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Gap Junction Channels: Distinct Voltage-Sensitive and -Insensitive Conductance States

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ABSTRACT All mammalian gap junction channels are sensitive to the voltage difference imposed across the junctional membrane, and parameters of voltage sensitivity have been shown to vary according to the gap junction protein that is expressed. For connexin43, the major gap junction protein in the cardiovascular system, in the uterus, and between glial cells in brain, voltage clamp studies have shown that transjunctional voltages (V_j) exceeding ±50 mV reduce junctional conductance (g_j). However, substantial g_j remains at even very large V_j values; this residual voltage-insensitive conductance has been termed g_{min}. We have explored the mechanism underlying g_{min} using several cell types in which connexin43 is endogenously expressed as well as in communication-deficient hepatoma cells transfected with cDNA encoding human connexin43. For pairs of transfectants exhibiting series resistance-corrected maximal g_j (g_{max}) values ranging from <2 to >90 nS, the ratio g_{min}/g_{max} was found to be relatively constant (about 0.4–0.5), indicating that the channels responsible for the voltage-sensitive and -insensitive components of g_j are not independent. Single channel studies further revealed that different channel sizes comprise the voltage-sensitive and -insensitive components, and that the open times of the larger, more voltage-sensitive conductance events declined to values near zero at large voltages, despite the high g_{min}. We conclude that the voltage-insensitive component of g_j is ascribable to a voltage-insensitive substate of connexin43 channels rather than to the presence of multiple types of channels in the junctional membrane. These studies thus demonstrate that for certain gap junction channels, closure in response to specific stimuli may be graded, rather than all-or-none.

INTRODUCTION

Voltage sensitivity was evident in some of the earliest electrophysiological studies of electrotonic coupling in excitable cells of invertebrates (Furshpan and Potter, 1959; Nicholls and Purves, 1972; Smith and Baumann, 1969). Subsequently, the analysis of gap junction currents in voltage-clamped pairs of amphibian embryonic cells (Spray et al. 1979, 1981) revealed voltage-dependent monoexponential relaxation to non-zero steady-state current levels, with time constants ranging from tenths of seconds to seconds, depending on the amplitude of transjunctional voltage V_j (Harris et al., 1981). After subtraction of the voltage-insensitive component, which was about 5% of the initial conductance in the case of the amphibian embryonic cells, steady-state conductance (G_{ss}) decreased symmetrically with V_j of either sign. This V_j-G_{ss} relation was found to be symmetric, as predicted for independent gating of each connexon (or hemichannel) by voltage of one polarity. The recent use of the dual whole cell recording method with patch pipettes (introduced by White et al. (1985) and Neyton and Trautmann (1985)) has revealed that gap junctions between all mammalian cells thus far evaluated are sensitive to V_j, and almost all exhibit a residual conductance that is insensitive to even very large V_j (Moreno et al., 1991a, c; Veenstra et al., 1992; Wang et al., 1992; Lal and Arnsdorf, 1992; Barrio et al., 1991; Willecke et al., 1991; Spray et al., 1991a, 1992, 1994; Bruzzone et al., 1993; Werner et al., 1989; Ebihara and Steiner, 1993; Giaume et al., 1991; Fishman et al., 1991; Rook et al., 1992; Chanson et al., 1993).

The origin of this residual conductance, and its relationship to the voltage-dependent component, have remained obscure. Several possibilities that have seemed to be the most likely are: (a) that the total junctional conductance (g_j) is composed of two (or more) types of junctional channels, some of which are voltage-sensitive and some of which are not; (b) that only one population of junctional channel with one channel size is present, but that its voltage sensitivity saturates at high potentials such that the channels continue to open and close with the same unitary conductance, i.e., that the non-zero open probability (P_o) of the channels at high voltage results in the residual macroscopic conductance; and (c) that the residual conductance is caused by a conductance substate of the larger channel size which exhibits less voltage sensitivity than the fully open state, i.e., that the P_o of the main conductance state of the channels declines to zero, whereas opening of the substate dominates at high V_j values. As is considered in more detail in the Discussion, there are precedents for each of the types of mechanism in the gap junction literature. For example, coexpression of multiple gap junctions proteins (connexins) is well documented between cell pairs (see Dermietzel et al. (1990) for review in the case of mammalian cells; see also Veenstra et al. (1992) for chick cardiocytes), non-zero P_o at high voltages with

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constant unitary conductance was previously hypothesized to be responsible for g_{min} (e.g., Harris et al., 1981), and reduced unitary conductance at high voltage has been attributed to channel gating by inside-outside potential (Weingart et al., 1993) or to V_j (Weingart and Bukauskas, 1993) in pairs of insect cells.

To distinguish among these possibilities, we have evaluated junctional properties in pairs of voltage-clamped cells transfected with human connexin43 and compared these properties with those of primary cultures of several cell types in which connexin43 appears to be the dominant gap junction protein (see Beyer et al., 1990; Dermietzel et al., 1990). Connexin43 was chosen for these studies because these gap junctions exhibit the largest voltage-insensitive residual g_j yet reported (on the order of 40–50% total g_j), which allowed us to perform tests of the underlying mechanism more readily than with channels formed of other connexins.

MATERIALS AND METHODS

Coupling-deficient human hepatoma (SKHep1) cells were stably transfected with cDNA that encodes rat or human connexin43 (Fishman et al., 1990). This parental cell line exhibits low level expression of another connexin (from conductance measurements, estimated to average about one channel per cell pair), which we now believe to be connexin45 (Moreno et al., 1991b, 1994a). Importantly, this endogenous channel is extremely voltage-dependent, with virtually no channel openings occurring at or above ±40 mV. After transfection, the conductance between the cells increased from a very low conductance background (less than one channel per cell pair: Moreno et al., 1991b) to a well coupled mean of 11 nS. Unitary conductance, voltage dependence, and phosphorylation characteristics of human connexin43 channels expressed in these cells have been published previously (Fishman et al., 1991; Moreno et al., 1992c, 1994b).

Methods for isolation of human smooth muscle cells from corpora cavernosa and identification of connexin43 as a major component of the junctional membrane have appeared (Campos de Carvalho et al., 1993; Christ et al., 1992). Unitary conductance and voltage dependence of these gap junctions have also been characterized recently (Moreno et al., 1993a).

Cardiocytes were isolated from neonatal (12–36 h old) rat hearts and cultured as previously described (Rook et al., 1988, 1992).

Cells attached to coverslips were transferred to the stage of a Nikon Diaphot microscope and constantly perfused (flow about 1 ml/min) with an external solution containing (in mM): 160 mM NaCl, 7 mM CsCl; 0.1 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM Hepes, pH 7.4. To determine junctional conductance between cell pairs, the dual whole cell voltage clamp configuration was used (White et al., 1985; Neyton and Trautmann, 1985). For each cell of the pair, access to the cell interior was achieved by gentle suction applied to the rear of a fire-polished glass pipette (usually 3–5 MOhm, filled with an internal solution containing: 130 mM CsCl; 0.5 mM $CaCl_2$; 2 mM Na_2ATP; 3 mM MgATP; 10 mM Hepes; 10 mM EGTA; pH 7.2) sealed with high resistance (>1 GOhm) to the cell membrane, as evaluated by brief pulses (−4 mV, 20 ms applied to each cell; holding potentials of both cells were generally 0 mV). Voltage steps (−4 to −10 mV, 200–500 ms duration) alternatively applied to each cell resulted in positive currents recorded in the nonstepped cell caused by junctional channels between the cells. This current (I_j), divided by the size of the voltage step, yields the junctional conductance (g_j) (Spray et al., 1979). To evaluate voltage dependence, sustained (>10 s, generally 70 or 80 mV) pulses of either polarity were applied to one cell while the other was held at 0 mV.

All cells were cultured in DMEM with 5% calf serum (except for the ES cells, where neonatal calf serum was used). For electrophysiological studies, pairs of cells were obtained by plating trypsin-dissociated cells at low density on 1 cm diameter glass coverslips. ES cells were distinguished from fibroblasts after staining the latter with 200 μM DiO before co-culturing. In freshly dissociated dishes, ES cells were easily recognized over the feeder fibroblast cells by their small size <10 μm and by their highly refringent membrane. To measure currents through individual junctional channels, g_j was reduced by halothane (2 mM) perfusion in the bath solution (Burt and Spray, 1989) and voltage steps (−30 to −90 mV as indicated) were applied to one of the cells.

RESULTS AND DISCUSSION

The phenomenon of voltage sensitivity of junctional conductance is illustrated in Fig. 1 *A* for a representative experiment performed on a pair of SKHep1 cells stably transfected with an expression plasmid encoding human connexin43. Traces illustrated correspond to V_j pulses of 0–100 mV in 20-mV increments. Note that for smaller V_j pulses, I_j remained relatively constant during the command step. For command potentials where V_j exceeded ±40 mV, I_j declined to reach a steady-state level at the end of the pulse. However, even at the highest V_j commands, substantial I_j remained; the underlying conductance (g_{min}) is thus described as being relatively insensitive to voltage. As has been described previously (e.g., Harris et al., 1981), the decline of I_j during the voltage pulse can be described by a single exponential fit (smooth curves superimposing data in Fig. 1 *A*) after the voltage-insensitive component is subtracted. Such behavior might be expected if the macroscopic conductance (g_j) were the sum of conductances contributed by two types of channels, some of which were voltage-sensitive and some which were not.

This first hypothesis, that g_{min} is caused by a channel population different from that comprising the voltage-sensitive component, was examined by comparing proportions of maximal junctional conductance (g_{max}) that g_{min} comprises (i.e., the ratio $g_{\mathrm{min}}/g_{\mathrm{max}}$) in several systems where connexin43 appears to be the major gap junction protein expressed. The rationale for this comparison was the expectation that if two channel types were expressed independently in these cells, the contribution of the residual conductance g_{min} would vary randomly in individual cell pairs. To evaluate voltage-dependent parameters for connexin43 expressed endogenously or exogenously, normalized steady-state junctional conductances (G_{ss}) were calculated by dividing I_j at the end of the command steps by the amplitude of V_j and then normalizing these values to the initial value of g_j obtained at the beginning of the voltage pulse. These G_{ss} values and their SDs are plotted as a function of V_j for each of several types of connexin43-expressing preparations in Fig. 1 *B*. Lines superimposed on the data points are Boltzmann relations, with best fits obtained for V_j of negative and positive polarities with $g_{\mathrm{min}}/g_{\mathrm{max}} = 0.38$ and 0.36, V_0 (voltage at which the voltage-sensitive component is reduced by half) $= -58$ and 64 mV, and $A = 0.12$ and 0.13 (corresponding to about 3 gating charges moving through the transjunctional field). Note that individual data sets for each preparation indicate that above ±60 mV, the ratio of steady-state to maximal G_j is between about 0.4 and 0.5. This fraction of g_j that is insensitive to V_j is consistent with previous reports on mammalian cardiac myocytes (Rook et al., 1988; Lal and Arns-

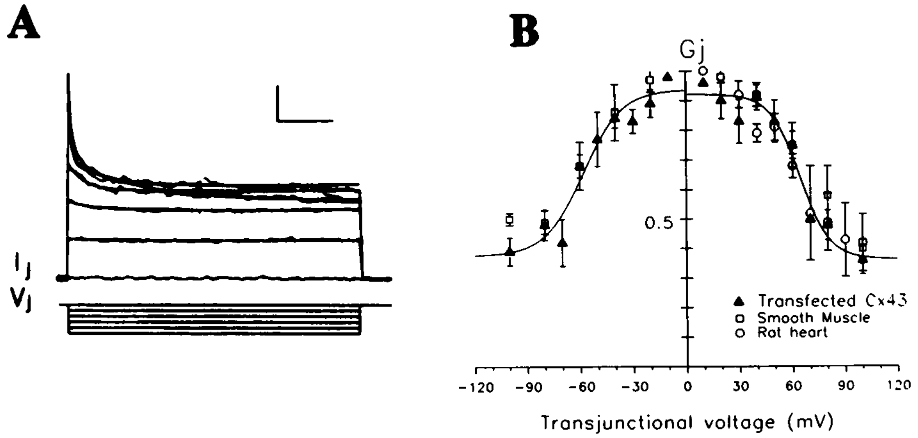
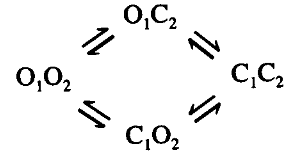


FIGURE 1 Residual macroscopic junctional conductance (g_{min}) in cells expressing connexin43. (*A*) Sample records of voltage dependence in SKHep1 cells transfected with cDNA encoding human connexin43. To determine the voltage sensitivity of connexin43 gap junctions, 20 s V_j steps between ±20 and ±100 mV were applied to one cell (only hyperpolarizing V_j values are shown in the figure). During steps ≤40 mV, junctional current (I_j) remained constant. For sustained $V_j > 40$ mV, initial junctional conductance (g_{max}) decreased to steady state values of g_j (g_{ss}). At $V_j >$ 70–80 mV, g_{ss} was constant and was not substantially reduced at higher V_j; this minimal g_{ss} value is designated g_{min}. Smooth curves superimposing the I_j data represent monoexponential declines obtained after subtraction of the steady-state current. (*B*) Dependence of g_j on V_j in various preparations expressing connexin43. g_{ss} values were normalized to initial conductances during the pulses for each experiment; normalized g_j is designated G_j. The smooth curve for V_j of each polarity corresponds to the best fit of a Boltzmann relation to normalized data obtained from human Cx43 transfected cells (▲, $n = 7$), after subtraction of the residual conductance in each experiment. Other symbols represent voltage dependence data obtained on human corporal smooth muscle (■, $n = 4$) and neonatal rat myocytes (○, $n = 5$).

dorf, 1992; Wang et al., 1992), rat astrocytes (Giaume et al., 1991), and human airway smooth muscle cells (L. DePalo and D. C. Spray, unpublished).

To determine further whether this ratio of voltage-insensitive to maximal g_j was an invariant property of connexin43, we analyzed the individual g_{min} values derived from Boltzmann relations obtained for 19 pairs of human connexin43-transfected SKHep1 cells. In these cell pairs, measured g_j varied from about 2 nS to nearly 25 nS, and in each case g_{min} was obtained as the steady state response to a 20 s 70 or 80 mV V_j command. As is shown in the major portion of Fig. 2, the data comparing these measured g_{min} and g_{max} values exhibited a curvi-linear relationship. As has been pointed out previously, the actual voltage dependence of g_j is underestimated at higher g_j values caused by series resistance of the pipettes (Moreno et al., 1991b; Wilders and Jongsma, 1993). Therefore, the measured g_{max} and g_{min} values were corrected, assuming electrode series resistance of 15 MOhm. As shown in the inset of Fig. 2, the relationship of the corrected g_{max} to g_{min} was linear ($r^2 = 0.97$), with a slope of 0.45. Together, these data show that the ratio g_{min}/g_{max} is independent of total g_j over a large range of conductance values and is similar in different cell types, providing strong evidence against the hypothesis that an independently expressed and independently gated gap junction channel population contributes the voltage-insensitive component of g_j (a similar argument was used to reach an identical conclusion for MIP26 channels in lipid bilayers (Ehring et al., 1990).

The second possible explanation of the residual conductance is that it arises from continued openings of the same population of channels (and with the same unitary conductance) that exhibit the voltage-sensitive component of g_j. One such mechanism that would give rise to non-zero open probability (P_o) at high V_j is that in the process of channel closure the dipole moment of the voltage sensor changes caused by a conformational change in the channel protein. (This explanation was proposed by Magleby and Stevens (1972) to account for the outward rectification in the ACh receptor. This possibility was also hypothesized to account for saturation of rate constants at high voltages for junctional conductance measured in amphibian embryonic cells (Harris et al., 1981) and in SKHep1 cells transfected with rat connexin32 (Moreno et al., 1991b), where the presence of g_{min} contributed a basal current at even high voltages.) Another possible contribution to g_{min} that would result in non-zero P_o, which was proposed entirely on theoretical grounds (Spray et al., 1984), is that the anatomical arrangement of gap junction channels with hemichannel gates in series could allow a circular reaction scheme, as illustrated below:



where O and C indicate whether a hemichannel's gate is open or closed, and 1 and 2 designate each of the series gates. In this scheme, a hemichannel's sensing one polarity of voltage field results in closure of its gate (either O_1C_2 or C_1O_2, depending on polarity), at which time the other hemichannel's gate can be either open or closed. If closed (C_1C_2), the transjunctional voltage drop can be entirely across it, and the first gate can reopen. The second gate will then open rapidly (O_1O_2), because of the high opening rate constant for this

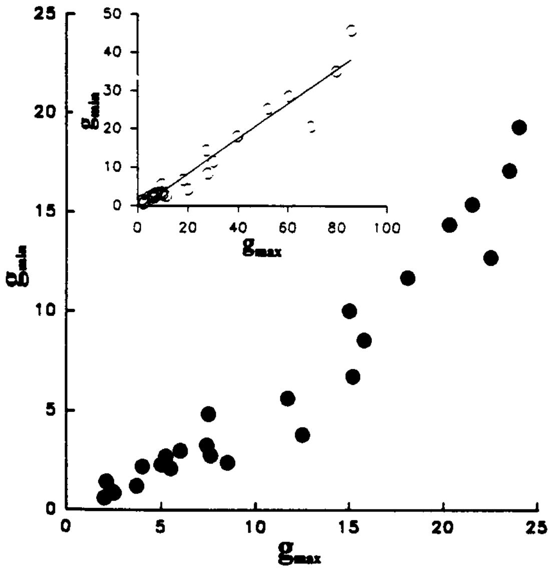


FIGURE 2 Correlation between the magnitudes of residual conductance (g_{min}) to total junctional conductance (g_{max}) in pairs of connexin43-transfected SKHep1 cells. Data were obtained for sustained V_j pulses of 70 or 80 mV. A major portion of this figure shows raw g_{max} and g_{min} data; inset shows corrected data set reflecting series resistance considerations (Moreno et al., 1991b; Wilders and Jongsma, 1993). Linear regression (r^2 = 0.97) describes a linear relationship with g_{min}/g_{max} = 0.45.

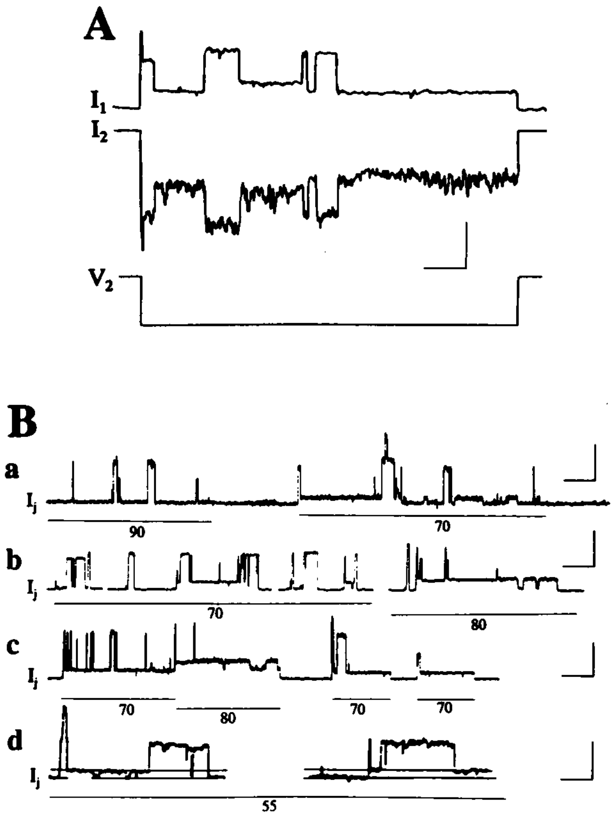


FIGURE 3 Multiple unitary conductances recorded at large V_j in cells expressing connexin43. (*A*) In response to a sustained 70-mV pulse, a poorly coupled pair of human Cx43 transfectants initially displays large channel fluctuations that cease by about the middle of the V_j pulse. Note that the level of steady-state current at the end of the pulse is smaller than that of the unitary fluctuations. Calibration bars represent 5 pA, and 2 s. (*B*) Four types of cells that express connexin43 were used to study the nature of the voltage-insensitive junctional conductance: a) SKHep1 cells transfected with human connexin43 cDNA, b) Primary cultures of human *corpus cavernosum* smooth muscle cells, c) mouse embryonic stem (ES) cell cultures that were sustained over a feeding layer of fibroblasts that secreted leukemia inhibitory factor to inhibit cell differentiation, and d) WB stem cells (modified from Spray et al., 1991b). Transjunctional voltage (mV) is indicated under each trace. At higher voltages, all cell types exhibited substate conductance of about 30 pS; events with higher conductance were brief at V_j > 50 mV, reflecting moderate voltage sensitivity of P_o. Calibration bars in A represent 5 pA, 2 s; in *B*, 5 pA, 50 mV, and 1 s.

voltage polarity, and the first will reclose. All this reopening and then closing (which was termed "steady state cycling around a circular reaction scheme") would be expected to give rise to a residual conductance because of the long rate constants involved.

If a mechanism such as these were to underlie g_{min}, unitary conductance should remain constant at high and low V_j, and P_o of the channels at the highest V_j should be reduced by no more than the g_{min}/g_{max} ratio. The rigorous analysis of P_o in the absence of pharmacological interventions has thus far only been possible in two systems in which g_j is very low (Chanson et al., 1993; Moreno et al., 1994a), and in neither of these systems is the channel formed of connexin43. Nonetheless, the very large g_{min}/g_{max} ratios for connexin43-expressing cells would require that P_o at high voltages be on the order of 0.4 to 0.5. Qualitative evidence against such an underlying mechanism for g_{min} is provided by examination of single channel currents in response to large V_j pulses in relatively poorly coupled cell pairs (Fig. 3 *A*) and after reduction of macroscopic conductance by halothane exposure (Figs. 3 *B* and 4). Fig. 3 *A* illustrates an example of a pair of transfected cells in which g_j was low. Note the behavior of the channels in the upper trace cell in response to a large V_j. Initially, multiple channels are present, and the first 10 s of the recording is dominated by large opening and closing events. Thereafter, transitions of the large size that characterize the initial decline in g_j are no longer detectable, yet substantial g_j remains at the end of the pulse. Note that the amplitude of this residual conductance is less than that of the unitary events apparent earlier in the trace and that transitions are virtually absent after the steady-state conductance level has been achieved. Both of these observations contradict the hypothesis that g_{min} is caused by non-zero P_o of the same channel size as g_{max}. A similar phenomenon was observed when g_j was rendered low by exposure of cells to halothane (Burt and Spray, 1989) and then large V_j pulses were applied; channel transitions of sizes typical for connexin43 gap junctional channels were quite brief and were superimposed on smaller background conductances with much longer open times. This behavior is illustrated in Fig. 3 *B* (*a*–*d*) for stable human connexin43 transfectants and three types of cell which exhibit endogenous expression of connexin43. Because steady-state g_j at high V_j is dominated by channel transitions with low unitary conductance, and because P_o of voltage-sensitive connexin43 channels appears to decrease to very low values at high V_j values, we conclude that the

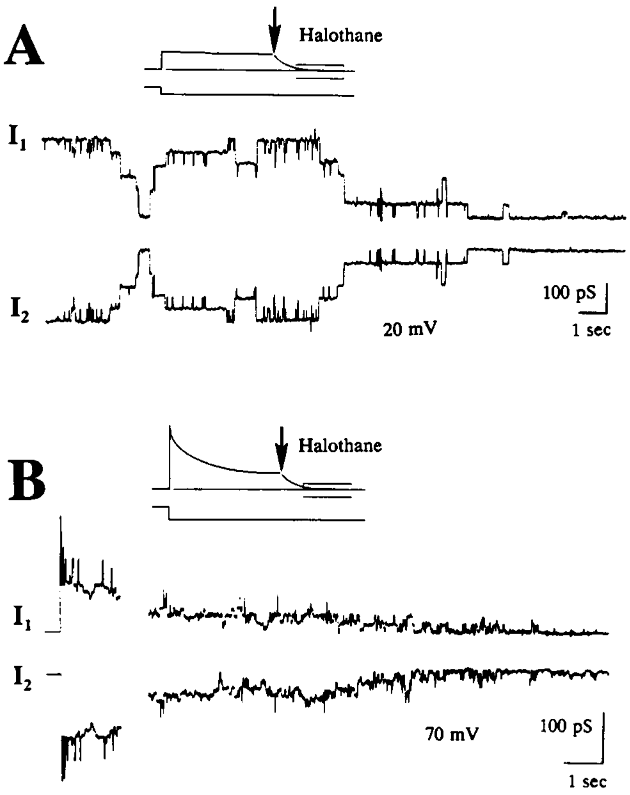


FIGURE 4 Unitary conductances of connexin43 channels in SKHep1 cells revealed during halothane (2 mM) application at two different transjunctional voltages (*A* and *B* at 20 and 70 mV, respectively). Insets demonstrate the protocols, with rectangles representing times examined in the main portion of the figure. Halothane was applied once g_{ss} was reached (arrow in inset). As cells uncoupled, currents were recognizable in recordings that occurred in both cells simultaneously and were of opposite polarity but of equal magnitudes. Unitary junctional conductance (γ_j) was calculated by dividing the amplitude of the unitary current by the amplitude of the transjunctional voltage. Note that at high V_j brief large events are initially present, but that the amplitudes of the unitary currents recorded at steady state (*B*) are much smaller than recorded at low voltage (*A*).

residual conductance is not attributable to saturation of rate constants for the voltage-dependent channel. Another possible mechanism is a voltage-dependent shift from modes of long and, thus, resolvable channel openings at low voltages to very brisk and unresolvable events at high voltages ("flickering"). If such a shift occurred, we would expect that event sizes would appear graded at intermediate V_j, which was not observed. We conclude that the hypothesis of a single channel population with non-zero P_o at high V_j does not account for the residual conductance (g_{min}).

The third hypothesis is that connexin43 channels possess a subconductance, or partially conducting state. The results described above (Fig. 3) suggest that the unitary conductance of gap junction channels in connexin43-expressing cells is smaller at high V_j than at lower potentials. To test this hypothesis more rigorously, we applied the protocol depicted in Fig. 4 *A* and *B* (*insets*) to pairs of SKHep1 cells transfected with human connexin43. First, sustained V_j of various amplitudes were applied. Subsequently (at *arrows* in Fig. 4 insets), g_j was reduced by the application of halothane, and the unitary conductances measured at different V_js were compared. At V_js $\leq$ 30 mV, all events were essentially either 60–70 or 90–110 pS in amplitude (Figs. 4 *A* and 5), which we have previously shown to be typical of channel sizes in this preparation when $V_j < 50$ mV (Moreno et al., 1992b, 1994). By contrast, application of halothane at $V_j = 70$ mV, a voltage at which G_{ss} approaches g_{min}, the channel events are predominantly much smaller after relaxation of I_j following voltage step (Figs. 4 *B* and 5). We conclude from these experiments that the unitary conductances comprising g_j at high V_js are different from those occurring at lower V_js. Together with the covariation in g_{min}/g_{max} in various cell types (Fig. 1) and in a single cell type over a wide range of g_j values (Fig. 2) which suggest that g_j is not the result of independent expression of different types of junctional channels, these data indicate that connexin43 gap junction channels possess a lower conductance state whose occupancy is favored at high V_js. Kinetic studies on the relaxation of junctional currents between cells connected by connexin43 gap junction channels have previously indicated the presence of multiple time constants (Moreno et al., 1992a; Wang et al., 1992; Lal and Arnsdorf, 1992). The least voltage-dependent component, with a time constant >tens of seconds, is most prominent at high V_js and seems likely to correspond to the macroscopic conductance contributed by 25–30 pS events.

Conductance substates appear to be a feature of many types of gap junction channels (see Ramanan and Brink, 1993; Churchill and Caveney, 1993; and references below). Moreover, in chick embryonic heart, multiple conductance states of junctional channels are recorded (Chen and DeHaan, 1992), although expression of multiple connexins is also evident at these developmental times and may contribute to the multiple unitary conductances observed (Veenstra et al., 1992). Our finding that g_{min} of connexin43 gap junction channels is ascribable to voltage dependent occupancy of a conductance substate may be extended to most, but not all, other types of gap junction channels. For example, in an insect cell line, residual conductance at high voltages has been attributed to substates (Weingart and Bukauskas, 1993; Weingart et al., 1993). Moreover, the endogenous gap junction channels of SKHep1 cells (Moreno et al., 1994a) and of *Xenopus* connexin38 expressed endogenously or in SKHep1 cells (Spray et al., 1979; Moreno et al., 1993b) both exhibit low conductance states at high V_j and their infrequent occurrence is consistent with the low g_{min} for these channels ($g_{min}/g_{max} = 0.05$ to 0.10). However, not all junctional channels have this property: rat Schwann cell pairs exhibit quite voltage-dependent, 40-pS channels, where $g_{min}/g_{max} = 0$ and substates are not seen even at V_j 4–6 times as high as V_0 (Chanson et al., 1993).

One question that arises from this study is whether the various conductance states of connexin43 channels reflect different effective pore radii. Lucifer Yellow diffusion between pairs of amphibian embryonic cells was not detected when g_j was reduced by high V_j, but interpretation was ambiguous due to the low g_{min} in this system (Spray et al., 1979). Using the same preparation, g_j was correlated with junctional

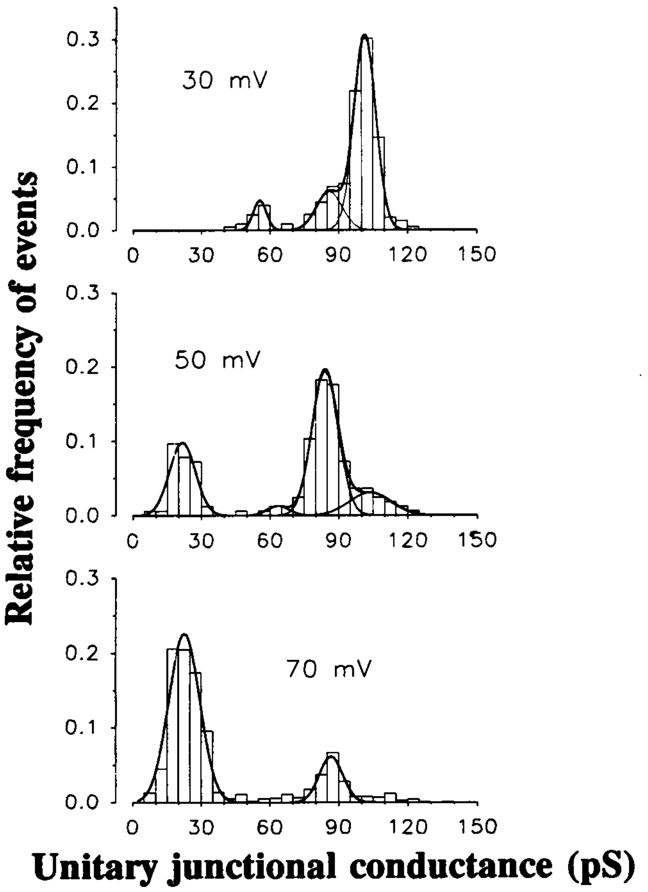


FIGURE 5 Histograms showing the relative frequency of events of unitary conductances obtained at low (30 mV), moderate (50 mV), and high (70 mV) transjunctional voltages in the experiment illustrated in Fig. 4. Amplitudes of current transitions were measured at each transjunctional voltage (206, 167, and 464 events recorded at 30, 50, and 70 mV, respectively) and converted to unitary conductance by dividing by V_j the relative frequency of each γ_j value was obtained after grouping values in 5-pS bins. Gaussian fits to frequency histograms (——) were obtained using Peak Fit software (Jandel Scientific).

permeability (P_j) to tetraethylammonium ions; g_j/P_j was found to be constant even when g_j was reduced to low levels by sustained large V_j pulses (Verselis et al., 1986). The conclusion that junctional channel closure was all-or-none rather than graded, together with recent observations that *Xenopus* connexin38 channels exhibit conductance substates (Moreno et al., 1992b), suggests that permeability of the substate and main state does not differ greatly despite the difference in unitary conductance. In cardiac myocytes and in SKHep1 cells transfected with human and rat connexin43, unitary conductances obtained using internal solutions containing KGluconate (Rook et al., 1992; Takens-Kwak et al., 1994, in press) can be compared with those obtained in this study using CsCl (Fig. 5). In each of these cell types, unitary conductance of each conductance state was lower by about 30% when measured using KGluconate internal solution; nevertheless, the g_{min}/g_{max} ratios obtained with the different internal solutions were very similar. Thus, either anionic permeabilities of the different conductance states of connexin43 channels are low enough to render this interpretation ambiguous, or the permeabilities of these states do not differ substantially.

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