Evidence for heteromeric gap junction channels formed from rat connexin43 and human connexin37


Evidence for heteromeric gap junction channels formed from rat connexin43 and human connexin37. Am. J. Physiol. 273 (Cell Physiol. 42): C1386–C1396, 1997.—Homomeric gap junction channels are composed solely of one connexin type, whereas heterotypic forms contain two homomeric hemichannels but the six identical connexins of each are different from each other. A heteromeric gap junction channel is one that contains different connexins within either or both hemichannels. The existence of heteromeric forms has been suggested, and many cell types are known to coexpress connexins. To determine if coexpressed connexins would form heteromers, we cotransfected rat connexin43 (rCx43) and human connexin37 (hCx37) into a cell line normally devoid of any connexin expression and used dual whole cell patch clamp to compare the observed gap junction channel activity with that seen in cells transfected only with rCx43 or hCx37. We also cocultured cells transfected with hCx37 or rCx43, in which one population was tagged with a fluorescent marker to monitor heterotopic channel activity. The cotransfected cells possessed channel types unlike the homotypic forms of rCx43 or hCx37 or the heterotypic forms. In addition, the noninstantaneous transjunctional conductance-transjunctional voltage (G/Vj) relationship for cotransfected cell pairs showed a large range of variability that was unlike that of the homotypic or heterotypic form. The heterotypic cell pairs displayed asymmetric voltage dependence. The results from the heteromeric cell pairs are inconsistent with summed behavior of two independent homotypic populations or mixed populations of homotypic and heterotypic channels types. The G/Vj data imply that the connexin-to-connexin interactions are significantly altered in cotransfected cell pairs relative to the homotypic and heterotypic forms. Heteromeric channels are a population of channels whose characteristics could well impact differently from their homotypic counterparts with regard to multicellular coordinated responses.

GAP JUNCTION CHANNELS provide an intercellular pathway between adjacent cells for ions and small solutes such as second messenger molecules. Unlike other ion channels, the gap junction channel consists of two hemichannels or connexons, each contributed by one of two adjacent cells. Hemichannels are oligomers, formed by six protein subunits called connexins. Connexins belong to a gene family with 13 identified members (3). On the basis of hydrophobic studies, the connexins are predicted to have four transmembrane domains, with the amino and the carboxy termini located on the cytoplasmic side. Adjacent cells can synthesize the same or different connexins, and in any one cell two or more connexins can potentially be coexpressed. Therefore, three different generic types of channels are possible: 1) homomeric/homotypic channels, in which both interacting hemichannels are composed of the same connexin, 2) heterotypic channels, in which the gap junction channel is formed by two hemichannels each composed of a different connexin, and 3) the heteromeric channel, in which each hemichannel contains at least two different connexins.

Macroscopic junctional currents for homotypic and heterotypic channels have been studied using the Xenopus laevis oocyte expression system. In general, homotypic channels show symmetric voltage dependence, whereas heterotypic forms generate asymmetric junctional currents in response to symmetric transjunctional voltage (Vj) steps (1, 2, 22, 29, 31–34). Heteromeric forms have been implicated in two studies using the oocyte system (1, 28) and in a study of connexin43 (CX43) containing osteoblasts transfected with connexin45 (11). Biochemical analysis of coexpressing cell systems has also indicated the presence of heteromeric forms (9, 21).

A few predictions are possible for the heteromeric case when both the unitary currents and macroscopic currents can be monitored. The number of heteromeric channel types possible with only two coexpressing connexins is large. If human connexin37 (hCx37) and rat connexin43 (rCx43) are coexpressed and are freely capable of mixing and forming heteromeric hemichannels, then in any individual cell there are 26 or 64 possible forms and 4,096 heteromeric gap junction channel types could, in theory, exist. The chances of forming a homotypic gap junction channel are then 1 in 4,096 (0.0002). If a sixfold symmetry axis is assumed and, furthermore, if interaction energies are rotationally symmetric, but not chiral, and if the energies of interaction for one hemichannel are not influenced by the configuration of another, then far fewer heteromeric forms are predicted. In this case, there are 12 possible distinct heteromeric hemichannel forms and 1 of each homomeric hemichannel form, 14 forms total within a cell. For any two coupled coexpressing cells, there are 14 × 14 possible combinations or 196 types of gap junction channel types possible, including the formation of a heterotypic type. For all 196 forms, only one would be a homotypic hCx37 and one a rCx43 and two configurations of a single heterotypic form would exist (hCx37-rCx43 vs. rCx43-hCx37). In addition, a homomeric hemichannel linked to any heteromeric channel in
an adjacent cell is considered to be a heteromeric gap junction channel. It is impossible to know whether there are significant differences in gating or conductance for so many potential forms. However, this scenario predicts the probability of observing a homotypic hCx37 channel or rCx43 channel to be \(<0.0052\) (1/196). The probability of observing heterotypic channel types would be only slightly better (0.0104). For the cases given, the observation of channel conductances unlike homotypic hCx37 or rCx43 provides strong evidence for the presence of heteromeric and/or heterotypic forms. Figure 1 shows a schematic for the 14 total forms possible in any one cell coexpressing 2 mixable connexins. There are 12 mixed hemichannels and 2 homomeric hemichannels. If the ratio of Cx43 to Cx37 is large, then the predicted forms would tend to arise from the heteromeres shown on the left of Fig. 1. The inverse would result in forms arising from the heteromeres shown on the right of Fig. 1.

Our aim was to investigate the effect of cotransfection of two connexins via dual whole cell patch clamp. We examined mouse neuroblastoma cells [Neuro-2a (N2a)] cotransfected with hCx37 and rCx43 and compared the data to those obtained from cells with homotypic channel's or heterotypic channels.

**MATERIALS AND METHODS**

Cell culture. Experiments were performed with mouse N2a neuroblastoma cells (American Type Culture Collection CCL-131), since these cells are normally devoid of any connexin expression and contain no endogenous gap junction channels that we have detected (19, 27). N2a cells were individually transfected with the hCx37 cDNA or with the rCx43 cDNA in the vector pSFFV-neo (7). This vector drives eukaryotic expression with the SV40 early gene promoter/enhancer, causes no splicing of the expressed mRNA, uses the SV40 polyadenylation signal, and allows selection with the non-cross-resistant antibiotic Zeocin (Invitrogen). N2a/hCx37 cells were transfected with linearized pZeoSV-rCx43 using lipofectin reagent (Life Technologies), and individual, single clones were selected with 0.25 mg/ml Zeocin. RNA was prepared from these cells and from cultured bovine aortic endothelial cells (BAECs) as a positive control and analyzed by RNA blotting with an equal mixture of 32P-labeled probes for rCx43 and hCx37 (19). Immunoblots of whole cell lysates (50 µg total protein/lane) were reacted with mouse monoclonal antibodies to a synthetic peptide representing amino acids 252–270 of rCx43 (Chemicon) or affinity-purified rabbit polyclonal antibodies directed against a bacterial fusion protein representing the carboxy-terminal tail of hCx37 (8), followed by incubation with peroxidase-conjugated secondary antibodies and detection by enhanced chemiluminescence (Amersham) as described in Ref. 13. Immunofluorescence microscopy was performed with these same primary antibodies according to Ref. 13.

Electrophysiology. Experiments were carried out on transfected N2a cell pairs with the dual whole cell voltage clamp method (5, 16, 25, 27). During the experiments, the cells were bathed in a solution containing (in mM) 180 CsCl or KCl, 1 CaCl2, 1.8 MgCl2, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) (pH 7.1–7.3). For the whole cell recording, the pipette solution contained (in mM) 180 CsCl, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid, 0.1 CaCl2, 1.8 MgCl2, and 10 HEPES (pH 7.0). In some experiments, the pipette and external bathing media contained 110 mM salt rather than 180 mM and the salts used were NaCl, LiCl, KCl, RbCl, or tetramethylammonium chloride. Flow of the intercellular junctional current (Ij) was induced by different voltage protocols. For the recording of the macroscopic current-voltage relationship, both cells were held at a holding potential of 0 mV. From this holding potential, the voltage of one cell was stepped to varying voltages (Vj of \(\pm 10–150\) mV or \(\pm 120\) mV, in 20-mV increments). For all experiments shown, the first voltage step was negative. After holding the potential for 400 ms or 4 s, respectively, the voltage was flipped to the equal but opposite polarity for the same time. For single-channel or multichannel recordings, one cell of the pair was stepped to different voltages for many seconds to minutes to observe a number of channel events. For current recording data and analyses, see Brink et al. (5). All macroscopic records were filtered at 1 kHz,
The term “noninstantaneous” is used to indicate the ms or 4 s were used to compute the junctional conductance. mRNA (3.3 kb) (Fig. 2, lane 4) and showed hybridization to the expected control, since these cells express both Cx37 and Cx43 initially determined by RNA blotting (Fig. 2). RNA derived from bovine aortic endothelial cells (lane 1), 2 independent cotransfected clones of Neuro-2a (N2a) cells (lanes 2 and 3), N2a cells solely transfected with Cx37 (lane 4), and untransfected N2a cells (lane 5) was hybridized with a mixture of probes for Cx37 and Cx43. Arrowheads, migration of the 18S and 28S rRNAs. Cx43 probe hybridizes to an endogenous mRNA of 3.3 kilobases (kb) and a transfection-derived mRNA of 2 kb. Cx37 probe hybridizes to an endogenous mRNA of 1.7 kb and a transfection-derived mRNA of 2.7 kb. Clones 9 and 10 (the 2 cotransfected clones) are shown and represent those used for dual whole cell patch clamp.

RESULTS

Coexpression in N2a cells. Expression of connexins in cells derived from clones of transfected N2a cells was initially determined by RNA blotting (Fig. 2). RNA derived from cultured BAECs was used as a positive control, since these cells express both Cx37 and Cx43 mRNAs (14) and showed hybridization to the expected endogenous Cx37 mRNA (1.7 kilobase (kb)) and Cx43 mRNA (3.3 kb) (Fig. 2, lane 1). Neither the Cx37 nor the Cx43 probe hybridized to the RNA derived from untransfected N2a cells (lane 5). Cells transfected solely with hCx37 showed hybridization to an mRNA of ~2.7 kb, as expected for the recombinant-derived mRNA (lane 4). This 2.7-kb hCx37 mRNA was also detected in total RNA derived from individual cotransfected clones (lanes 2 and 3), which also showed hybridization to a band of ~2 kb (the size expected for the mRNA derived from pZeoSV-rCx43). The identity of the rCx43 and hCx37 bands was confirmed by blots hybridized with each probe alone (data not shown). The intensity of hybridization of hCx37 and rCx43 probes was approximately equivalent; because this pointed to expression of a similar magnitude, these clones were chosen for further experiments. Production of connexin proteins in the serially transfected N2a cells was examined immunochemically. Immunoblots of whole cell lysates of the coexpressing N2a cells showed a major immunoreactive Cx37 band of ~37 kDa and an immunoreactive Cx43 band of ~45 kDa, which were not detectable in untransfected N2a cells or N2a cells transfected with the plasmid vectors alone (Fig. 3). There was relatively little Cx43 produced by these cells, as indicated by comparison with blots of heart homogenates (not shown), requiring substantial exposure of the N2a blots and an increase in the background of nonspecific bands (compare lanes 1 and 2 in Fig. 3). We attempted to visualize the connexin proteins produced in these cells by immunofluorescence. Staining with either anti-Cx37 or anti-Cx43 antibodies (which will intensely stain other Cx37- and Cx43-expressing cells such as aortic endothelial cells) yielded only occasional small spots of fluorescence between cells, suggesting that there were only very small gap junctions between these cells (data not shown).

Single-channel conductance in homomeric/homotypic rCx43 and hCx37 cell pairs. To examine the effect of the coexpression of two different connexins on junctional coupling, experiments were performed on cells expressing solely hCx37 or rCx43 and then compared with cells coexpressing hCx37 and rCx43. Weakly coupled N2a cell pairs allowed the observation of single-channel events. The hCx37 channel was previ-
ously described as a 330- to 400-pS channel with a 63-pS substate (27). rCx43 has also been characterized and has a unitary conductance of 80–90 pS in 150 mM salt (25) and 96 pS in 120 mM KCl (24). Figure 4 illustrates the homomeric forms of hCx37 and rCx43 transfected into N2a cells. In both cases, the pipette solution contained 180 mM CsCl, as described in MATERIALS AND METHODS. In this study, the unitary conductance of hCx37 was 360 pS and for rCx43 it was 115 pS. Both of these values are in the same range as previous reports for hCx37 (27) and rCx43 (Refs. 24, 25, 30; see Ref. 5 for hCx43). Both records illustrate the typical gating found for these two homomeric connexin types. A substate is present in the recording showing the hCx37 homotypic channel. Its conductance was 66 pS [1/(25 mV/1.65 pA)]. rCx43 shows few substates when Vj values of 70 mV or less are used.

Single-channel conductance in heterotypic cell pairs of rCx43 and hCx37. Heterotypic cell pairs also revealed unitary activity, as shown in Fig. 5. At the single-channel level, the gating of the heterotypic channel is asymmetric. For Fig. 5, a step from Vj of 0 to −60 mV was applied and subsequently stepped briefly back to Vj = 0 mV and then to +60 mV. The recordings are from the Cx43 cell that was held at a Vm = 0 mV. The stepping cell was the Cx37 cell. The pipette solution was the 180 mM CsCl solution. Stepping the potential of the Cx37 cell to positive values resulted in a gating behavior for the heterotypic channel that was similar to homotypic Cx37. The opposite polarity revealed gating behavior that appears almost voltage independent. The unitary conductance for the positive step shown in Fig. 5, bottom, revealed a transition of 175 pS and a substate of 55 pS. We never observed a complete closure from the 175-pS state to zero conductance. Instead, the closure to the 55-pS substate and a subsequent opening back to the 175-pS level were common. The conductance of the intermediate state was 120 pS. The opposite polarity generated a unitary conductance of 98 pS. Some substate activity is also apparent. This result is the same as that found for three other experiments of paired heterotypic channels that were weakly coupled. These conductances and the asymmetric behavior are very different from the homotypic forms shown in Fig. 4 and the heteromeric forms shown in Fig. 6.

Single-channel conductance in cell pairs cotransfected with rCx43 and hCx37. In weakly coupled pairs of cotransfected cells, unitary channel events could be observed. Figure 6 shows representative current traces recorded from a multichannel preparation. The pipette solution was 180 mM CsCl. Six different conductance levels can be observed in Fig. 6A: 280, 220, 200, 150, 70, and 35 pS. Again, the channel activity was monitored from the cell held at Vm = 0 mV. Three of those conductance states were also observed in the current traces presented in Fig. 6B (opposite polarity). The majority of these conductance states are unlike the unitary conductance levels of the homomeric hCx37 or rCx43 channels (24, 25, 27). Another feature of the cotransfected cell pairs is illustrated in Fig. 6, A and B. During the application of voltage steps, no transitions to Ij = 0 pA could be observed. This indicates that one or more channels were open at all times. Assuming there is only one constantly open channel that means a unitary conductance of 130 pS (50 mV/6.4 pA) is found for that channel. The conductance states recorded from other cotransfected cells yielded similar observations (n = 5). The multichannel recordings are inconsistent with a homomeric/homotypic population of hCx37 and rCx43 channels. They are also inconsistent with heterotypic behavior. The data provide strong evidence for the presence of heteromeric and/or heterotypic forms. The 280-, 200-, and 150-pS channels have no identifiable counterparts for any of the conductive states of the
homotypic forms or the heterotypic forms. The 70- and 35-pS conductances cannot unequivocally be attribut-
able to substates of Cx37 or Cx43 or as substates or main states of heteromeric forms.

Macroscopic transjunctional current in homomeric, heterotypic, and heteromeric gap junctions. Many of the cell pairs examined generated macroscopic $I_j$ in which unitary activity was not observable. These data provided a monitor of the voltage-dependent behavior for the homotypic, heterotypic, and heteromeric forms.

The range of junctional conductance was 1.0–10 nS for hCx37, 0.3–11 nS for rCx43, 0.1–9 nS for the heterotypic forms, and 0.25–11 nS for the cotransfected cells. In cell pairs that produced macroscopic $I_j$, the voltage-dependent inactivation of the current was examined. Figure 7 shows original macroscopic current traces recorded from the homotypic hCx37 and rCx43 cell lines. The hCx37 recording (Fig. 7A) exhibits strong voltage dependence. Only at a $V_j$ of 20 mV or less is the $I_j$ constant over the duration of the voltage pulse. The data shown for hCx37 demonstrate the strong voltage-dependent inactivation of $I_j$. Neither a variation of the main cation in the pipette solution (Cs⁺, Na⁺, K⁺, Li⁺, tetramethylammonium cation, Rb⁺) nor a prolongation of the voltage pulse influenced the shape of the curve. Weaker voltage dependence is observed for rCx43 (Fig. 7B). Here, a time- and voltage-dependent decay of the current can first be observed at a $V_j$ of 50 mV. Figure 7, C (n = 12) and D (n = 5), shows the noninstantaneous conductance (400 ms after the initiation of the step) plotted against $V_j$. The data from each experiment are displayed for both cell types. For both homotypic forms, the $G_j$ vs. $V_j$ relationships are the same as reported previously (25, 30, 33, 34).

Heterotypic hCx37 and rCx43 junctional conductance shows voltage-dependent asymmetry that is consistent with previous observations in oocyte pairs (32–34). The data shown in Fig. 8, A and B, show the two recording modes. Either the rCx43-transfected cell was stepped (Fig. 8A) or the hCx37-transfected cell was stepped using the short protocol (0.4 s) described in MATERIALS AND METHODS. The records shown in Fig. 8A are measurements of $I_j$ recorded in the hCx37 cell held at $V_m = 0$ mV, whereas Fig. 8B shows currents recorded in the rCx43 cell held at $V_m = 0$ mV. The data are from two different experiments. Figure 8C summarizes the data from six experiments, which are all plotted relative to the rCx43 cell being stepped and the hCx37 cell being held at $V_m = 0$ mV. The $V_o$ (which is the $V_j$ where $G_j$ is one-half the maximal measured conductance for $G_j$) was ~70–80 mV for the negatively gated rCx43 positively gated hCx37 heterotypic case. Pipette solu-
tions of 110 mM KCl and 180 mM CsCl were used in experiments for Fig. 8C. The data in Fig. 8 are consistent with previous studies that indicate that Cx37 gates positively and Cx43 gates negatively (15, 18).

Data obtained from cell pairs transfected with both hCx37 and rCx43 are much more scattered than those obtained from homotypic or heterotypic cell pairs, especially when the short protocol (400-ms step duration) is employed (Fig. 9). The voltage-dependent decrease of the normalized conductance is shifted to higher voltages than those observed for homotypic or heterotypic when a short (400 ms) protocol is used. The longer protocol reveals a $G_j/V_j$ relationship that approximates that obtained for rCx43.

**DISCUSSION**

Morphological studies have shown that different connexins are not only coexpressed in the same tissue but that they also may occur in identical gap junctional plaques (10, 17, 18). Stauffer (21) demonstrated, after coexpression of Cx32 and Cx26 in an insect cell line, the occurrence of both proteins in the same gap junctions. Although this evidence is compelling, mass measurements have not been able to confirm Cx32-Cx26 heteromeric forms (20). Our approach was to examine if coexpression of hCx37 and rCx43 in the same cell line results in conductance and gating properties atypical of homotypic and heterotypic gap junction channels. We performed these studies by stable transfection of N2a cells with individual connexin sequences and cotransfection with two connexins, since the parental N2a cells are devoid of connexin expression and detectable gap junction channels. Previous studies of gap junction channels in N2a cells transfected with hCx37 and rCx43 (19, 25, 27), in which macroscopic and microscopic records allowed determination of voltage-dependent behavior and illustration of unitary activity, are consistent with our present findings. N2a transfectants produce relatively small amounts of gap junction proteins; therefore, detection of single-channel events are facilitated. We typically detected <100 channels in a cell pair. We were able to confirm expression of Cx37 and Cx43 in the cotransfected N2a clones by RNA blotting and immunoblotting. We could not compare localization of these connexins by immunofluorescence, possibly because the amounts of connexin produced...
were below the detection limits of this system. Estimates by others of the sensitivity of immunostaining (23) suggest that connexin immunoreactivity would only be visualized if all of the connexin channels were concentrated in one or two plaques and, furthermore, each channel needed to be labeled. Unfortunately, the ratios of the two connexin types can neither be modulated nor rigorously determined. The biochemical data illustrate that both connexins are synthesized. The electrophysiological data indicate that the two connexins in question are capable of mixing, based on channel conductances and different voltage dependence relative to homotypic and heterotypic forms.

Cx37 and Cx43 were chosen because they are known to coexpress in vivo in endothelial cells (19). Cx37 and Cx43 are known to form heterotypic channels as well (33, 34), a finding confirmed in this study. On the experimental level, hCx37 and rCx43 offer the possibility that they exhibit distinctive electrophysiological properties. Cx37 is the most voltage-sensitive connexin, with a half-maximal inactivation at ±25 mV and a single-channel conductance of 350–400 pS (25, 27). On the other hand, Cx43 exhibits a weak voltage dependence, with a half-maximal inactivation at Vj values of ±60–70 mV (30). Figure 4 shows examples of hCx37 and rCx43 under identical Vj and ionic conditions. The unitary conductance of hCx37 is in the range previously reported, as is that of rCx43. The records also illustrate the gating behavior of the two homotypic gap junction channels. The heterotypic channels of hCx37 and rCx43 represent a novel observation. Our data indicate that the heterotypic channel has configuration-dependent conductance.

The coexpression of hCx37 and rCx43 resulted in a channel population in which gating behavior could not be predicted by the two connexins alone. In the absence of any heteromeric gap junction channel, only three types of gap junction channels may occur. Two cases
have both hemichannels of a gap junction channel formed of either rCx43 or hCx37 (homotypic), and the other type has two heterotypic forms (mirror images). The \( V_o \) for these cases are 70, 25, and 80 mV for 400-ms protocol, respectively. Although one would expect a variability depending on the expression level of the different channels, the resulting \( G/V_j \) relationship should display a \( V_o \) between 25 and 80 mV. This was not the case. The N2a cell line cotransfected with hCx37 and rCx43, therefore, did not exhibit the voltage-dependent characteristics of either homotypic or heterotypic forms. This result implies that the homomeric voltage gate requires some form of interaction between the individual connexins, which might not be implemented or is impaired in the heteromeric channels.

In those experiments for which unitary activity was observed for cotransfected cell pairs, no unitary event like hCx37 or rCx43 could be observed. A number of other channel conductances were observed (Fig. 4). Comparison with Fig. 6 indicates that there are channel types with conductances intermediate between the two homotypic forms and smaller conductance states as well. Two general questions arise. How many different types of channels can be predicted to occur when hCx37 and rCx43 are freely capable of mixing and forming heteromeric hemichannels and what is the probability of occurrence for homotypic channels under such conditions? With the assumption of sixfold symmetry, 196 types of gap junction channel are then possible between two coexpressing cells. If only a limited number of heteromeric hemichannel types were allowed, 3 vs. 12 for example, then the total number of combinations is 3 \( \times 5 \) or 25 (3 heteromeric forms and 1 each of the homomeric hemichannels equals 5). The total number of gap junction channel types is 25 vs. 196. With this scheme, the probability of observing a homotypic channel of either type is 0.04 and the probability of observing heterotypic channels becomes 0.08. For the case of only two heteromeric forms, the probabilities of observing homotypic or heterotypic channels are 0.063 and
0.13, respectively. For the case of one heteromeric form with one each of the homomeric hemichannel forms (3 \times 3 = 9 total combinations), the probabilities of observing a homotypic channel are 0.1 and 0.2 for the heterotypic mirror image forms.

In our experiments, homotypic rCx43 exhibited a single-channel conductance of 115 pS in 180 mM CsCl, which is in good accordance with previous results (5, 25, 27). For homotypic hCx37, the single-channel conductance was 360 pS in 180 mM CsCl, which is similar to previous reports (27). Our experimental data for the cotransfected cells show channel conductance states of 280, 220, 200, 200, 150, 70, and 35 pS, with 180 mM CsCl as the major solute in the pipette. The majority of single-channel conductances cannot be described by a homotypic/heterotypic population of hCx37 and rCx43 channels. Instead, it points to a free combination of connexins in one hemichannel. We observed multichannel activity in a number of cotransfected cell pairs and, as Fig. 5 illustrates, could identify a number of conductive states.

Even with the assumption that each conductive state arises from a distinct heteromere, this does not represent an upper limit for the number of heteromeric forms but does equate to a lower boundary. It is entirely possible that many heteromeric forms have similar or identical conductances and/or gating properties and would thus be indistinguishable from one another using dual whole cell patch clamp methods.

Could the unitary conductances seen in the coexpressing cell pairs be substates of homotypic hCx37 or rCx43? If this were the case, then the macroscopic records would reflect \( G/V \) relationships intermediate between homotypic hCx37 and rCx43. Furthermore, the multitude of states seen in the weakly coupled pairs is not consistent with the magnitude or frequency of subconducting states for either homotypic form. hCx37 has a pronounced 63-pS substate (25, 27) that persists for seconds, whereas rCx43 has two easily observed substates of \( \sim 60 \) and \( \sim 30 \) pS that are usually infrequent unless large \( V_J \) steps are employed (26, 30).
The macroscopic Ij and unitary current data illustrated here are consistent with the formation of functional heteromeric gap junction channels by two members of group II or α-type connexins (3, 12). The single-channel data shown here provide evidence for a number of conductive states that cannot be identified as either homotypic main states or substates. The same is true for the heterotypic channels. The existence of heteromeric forms implies that cells coexpressing connexins may produce a spectrum of channel types, each form potentially having unique specific permselective and gating characteristics. This potential plethora of channel types could well be critical to multicellular processes such as differentiation or coordinated contraction in both excitable and nonexcitable cells. For example, alterations in the ratios of two connexins, leading to possible changes in distribution of heteromeric channels, may have functional consequences for intercellular communication in endothelial cells. An example is reported in Larson et al. (14), which recently demonstrated that abundances of Cx37 and Cx43 are differentially affected by cellular growth, density, and tumor growth factor-β.

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