

Cell coupling in the supporting cells of Corti's organ: Sensitivity to intracellular H^+ and Ca^{+2}

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Abstract

The input capacitance of cell pairs or small groups can be used to gauge the degree of electrical coupling via gap junctions (Santos-Sacchi, 1991). In order to estimate junctional sensitivity to intracellular Ca^{+2} and H^+ concentration, the input capacitance of supporting cell syncytia of the organ of Corti was measured with the whole cell voltage clamp technique, while directly modifying the cation concentrations via the patch pipette. Typically, a pH below 6.5 was capable of uncoupling Hensen cells. On the other hand, pCa levels as low as 3 were ineffective.

Keywords: Gap junctions; Organ of Corti; Supporting cell; Homeostasis; Ionic concentration

1. Introduction

The supporting cells of the organ of Corti not only serve to physically support the hair cells, but probably to maintain the homeostasis of the organ. It is significant, therefore, that the supporting cells are joined together by gap junctions (Jahnke, 1975; Gulley and Reese, 1976; Iurato et al., 1976), and are consequently electrically and metabolically coupled (Santos-Sacchi and Dallos, 1983; Santos-Sacchi, 1986). In our previous work, we demonstrated that uncoupling was induced reversibly by treatments which lead to a rise in $[Ca^{+2}]_i$ or $[H^+]_i$, either by direct intracellular iontophoresis of these ions or in the case of H^+ , by perfusion of the cells with CO_2 saturated media (Santos-Sacchi, 1984, 1985, 1991). During those experiments the degree of intracellular ionic concentration changes was unknown. The present work was carried out in order to estimate the relative uncoupling abilities of these two ions, by intracellular perfusion with known cation concentrations. We report that supporting cell coupling is more sensitive to alterations of $[H^+]_i$.

A preliminary account of this work has been presented previously (Sato and Santos-Sacchi, 1991).

2. Methods

Albino guinea pigs were decapitated under halothane anesthesia. The cochleas were removed, the top two turns separated, and placed in calcium free Leibovitz salt solution containing 1 mg/ml trypsin. The tissue was agitated gently in a modified test tube shaker for about 15 min, where upon isolated cells were harvested and allowed to settle onto the coverslip bottom of a 750 μ l perfusion chamber. The cells were continuously perfused with an ionic blocking solution containing (in mM) 100 NaCl, 20 TEA, 20 CsCl, 2 $CoCl_2$, 1.52 $MgCl_2$, 10 HEPES and 5 dextrose, pH 7.2, 300 mOsm. Patch electrodes contained (in mM) 140 CsCl, 10 HEPES, 10 EGTA, and 2 $MgCl_2$, with the pH ranging from 4.03 to 8.14, adjusted using CsOH. Alternatively, at a fixed pH of 7.2, pCa was altered between 2.3 and 8. EGTA buffering was used only for levels of Ca at and below 1 μ M (Imai and Takeda, 1967). Calcium levels in bulk solution were verified using a Ca-sensitive electrode (Orion Research). Initial resistances of the patch electrodes ranged from 3–4 $M\Omega$. The ionic blocking solution was used to reduce the

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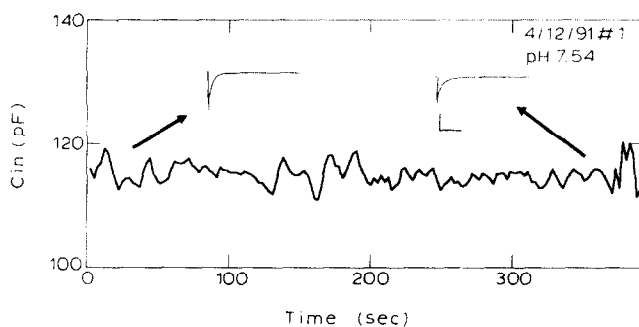


Fig. 1. Group of four cells, one cell of which was whole cell recorded. Pipette pH was 7.54. The input capacitance is stable during the 6.5 min recording, indicating that no uncoupling had occurred. Insets: Capacitive current traces due to -10 mV steps collected at beginning and end of recording session. Changes in trace shape is indicative of changes in cell parameters. Scale: 1.4 nA/4 ms.

voltage dependent ionic conductances so that capacitive currents were recorded in isolation. Blockers of plasmalemma voltage dependent ionic channels do not interfere with junctional communication (Fig. 1; Santos-Sacchi, 1991).

Pairs or small groups of isolated Hensen cells were easily identified under Hoffmann optics and one cell of the pair or group was whole cell voltage clamped. The measurement of input capacitance is a sensitive indicator of cell coupling because by definition the input capacitance will correspond to the amount of electrically contiguous membrane. Changes in input capacitance can therefore indicate the degree of cell coupling (Santos-Sacchi, 1991). However, because the other whole cell parameters (i.e., R_s and R_m) can change as well during alterations in coupling, it is necessary to estimate the input capacitance corrected for these changes. For a single cell the proper method of measuring membrane capacitance can be obtained by modelling the cell-clamp as an electrode resistance (access resistance, R_s) in series with a parallel combination of a membrane resistance (R_m), and a membrane capacitance (C_m). Solving for membrane capacitance (Santos-Sacchi, 1993),

$$C_m = \frac{(R_m + R_s)^2 Q}{R_m^2 V_c} = \frac{(R_{tot})^2 Q}{R_m^2 V_c} \quad (1)$$

The charge (Q) is obtained by integration of the decaying current transient induced by the command voltage step (V_c : 10–20 mV), at a holding potential of -80 mV. R_{tot} is equal to the sum of membrane and electrode resistances, and is obtained from the steady state current response. R_s is estimated from the current decay time constant (Santos-Sacchi, 1993). To a first approximation, for pairs or small groups of cells, R_{in} and C_{in} replaced R_m and C_m , respectively. The capacitance determination is actually only exact when either the cells are highly coupled (if voltage drops are equal in coupled cells) or uncoupled. Nevertheless, the

intermediate capacitance values during uncoupling are useful inasmuch as they reflect changes in cell-to-cell interaction, i.e., in the electrical contiguity between the membranes of adjacent cells. Cell uncoupling was judged to occur if input capacitance values approached single cell levels (25 ± 9 pF; Santos-Sacchi, 1991), within recording periods of 6 min. Syncytia with an initial R_{in} less than 70 M Ω were abandoned. Electronic series resistance compensation was not performed in order to gauge pipette plugging.

The care and use of the animals in this study were approved by the Yale Animal Care and Use Committee under NIH grant DC00273.

3. Results

Whole cell recordings were made 30 min to 2 h after tissue isolation, sufficient time for residual halothane, a potent uncoupler, to be washed away. The input capacitance of a syncytium of supporting cells depends upon the number of coupled cells. In Fig. 1, a group of four Hensen cells, one cell of which was whole-cell recorded, possesses an input capacitance of about 120 pF. This is roughly four times the value of single cells (Santos-Sacchi, 1991). In this case, the pipette solution pH was 7.54, and the Ca concentration was less than 10 nM. The input capacitance is stable during the 6.5 min recording period, indicating that the recorded cell remains coupled to adjacent cells. When pH is lowered below 6.5, an increase in the probability of uncoupling occurs (Figs. 2 and 3). Note in Fig. 2 that the input capacitance begins to decrease during uncoupling before the input resistance changes. This indicates that capacitance measures are more sensitive than DC resistance measures in evaluating junctional conductance changes. Between pH 7 and 8, only one cell uncoupled ($N = 7$), possibly due to injury during recording. Between pH 5 and 7, five cells uncoupled ($N = 13$), and between pH 4 and 5, 6 cells uncoupled ($N = 12$).

Unlike H^+ effects, Ca^{+2} induced uncoupling was observed in only one out of two cases at pCa 2.27 (Fig. 4). At lower concentrations ($N = 18$; pCa 3–8) no uncoupling was observed throughout the recording period, which typically lasted for greater than 6 min. Pooling all data for cells into two groups where pH was either above or below 7, we obtained 2 uncouplings / 26 observations, and 11 uncouplings / 25 observations, respectively. There were significantly fewer uncoupled cells in the high pH condition ($\chi^2 = 6.2$; $P = 0.01$).

4. Discussion

The classic experiments of Loewenstein (e.g., Loewenstein et al., 1967) implicated Ca^{+2} as a control-

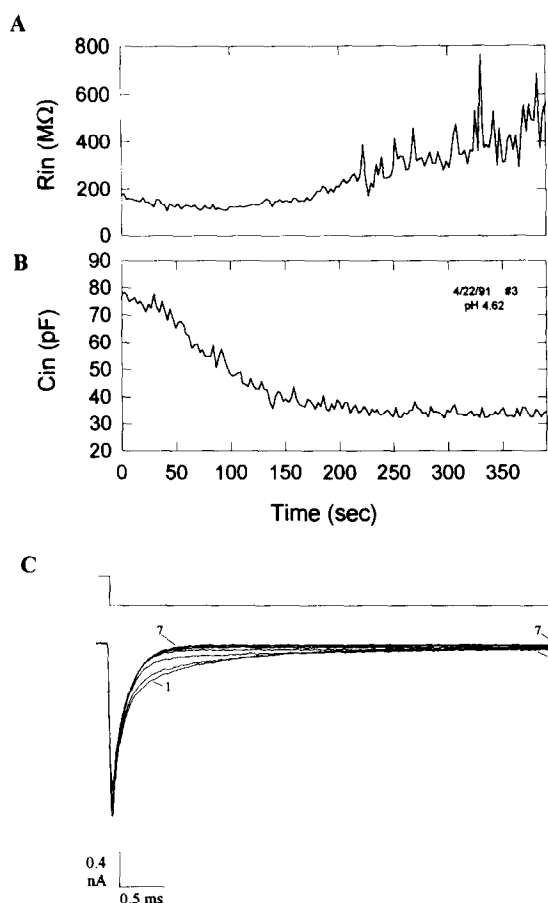


Fig. 2. Two cells with patch pipette at pH 4.62. (A): Input resistance increases as cells uncouple. Note that R_{in} begins to rise following changes in C_{in} . (B): Input capacitance begins to decrease almost immediately after establishment of whole cell configuration. It eventually approaches single cell values as the recorded cell's junctional conductance decreases. (C): Capacitive transients recorded at 50 s intervals (1–7). Note the decrease of steady state values over time indicative of increasing input resistance; the reduction of the charge (area under curves) over time corresponds to the decrease in C_{in} .

ling factor in gap junctional communication. Subsequently, pH was implicated as well (Turin and Warner, 1977). Considerable controversy still exists concerning

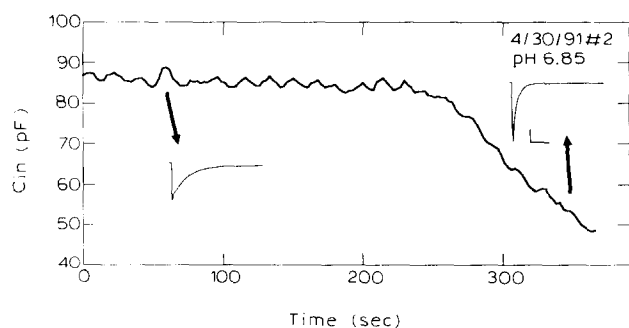


Fig. 3. Two or three cells. Patch pipette pH was 6.85. Cell uncoupling occurred only after prolonged recording. Insets: Capacitive current traces due to -10 mV steps collected at beginning and end of recording. Changes in trace shape is indicative of changes in series resistance, as well as R_{in} and C_{in} . Scale: 0.4 nA/ 0.8 ms.

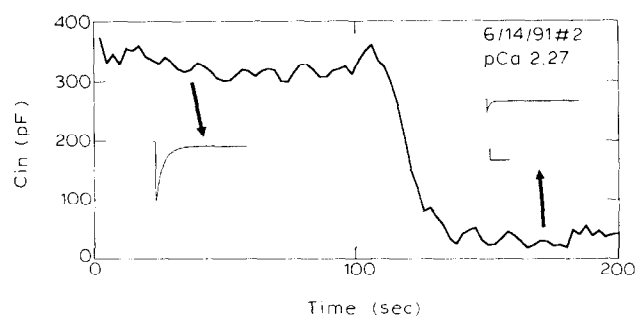


Fig. 4. Large group of cells, one cell of which was whole cell recorded. Pipette pH was 7.2 and pCa was 2.27. The input capacitance drops after one hundred seconds, indicating that uncoupling had occurred due to this high level of Ca^{+2} . Insets: Capacitive current traces due to -10 mV steps collected at beginning and end of recording. Scale: 0.2 nA/ 6 ms.

the sensitivity of gap junctional communication to intracellular Ca^{+2} and H^{+} levels. For example, Spray et al. (1981) found in fish blastomeres that uncoupling due to pH alterations occurred near physiological levels whereas calcium effects were observed only in the millimolar range. Indeed, Ca^{+2} has been shown to permeate both hepatocyte and smooth muscle cell gap junctions (Saez et al., 1989; Christ et al., 1993). On the other hand, others have found that Ca^{+2} can uncouple cells at concentrations approaching physiological levels, viz., in the low to sub-micromolar range (Neyton and Trautmann, 1986; Noma and Tsuboi, 1987; Perachia, 1990). The intracellular regulation of Ca^{+2} and H^{+} are coupled, so that it is important to have control of each ion during experimental manipulations. In the present study, direct perfusion of small Hensen cells with buffered solutions provided a convenient means to study the differential effects of these ions on supporting cell junctional conductance.

It is demonstrated here that an individual Hensen cell is more likely to uncouple from its neighbors during alterations of pH near physiological levels than during alterations of calcium near physiological levels. Indeed, only when calcium levels were clearly pathologic, did uncoupling occur. It should be noted that the ability to maintain the cell's intracellular H^{+} and Ca^{+2} concentrations at that of the electrode's solution depends upon the efficacy of the intracellular perfusion, and intracellular buffering capacity. The latter is an especially important factor at sites proximal to gap junctions which may be distal to the perfusion site. In this regard, it is important to note that Hensen cells are relatively small, with single cell capacitance values of about 25–30 pF (Santos-Sacchi, 1991). Intracellular concentration variability may also occur due to pipette plugging. One difference noted between the two experimental protocols was a greater series resistance for high Ca^{+2} containing pipettes. In the Ca^{+2} modification experiments the average series resistance was 29

$M\Omega$ as compared to $17 M\Omega$ for the H^+ modification experiments. Typically, as the concentration of either ion was increased, the series resistance increased. Nevertheless, there are indications that the experimental differences between H^+ and Ca^{+2} sensitivities are real, and not related to perfusion problems. That is, in a group of cells ($N = 5$) near pH 6, where the probability of uncoupling is great, the series resistance was $21.6 M\Omega$. At a comparable concentration of calcium, (pCa 6 [$N = 5$]), where the probability of uncoupling is negligible, the series resistance was $10.1 M\Omega$. Thus, in cases of similar perfusion efficiencies, intercellular communication in the supporting cells appears more sensitive to the effects of H^+ than Ca^{+2} . Nevertheless, other means of raising intracellular Ca^{+2} levels should be explored.

The supporting cells of the organ of Corti are extensively coupled to one another via gap junctions (Iurato et al., 1976; Jahnke, 1975). It is probable that such coupling is important for sinking of K^+ released during hair cell and neural activity, even in relative quiet. Indeed, it has been shown that Henson cells 'share' ionic channels among each other via gap junctions; in particular, an inward rectifier is active near normal resting potentials (Santos-Sacchi, 1991). The sensitivity of the inner ear to metabolic insult is high and it is likely that resulting H^+ imbalances contribute to this insult by uncoupling supporting cells.

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