to note that Brownell et al. [5] detected structural changes in response to current only in outer hair cells and not in inner hair cells or supporting cells. However, they noted that changes in supporting cell shape may have been below their limit of resolution. It is difficult to imagine that even slight changes in supporting cell tonus would not affect organ of Corti function, based on numbers of supporting cells and their structural role.

If indeed any of these above mentioned roles exist, the results presented here demonstrate that they could be modified by certain changes in the organ of Corti's microenvironment. Thus in cases where the supporting cell length constant may increase, a possible increase in $K^+$ buffering and shunting capacity may occur. On the other hand, uncoupling of supporting cell gap junctions might compromise $K^+$ shunting and metabolic cooperation which could detrimentally affect the organ.

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## References

1 Audesirk, G., Audesirk, T. and Bowsher, P. (1982): Variability and frequent failure of Lucifer yellow to pass between two electrically coupled neurons in *Lymnaea stagnalis*. J. Neurobiol. 13, 369–375.

1 Bennett, M.V.L. (1966): Physiology of electrotonic junctions. Ann. N.Y. Acad. Sci. 137, 509–539.

3 Bernardini, G., Peracchia, C. and Venosa, R.A. (1981): Healing-over in rat crystalline lens. J. Physiol. (London) 320, 187–192.

4 Bosher, S.K. (1979): The nature of the negative endocochlear potentials produced by anoxia and ethacrynic acid in the rat and guinea pig. J. Physiol. (London) 293, 329–345.

5 Brownell, W.E., Bader, C.R., Bertrand, D. and de Ribaupierre, Y. (1985): Evoked mechanical responses of isolated cochlear outer hair cells. Science 227, 194–196.

6 Connors, B.W., Benardo, L.S. and Prince, D.A. (1984): Carbon dioxide sensitivity of dye coupling among glia and neurons of the neocortex. J. Neurosci. 4, 1324–1330.

7 De Mello, W.C. (1980): Influence of intracellular injection of $H^+$ on the electrical coupling in cardiac Purkinje fibres. Cell Biol. Int. Rep. 4, 51–57.

8 De Mello, W.C. (1983): The influence of pH on the healing-over of mammalian cardiac muscle. J. Physiol. (London) 339, 299–307.

9 Duvall, A.J. and Sutherland, C.R. (1970): The ultrastructure of the extrasensory cells in the cochlear duct. In: Biochemical Mechanisms in Hearing and Deafness, pp. 149–170. Editor: M. Paparella. C. Thomas, Springfield, IL.

10 Erulkar, S.D. and Maren, T.H. (1961): Carbonic anhydrase in the inner ear. Nature (London) 189, 459–460.

11 Gardner-Medwin, A.R. (1982): Analysis of potassium dynamics in mammalian brain tissue. J. Physiol. (London) 335, 393–426.

12 Giaume, C., Spira, M.E. and Korn, H. (1980): Uncoupling of invertebrate electrotonic synapses by carbon dioxide. Neurosci. Lett. 17, 197–202.

13 Gulley, R.S. and Reese, T.S. (1976): Intercellular junctions in the reticular lamina of the organ of Corti. J. Neurocytol. 5, 479–507.

14 Hertzberg, E.L., Spray, D.C. and Bennett, M.V.L. (1984): An antibody to gap junctions blocks junctional conductance. J. Cell Biol. 99, 343a.

15 Hess, P. and Weingart, R. (1980): Intracellular free calcium modified by pH in sheep cardiac Purkinje fibres. J. Physiol. (London) 307, 60–61P.

16 Hilding, D.A. and Sugiura, A. (1970): Electronmicroscopic histochemistry of the cochlea. In: Biochemical Mechanisms in Hearing and Deafness, pp. 137–147. Editor: M. Paparella C. Thomas, Springfield, IL.

17 Iurato, S., Franke, K., Luciano, L., Wermbter, G., Pannese, E. and Reale, E. (1976): Intercellular junctions in the organ of Corti as revealed by freeze fracturing. Acta Otolaryngol. 82, 57–69.

18 Jahnke, K. (1975): The fine structure of freeze-fractured intercellular junctions in the guinea pig inner ear. Acta Otolaryngol. 82, 57–69.

19 Jarrell, J.A. (1983): Reversible carbon dioxide-induced inhibition of dye coupling in *Necturus* gallbladder. Am. J. Physiol. 244, C419–C421.

20 Mountain, D.C. (1980): Changes in endolymphatic potential and crossed olivo-cochlear bundle stimulation alter cochlear mechanics. Science 210, 71–72.

21 Obaid, A.L., Socolar, S.J. and Rose, B. (1983): Cell-to-cell channels with two independent regulated gates in series: analysis of junctional channel modulation by membrane potential, calcium and pH. J. Membr. Biol. 73, 69–89.

22 Reber, W.R. and Weingart, R. (1982): Ungulate cardiac purkinje fibres: the influence of intracellular pH on the electrical cell-to-cell coupling. J. Physiol. (London) 328, 87–104.

23 Santos-Sacchi, J. (1984): An increase in the intracellular activities of $H^+$ and $Ca^{++}$ uncouples the supporting cells of Corti's organ. In: Midwinter Meeting of the Association for Reasearch in Otolaryngology, St. Petersberg, FL.

24 Santos-Sacchi, J. (1984): A re-evaluation of cell coupling in the organ of Corti. Hearing Res. 14, 203–204.

25 Santos-Sacchi, J. and Dallos, P. (1982): Intercellular coupling in the supporting cells of the guinea pig organ of Corti. In: Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg, FL.

26 Santos-Sacchi, J. and Dallos, P. (1983): Intercellular communication in the supporting cells of the organ of Corti. Hearing Res. 9, 317–326.

27 Schuetze, S.M. and Goodenough, D.A. (1982): Dye transfer between cells of the embryonic chick lens becomes less sensitive to $CO_2$ treatment with development. J. Cell Biol. 92, 694–705.

28 Somjen, G.G. (1979): Extracellular potassium in the mammalian central nervous system. Annu. Rev. Physiol. 41, 159–177.

29 Spray, D.C., Harris, A.L. and Bennett, M.V.L. (1979): Voltage dependence of junctional conductance in early amphibian embryos. Science 204, 432–434.

30 Spray, D.C., Harris, A.L. and Bennett, M.V.L. (1981): Gap junctional conductance is a simple and sensitive function of intracellular pH. Science 211, 712–715.

31 Spray, D.C., Stern, J.H., Harris, A.L. and Bennett, M.V.L. (1982): Gap junctional conductance: Comparison of sensitivities to H and Ca ions. Proc. Natl. Acad. Sci. U.S.A. 79, 441–445. Editors: R. Nuccitelli and D.W. Deamer. Alan R. Liss, New York.

32 Spray, D.C., Harris, A.L. and Bennett, M.V.L. (1982): Comparison of pH and calcium dependence of gap junctional conductance. In: Intracellular pH: Its Measurement, Regulation, and Utilization in Cellular Functions, pp. 445–461.

33 Spray, D.C., White, R.L., Campos de Carvalho, A., Harris, A.L. and Bennett, M.V.L. (1984): Gating of gap junction channels. Biophys. J. 45, 219–223.

34 Sterkers, O., Saumon, G., Tran Ba Huy, P. and Amiel, C. (1983): pH of endolymph and perilymph of the rat: effect of acetazolamide. In: Midwinter Meeting of the Association for Research in Otolaryngology, St. Petersburg, FL.

35 Strelioff, D. and Flock, Å. (1984): Stiffness of sensory-cell bundles in the isolated guinea pig cochlea. Hearing Res. 15, 19–28.

36 Tajkato, M. and Goldring, S. (1979): Intracellular marking with lucifer yellow CH and horseradish peroxidase of cells electrophysiologically characterized as glia in the cerebral cortex of the cat. J. Comp. Neurol. 186, 173–188.

37 Turin, L. and Warner, A.E. (1977): Carbon dioxide reversibly abolishes ionic communication between cells of early amphibian embryo. Nature (London) 270, 56–57.

38 Turin, L. and Warner, A.E. (1980): Intracellular pH in early *Xenopus* embryos: its effect on current flow between blastomeres. J. Physiol. (London) 300, 489–504.