Cardiac Gap Junction Channels Show Quantitative Differences in Selectivity

Virginijus Valiunas, Eric C. Beyer, Peter R. Brink

Abstract—Several proteins including connexin40 (Cx40) and connexin43 (Cx43) form gap junctions between cells of the heart; they may be found separately or may be coexpressed. These connexins form channels with differing conductance and permeability properties. Cx40 and Cx43 are each required for normal electrical conduction between cells in different regions of the heart. We hypothesized that the major difference between these connexins might be in their selective intercellular passage of small molecules such as second messengers, which can be assessed using biologically inert fluorescent probes. Therefore, we designed experimental paradigms to quantitate the permeability properties of these cardiac connexins using simultaneous measurement of junctional conductance (gj) by the double whole-cell patch-clamp technique and intercellular transfer of Lucifer Yellow (LY) by fluorescence microscopy. These studies were performed in HeLa cells stably transfected with Cx40 or Cx43 or cotransfected with both connexins. We found that homotypic Cx43 channels were about 5 times more permeable to LY than homotypic Cx40 channels (flux of ≈1560 versus ≈300 molecules/channel per second). Channels between heterotypic (Cx40-Cx43) cell pairs and between pairs of coexpressing cells exhibited intermediate LY permeability. The permeability ratio for LY relative to monovalent cation (K+) ranged from 0.0025 for Cx40 to 0.028 for Cx43. These permeability ratios suggest that the connexins are highly selective for solutes in the size and charge range of many second messengers. Moreover, the data indicate that coexpression of connexins does not generate unique permeability characteristics, but rather results in an intermediate permeability for solutes involved in metabolic/biochemical coupling. (Circ Res. 2002;91:104-111.)

Key Words: connexin40 ■ connexin43 ■ electrophysiology ■ gap junction ■ permselectivity

Gap junction channels play a critical role in the propagation of action potentials in myocardium by allowing intercellular passage of current-carrying ions. They also are essential for the exchange of larger molecules in both excitable and nonexcitable multicellular tissues. Each gap junction channel is composed of two hexameric hemichannels (connexons) formed of subunit proteins called connexins. Three different connexins (Cx43, Cx40, and Cx45) are expressed by cardiac myocytes.1–3 These connexins exhibit different, but overlapping distributions in various regions of the heart.4 Each of the cardiac connexins forms channels by itself (homomeric/homotypic) that have some differing biophysical properties, including unitary conductances and voltage gating.5–7 Pairing of cells expressing different connexins (to form a heterotypic channel) or coexpression of multiple connexins in the same cell (leading to the potential formation of heteromeric channels) may produce additional unitary conductances and differences in voltage-dependent gating.8,9 However, it is not clear that these differences would lead to significant differences in cardiac electrical conduction. It is quite possible that the most physiologically consequential difference between different connexin channels is their differing abilities to permit intercellular passage of molecules larger than current carrying ions (such as second messengers). However, direct measurement of the transit of specific endogenous messengers is problematic, because they have finite life-spans of seconds to minutes. The experimental study of biologically inert exogenous fluorescent probes eliminates this difficulty. Many gap junction channels have been shown to pass charged probes with minor diameters of ≈1 nm,10 but neither the flux of probe per channel nor the permeability ratios have been determined. One potentially useful parameter for dissecting biophysical properties of gap junction channels is the permeability ratios for large charged solutes relative to monovalent ions.

To obtain a clearer picture of gap junction channel selectivity, we studied HeLa cells transfected with Cx43 (HeLaCx43) or Cx40 (HeLaCx40) or cotransfected with Cx43 and Cx40 (HeLaCx40/Cx43).9 We measured the flux of Lucifer Yellow (LY) while simultaneously determining junctional conductance. Measurement of junctional conductance...
allowed estimation of the number of active gap junction channels passing the probe and further, allowed the determination of LY flux/channel. Our approach facilitated a quantitative comparison of the channel selectivity properties of homotypic, heterotypic, and coexpressing cell pairs.\textsuperscript{5,6} Our data show that Lucifer Yellow flux is significantly lower in homotypic Cx40 channels relative to Cx43. Further, LY flux in heterotypic Cx43-Cx40 and coexpressing cell pairs is intermediate between the two homotypic forms.

Materials and Methods

Cells and Culture Conditions
Experiments were carried out using HeLa cells that were transfected with rat Cx40 or rat Cx43 or sequentially transfected with both connexins.\textsuperscript{6,9} Production and characterization of these cells, culture conditions, and staining methods for identification of specific cells have been described previously.\textsuperscript{6,9} Electrophysiological measurements and dye flux studies were carried out on cell pairs cultured for 1 to 3 days.

Electrophysiological Measurements
For simultaneous electrical and fluorescence recording, glass coverslips with adherent cells were transferred to an experimental chamber mounted on the stage of an inverted microscope (Olympus IMT2)\textsuperscript{6} equipped with a fluorescence imaging system. The chamber was perfused at room temperature (\(\sim 22^\circ\text{C}\)) with bath solution containing (in mmol/L) NaCl 150; KCl 10; CaCl\(_2\) 2; HEPES 5 (pH 7.4); and glucose 5. CsCl and BaCl\(_2\) (2 mmol/L) were added. The patch pipettes were filled with solution containing (in mmol/L) K\(^+\) aspartate 120, NaCl 10; MgATP 3; HEPES 5 (pH 7.2); EGTA 10 (pCa \(\sim 8\)); filtered through 0.22-\mu m pores. In perforated patch experiments, the pipette solution contained 30 to 50 mmol/L \(\beta\)-escin.\textsuperscript{11} Patch pipettes were pulled from glass capillaries (code 7052; A-M Systems) with a horizontal puller (Sutter Instruments). When filled, the resistance of the pipettes measured 1 to 1.5 M\(\Omega\).

A dual voltage-clamp method and whole-cell recording were used to control the membrane potential of both cells and to measure currents.\textsuperscript{8}

In perforated patch experiments, pipette solution containing \(\beta\)-escin was brought in contact with the cell and high resistance seal of 10 G\(\Omega\) or more was formed (cell-attached configuration). With time (5 to 10 minutes), the \(\beta\)-escin diffuses into the membrane facing the pipette solution and establishes channels that are relatively nonselective with regard K\(^+\), Na\(^+\), and Cl\(^-\).\textsuperscript{11}

Dye Flux Studies
Dye transfer through gap junction channels was investigated using cell pairs. Lucifer Yellow (LY) (Molecular Probes) was dissolved in the pipette solution to reach a concentration of 2 mmol/L. Fluorescent dye cell-to-cell spread was imaged using a 16-bit 64 000 pixel gray scale digital CCD-camera (LYNX2000T, Spectra Source Instruments), LY concentration is directly related to fluorescence intensity. A calibration curve for the camera was constructed by placing a 10-\(\mu\)L volume of known LY concentration on a coverslip and subsequently placing another coverslip on top. An image was taken in the center of the fluid drop for a number of concentrations using a fixed exposure time. The background intensity was subtracted from the respective image. The resultant curve showed the camera output to be linear over the range of concentrations used (2 mmol/L and less, see Figure 2).

Signal Recording and Analysis
Voltage and current signals were recorded using patch clamp amplifiers (Axopatch 200). The current signals were digitized with a 12 bit A/D-converter (DT212EZ, Data Translation) and stored with a personal computer. Data acquisition and analysis were performed with custom-made software.\textsuperscript{12,13} Curve fitting and statistical analyses were performed using SigmaPlot and SigmaStat, respectively (Jandel Scientific). The results are presented as mean\(\pm\)1 SEM.

Results
We sought to compare directly the permeability of a larger solute for gap junction channels formed of the cardiac connexins, Cx40 and Cx43, or combinations of those connexins. We examined homotypic channels using pairs of HeLaCx40 or HeLaCx43 cells, heterotypic channels using HeLaCx40-HeLaCx43 cell pairs, and the variety of channels formed in coexpressing cells using pairs of HeLaCx40/Cx43 cells. We used 3 different approaches to facilitate simultaneous monitoring of junctional conductance (\(g_j\)) and the flux of Lucifer Yellow (Figure 1).

In Method 1, a cell pair was studied using one electrode in the whole-cell mode to deliver a known concentration of LY and another electrode on the adjacent cell in the perforated-patch mode. The amount of transfer of LY was determined over time by fluorescence microscopy (illustrated for a pair of HeLaCx43 cells in Figure 1A). In this case, 3 time points are shown (1 minute, 5 minutes, and 10 minutes) after dye injection. In addition, \(g_j\) was monitored at 5-minute intervals by recording of transjunctional current, \(I_j\) (Figure 1B, left). The \(I_j\) was generated by short (200 ms) 20-mV \(V_j\) steps delivered every second.

Fluorescence intensity was subsequently determined at different time intervals for each experiment in both the source cell and recipient cell. Figure 1C summarizes the fluorescence intensity data illustrated in Figure 1A with the relative fluorescent intensities for source cell (●) and recipient cell (○) plotted versus time. The maximum (steady state) intensity attained in the source cell represents the equivalent of the concentration in the pipette.

In Method 2, two patch electrodes were employed in perforated-patch mode with a third electrode in whole-cell mode, used to deliver LY. This method is illustrated in Figure 1D.

In Method 3, \(g_j\) was assessed at the end of the experiment, rather than periodically throughout the experiment (Figure 1E). In this case, one patch electrode was used in the whole-cell mode (Figure 1E, left) and contained LY as in Method 1. The second patch pipette was left in cell-attached recording mode for 15 to 20 minutes, and after some LY transfer had occurred, the patch was opened and \(g_j\) was measured.

Application of \(CO_2\) was used routinely to prove that dye transfer occurred through gap junction channels and that cytoplasmic bridges\textsuperscript{14} were not responsible for the observed cell-to-cell communication. At the end of the dye spread experiment, 100% \(CO_2\) was bubbled into the bath, while monitoring \(I_j\); the \(g_j\) was rapidly reduced to zero (within 150 seconds of the onset of acidification in the example shown in Figure 1B, right). Only data sets where complete cell-cell uncoupling was demonstrated were analyzed.

All 3 approaches allowed dye spread to the neighboring cell without loss caused by cell dialysis via patch pipette and yielded similar results. Therefore, data from these methods will be considered together.
The number of molecules ($MN$) that transfer from the injected cell to the recipient cell can be determined by the following: 

$$MN Vc Ci \cdot N A,$$

where $Vc$ indicates cell volume; $Ci$, flux concentration; and $NA$, Avogadro’s number. By assuming the cell volume to be $\approx 0.5$ to 1 pL and determining the amount of LY in the recipient cell relative to the source cell at time $t$, a calculation of the number of LY molecules transferred per unit time can be made. For the example shown in Figure 1A, at the 12-minute time point, the fluorescent signal in the recipient cell was 37% of that in the source cell. Using a 2 mmol/L source concentration and assuming a cell volume of 0.75 pL, yields $1.5 \times 10^{-15}$ moles or $9.0 \times 10^4$ LY molecules in the source cell. Over 12 minutes, $3.3 \times 10^6$ LY molecules have traversed the junction to accumulate in the recipient cell; therefore, the junctional flux is $3.3 \times 10^6$ LY molecules per 720 seconds or $4.6 \times 10^5$ dye molecules per second. For these calculations, it is assumed the nonjunctional permeability of LY is insignificant, based on the observation that LY leak across the plasma membrane in HeLa cells is less than 3% over the initial 15-minute interval and only 15% over a 2-hour interval.\textsuperscript{15}

To look for possible differences in selectivity, LY flux was compared in pairs of HeLaCx40, HeLaCx43, or HeLaCx40/Cx43 cells with similar macroscopic junctional conductances. Figure 2A illustrates LY permeability for 3 such cell pairs with comparable $g_j$ of 22 to 23 nS. Note that all micrographs were obtained using the same exposure time. The lower image of the Cx40 cell pair at the 15-minute mark has been contrast enhanced to demonstrate that the recipient cell contains LY. Plots of the fluorescence intensity (in source and recipient cells) versus time for all 3 cases (Figure 2B) showed that there was a difference in LY permeability for homotypic Cx43 and Cx40 channels. Coexpression of Cx40 and Cx43 resulted in an intermediate permeability. Using the calculation illustrated for Figure 1, the numbers of LY molecules traversing the junction per unit time were as follows: $5.5 \times 10^5$ dye molecules per second for the HeLaCx43 cell pair; $5.0 \times 10^4$ dye molecules per second for the HeLaCx40 cell pair; and $2.5 \times 10^5$ dye molecules per second for the coexpressing cell pair.

We simultaneously examined LY dye flux and $g_j$ in many cell pairs with differing total conductances. A summary of these data are presented in Figure 3, which shows a plot of junctional conductance ($g_j$) versus the relative intensity in the recipient cell (at 12 minutes after introduction of LY into the
source cell) for pairs of HeLaCx40, HeLaCx43, and HeLaCx40/Cx43 cells and for the heterotypic combination of HeLaCx40-HeLaCx43 cells. The homotypic Cx43, homotypic Cx40, and heterotypic Cx40-Cx43 could all be closely fit by first order regressions. In contrast, the data from the coexpressing HeLaCx40/Cx43 cell pairs were best fit by a 2nd order regression, which suggests variation of the channel type or types present in this data set over the $g_j$ range studied. Such a relationship was not predicted nor observed for the other cases, where only one channel type is possible: homotypic or heterotypic.

The slopes of the dye intensity-conductance curves were very different. The curve for homotypic Cx43 was much steeper than for homotypic Cx40, consistent with a much greater LY permeability of Cx43 channels. The curves for both the heterotypic and the coexpressing combinations of Cx40 and Cx43 were intermediate between those for homotypic Cx40 and Cx43. The curve for heterotypic Cx40-Cx43 was shorter than the others, because no high conductance heterotypic pairs were observed (possibly reflecting the inefficiency of channel formation in this combination).

The amount of LY flux per channel can be calculated from our experimental data if the open probability and the unitary conductance of a channel are known. To assess these variables, we obtained long single-channel recordings of homotypic HeLaCx43 (Figure 4A) and HeLaCx40 (Figure 4B) using conditions similar to those used for the simultaneous $g_j$/LY flux experiments (pipette solution containing 120 mmol/L K+ aspartate). As shown in these recordings, Cx43 and Cx40 gap junction channels had open probabilities of near 1 (0.95 and 0.91, respectively) even during sustained transjunctional voltages ($V_j$) of 40 mV. The LY diffusion in the present study was observed when no $V_j$ was applied. We
would only expect to find higher \( P_o \) values at \( V_f = 0 \). The open probabilities for homotypic Cx43 and Cx40 have previously been shown to approach 1.0, when \( V_f = 0 \text{ mV} \). For the coexpressing cell pairs and the heterotypic Cx43-Cx40 pairs, we assumed that the open probability was similar to that of the homotypic forms when \( V_f = 0 \text{ mV} \). Thus, at any one time the macroscopic conductance measured divided by the unitary conductance yields the total number of functioning channels. We also assumed that the dye transfer depended entirely on the cumulative open time of the main channel state, because (where they have been examined) the residual state or substates are less permeable or impermeable to dye.19,20

In our recordings using 120 mmol/L K\(^+\) aspartate\(^-\), the homotypic Cx43 single channel conductance was 54 pS and the homotypic Cx40 unitary conductance was 125 pS (Figure 4). Previous studies that determined unitary conductance are consistent with these values when corrected for the salt concentrations.7,8,17,21,22 The average unitary conductances observed by Valiunas et al.\(^{8,9} \) were 80 pS for heterotypic Cx40-Cx43 channels and 67 pS for the channels in pairs of Cx40 and Cx43, where the major salt was 110 mmol/L CsCl. Assuming that aspartate is not a significant current carrier, the calculated conductances become 61 pS and 51 pS, respectively (ie, Cl\(^-\) is assumed to have carried \( \approx 20\% \) to 30\% of the current, which is the estimated percentage for Cx43.\(^{23} \) For Cx40, less than 10\% of current is carried by Cl\(^-\).\(^{21} \)

The calculated unitary conductances are given in the Table and were used to determine the total number of functioning channels. The additional assumption that open probability is near unity\(^{12,16–18} \) or the product of the number of channels (n) and open probability (\( P_o \)) remains constant throughout the duration of the measurement yields the following numbers of channels for the example shown in Figure 2: \( \approx 407 \) channels in Cx43 cell pair; \( \approx 184 \) channels in Cx40 cell pair; and \( \approx 431 \) channels in coexpressed Cx43/Cx40 cell pair. The calculated LY flux yields 1351 LY molecules/channel per second for homotypic Cx43 channels, 272 molecules/channel per second for homotypic Cx40 channels, and 580 molecules/channel per second for the channels in the Cx40/Cx43 coexpressing cells.

To calculate the permeability ratio of LY to K\(^+\), we must first estimate the K\(^+\) flux/channel per second for Cx43 and Cx40 by dividing the current passing in response to a 10-mV step by the quantity of charge, ie, elementary charge \( e = 1.6 \times 10^{-19} \text{ C} \), where we assume that the 10-mV step is a rough equivalent to a concentration gradient of 10\( \times \).

Thus, for Cx43, a 10-mV step yields a 0.54-pA current or \( 3.8 \times 10^6 \) ions/channel per second, and for Cx40, a 1.25 pA translates into \( 7.8 \times 10^6 \) ions/channel per second in 120 mmol/L K\(^+\) aspartate\(^-\) pipette solution. The data from Figure 2 were used to illustrate calculation of the LY/K\(^+\) flux ratio. In Figure 2A, \( g_j \) was 22 nS (corresponding to 407 Cx43 channels at 54 pS each). The total number of LY dye molecules traversing the junction per second is \( 5.5 \times 10^8 \) molecules/sec. The correction factor for the concentration difference between K\(^+\) and LY is 60\% (2 versus 120 mmol/L). Thus, normalizing LY to K\(^+\) gives \( 3.5 \times 10^7 \) dye molecules per second traversing the junction or \( 8.1 \times 10^7 \) dye molecules/channel per second (3.3 s\(^{-1}\)). This allows the determination of the K\(^+\)/LY permeability ratio for Cx43, (8.1 \( \times 10^7 / \) \( 3.38 \times 10^8 \)) = 0.024. In case of Cx40, the \( g_j \) was 23 nS (Figure 2), which corresponds to 184 functioning channels yielding a K\(^+\)/LY ratio of 0.0021. For coexpressing Cx43/Cx40 cells, \( g_j \) was 22 nS (Figure 2), which turned out in to 431 functioning channels yielding a K\(^+\)/LY ratio of 0.011.

Using the same calculations as presented for homotypic and cotransfected cells in Figure 2, the LY/K\(^+\) ratio for all types of cells was obtained. The LY flux rate and the permeability ratio for LY relative to K\(^+\) for the homotypic, heterotypic, and coexpressed combinations of Cx43 and Cx40 are summarized in the Table. The summarized LY flux and LY/K\(^+\) ratios were calculated using the slopes obtained from data in Figure 3, ie, Cx43 = 0.023/nS; Cx40 = 0.00187/nS; Cx40-Cx43 = 0.00713/nS. Because of the nonlinear relationship between the relative fluorescence in recipient cells and \( g_j \) in pairs of HeLaCx40/Cx43 cells, we computed the flux and LY/K\(^+\) ratio at both 5 nS and 69 nS, yielding a range of values for each. The Table also contains the LY/K\(^+\) ratio corrected for series resistance as defined by van Rijen et al.\(^{24} \)

This correction results in reduction of LY flux relative to K\(^+\) for all the connexins studied. Regardless, the difference in the LY/K\(^+\) ratio for Cx43 compared with Cx40 remains greater than an order of magnitude whether corrected or not.

**Discussion**

The data presented in this manuscript have shown that homotypic Cx43 gap junction channels permit a LY flux that is 5.3 times that of Cx40 channels. Such a difference might result from significant differences in pore diameters approaching the minor diameter of LY (0.95 nm). However, the
differences might also result from differences in charge selectivity, because LY possesses two negative charge groups and previous studies have suggested that Cx40 is more highly selective than Cx43 with regard to anions.21

To distinguish between pore size and selectivity we applied the equation derived by Levitt: 25

\[
\frac{D(x)}{D_0} = \frac{1 - 2.1054p + 2.0805p^3 - 1.7068p^5 + 0.72603p^6}{1 - 0.75857p^2},
\]

where \( p = a/r \) (\( a \) indicates solute diameter; \( r \), pore diameter); and \( D_0 \) indicates the diffusion coefficient in free solution, whereas \( D(x) \) is the diffusion coefficient at position \( x \) within the pore.

This equation was used to estimate the pore diameters of Cx43 as \( \approx 1.2 \) nm 23 and \( \approx 1.3 \) nm for Cx40.21 For Cx43, using 0.95 nm as the LY diameter and 1.2 nm for the pore yields a \( D(x)/D_0 \) ratio of 0.02. This calculation is consistent with the LY permeability in Cx43 being a result of size alone as it is similar to the ratio given in the Table for the LY/K* ratio but it is not unequivocal. To compare LY/K* with LY(Dx)/LY (\( D_0 \)), it is assumed that the diffusion coefficient in free solution for LY is within a factor of 2 of the K* diffusion coefficient.26,27 For Cx40, the calculation yields another result. The pore diameter must be 1.0 nm to yield a ratio of 0.0025 to 0.0018 (Table). This is inconsistent with the estimates of Cx40 pore size given by Beblo and Veenstra.21 Further, if we assume the pore diameter is 1.0 nm, then to achieve the unitary conductance of 125 pS as shown in this study would require the channel length be reduced by at least half. These results cannot eliminate a size limitation effect for Cx43 or Cx40, but clearly show that for Cx40 the simplest explanation is a difference in selectivity relative to Cx43.

Our data also establish the LY permeability relative to K* (LY/K*) for homotypic Cx43, homotypic Cx40, and heterotypic Cx43-Cx40 channels; they demonstrate the range of values for cells coexpressing Cx43 and Cx40 (Table). The LY/K* permeability ratio for Cx43 channels is 11.2-fold higher than for Cx40 channels, whereas the LY flux rate is only 5.3-fold higher than Cx40. This difference (≈2-fold) reflects a difference in the unitary conductances, ie, 125 pS for Cx40 versus 54 pS for Cx43 or Cx40 allows passage of \( \approx 2.3 \) times more current carrying K* ions than Cx43.

The LY flux observed in either heterotypic pairs or pairs of cells coexpressing both connexins was intermediate between that observed for homotypic Cx40 and for homotypic Cx43 (Figure 3). Thus, mixing of connexins does not lead to any novel permeability properties. This is consistent with our previous observation where heteromeric Cx40/Cx43 channels were detected biochemically, but showed no novel voltage-dependent gating properties.9 The data from the heterotypic and the coexpressing cell pairs do not clearly demonstrate any dominating effect of Cx40 or Cx43 on permeability; the results suggest that as the ratios of two coexpressed connexins vary, the permeability will tend toward that of the more abundant connexin. The nonlinearity of the plot of flux versus conductance for the coexpressing cells (Figure 3) likely reflects the multiple different channels present in such cell pairs (two different homotypics, heterotypics, and any functional heteromeric).

We directly compared the selectivity properties for homotypic Cx43 and Cx40 for various monovalent cations using the data from Wang and Venstra25 and Beblo and Veenstra21 and those for LY determined in the present study by plotting the log of the flux for the various ions (normalized to that of K*) versus their diffusion coefficient (Figure 5). This analysis shows that the selectivity properties for the two homotypic gap junction channels decline in proportion to mobility for the monovalent cations, indicating that the channels are not highly selective for any of these ions. This was not a case for LY, where gap junction channels become selective.

For all the data summarized in Figure 5, an effective K* diffusion coefficient of 1.1 \times 10^{-6} \text{cm}^2/\text{s} was used. This value is 50% of the value determined in water. Others have shown that the K* diffusion coefficient is about 50% less in cytoplasm than in water mainly due to the increased viscosity of cytoplasm. 28

The minor diameter of LY is 0.95 nm, and it possesses 2 negative charges as well as a primary amine, all of which will be charged at physiological pH. cAMP and IP3 are 2 messengers of slightly smaller size, but in the case of IP3, there are 6 negative charges. The measured diffusion coefficient for LY is 15% of K* in free solution.26 The measured diffusion coefficient is \( \approx 2.7 \times 10^{-6} \text{cm}^2/\text{s} \), (0.24 on the x-axis in Figure 5) for either cAMP or IP3 in cytoplasm or cell extracts.30,31 These values are in the same range as that estimated for LY.26

Although the two homotypic connexin channels have very different selectivity properties for solutes, both appear to be
discriminatory for solutes with mobilities (diffusion coefficients) that are ≤20% of that for K⁺ (eg, LY; see Table and Figure 5). For comparison, the maxi-K⁺ channel has a Na⁺/K⁺ permeability ratio of ≈0.01.²⁹ LY transits a connexin channel about as efficiently as Na⁺ transits a maxi-K⁺ channel; it falls into the category of relatively impermeable. This analysis suggests that the selectivity properties of connexins might exclude the rapid transit of specific solutes such as cAMP and IP₃. From our studies, it is clear that Cx43 channels will be the most effective in allowing permeation, whereas Cx40 will be least.

The low permeation rates relative to K⁺ raise the question of whether this is an effective pathway when considering physiological concentrations for these second messengers. Both cAMP and IP₃ are found in cells at micromolar concentrations, and both are labile with life times of seconds to minutes. The LY flux of Cx43 channels corrected for concentration (120 mmol/L) yields ≈9×10⁹ dye molecules/channel per second. Therefore, the flux for a 120 µmol/L concentration would be ≈90 dye molecules/channel per second; for a 1 µmol/L concentration the flux would be <1 dye molecule/channel per second. The latter two concentrations are in the physiological range expected for second messengers and metabolites. When considering the expected life cycle of solutes with poor permeation properties, it is hard to envision the effectiveness of gap junction mediated messenger pathways unless (1) a solute is produced near the gap junction channel, resulting in a transient but high local concentration and (2) the transiting solute acts on sites within the adjacent cell close to the gap junctions.

Our studies may have significant implications for understanding the roles of multiple connexins in differing functions in the cardiovascular system. Our analysis implies that either Cx40 or Cx43 (or likely any other connexin) should be adequate to pass ions that carry intercellular current. This is consistent with the observations of Thomas et al.²² who showed that channels formed by coexpressed connexin40 and connexin43. Am J Physiol. 2001;281: H1675–H1689.

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