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Isolated supporting cells from the organ of Corti: Some whole cell electrical characteristics and estimates of gap junctional conductance

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Whole cell voltage clamp studies were performed upon isolated and small groups of supporting cells from the guinea pig organ of Corti in order to evaluate junctional and non-junctional membrane characteristics. Single Hensen cells have an average input resistance and capacitance of 1.03 GΩ and 24.9 pF, respectively. I-V functions indicate an outward K^+ rectification, which is blocked by external TEA, intracellular Cs, or photo-irradiation of intracellularly injected fluorescent dye. Voltage clamping of pairs or small groups of cells indicates that supporting cells 'share' K^+ channels within the syncitium. The input impedance of coupled cells was studied during uncoupling with CO_2 or octanol media. As expected, coupled cells showed an increase in input capacitance and a decrease in input resistance over single cell values. Input capacitance is a more sensitive indicator of cell coupling than dc input resistance. During uncoupling, input capacitance values drop to single cell levels prior to an increase of dc input resistance to single cell levels. Modeling the results indicates that Hensen cells are well-coupled under normal conditions and may have junctional resistances with values less than 0.1% of the non-junctional resistance. The sensitivity of the supporting cell syncitium's input impedance to small changes in junctional resistance markedly influences the syncitium's RC filter characteristics, and thus may control the frequency response of sound evoked electrical activity measurable in supporting cells in vivo.

Voltage clamp; Organ of Corti; Supporting cells; Gap junctions; Cell coupling

Introduction

The mammalian organ of Corti is comprised of sensory (inner and outer hair cells) and supporting cells (e.g., Hensen, Deiter, Pillar cells). Ultrastructural studies established that the various supporting cells are joined to one another by gap junctions (Iurato et al., 1976; Jahnke, 1975; Gulley and Reese, 1976; Hama and Saito, 1977). Subsequently, it was demonstrated that these supporting cells are dye and electrically coupled, presumably via gap junctions (Santos-Sacchi and Dallos, 1983; Santos-Sacchi, 1984b; 1986a). Electrical coupling among these cells can be modulated by a variety of treatments, including alteration of intracellular pH, Ca^{2+} and temperature (Santos-Sacchi, 1984a; 1985; 1986a). A possible physiological role for such coupling may involve K^+ uptake and buffering in areas of the organ where hair cell and neuronal activity is high. Although electrical coupling between sensory and supporting cells may occur in the lower vertebrate inner ear (Weiss et al., 1975), ultrastructural studies (cited above) as well as indirect electrophysiological evidence (Oesterle and Dallos, 1988; 1989) indicate an absence of electrical communication between supporting and sensory cells in the mammal.

Previous measures of electrical coupling in the supporting cells have relied upon coupling ratio measurements (the ratio of voltage drops in adjacent cells due to current injection in one cell; Bennett, 1966) in the intact organ of Corti, both in vitro and in vivo (Santos-Sacchi, 1987). Because these measures were made in a coupled syncitium, no estimates of junctional conductance could be made. The present report relies upon the analysis

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of input impedance measures from isolated and small groups of supporting cells using whole cell voltage clamp to provide estimates of junctional and non-junctional conductances. Preliminary accounts of this work have been reported (Santos-Sacchi, 1988a, b).

Methods

Isolated Hensen cells or cell aggregates were obtained from the excised guinea pig organ of Corti by agitation for 15 min in Ca^{2+}-free Leibovitz medium containing 1 mg/ml of trypsin. The cell enriched supernatant was then transferred to a 700 μl perfusion chamber, and cells were allowed to settle and attach to the untreated glass bottom of the chamber at room temperature. A modified Leibovitz medium (NaCl 136.9 mM, KCl 5.37 mM, $CaCl_2$ 1.25 mM, $MgSO_4$ 0.81 mM, $MgCl_2$, 0.98 mM, KH_2PO_4 0.44 mM, $NaHPO_4$ 1.54, Dextrose 5.00 mM, pH 7.0) was used as the normal perfusate. In some experiments, 10 mM TEA was added to the culture media (NaCl adjusted) to block K^+ currents. The cells were washed with medium to remove the enzyme and cell debris ($\approx$ 15 min) prior to recording, and were continuously perfused for the duration of each experiment. Pipette solutions were composed of 140 mM KCl or CsCl, 1 mM EGTA, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.0. Patch electrodes (flint glass) had initial resistances of 3–5 MΩ. Subsequent to the formation of gigohm seals, cells were whole cell voltage clamped at a holding potential near -70 mV and pulsed to various potentials for 200 ms at 1 s intervals, under computer control. Current traces sampled at 25–30 μs and filtered at 3 kHz with an eight pole bessel filter were observed on a D6000 waveform analyzer (Analogic, MA) and stored to disk for subsequent analysis. Actual voltages were obtained by correcting for the electrode series resistance (10–15 MΩ) which was estimated from the capacitative current transient at the onset of the voltage pulse (Marty and Neher, 1983). Current values for I-V plots were obtained by averaging the final 20 ms of each current trace. Chord conductance [$g_K = I/(V_m - V_{eq})$] for outwardly rectifying K^+ currents between -30 and 50 mV was calculated using a K equilibrium potential (V_{eq}) of -81 mV. Linear leakage current was subtracted for these calculations.

Specific membrane capacity of biologic membrane is a fairly robust quality, which according to Cole (1971) is 'largely independent of cell physiology, pathology and pharmocology, and probably life itself.' Measurements of membrane capacitance have been used extensively during this cen-

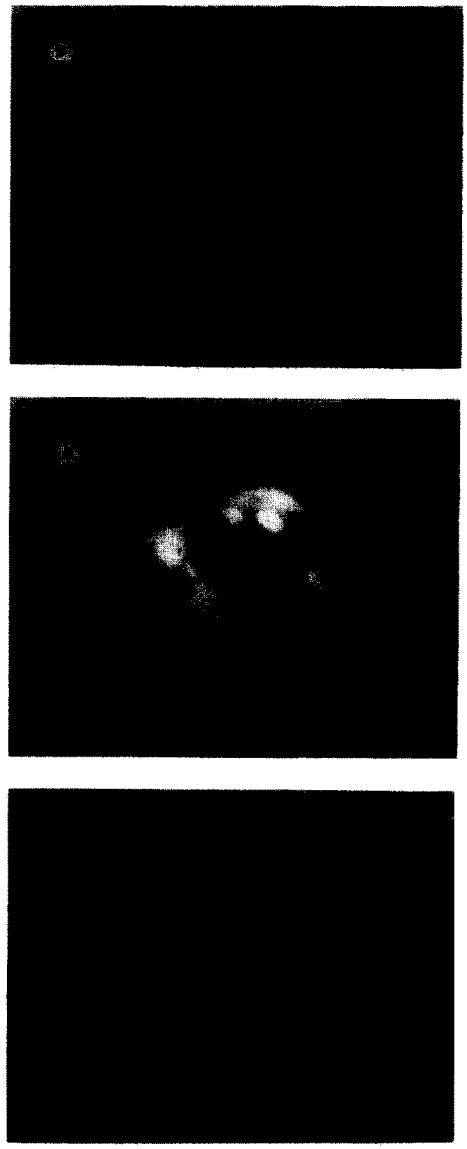

Fig. 1. Video prints of isolated Hensen cells viewed with Hoffman optics. (a) single cell; (b) cell pair; (c) three cell group. Note lipid inclusions. Scale bar: 10 μm.

tury to estimate the amount of electrically contiguous membrane surface area of cells, a technique which has its origins in the classical experiments of Fricke (1925). In the present report, membrane capacitance measures are used in conjunction with input resistance measures to estimate changes in electrical coupling between and among supporting cells, since by definition, membranes of coupled cells are electrically contiguous.

In order to evaluate the changes of input capacitance and resistance during Hensen cell uncoupling, the cells were treated with the known uncoupling agents, CO_2 and octanol (Turin and Warner, 1977; Johnston et al., 1980). CO_2 saturated media was perfused through the chamber. Saturated octanol media was perfused directly on the cells via a separate small tipped pipette. At the holding potential, input capacitance and resistance were determined using 10 mV pulses of 25–30 ms duration, by integration of the capacitative current transient and measuring steady state current, respectively (Marty and Neher, 1983; Ogden and Stanfield, 1987). These measures were performed online every 3.5–4.5 s, and the results saved to disk. Data were smoothed using a three point running average, and for presentation purposes, sigmoidal fits were made.

Fluorescein was delivered through patch pipettes which contained 1–5% dye. Epi-illumination was provided by a Nikon Diaphot filter system with a 50 watt high pressure mercury lamp. All experiments were video taped with a Panasonic AG6300 recorder, and photographs were made with an Hitachi video printer.

Results

Hensen cells isolated from the apical turns of the guinea pig cochlea are readily identified by light microscopy due to their lipid inclusions (Fig. 1). Groups of any number of cells can be obtained, although with larger groups it is difficult to determine exact numbers. The size of the cells is quite variable as is indicated by input resistance and capacitance measures. Isolated cells have input resistances much higher than those determined from the intact organ of Corti (1.03 ± 0.585 GΩ [$N = 9$] vs. 0.5 MΩ [Santos-Sacchi, 1987a]), indicating the high degree of electrical coupling present in the intact in vitro syncitium. The average capacitance of these isolated cells was 24.9 ± 9.19 pF. Fig. 2 shows averaged current traces and an I-V plot (open circles) obtained from 7 isolated Hensen cells in response to a series of voltage steps. Delayed outward currents were measured

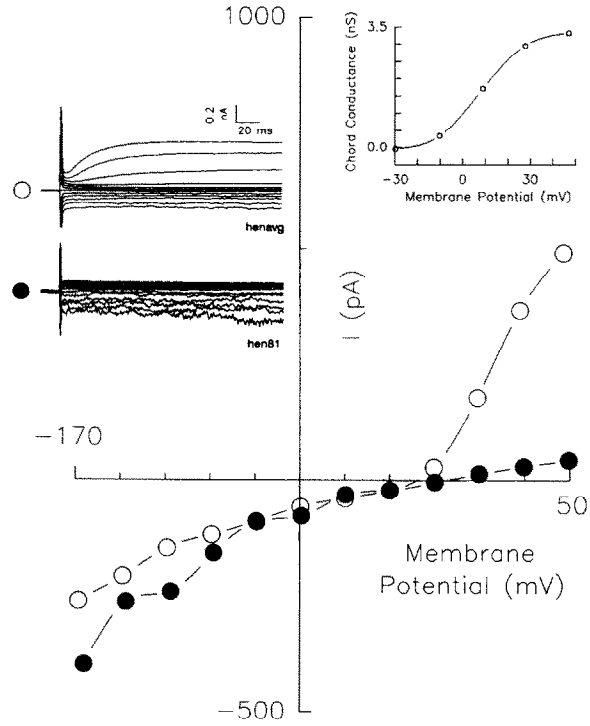

Fig. 2. Seven single isolated Hensen cells were individually whole cell voltage clamped at a holding potential of −70 mV, with normal perfusion media and KCl electrode solutions. The averaged traces (open circles) depict currents generated by pulsing the membrane potential to potentials nominally ranging from −170 to 50 mV, in steps of 20 mV. Outward current is upward. Linear leakage currents have not been subtracted in any of the figures. The I-V plot represents the averaged currents obtained during the last 20 ms of the traces, corrected for residual series resistance. Note the delayed outward rectification at potentials greater than −30 mV. Average resting potential estimated from zero current is −20 mV. Traces and I-V plot labeled with closed circles represent responses from a single isolated Hensen cell as above, except that CsCl replaced KCl in the patch pipette. Note the absence of outward rectification, indicating that K^+ ions are the charge carriers. Resting potential estimated from zero current is +2 mV. Insert: Average K chord conductance after subtraction of linear leakage currents for the seven Hensen cells exhibiting outward rectification above (see Methods). Probability of K channel activation is very low at potentials more hyperpolarized than −30 mV and reaches a maximum value near +50 mV.

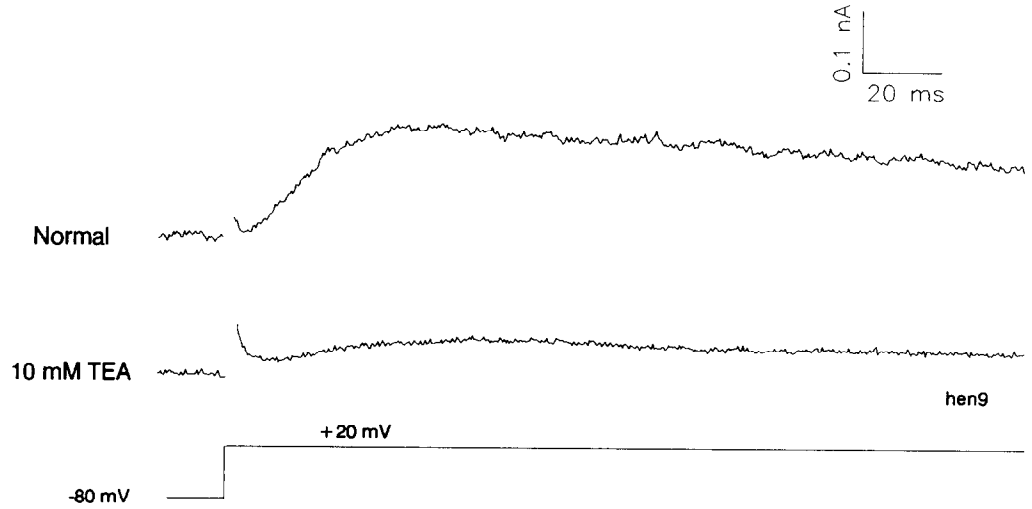

Fig. 3. Outward K^+ current trace obtained from a single Hensen cell before and after perfusion of the cell with 10 mM TEA solution. Note marked reduction of outward current in response to treatment with the K^+ channel blocker. Outward currents returned following washout of TEA. Zero current potentials before, during and after TEA treatment were -24, -8, -17 mV, respectively.

upon depolarizations greater than -30 mV, and were blocked by intracellular CsCl (closed circles), indicating that they are K^+ currents. Reversible block of these outward currents by 10 mM TEA provides further evidence that these currents are carried by K^+ (Fig. 3). Zero current levels for isolated cells recorded with the KCl intracellular solution indicate a resting potential of -20 mV. After subtraction of linear leakage currents, the average chord conductance for the outwardly rectifying K^+ currents increased sigmoidally from very low levels at -30 mV to a near maximum value of 3.3 nS at a potential of -47.3 mV (Fig. 2, insert). The inward currents noted upon hyperpolarization probably represent K^+ currents through the inward rectifier as has been demonstrated in the vestibular hair cell (Ohmori, 1985).

Interestingly, the outward K^+ currents were also blocked by photo-irradiating cells which had been perfused intracellularly with fluorescein (Fig. 4), a procedure which is known to electrically uncouple Hensen cells (Santos-Sacchi, 1986b). TEA, intracellular cesium, and photo-irradiation also produced a depolarization of isolated Hensen cells, as estimated from zero current levels (Figs. 2, 3 and 4); in the case of TEA blockade, repolarization follows washout.

Cell coupling in isolated cell aggregates was studied by noting cell input resistance and capacitance changes during uncoupling with CO_2 saturated media. Fig. 5 demonstrates uncoupling-induced changes in the I-V characteristics of a large group (≈ 10) of Hensen cells, one of which was whole cell recorded. The tremendous outward current depicted in Fig. 5 prior to uncoupling (note scales) indicates the summation of outward K^+ currents from the electrically coupled cells. Also note the summation of hyperpolarization-induced inward currents, and the large capacitive transients, indicating a large membrane surface area. Due to the large cytoplasmic volume resulting from coupling, and the attenuating effects of the junctional resistances, it is unlikely that all cells were voltage clamped equally; despite this, however, elicited currents indicate substantial voltage alterations in adjacent cells. After perfusion of the cells with CO_2 saturated medium, the cells uncouple from each other and the current traces approach those of a single cell. Fig. 6 depicts similar results obtained with a group of four Hensen cells. As expected, the currents and capacitative transients are smaller in magnitude than the 10 cell group;after CO_2 treatment, values approach that of a single Hensen cell. These data

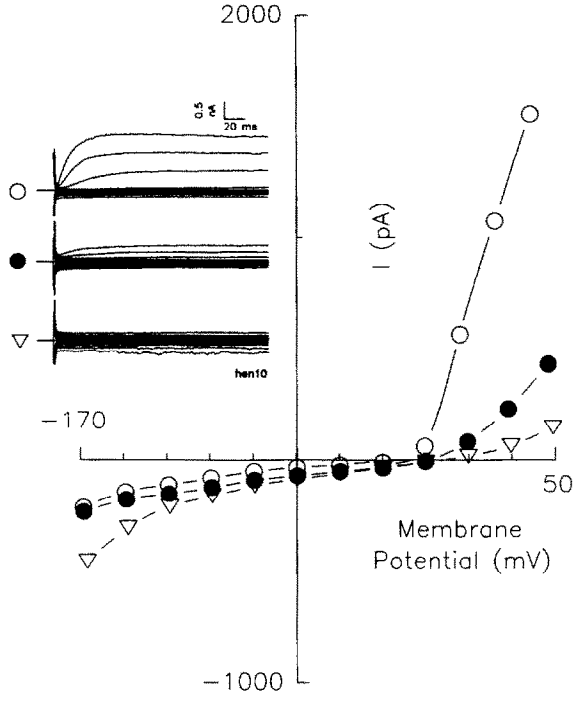

Fig. 4. Single isolated Hensen cell with KCl pipette solution, except that 5% fluorescein was added. I-V plot of steady state current vs. membrane potential corrected for series resistance. Open circles represent currents obtained after fluorescein had entered the cell, but prior to epi-illumination. Note outward rectification similar to cells not containing fluorescein. After 12 s of epi-illumination, the outward K^+ current is partially reduced (closed circles). After a total of 28s exposure, the K^+ current is markedly reduced, probably due to release of free radicals during fluorescence of the dye (open triangles). Membrane potential of the cell, estimated from zero current levels, decreased during the channel inactivation [0 s, −27 mV; 12 s, −8 mV; 28 s, −2 mV], indicating that K^+ permeability is involved in the maintenance of membrane potential in isolated cells.

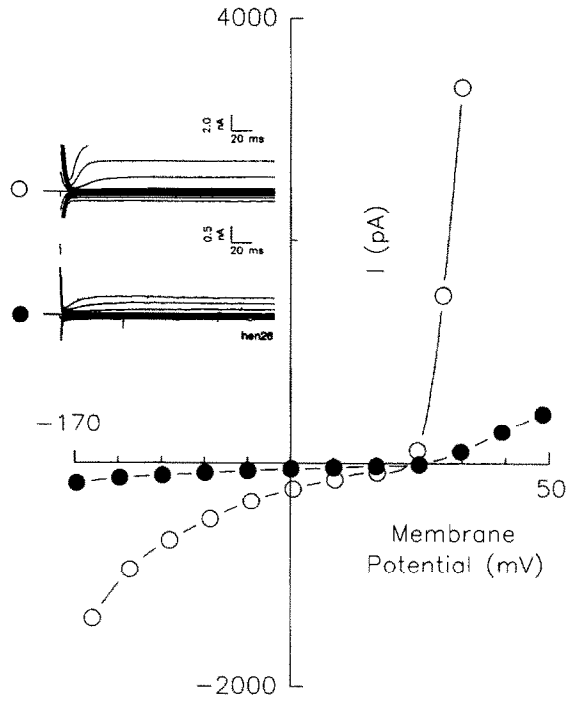

Fig. 5. Voltage clamp of single Hensen cell within a large group ($\approx$10) of cells. I-V plot of steady state current vs. membrane potential corrected for series resistance. The tremendous outward current indicates the summation of outward currents from electrically coupled cells (open circles). Due to the large cytoplasmic volume resulting from coupling, it is unlikely that all cells were voltage clamped equally. After perfusion of the cells with CO_2 saturated medium, the cells uncouple from each other and the I-V response approaches that of a single cell (closed circles).

suggest that coupled supporting cells 'share' K^+ channels via functional gap junctions. To provide clearer proof of this phenomenon, Fig. 7a shows the results of a similar treatment on a group of three Hensen cells; however, in this case, the K^+ channels of the recorded cell were blocked by the use of a CsCl electrode solution. Note that prior to uncoupling, outward K^+ currents are present, and represent only the currents generated in the adjacent coupled cells, since upon uncoupling by CO_2 treatment, the current response is solely from the recorded cell, and lacks outward currents due to the cesium block. Apparently, sufficient amounts of Cs^+ did not diffuse into the adjacent cells through gap junctions during the 5 min period required to collect the data. Fig. 7b shows the time course of input resistance and capacitance changes which accompany uncoupling. During CO_2 perfusion, input capacitance decreases and input resistance increases, until single cell values are attained.

Fig. 8a demonstrates the effect of octanol perfusion on a coupled Hensen cell pair. Octanol rapidly abolishes cell communication. During the process of uncoupling, it is typically found that input capacitance decreases to single cell values

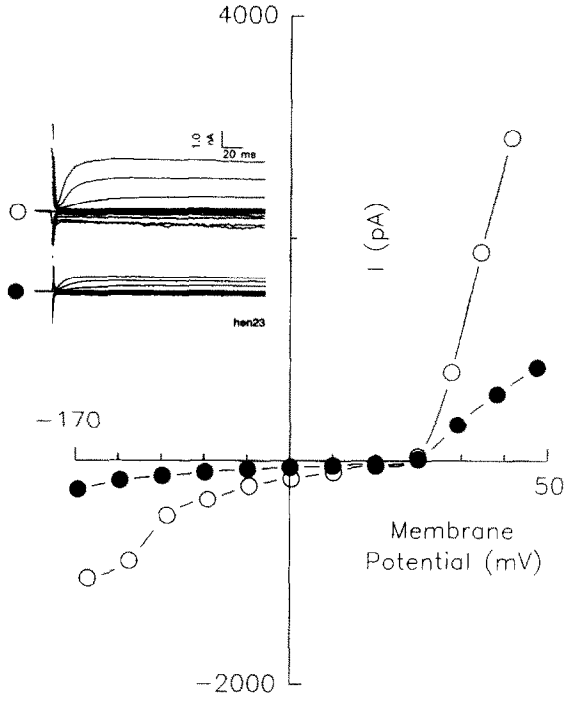

Fig. 6. Single Hensen cell within a group of four cells voltage clamped as in Fig. 5. Currents are smaller than that of the 10 cell group in Fig. 5, but larger than that of single cells, indicating that these cells are electrically coupled (open circles). After perfusion of CO_2 saturated medium, the I-V response is reduced to that of a single cell, confirming that the cells were coupled and that channel 'sharing' among the cells occurs (closed circles).

before dc input resistance reaches single cell values. The reversibility of octanol uncoupling is readily demonstrated by monitoring input capacitance during treatment and washout (Fig. 8b).

Discussion

Hensen cells display an outward rectification upon depolarization, which is mediated by K^+ channels. For individual Hensen cells, this current is small and is activated at potentials far removed from the normal in vivo resting potential. Deiter cells, supporting cells which are closely apposed to outer hair cells and are also coupled to Hensen cells (Santos-Sacchi, 1986b), exhibit larger outward currents (Santos-Sacchi, 1989). Supporting cells within the organ of Corti typically have resting potentials ranging from -60 to -100 mV, and are dependent upon extracellular K^+ concentration, since high extracellular potassium solutions produce reversible depolarizations in the in vitro organ (Santos-Sacchi, 1986a). The membrane potential of isolated supporting cells as determined from zero current levels is depolarized compared to normal resting levels, being on average about -20 mV. It has been demonstrated that the potential of Hensen cells in the intact (stria removed) in vitro organ of Corti is about -32 mV immediately upon removal from the animal, but increases to in vivo levels (-59 mV) over the course of a few hours only when the temperature is maintained at 37°C (Santos-Sacchi, 1985a). In that study, it was also shown that metabolic inhibitors can induce depolarization. Interestingly, whole cell recordings from isolated OHCs indicate that their membrane potentials are depolarized compared to in vivo values (Santos-Sacchi and Dilger, 1988; Dallos et al., 1982); but in many OHCs the potential approaches in vivo levels as the KCl patch pipette solution enters the cell over the course of a few minutes. This has not been observed with isolated Hensen cells. A variety of factors may be responsible for the depolarized state of isolated Hensen cells in this study, including temperature, use of simple salt solutions as opposed to culture media, and isolation procedure. Nevertheless, it has been shown that uncoupling agents will produce a depolarization in the Hensen cell syncitium (Santos-Sacchi, 1985; 1986a, b), and obviously, isolated cells and small groups of cells are uncoupled from the very large number of supporting cells normally forming the intact syncitium. It is demonstrated here that membrane potentials of isolated supporting cells are decreased by treatments (TEA, intracellular cesium, photoirradiation of intracellularly inject dye) which block K^+ conductance, indicating the dependence of membrane potential in these isolated cells upon a susceptable K^+ conductance. Conceivably, photoirradiation may produce free radicals which destroy K^+ channel function; Hensen cells have been shown to be uncoupled by the same treatment (Santos-Sacchi, 1986b). Clearly, the present results indicate that the supporting cells can 'share' ionic channels with neighboring cells, and for a cell type whose membrane potential is to some degree K^+ dependent, this may play an important role in maintaining the stable

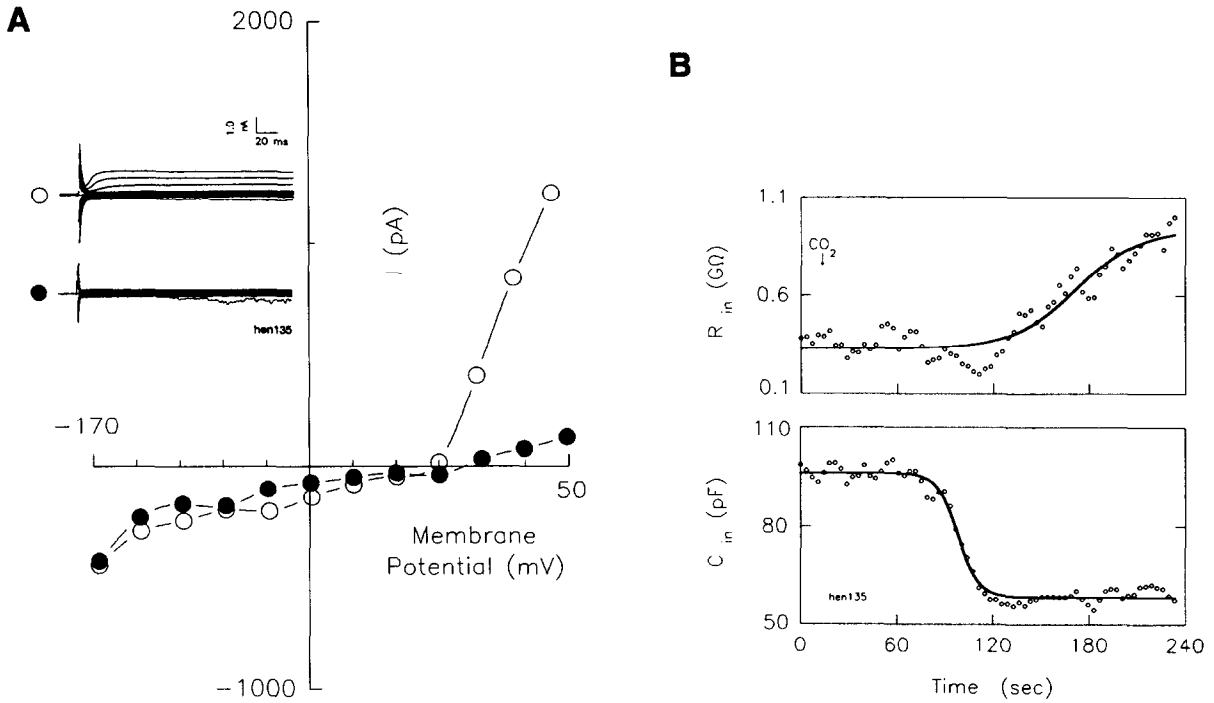

Fig. 7. (A) Single Hensen cell in a group of 3 Hensen cells was voltage clamped with a patch electrode containing the K^+ channel blocker CsCl. Normally, the CsCl patch solution will replace intracellular fluid and block the K^+ channels, thus eliminating outward rectification as in Fig. 2. In this case, however, outward rectification is present (open circles), because the pipette solution has not replaced the intracellular fluid of the other two coupled cells. Thus, the cell which is recorded from is utilizing the K^+ channels of its neighbors. After perfusion of CO_2 saturated medium, the cells uncouple and the cell recorded from appears as a single cell whose K^+ channels are blocked by Cs^+ (closed circles). (B) Time course of CO_2 uncoupling as indicated by changes in input resistance and capacitance. At onset of recording, R_{in} is low relative to isolated single cells and C_{in} is high relative to isolated single cells (see Results). During CO_2 medium perfusion, there is a dramatic change in these values, such that they approach the values of single cells, as would be expected in the uncoupled state. Note that capacitance values reach a minimum before resistance values reach a maximum.

resting potentials found in vivo. Maruyama et al. (1983) have estimated that isolated salivary acinar cells have few potassium channels per cell, numbering about 50. Open channel probability is very low at normal resting potentials, and since the possibility exists that no K^+ channels will be open at a given point in time, it was speculated that the stability of the K^+-dependent resting potentials noted in the intact acinar syncitium is due to the sharing of these channels among coupled cells. The input conductance of isolated Hensen cells is less than 1 nS at the holding potential of -70 mV, and it appears that a major component of this may be due to linear leakage current. Conceivably, a non-specific leakage current may dominate any potential generating effects of a small K^+ conductance at normal resting potentials. Thus, it is possible that the highly coupled nature of the supporting cells of the organ of Corti, with the attendent 'sharing' of K^+ channels, contributes to the stable, highly negative, K^+-dependent resting potentials encountered in the intact syncitium. This would indicate that the drop in membrane potential noted upon uncoupling of Hensen cells (Santos-Sacchi, 1985; 1986a) may have been due to the reduction of utilizable neighboring cell K^+ channels.

The change in input resistance and capacitance which occurs upon uncoupling of cell groups or pairs is interesting in that the increase in input resistance is preceded by a reduction of input capacitance values to single cell levels. This type of behavior may not appear intuitive since one might expect both input capacitance and input

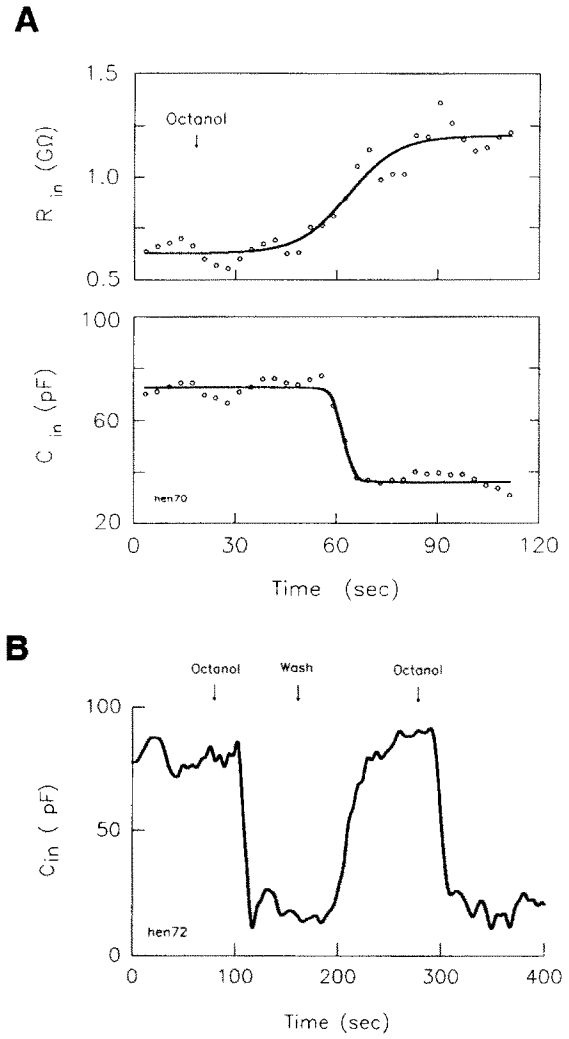

Fig. 8. (a) Demonstration that octanol is capable of uncoupling a pair of Hensen cells. Note similarities with CO_2 uncoupling, i.e., the capacitance decreases to single cell values before resistance change is complete. Fig. 9 presents an electrical model based on these data. (b) Reversibility of uncoupling by octanol in a group of 4 Hensen cells. C_{in} decreases to single cell levels during perfusion with octanol, and returns to coupled state levels during washout. Further treatment with octanol uncouples the cells again.

resistance to change reciprocally. The results, however, are not aberrent, as is demonstrated by an analysis of cell models comprised solely of resistive and capacitative elements. In fact, these alterations of input impedance provide information which allows the estimation of gap junctional conductance between the Hensen cells. Fig. 9 depicts a circuit model and a voltage step analysis of a cell pair, with single cell component values of 1.1 GΩ and 35 pF, and a series resistance of 10 MΩ, similar to the values obtained from the cell pair of Fig. 8a, after uncoupling.

Fig. 9 indicates the results of a computer simulation where the junctional resistance, R_j, is varied over ten orders of magnitude, and the input capacitance and dc resistance are plotted. It can be seen that the resultant changes in impedance are similar to those of the actual cells, and corroborates the observation that input capacitance is a more sensitive indicator of the state of coupling at low R_j values than dc input resistance. Analyses such as these indicate that junctional conductance in the supporting cells of the organ of Corti can be 3 orders of magnitude larger than non-junctional resting conductance, in this case about 1 μS vs. 900pS, respectively. Freeze fracture studies have indicated expansive areas of gap junctional mem-

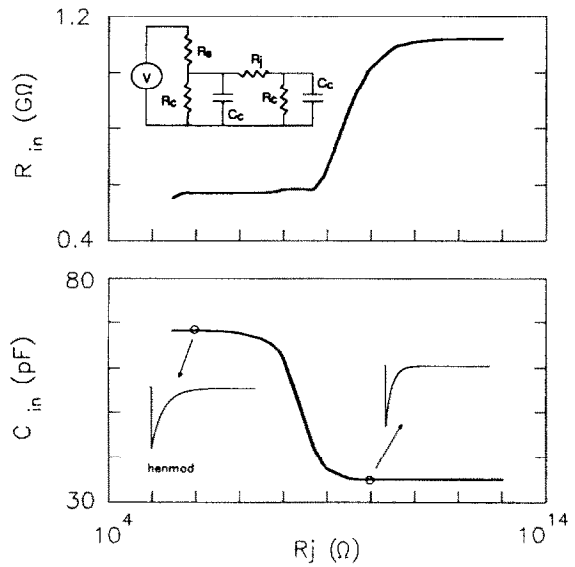

Fig. 9. Two cell model of uncoupling based on data from Fig. 8a. Values for individual cell resistance (R_c) and capacitance (C_c) are 1.1 GΩ and 35 pF, respectively. Electrode series resistance (R_s) is 10 MΩ. Uncoupling was simulated by varying the junctional resistance (R_j) and a voltage step analysis of the network R_{in} and C_{in} was performed. The resulting plots are similar to actual data in that values approach single cell levels as junctional conductance is decreased. For these cells, R_j in the normal coupled state appears to be less than 1×10^6 Ω. Capacitive current traces are shown for R_j values of 1×10^6 Ω and 1×10^{10} Ω.

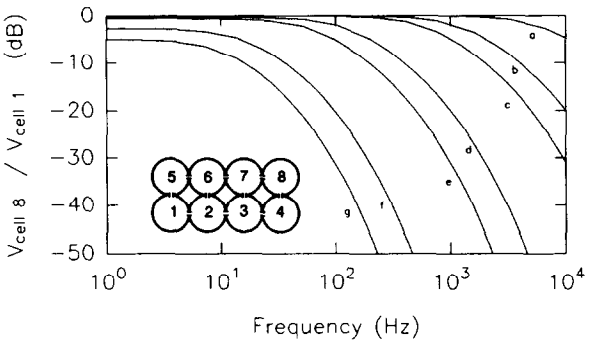

Fig. 10. Simulated ac response in cell 8 in response to a constant voltage injection into cell 1, in a coupled eight cell model (insert). Individual cell capacitances and resistances were 35 pF and 1 GΩ, respectively. Junctional resistances were varied: a, 0.1 MΩ; b, 0.5 MΩ; c, 1.0 MΩ; d, 5.0 MΩ; e, 10 MΩ; f, 50 MΩ; g, 100 MΩ. Note the dramatic drop in cutoff frequency as the junctional conductance is reduced, whereas very low frequency coupling is little affected.

brane which may account for such high junctional conductance (Jahnke, 1975; Gulley and Reese, 1976; Iurato et al., 1976).

Various investigators have recorded sound evoked electrical activity from mammalian supporting cells (Dallos et al., 1982; Goodman et al., 1982; Russell and Sellick, 1978; Oesterle and Dallos, 1986; 1989). In the mammal, this activity is thought to be passively picked up from hair cells (Russell and Sellick, 1978; Oesterle and Dallos, 1988, 1989). Regardless of the mechanism whereby acoustically evoked potentials are established in the supporting cells, it is important to understand the effects of junctional conduction upon such measures. The presently reported sensitivity of supporting cell input impedance to small changes in junctional conductance may indicate that the spectral content of evoked ac activity in supporting cells is sensitive to modifications of gap junctional communication. That is, small fluctuations in gap junctional conductance may alter the low pass RC filter effect of the coupled syncitium. This is examined in Fig. 10 where the ac frequency response is simulated in a model of eight coupled supporting cells. The ratio of the response measured in cell 8 to a constant voltage input into the system via cell 1 is plotted against frequency. The family of curves (a–g) represents the result of a decreasing junctional conductance (non-junctional membrane conductance and capacitance kept constant, see figure legend for values), and indicates that the frequency response of a coupled cell in the syncitium falls dramatically with relatively small changes in junctional conductance. For example, a change in junctional conductance from 10 μS (response a) to 0.1 μS (response e) results in a two octave shift (reduction) in the high frequency cut-off, but only results in about a one dB drop in the dc (0.001 kHz) response. Clearly, good dc coupling can remain during dramatic changes in ac coupling. Since supporting cell coupling can be perturbed by a variety of micro-environmental influences (Santos-Sacchi, 1984a; 1985; 1986a) it is conceivable that fluctuations in organ of Corti homeostasis are more readily expressed as changes in ac coupling rather than dc coupling. This observation may prove useful in future evaluations of coupling in the organ of Corti. For example, in vivo studies of electrical coupling using a dc paradigm to measure cell-to-cell communication alterations during manipulation of the endolymphatic potential have demonstrated little effect upon coupling (Santos-Sacchi and Marbey, 1987). Perhaps an ac analysis would provide the required sensitivity to detect changes in junctional communication during such manipulations.

Finally, the sharing of K^+ channels of the inwardly rectifying type may help buffer K^+ levels in the extracellular spaces of the organ of Corti, where K^+ levels may increase over perilymphatic levels due to sensory and neural activity. Increased K^+ in the spaces of Nuel may induce slow length changes of outer hair cells (Goldstein and Mizukoshi, 1967; Zenner et al., 1985) and affect the rapid motility characteristics of these cells by its depolarizing effects (Santos-Sacchi, 1989). Obviously, extracellular K^+ levels must be controlled. It is conceivable that the high resting membrane potentials of the supporting cells (up to −100 mV) may induce a steady state activation of the inward rectifier, thereby promoting K^+ uptake; gap junctions may contribute to the overall buffering as is speculated in the CNS (Smojen, 1979).

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