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Voltage Gating of Gap Junctions in Cochlear Supporting Cells: Evidence for Nonhomotypic Channels

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Abstract. The organ of Corti has been found to have multiple gap junction subunits, connexins, which are localized solely in nonsensory supporting cells. Connexin mutations can induce sensorineural deafness. However, the characteristics and functions of inner ear gap junctions are not well known. In the present study, the voltage-dependence of gap junctional conductance (G_j) in cochlear supporting cells was examined by the double voltage clamp technique. Multiple types of asymmetric voltage dependencies were found for both nonjunctional membrane voltage (V_m) and transjunctional (V_j) voltage. Responses for each type of voltage dependence were categorized into four groups. The first two groups showed rectification that was polarity dependent. The third group exhibited rectification with either voltage polarity, i.e., these cells possessed a bell-shaped G_j-V_j or G_j-V_m function. The rectification due to V_j had fast and slow components. On the other hand, V_m-dependent gating was fast (<5 msec), but stable. Finally, a group was found that evidenced no voltage dependence, although the absence of V_j dependence did not preclude V_m dependence and vice versa. In fact, for all groups V_j sensitivity could be independent of V_m sensitivity. The data show that most gap junctional channels in the inner ear have asymmetric voltage gating, which is indicative of heterogeneous coupling and may result from heterotypic channels or possibly heteromeric configurations. This heterogeneous coupling implies that single connexin gene mutations may affect the normal physiological function of gap junctions that are not limited to homotypic configurations.

Key words: Voltage dependence — Gap junction — Hybrid coupling — Supporting cell — Inner ear — Hearing loss

Introduction

Connexins, the basic protein subunits of vertebrate gap junction channels, derive from a homologous gene family. More than 15 connexin genes have been cloned. Gap junctions comprised of different members of the connexin family have differing voltage gating properties either to transjunctional voltage (V_j) or to membrane potential (V_m or $V_{i\text{-}o}$) (*see* reviews: Bennett et al., 1991; Dahl, 1996; Kumar & Gilula, 1996). Each gap junction channel is composed of two hemichannels (connexons) and each hemichannel is composed of six connexin subunits (Perkins et al., 1997). A homotypic channel consists of two identical connexons and has symmetric voltage gating on either side owing to its symmetric structure. A heterotypic channel is formed by two different connexons and its voltage gating can be asymmetrical owing to its asymmetrical constitution (Barrio et al., 1991; Rubin et al., 1992*a;* Verselis et al., 1994; White et al., 1994*a*). A heteromeric channel possesses hemichannels formed by different connexin subunits and little is known about its voltage gating (Brink et al., 1997; Lee & Rhee, 1998).

Physiological and anatomical evidence for gap junctional coupling among the supporting cells of Corti's organ has been obtained both in vivo and in vitro (Jahnke, 1975; Gulley & Reese, 1976; Iurato et al., 1976; Hama & Saito, 1977; Santos-Sacchi & Dallos, 1983; Santos-Sacchi, 1987; Zwislocki et al., 1992; Kikuchi et al., 1995; Forge et al., 1997; Zhao & Santos-Sacchi, 1998). Connexin 26 (Cx26) is extensively distributed among cochlear nonsensory cells, including the supporting cells of Corti's organ (Kikuchi et al., 1995; Forge et

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al., 1997). More recently, Lautermann et al. (1998) reported that cochlear supporting cells also contained Cx30 and Cx43; moreover, Cx30 was extensively expressed in a pattern similar to that of Cx26. Ultrastructural, immunohistochemical and physiological studies have found no evidence indicating that sensory cells (inner and outer hair cells) have gap junctions. However, mutations of connexin genes, for example, *GJB2* which encodes Cx26, have been identified in association with a high incidence of nonsyndromic sensorineural deafness (Kelsell et al., 1997; Denoyelle et al., 1998; Estivill et al., 1998; Xia et al., 1998). Little is known about the mechanism by which such a mutation may cause deafness. To some extent, the lack of a complete characterization of the biophysical properties of inner ear gap junctions limits our understanding.

Inner ear gap junctions can be modified by a variety of treatments, such as altering the intracellular activities of Ca^{++} and H^{+}, temperature and membrane tension (Santos-Sacchi, 1985, 1986, 1991; Sato & Santos-Sacchi, 1994; Zhao & Santos-Sacchi, 1998). In the present report, the effects of voltage on inner ear gap junctions were explored by a double voltage clamp technique. The principal finding is that cochlea supporting cells display a variety of voltage dependencies, typified by asymmetrical rectification. The variety of asymmetrical voltage gating is indicative of complex heterotypic and/or heteromeric coupling. This heterogeneous coupling implies that single connexin gene mutations may affect the normal physiological function of gap junctions that are not limited to homotypic configurations.

Preliminary accounts of this work have been presented (Zhao & Santos-Sacchi, 1997, 1999).

Materials and Methods

The guinea pig's organ of Corti was freshly isolated and subsequently dissociated by shaking for 5–15 min in nominally Ca^{++}-free Leibovitz medium containing 1 mg/ml of trypsin. The dissociated cells were transferred into a recording chamber. Hensen cells are distinguishable by their bright lipid vacuoles under Hoffman optics (Fig. 1*A*). A classical double voltage-clamp technique was used to measure transjunctional conductance (G_j). Each cell in a Hensen cell pair was separately voltage-clamped using an Axopatch 200A and 200B (Axon, CA) (Fig. 1*A*). Test voltages were applied to one cell (cell 1) and transjunctional current (I_j) was measured in the adjacent cell (cell 2). To accurately obtain G_j–voltage relations, calculations were made with corrections for electrode access resistance (R_s) and nonjunctional membrane resistance (R_m) (Neyton & Trautmann, 1985):

$$V_j = V_{c1} - V_{c2} - (\Delta I_1 R_{s1} - \Delta I_2 R_{s2}) \quad (1)$$

$$G_j = \frac{I_j}{V_j} = \frac{-\Delta I_2 \left(1 - \frac{R_{s2}}{R_{m2}}\right)}{V_j} \quad (2)$$

Where V_{c1} and V_{c2} are the clamp voltages applied to cell 1 and cell 2, respectively; ΔI_1 and ΔI_2 are the holding current changes in cell 1 and cell 2 caused by the test voltage in cell 1. R_{m2} is the nonjunctional membrane resistance of cell 2, where I_j was measured (Fig. 1*A*). It is clear from recent work that in cases were R_m is greater than 500 MΩ, R_s dominates errors in junctional estimates (Van Rijen et al., 1998), and is readily corrected for. Our residual series resistance is quite small and stable, and membrane resistance is typically greater than 1 GΩ (*see below*). Furthermore, cell pairs that had G_j greater than 20 nS were not included in the data analysis. Data collection and analysis were performed with an in-house developed Window's based whole-cell voltage clamp program, **jClamp** (http://www.med.yale.edu/surgery/otolar/santos/jclamp.html), utilizing a Digidata 1200 board (Axon, Foster City, CA.) All experiments were tape recorded and performed at room temperature.

To limit interference from nonjunctional voltage-dependent ionic currents, cells were continuously perfused by an extracellular ionic blocking solution that was composed of (in mM): NaCl 100, TEA 20, CsCl 20, $CoCl_2$ 1.25, $MgCl_2$ 1.48, HEPES 10, pH 7.2 and osmolarity 300 mOsm (Santos-Sacchi, 1991; Zhao & Santos-Sacchi, 1998). Patch pipettes were filled with an intracellular solution that contained (in mM): 140 CsCl, 5 EGTA, 2 $MgCl_2$, and 10 HEPES, pH 7.2. After blocking nonjunctional voltage dependent ionic channels, Hensen cells had linear *I-V* relationships and high membrane resistances ($R_m > 1$ GΩ) (Fig. 1*C*).

Patch pipettes (Borosilicate Glass, MTW150-4, World Precision, FL) were pulled by a laser, based pipette puller (P-2000, Sutter, CA). A pair of pulled pipettes was used in each double voltage-clamp recording to promote symmetrical recording conditions. Pipettes had initial resistance of 2.5–3.5 MΩ in bath solution. After whole cell configuration was established, the residual series resistance of the electrode was in a range of 3.9–9.8 MΩ, which was measured by the peak of the uncompensated whole cell capacitance current (Huang & Santos-Sacchi, 1993) as both cells were simultaneously stimulated by an identical test pulse. R_s was stable and was 6.09 ± 0.22 MΩ and 6.23 ± 0.34 MΩ (mean ± SE, $n = 64$) before and after experiments, respectively. In 11 cell pairs, R_s was further validated after one cell of the cell pairs was broken or pulled away by an electrode so that R_s could be unequivocally determined under single whole cell configuration. The measured R_s was 6.17 ± 0.62 MΩ consistent with those values which were determined under double voltage-clamp configuration.

Results

Gap junctions in Hensen cells had asymmetric responses to positive and negative transjunctional voltages (V_j). Macroscopic transjunctional currents were both time and voltage dependent (Fig. 1), and consequently, nonlinear I_j-V_j relationships were obtained at voltage onset and steady state. G_j–V_j relations could be classified into four groups, including one with no apparent voltage-dependence (Fig. 2*D*). The purpose of our categorizing junctional (and nonjunctional—*see below*) voltage dependencies was to simplify our dealings with the data, emphasize the diversity of response, and make some comparisons to published data.

Two basic asymmetrical V_j-dependencies were identified which showed opposite polarity sensitivities. The one whose conductance increased as V_j was made positive (Fig. 2*A*) is similar to that obtained from heterotypic channels containing Cx26 components (Barrio et al., 1991; Rubin et al., 1992*a*,*b;* Verselis et al., 1994; Dahl et

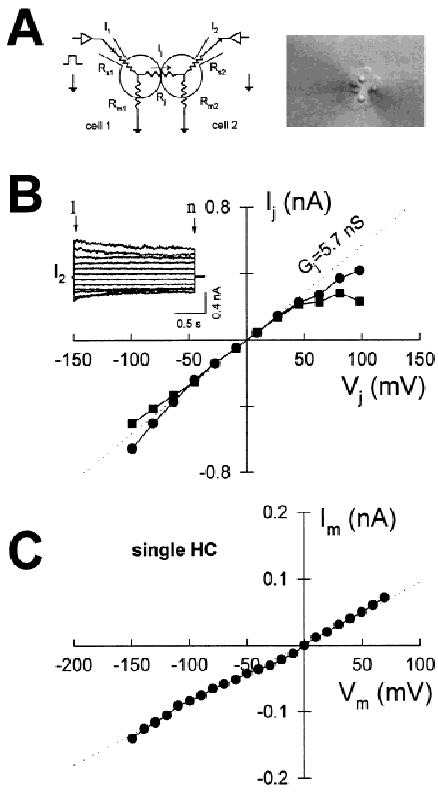

Fig. 1. (*A*) Schematic drawing and video-captured image illustrate the double voltage-clamp recording in a Hensen cell pair. Scale bar is 20 μm. Both cells were initially voltage-clamped at −40 mV. V_j was produced by 2 sec voltage steps (V_{c1}) applied to cell 1 in 20 mV increments from −150 to +70 mV and cell 2 (V_{c2}) continuously held at −40 mV; the change in V_j is equal to ±110 mV. Depolarization of the stepped cell corresponds to positive V_j and produces a downward current in cell 2 (I_2). (*B*) Transjunctional currents in response to V_j stimulation were obtained from a Hensen cell pair. Initial and steady-state transjunctional currents (I_j) were measured at onset (5 msec) and end (2 sec) of voltage steps and are represented by solid circles and squares, respectively. Initial I_j as well as steady-state I_j increased nonlinearly for V_js greater than +50 mV. The dotted line indicates a conductance of 5.7 nS. (*C*) Typical example of membrane current (I_m) of single isolated Hensen cell as a function of membrane potential (V_m) obtained with the ionic blocking solutions used for coupling studies (*see* Materials and Methods). Membrane resistance is greater than 1 GΩ, and the $I_m - V_m$ curve is linear.

al., 1996). The other had a conductance that decreased with positive V_j but changed less with negative V_j (Figs. 1 and 2*B*). The last group evidenced a slightly asymmetric bell-shaped G_j–V_j relationship (Fig. 2*C*). While V_j–induced G_j changes typically ranged between 40–50%, in some cases G_j decreased below 20%; this variability probably reflects the heterogeneity of the channel population.

In most cell pairs, the V_j asymmetry was characterized by alternating the stimulus configuration, such that transjunctional conductance was measured in both directions between a cell pair (cell 1 → cell 2 and cell 2 → cell 1). An example is shown in Fig. 3. When cell 1 of the pair was stepped to positive potentials, while holding cell 2 constant, steady-state transjunctional conductance, G_{jss}, decreased. On the other hand, when cell 2 was stepped to negative potentials, G_{jss} decreased. Unlike the asymmetry of G_{jss}, G_{j0} was insensitive to V_j gradients. These data illustrate that the asymmetric response to V_j in this cell pair resulted from a junctional conductance decrease only when cell 1 was depolarized, a clear transjunctional effect, and not due to possible asymmetric recording conditions. Furthermore, the conductance plots are nearly mirror images of each other, indicating an absence of V_m contribution in this case. This V_m insensitivity was independently verified by direct measures (*see below*).

To rule out fast gating effects, we determined the ratio of G_{jss}/G_{j0} in all cells that showed V_j dependence (Fig. 2 *A–C*). From these cells we found indications of slow (steady state) rectification in 9 out of the 28 cell pairs (Fig. 4). The ratio shows no voltage dependence until positive V_j values are reached, whereupon the ratio falls. This type of behavior strongly indicated that Hensen cells have heterotypic gap junctions, such as those containing Cx26 subunits as observed in oocyte pairs (Barrio et al., 1991; Rubin et al., 1992*a*,*b;* Verselis et al., 1994; Dahl et al., 1996).

Interestingly, Hensen cells demonstrate rapid voltage sensitivity of gap junctional coupling with the V_j protocol (Figs. 1 and 2). V_j–G_j functions obtained from onset currents displayed the same asymmetrical behavior as steady state functions. In fact, in some cell pairs, no slow V_j-sensitive gating was observed, and it should be noted that nonlinear onset responses such as these might result not only from V_j sensitivity, but also from V_m sensitivity. This is further investigated below.

V_m-dependent gating was found in most (29/45) Hensen cell pairs (Figs. 5 and 6). In Fig. 5, G_j changes occurred within 5 msec or less after altering V_m, and remained stable for the full recording period. Slow V_m-dependent gap junction gating was never detected in Hensen cells. However, we only explored time periods up to 5 sec.

As with the V_j dependent conductance data, V_m dependent junctional sensitivities could be categorized into four groups, including one that showed no dependence (Fig. 6). One group showed a decrease in coupling upon depolarization, while another showed the opposite effect (Fig. 6 *A* and *B*). The third group demonstrated a bell shaped G_j–V_m function (Fig. 6*C*).

V_m gating was independent of V_j-gating. This is illustrated in Fig. 7, where comparisons between V_j and V_m dependence are made within the same cell pairs. Figure 7*A* shows data from a cell pair that lacked V_j-dependent gating; neither G_{j0} nor G_{jss} showed V_j sensitivity. However, G_{jm} decreased with cell depolarization. In another

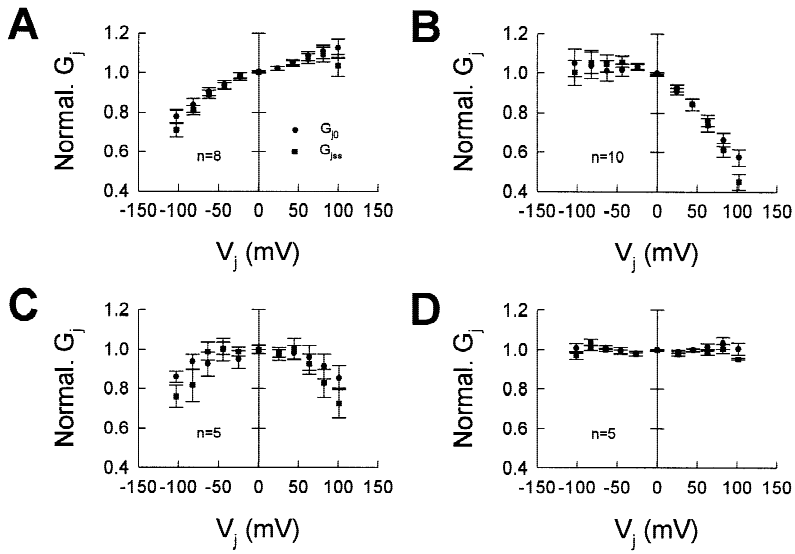

Fig. 2. Gap junctional coupling of Hensen cells presented differing voltage sensitivities for V_j stimulation. Initial transjunctional conductance (G_{j0}) and steady state conductance (G_{jss}) were normalized to the mean conductance at the average of V_j at ±10 mV. G_j (mean ± SE; $n = 28$) was 11.8 ± 1.31 nS. Cell pairs that had G_j greater than 20 nS were not included. Holding potential was −40 mV. Error bar represents SE.

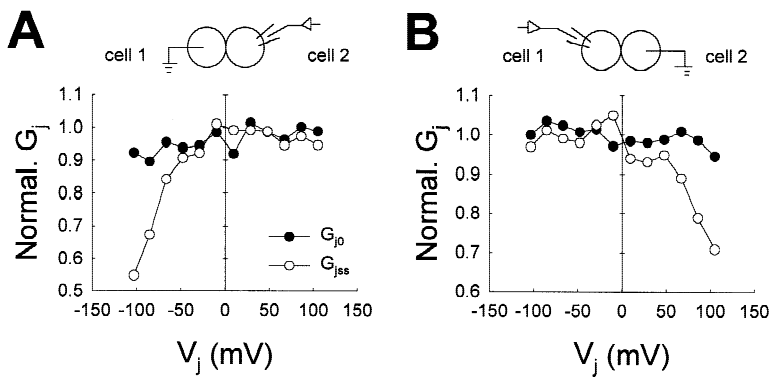

Fig. 3. V_j applied to either cell in the same cell pair identified rectified V_j-dependence. Positive V_j polarity corresponds to depolarization in the stepped cell. (*A*) Cell 1 was held and cell 2 was voltage-stepped. Rectification occurred at negative V_j, i.e., during cell 2 hyperpolarization. (*B*) Voltage steps were delivered to cell 1 while cell 2 voltage was held constant. At positive V_j (cell 1 depolarized), G_{jss} decreased. In this cell pair, G_{jss} always rectified at relatively positive polarity of V_j referring to cell 1. For each case onset conductance was fairly stable across voltage. The transjunctional conductance in this cell pair was about 13 nS. $R_{s1} = 4.3$ MΩ, $R_{s2} = 4.9$ MΩ, $R_{m1} = 1591$ MΩ, and $R_{m2} = 962$ MΩ.

cell pair the opposite behavior is illustrated (Fig. 7*B*). In this case, transjunctional conductance exhibited an asymmetric V_j-dependence, but was insensitive to V_m. Additionally, slow V_j-sensitive gating was undetectable since G_{jss} mirrored G_{j0}; this further indicates that only a pure fast V_j-dependent gating is present.

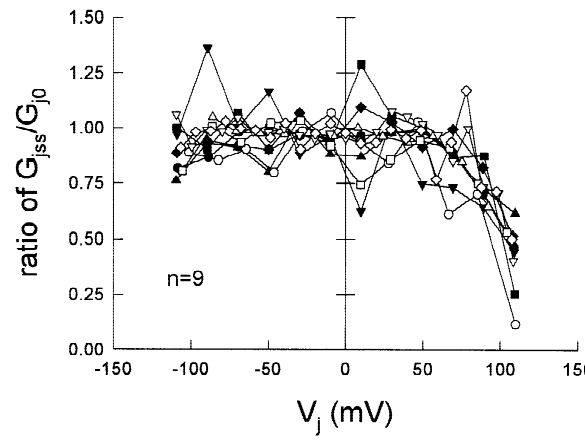

Fig. 4. Ratio of G_{jss}:G_{j0} indicates slow V_j-dependent gating in response to V_j stimulation. Each symbol and line represents a cell pair.

Discussion

The most pronounced property of gap junctional voltage gating within cochlear supporting cells is its diverse asymmetry. This asymmetric gating is likely indicative of asymmetric channel structure within each cell, i.e., heterotypic and/or heteromeric junctional channels. Evidence is mounting that nonsyndromic deafness is associated with connexin gene mutations, such as Cx26 (Kelsell et al., 1997; Denoyelle et al., 1998; Estivill et al., 1998; Xia et al., 1998). While a variety of connexin types are present in supporting cells (Lautermann et al., 1998), functional impairments induced by single gene mutations can not be rescued by other coexisting connexins. Our data provide evidence for the occurrence of hybrid gap junction channels in cochlea supporting cells,

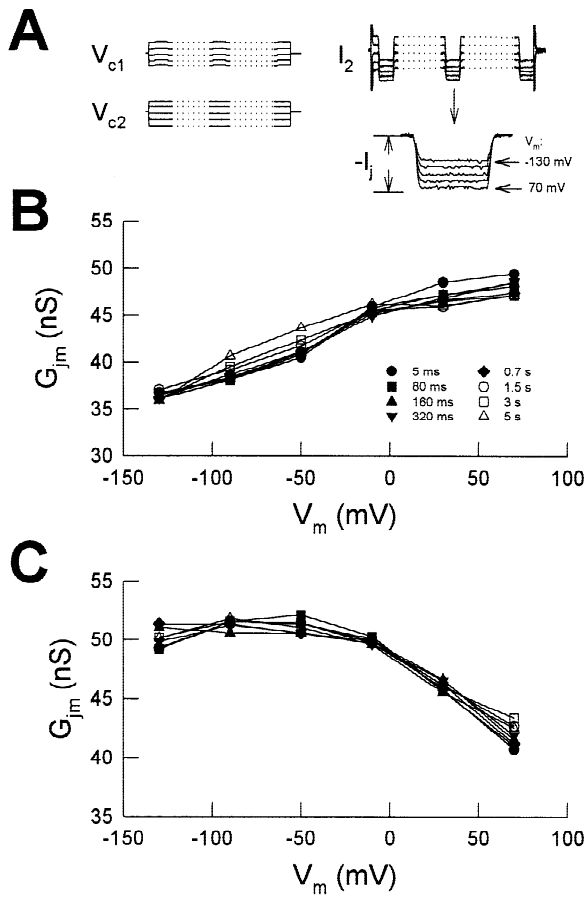

Fig. 5. Gap junction channels in Hensen cells are sensitive to membrane potential (V_m or V_{i-o}). *A* Voltage protocols. Both cells were simultaneously stimulated with voltage steps (V_{c1} and V_{c2}) from −130 to +70 mV in 40 mV increments, for a 5 sec duration. A test pulse (10 mV, 10 msec) was superimposed on cell 1 at different times to elicit transjunctional current. For clarity, the full length of waveforms is not shown. Example current traces at different membrane potentials are zeroed and superimposed. (*B* and *C*) V_m-dependent transjunctional conductance (G_{jm}) at different times is plotted. G_{jm} of the cell pair in panel *B* increased as the cells were depolarized, whereas in panel *C* G_{jm} decreased as the cells depolarized. Despite the opposite polarity dependence of the two coupled pairs, G_{jm} remained stable throughout the recording period.

and indicate that single gene mutations will impair more than just those channels destined to be homotypic. In heterotypic or heteromeric channels, the presence or absence of a single subunit type can have dramatic effects on junctional voltage dependence (Dahl et al., 1996; Brink et al., 1997; He et al., 1999; Li & Simard, 1999) and possibly other gating characteristics (Lee & Rhee, 1998). Thus, the potentially pervasive influence of single connexin gene mutations on diverse gap junction channel types may contribute to its devastating effects on hearing. This indicates, as well, that the diversity of channel types is important for cochlea function.

Cx26 and Cx30 are colocalized in cochlear supporting cells (Lautermann et al., 1998). In the paired oocyte expression system, Cx26 can form heterotypic gap junction channels with Cx30 (Dahl et al., 1996). The voltage gating of this heterotypic channel demonstrates remarkable differences from those of the corresponding homotypic channels (Jarillo et al., 1995; Dahl et al., 1996). One of the most distinguishable properties of this heterotypic channel is its slow V_j-dependent gating, rectifying with positive V_j on the Cx30 side of the junction (Dahl et al., 1996). It is likely that this type of heterotypic channel exists in the organ of Corti, since we find similar gating characteristics in cochlear supporting cells (Figs. 1, 3 and 4).

However, in addition to characteristics that are found in known heterotypic channels, we observed properties that are atypical, possibly indicating the existence of heteromeric channels. Although few physiological studies have suggested the existence of heteromeric channels (Brink et al., 1997; Lee & Rhee, 1998), biochemical analyses clearly reveal their presence (Stauffer, 1995; Jiang & Goodenough, 1996; Brink et al., 1997). In our experiments, several observed gating properties might arise from heteromeric channel behavior. First, the existence of fast asymmetric V_j-dependent gating that is distinct from slow V_j-dependent gating and V_m-dependent gating (Figs. 2 and 7*B*) is inconsistent with behaviors of known heterotypic and homotypic channels (Rubin et al., 1992*a*,*b;* Verselis et al., 1994). Second, the occurrence of multiple V_m-sensitivities found in coupled Hensen cells is unusual. While a depolarization-induced increase in conductance (as in Fig. 6*A*) has been observed in oocyte preparations of vertebrate Cx26 connexins (Rubin et al., 1992*a*,*b*), decreases in conductance, as in Fig. 6*B*, are only seen in insect preparations (Obaid et al., 1983; Verselis et al., 1991; Bukauskas et al., 1992). Bell-shaped V_m dependencies (Fig. 6*C*) have never been previously reported. Finally, the independence of V_m and V_j gating within the same cell pair is unique (Fig. 7). Considering this evidence and the high probability of heteromeric connexon formation within supporting cells (since at least Cx26, 30 and 43 are coexpressed), heteromeric channels likely exist in inner ear gap junctions.

Although contrary evidence initially existed (Spray et al., 1984, 1986; Riverdin & Weingart, 1988), it is currently accepted that vertebrate gap junctions are gated by voltage, either V_j or V_m (Moreno et al. 1991; 1992; Kumar & Gilula, 1996). Interestingly, although most Hensen cells present voltage-gated junctional conductances, we find an absence of voltage dependence, either to V_j or V_m, in a large proportion of Hensen cells. While this might be expected for coupling derived from cytoplasmic bridges between cells, we typically find that Hensen cell coupling is sensitive to agents that uncouple gap junctions, e.g., octanol or low pH (Santos-Sacchi, 1985; 1991; Sato & Santos-Sacchi, 1994). It should also

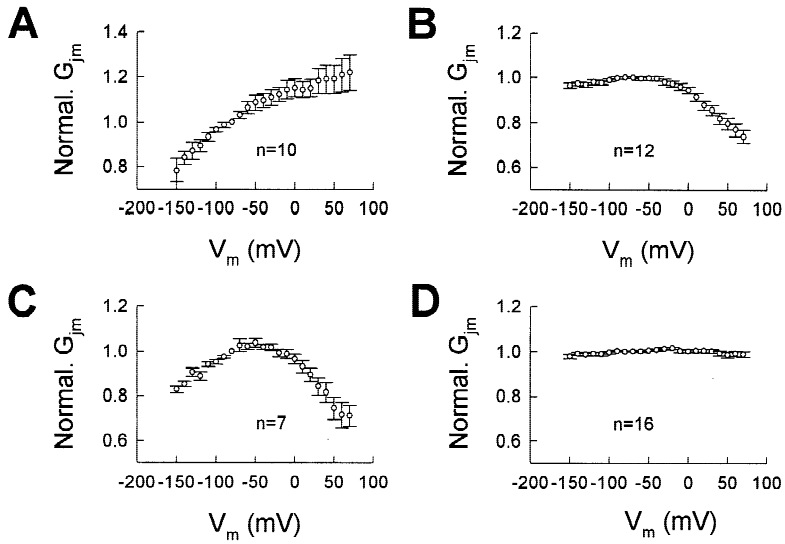

Fig. 6. Coupling of Hensen cells showed multiple V_m-dependences. V_m-dependent G_{jm} was normalized to that at the Hensen cell's holding potential of −80 mV. Error bar represents SE. *(A)* Conductance increases during depolarization. *(B)* Above −40 mV, conductance decreases. *(C)* Conductance is a bell-shaped function of membrane voltage.

be noted that we have used techniques (*see* Materials and Methods) that enable us to accurately deliver voltage and assess its delivery to the cells, so that we can be sure that voltage-clamp problems are minimized. The voltage-gating properties of homotypic and heterotypic channels comprised of connexins identified in supporting cells (Cx26, 30 and 43) show clear voltage dependence. Channels derived from Cx26 and 30 exhibit marked V_j-dependence, whereas homotypic channels derived from Cx43 exhibit weak steady-state voltage dependence (Moreno et al., 1992; Veenstra et al., 1992; Miyoshi et al., 1996). In the inner ear, the expression of Cx43 in cochlear supporting cells was described as relatively weak (Lautermann et al., 1998). Indeed, Forge et al. (1997) did not detect Cx43 antibody labeling in the supporting cell region in gerbil and guinea pig cochlea. Thus, the lack of voltage dependence in many supporting cells is possibly due to either the summed opposed characteristics of the channel populations found in the cell pair or due to heteromeric configurations. Voltage sensitivity may be modulated by allosteric interactions between opposing connexons (White et al., 1994*a*). It may also be that voltage sensitivity can be modulated by heteromeric interactions within a connexon.

It is well established that V_m-sensitive gating occurs in invertebrate gap junctions (Obaid et al., 1983; Verselis et al., 1991; Bukauskas et al., 1992). Only recently, however, has V_m-dependence been documented in vertebrate gap junctions either in natural tissues (Verselis et al., 1993) or in the paired oocyte expression system (Barrio et al., 1991, 1997; Rubin et al., 1992*a;* White et al., 1994*b;* Jarillo et al., 1995). The conductance of channels possessing Cx30 and 43 decreases with depolarization (White et al., 1994*b;* Jarillo et al., 1995), similar to what we observed in Hensen cells. A V_m dependent conductance also arises when channels possess either Cx26 or

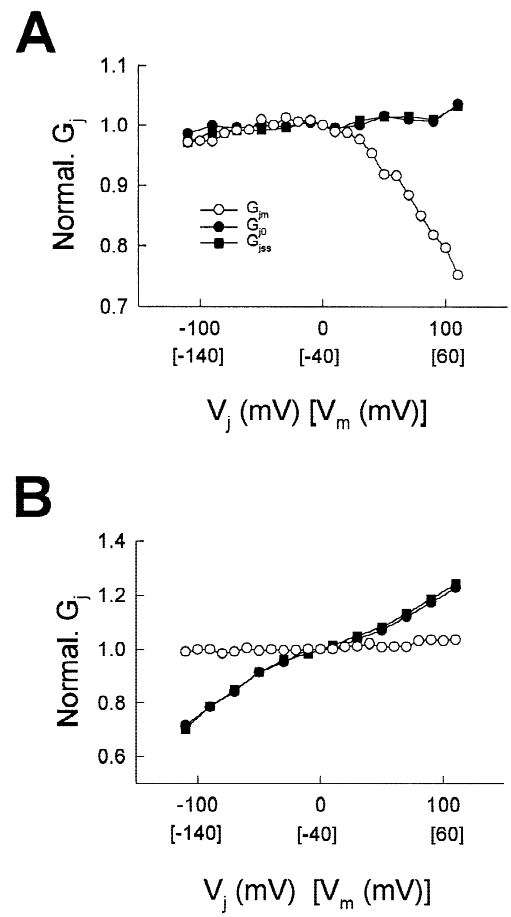

Fig. 7. Voltage-dependent Hensen cell coupling shows independent V_j and V_m sensitivities. (*A*) In the same cell pair, V_m depolarization reduced junctional conductance but V_j was without effect. (*B*) In this cell pair the sensitivity is reversed. Coupling was sensitive to V_j stimulation but insensitive to V_m (or V_{i-o}). Note that this pair had fast V_j-dependent gating, but no slow V_j-dependent gating.

45; however, conductance increases upon depolarization (Barrio et al., 1991, 1997; Rubin et al., 1992*a*). Some Hensen cell pairs have similar gating characteristics to that of Cx26 and 45 (Figs. 5*B* and 6*A*). However, while the voltage sensitivity expressed in Hensen cells is greater than that of Cx26, it is quite similar to that of Cx45 (Barrio et al., 1997). Whether Cx45 is present in the inner ear remains to be seen; nevertheless, mutation studies indicate that connexins other than those already identified histochemically also exist (*see below*).

Hensen cells, in vivo, are bathed in different media, endolymph (high K^+ and +80 mV) apically and perilymph (low K^+ and 0–10 mV) basally. They contribute to a barrier against ionic diffusion from the endolymph to perilymph caused by the gradients of ions and voltage. These gradients are vitally important for normal sensory function, and it has been proposed, though still remains unproved, that supporting cells help maintain these gradients by sinking K^+ released during hair cell and neural excitation (Santos-Sacchi, 1987; 1991). Recently, an anatomical substrate for the directional sinking of ions through gap junctions has been proposed (Kikuchi et al., 1995). It is currently held that differing connexins may contribute to the formation of functional junctional pathways with variable gating sensitivities and permeabilities (Elfgang et al., 1995; Mills & Massey, 1995; Brink, 1996; Trexler et al., 1996). In fact, non-uniform, directionally limited dye spread in the intact organ of Corti has been found (Santos-Sacchi, 1986; Oberoi & Adams, 1998). Our data that establishes the likelihood of hybrid gap junction channels underscores the possibility of functional pathways within the organ of Corti. It may be that the nonsyndromic hearing loss that arises from connexin mutations occurs because functional pathways are disturbed. So far, three connexin genes, *GJB1* (encoding Cx32), *GJB2* (encoding Cx26) and *GJB3* (encoding Cx31), have been identified in association with hearing impairment (Bergoffen et al., 1993; Kelsell et al., 1997; Denoyelle et al., 1998; Estivill et al., 1998; Xia et al., 1998). These data indicate that the potential variability in heteromeric combinations is even greater than the immunohistochemical data suggest. Furthermore, it is likely that mutations of Cx30 and 43 could also alter heterotypic- or heteromeric-like gap junctional coupling in the inner ear, thus leading to hearing impairment.

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