Review

Selective permeability of gap junction channels

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Abstract

Gap junctions mediate the transfer of small cytoplasmic molecules between adjacent cells. A family of gap junction proteins exist that form channels with unique properties, and differ in their ability to mediate the transfer of specific molecules. Mutations in a number of individual gap junction proteins, called connexins, cause specific human diseases. Therefore, it is important to understand how gap junctions selectively move molecules between cells. Rules that dictate the ability of a molecule to travel through gap junction channels are complex. In addition to molecular weight and size, the ability of a solute to transverse these channels depends on its net charge, shape, and interactions with specific connexins that constitute gap junctions in particular cells. This review presents some data and interpretations pertaining to mechanisms that govern the differential transfer of signals through gap junction channels.
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1. Introduction

There are at least 20 different mammalian connexin genes. Connexins form an intercellular channel, the gap junction channel [1–3]. Knowledge of signals transmitted by gap junctions composed of connexins is still an area of some controversy. Defining the properties that govern cell-to-cell movement of solutes is a necessary step in elucidating how gap junctions affect cell growth and physiology. This information should also provide insights into several human diseases that are caused by connexin mutations [4–7].

In some tissues, such as the heart, the role of gap junctions in aiding the propagation of an action potential to promote the coordinated contraction of millions of cells over a few tenths of a second is well understood [8,9]. In contrast, gap junctions also play a critical, yet less defined, role in many non-excitable (i.e., an absence of regenerative electrical events) tissues such as vascular smooth muscle cells or hepatocytes. In both vascular smooth muscle cells and hepatocytes, diffusion of second messenger molecules/

ions through gap junctions appears to be paramount to coordinated function [10–12]. To develop an understanding of how gap junctions might function in such tissues, a primary goal is to determine biophysical properties that are most relevant to the transit of a solute through a gap junction channel between cells. Properties such as voltage- and pH-dependent gating, open probability, and selectivity/permselectivity come to mind.

2. Gating and open probability of gap junction channels

As mentioned above, gap junction channels are composed of connexin proteins. Homotypic gap junctions are composed of 12 identical connexin proteins, with each cell of an adjacent cell pair contributing two identical halves referred to as hemichannels or connexons. Heterotypic gap junction channels are composed of two hemichannels, each containing identical connexins, but the connexin type in each half is different. Heteromeric gap junctions are composed of hemichannels which contain more than one type of connexin [13–16].

Gap junction channels, regardless of type (homotypic, heterotypic, or heteromeric), display voltage-dependent gating, but the kinetics and sensitivity vary over a wide range

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[17–24]. While voltage dependence has proved to be a useful biophysiological tool, its relevance under physiological conditions is questionable.

Ultimately, defining the mechanisms of gating is a means for determining the open probability of a channel, and tools like voltage dependence and chemical or pH gating have proved useful toward that end. Open probability is a measure of the percentage of time a channel remains open versus being closed. For any channel, experimental determination of the mean open time (MOT) and closed time (MCT) will allow calculation of open probability (Po) via the following formula: (Po) = MOT/(MOT + MCT). In fact, this determination can be made under steady state conditions at various voltages and pH conditions. Even at transjunctional voltages of 40 mV and pH levels of 6.8, gap junction channels have very high open probabilities in the range of 0.6–0.9 [11].

Taken together, these observations suggest that, under a wide range of intracellular conditions, most gap junction channels are gated open more often than closed. In determining selectivity properties or permeability ratios, it is not necessary to know open probability values or, as is the case with the gap junction channel, to have a Po near unity. However, because gap junction channels are open more often than not, it is the selectivity of the channel that dictates what passes from cell to cell. Realizing this, it becomes obvious that selectivity is a major determinant able to affect and coordinate multicellular systems. Investigations have been made into the selective transfer of a variety of molecules through gap junction channels.

3. Monovalent cations: a lack of selectivity

A number of studies have illustrated the selectivity properties of homotypic gap junctions for monovalent ions, particularly Cx43 and Cx40 and, to a lesser degree, Cx37 and Cx45 [25–28]. While unitary conductances vary widely (25 pS for Cx45 and 350 pS for Cx37), the sequence of monovalent cation selectivity is the same (K>Na>Li>TMA>TEA). This sequence is reminiscent of a nonselective pore such as Porin [29]. In contrast, the same is not true for larger solutes, endogenous or exogenous.

4. Selective transfer of endogenous solutes

Using a technique called transport specific fractionation, Bevens and Harris first reported differential permeabilities of connexins to biological signaling molecules. They reconstituted liposomes with connexin hemichannels, loaded them with urea and radioactive transjunctional molecules, and then fractionated them by sucrose density gradient centrifugation. In this technique, movement of urea and other molecules through connexin hemichannels causes the liposomes to move toward the bottom of the tube during centrifugation [30].

By employing tritiated cyclic nucleotides, results from transport specific fractionation experiments demonstrate that cGMP and cAMP pass equally well through homomeric hemichannels formed by Cx32. However, cGMP passes more efficiently through heteromeric hemichannels composed of Cx32 and Cx26. Thus, Cx26 appears to preferentially restrict the passage of cAMP over cGMP [30].

In subsequent work, Bevans and Harris showed that cyclic nucleotides can block the transfer of molecules through reconstituted homomeric hemichannels formed by Cx32, or heteromeric hemichannels formed by Cx32 and Cx26. Nanomolar levels of cAMP or cGMP effectively shut these channels. Interestingly, this effect is specific in that other nucleotides including AMP, ADP, ATP, cTMP, and cCMP are not effective. These data suggest that specific high affinity interactions take place between connexins and particular cyclic nucleotides [31].

Results from transport specific fractionation experiments indicate that connexins contain binding sites for natural metabolites to control the intracellular flow of specific molecules. However, this technique presents some questions in that about half of the connexins in reconstituted channels are in reverse orientation with respect connexin topology found in living cells. In addition, since no docking occurs, the technique assays permeability of hemichannels as opposed to gap junctions [30–32].

Studies by Goldberg et al. have utilized fluorescence activated cell sorting [33,34] and a layered culture system [35,36] to examine the transfer of endogenous metabolites between cells through gap junctions. The data reveal that Cx32 shows very suppressed passage of AMP and ATP relative to Cx43. These data do not establish how selective Cx43 is to nucleotides relative to any monovalent ions such as K$^+$, but they do establish that the different connexins have very different permeability properties for endogenous compounds.

Other species transferred by channels composed of Cx43 and Cx32 include glucose, glutamate, glutathione, adenosine, and ADP. In general, Cx43 mediates the transfer of most of these molecules several fold more efficiently than Cx32. Two exceptions to this rule have been identified; adenosine transfers severalfold better through channels formed by Cx32, while ATP transfers over 100-fold better through channels formed by Cx43. This implies that that phosphorylation of adenosine can shift its permselectivity from Cx32 to Cx43 by about 2–3 orders of magnitude [35]. Therefore, channels formed by Cx32 may restrict the transfer of a particular compound, such as adenosine, based on negative charge, such as that conferred by phosphate, more rigorously than channels formed by Cx43.

However, the selective transfer of molecules through gap junction channels is not dictated by charge alone. In contrast to ATP, some anionic metabolites may pass better through channels formed by Cx32 than channels formed by Cx43. For example, Niessen et al. [37] utilized FURA-2 to analyze the propagation Ca(2+) waves from cells injected with

1,4,5-trisphosphate to neighboring cells. Data from these experiments suggest that IP3 moves better through channels formed by Cx32 than channels formed by Cx26 or Cx43.

5. Selective transfer of exogenous probes

A comparative study of the relative permeability of specific connexins to fluorescent probes of positive and negative charge was first performed by Elfgang et al. [38]. HeLa cells were transfected with specific connexins and the electrical coupling between cell pairs from each transfected clone was shown to be roughly equivalent. The spread of probe in a monolayer of cells transfected with a specific connexin was then performed, and comparison of the extent of spread from cell to cell in each clone was made. The extent of dye spread was dependent on the type of connexin transfected. This clearly established that specific connexins
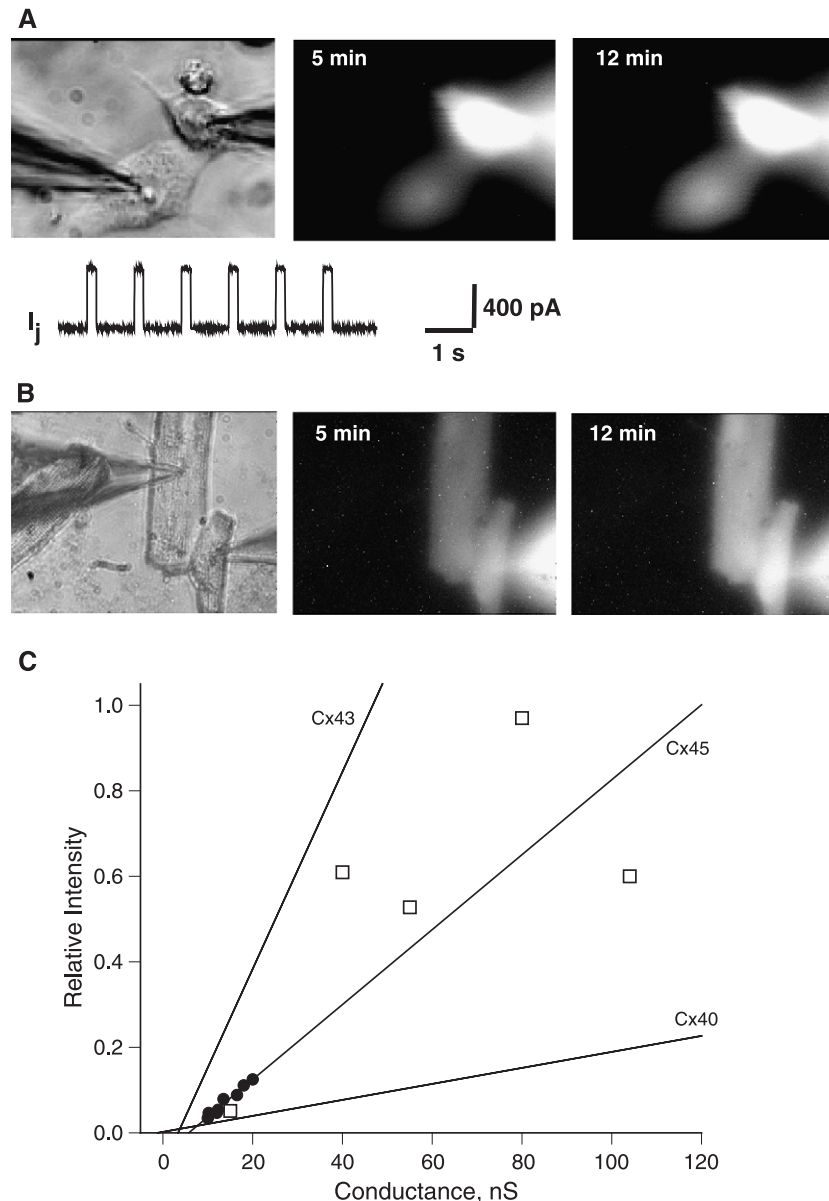

Fig. 1. Coordinated measurements of electrical conductance and dye transfer through gap junctions. Simultaneous measure of gap junction conductance (gj) and dye flux are shown between HeLa cells transfected with Cx43 (A) and guinea pig cardiac myocytes (B). In both cases a pipette containing 2 mM Lucifer yellow was attached to the cell on the right in whole-cell configuration. The cell on the left was patched using the perforated patch configuration. Epifluorescent micrographs taken at 5 and 12 min after dye injection into right-sided cell show a progressive fluorescence intensity increase in the recipient cell. Bottom panel in A shows gap junction currents elicited by Vj pulses of 20 mV for Cx43 HeLa cell pair. The gj measured was ~ 25 nS for Cx43HeLa cell pair (A) and ~ 40 nS for cardiac myocyte pair (B). (C) Summary of Lucifer yellow flux data versus gj for cardiac connexins. Each data point is an experiment where the flux at 12-min time point was plotted versus measured gj for homotypic Cx45 (closed circles) and guinea pig cardiac (open squares) pairs. The solid lines correspond to first order regressions. See text for references on derivation of regression lines for Cx43 and Cx40.

have different selectivities for charged probes in the 1.0-nm range, but did not establish flux per channel.

A similar study of Cx32 and Cx26 by Cao et al. [39] using oocyte pairs and HeLa cell pairs showed that heterotypic Cx32-Cx26 channels have a permeability to Lucifer yellow that is intermediate between homotypic Cx32 and Cx26. Again, this established that permeability of connexins is not a simple function of size, but is affected by other parameters such as charge. These studies, like that of Elfgang et al. [38], did not determine the open probability of the channels and did not make simultaneous measurements of conductance and permeability to Lucifer yellow. Thus, flux of Lucifer yellow per channel could not be determined. It is clear, though, from these data that each connexin possesses different permeability characteristics for probes of similar size and charge to messenger molecules.

A recent study by Valiunas et al. [40] has determined the permeability of Lucifer yellow relative to the ubiquitous monovalent cation K^+. This was accomplished by the simultaneous measurement of fluorescence intensity over time in both a source and recipient cell of a cell pair, while also monitoring junctional conductance. Using this method, two connexins, Cx43 and Cx40, were studied. The permeability ratio of Lucifer yellow/K^+ was 0.028 for Cx43 and 0.0025 for Cx40. These data clearly demonstrate that Cx43 and Cx40 have different selectivity properties for solutes with minor diameters of 0.9–1.0 nm. Furthermore, the permeability ratios for Lucifer yellow and K^+ are similar to those for Na^+ or Cs^+ in potassium channels (0.01–0.005) [41]. Thus, Cx43 and Cx40 channels are as selective towards Lucifer relative to K^+ as potassium channels are towards Na^+ or Cs^+ ions.

Fig. 1 presents a summary of data from Valiunas et al. [40] for Cx43 and Cx40 where the ratio of recipient cell fluorescence intensity relative to the source cell is plotted for a given time interval against the measured junctional conductance for a cell pair. Cardiac myocytes and HeLa cell pairs transfected with Cx45 are also shown as individual data points. The data for the myocytes are indicative of cell types that co-express Cx43 and Cx40 [40]. The calculated permeability ratio for Lucifer yellow relative to K^+ for Cx45 is 0.01, which is greater than the value for Cx40 (0.0025). Cx45 is a lower conducting channel than Cx40, but is less selective to Lucifer yellow relative to K^+. This is yet another indication that individual connexins form gap junction channels that are quite discriminating for solutes in the size range of 0.9–1.0 nm.

6. Gap junction permselectivity: interpretation and theory

A useful relationship for determining the mobility or diffusion coefficient within a channel was derived by Levitt [42]. The relationship predicts that as the diameter of the solute and the channel approximate each other, the diffusion

coefficient or mobility within the channel drops off rapidly. Wang and Veenstra [26] and Beblo and Veenstra [25] used this relationship to fit their unitary conductance data using monovalent cations for Cx43 and Cx40, and found that the data where best fit using a channel radius of ~1.2 nm for both connexins. The largest cation used had a diameter of 0.8 nm. A similar analysis was done by Valiunas et al. [40] adding Lucifer yellow. The Levitt relationship did not predict the Lucifer yellow/K^+ permeability ratio for Cx40 using the radius predicted from the K^+ conductance data [25]. In fact, the Levitt model predicted a radius of ~0.5 nm for Lucifer yellow as opposed to a predicted radius of 1.2 nm for K^+. A similar trend was observed for Cx43, 0.6 nm, as opposed to 1.2 nm for K^+. The discrepancies in apparent channel width for Lucifer yellow versus K^+ arise because the channels are both selective and the Levitt relationship manifests this as a change in diameter. These observations and analysis yielding apparent pore sizes fall within the range predicted from X-ray crystallography data obtained by Unger et al. [43].

An illustration of connexin selectivity combining data from Wang and Veenstra [26], Belbo and Veenstra [25], Goldberg et al. [34,35], Tsien and Weingart [44], Weidman [45,46] and Valiunas et al. [40] is shown in Fig. 2. Diffusion coefficients for a particular species normalized against K^+ are given on the X-axis. Values on the Y-axis represent the log of either normalized unitary conductance relative to K^+ or the permeability ratio for a particular solute. For all the
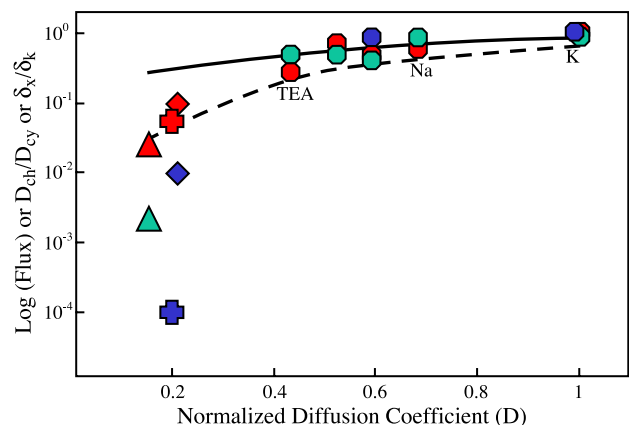

Fig. 2. Comparison of connexin permselectivity of some ions, dyes, and metabolites. Data for Cx32, Cx40, and Cx43 are shown in blue, green, and red, respectively. A semi-log plot of the normalized unitary conductance for Na, Li, TMA and TEA relative to K, and K relative to Cs (circles), and the flux of Lucifer yellow relative to K^+ (triangles) is shown versus the normalized diffusion coefficient. Data for AMP and cAMP (diamonds) are plotted for Cx43 and Cx32. These data are normalized to K^+ permeability, while ATP data (crosses) are normalized to AMP permeability. The normalized diffusion coefficient relative to K^+ for each species is shown along the X-axis. The solid and dashed lines represents the Levitt relationships for the same species using a channel diameter of 3.0 or 1.5 nm, respectively. The monovalent cations fit the data well, but the relationship fails to fit the data for the larger species. All solute radii were taken as unhydrated. See text for references on derivation of data.

species, an experimental measure or estimate of the diffusion coefficient within the cytoplasm is used.

In Fig. 2, the solid line represents the Levitt relationship using the cytoplasmic diffusion coefficients for the respective probes and a pore diameter of 3.0 nm, while the dashed line uses a diameter of 1.5 nm. The figure combines data for the transfer of ions, Lucifer yellow, AMP, ATP, and cAMP through channels composed of Cx32 and/or Cx43 [34,35,44,47]. The three channel types shown in Fig. 2 display little or no discrimination with regard to cations. The cations appear to move as a function of their free solution mobility. Cx32 though is highly selective against phosphorylated nucleotides (ATP). This data is consistent with that of Nicholson et al. [48] where Cx43 was shown to be more permissive than Cx32 using Alexa dyes. The fact that the Levitt derivation is not able to predict the permeabilities/mobilities that are experimentally determined is a sure indicator that selectivity is at work for solutes that have a size of ~0.9 nm in diameter or larger.

Limitations of present techniques make investigations of gap junction permselectivity difficult. For example, gradients introduced by dye transfer assays can be much higher than those encountered with endogenous molecules. The quantitative analysis thus far published has, in fact, taken advantage of the uniform distribution of exogenous probes as it allows for simplified analysis [40]. Indeed, other procedures including photobleaching and metabolic transfer assays are equally unable to determine the cytoplasmic distribution of a substance or the gradient across a junctional interphase under physiologically relevant conditions with any quantitative rigor [36]. In addition, formation of heterotypic and heteromeric channels by multiple connexins expressed in vivo would further complicate the scenario. Nonetheless, the data reviewed here provide proof for selectivity of gap junction channels derived from the connexin family. Such specificity with regard to selectivity is of potential importance in a variety of processes including cell growth control, proliferation in cell populations with rapid turnover rates such as the epithelial lining of the gastrointestinal tract, and coordinated syncytial behaviors such as the dynamic modulation of vascular tone [1,4,6,7,36].

Acknowledgements

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