Journal of Bioenergetics and Biomembranes, Vol. 28, No. 4, 1996

Gap Junctions in Excitable Cells

Peter R. Brink,[1] Kerry Cronin,[1] and S. V. Ramanan[1]

Received February 27, 1996; accepted March 13, 1996

Gap junction channels are an integral part of the conduction or propagation of an action potential from cell to cell. Gap junctions have rather unique gating and permeability properties which permit the movement of molecules from cell to cell. These molecules may not be directly linked to action potentials but can alter nonjunctional processes within cells, which in turn can affect conduction velocity. The data described in this review reveal that, for the majority of excitable cells, there are two limiting factors, with respect to gap junctions, that affect the conduction/propagation of action potentials. These are (1) the total number of channels and (2) the selective permeability of the channels. Interestingly, voltage dependence and the time course of voltage inactivation (kinetics) are not rate limiting steps under normal physiological conditions for any of the connexins studied so far. Only specialized rectifying electrical synapses utilize strong voltage dependence and rapid kinetics to permit or deny the continued propagation of an action potential.

KEY WORDS: Gap junctions; action potentials; propagation; conduction; excitable cells.

OVERVIEW

Gap junctions are the structural elements which comprise a low-resistance pathway between adjacent cells. In addition, it has become increasing clear that they provide a conduit for small solutes which include second messenger molecules. The traditional role of gap junctions, or electrical synapses, in nervous tissue is to allow the rapid transmission of information, usually in the form of a conducting action potential. In addition, the nervous system utilizes gap junctions in specific circumstances to mimic the functions of chemical synapses. In multicellular tissue such as muscle, specifically cardiac and smooth muscle, the conducting action potential is also able to propagate from one cell to another via gap junctions. Here excitable cells are defined as cells which generate and conduct action potentials.

WHAT IS A GAP JUNCTION?

Gap junctions are composed of subunit proteins called connexins (Goodenough, 1975). Each cell of an adjacent pair contributes one hemichannel to form a gap junction channel. Each hemichannel is composed of 6 connexins. One gap junction channel contains 12 subunit connexins (Makowski *et al.*, 1984; Sosinsky, this volume). There are some 18 connexin subunit proteins identified to date (Bennett *et al.*, 1995). The molecular weight of the connexins ranges from 26 to 70 kDA. Each connexin is believed to have four membrane spanning regions (M1–M4). The M3 membrane spanning domain is thought to be the most significant contributor of each connexin with regard to the formation of the hydrophilic channel (Bennett and Verselis, 1992). It is possible that M1, M2, or M4 might contribute to the channel wall as well, but there is presently no evidence one way or the other. The extracellular loops, namely E1 and E2, are necessary to the docking of any two hemichannels and are also suspected to contribute to the structure of the channel wall interior within the "gap" region of the gap junction channel. Indeed much of the present research effort centers on determining the roles of the different domains in channel gating, selective permeability, and pore architecture.

The gap junction channel is the structure which allows the movement of small solutes from cell to

[1] Department of Physiology and Biophysics, State University of New York at Stony Brook, Stony Brook, New York 11794.

0145-479X/96/0800-0351$09.50/0

cell down their concentration gradients (Loewenstein, 1981). Thus one cell in a gap junction-linked network of cells can generate and then disseminate information in the form of a specific diffusible molecule. This feature is a general characteristic of gap junction channels whether in excitable cells or nonexcitable cells. In excitable cells it represents a pathway by which specific molecules can affect action potential conduction via activation or inactivation of nonjunctional membranes, as well as providing a path for current flow associated with the action potentials.

WHAT GETS THROUGH A GAP JUNCTION CHANNEL?

Early observations in lower vertebrates and invertebrates indicated that negatively charged water-soluble fluorescent dyes less than 1 kDa could pass from cell to cell (Loewenstein, 1981; Spray and Bennett, 1985). These observations indicated that gap junctions were permeable to anions. Analysis showed anionic dye transfer to be governed by simple diffusion (Brink and Ramanan, 1985). Even the rectifying synapse of the crayfish allows Lucifer Yellow to pass in either direction when transjunctional voltage is near 0 mV (Margiotta and Walcott, 1983). There are, in fact, no examples where unidirectional flux of dye has been proven to occur; in fact such unidirectional diffusion would contradict the second law of thermodynamics (Little *et al.*, 1995; Robinson *et al.*, 1993).

Electrophysiological studies which focused on gap junctions in the propagation of action potentials indicated a preference for cations, principally K^+. The rationale for K^+ preference was based on its ubiquity and the lesser amounts of small mobile anions like Cl^- (Barr *et al.*, 1965, 1968). A better understanding of the permeability characteristics or selectivity properties was not forthcoming until the implementation of dual whole cell patch clamp. This method allowed the observation of single-channel events under a variety of ionic conditions; thus, the selective permeability could be determined. Neyton and Trautmann (1985) were the first to make such determinations in lacrimal acinar cells of rat. They reported unitary conductances ranging form 70–180 pS and determined the selective permeability sequence for K^+, Na^+, and Cl^- to be $K^+ > Na^+ > Cl^-$. The permeability properties were determined by establishing asymmetric ionic conditions across the junctions and measuring the reversal potential. Two connexin types are thought to be present in these cells; therefore, these initial estimates must be viewed as an average of the two connexin types in terms of their characteristic selective permeabilities. Recent studies, where specific connexins were transfected into mouse N2A cells, have allowed the determination of the permeability characteristics of a number of connexins (Veenstra *et al.*, 1995; Veenstra, this volume). While the individual channels are all relatively nonselective with regard to cations, there are differing anion permeabilities from connexin to connexin relative to cations. The gap junction channels of invertebrate septate axons have also been shown to be relatively nonselective ($Cs^+ = K^+ > Na^+ > TMA^- > Cl^-$; Brink and Fan, 1989). Anionic permeability has been inferred by comparison of unitary conductance with and without Cl^- present (Veenstra *et al.*, 1994a, 1995; Veenstra, this volume), or by monitoring shifts in reversal potentials (Brink and Fan, 1989). The data argue for a nonselective or poorly selective channel type that presumably allows the passage of almost any small solute including second messenger (cell-to-cell) molecules. However, there are potentially significant differences in the anion permeability from connexin to connexin to warrant consideration of connexin-specific effects on the ability of negatively charged second messengers to diffuse throughout a tissue via gap junctions.

Two channel types worth mentioning for comparative reasons are Porin Ompf ($Cs^+ > K^+ > Cl^- > TMA^- > TEA^-$; Benz, 1986) and the Maxi-K channel ($K^+ >>>> Na^+ >> Cs^+$; 1:0.05:0.01; Blatz and Magleby, 1984). The Porin Ompf channel appears functionally the same as the gap junction channels (poor selectivity) but has no sequence homology. The Maxi-K channel data show that channel to be highly selective for K^+ over other cations. It has no measurable permeability to anions. Both have voltage sensitivities that differ from the connexins.

CONNEXIN TYPES WITHIN EXCITABLE CELLS

There are six types of connexins found in excitable cells, nervous tissue, and muscle. In the case of the nervous system, the transmission of information such as the conduction of an action potential from one cell to another can occur via unidirectional chemical synapses or unidirectional or bi-directional electrical synapses. Both types of electrical synapses are gap junctions. In muscle, both cardiac and smooth muscle

contain gap junctions. The gap junctions in these cells allow bi-directional conduction/propagation. The types of connexins found in nervous tissue are Cx26, Cx32, and Cx43 (Dermietzel and Spray, 1993; Fushiki and Kinoshita, 1995). Both myocardium and smooth cells contain Cx43. In addition, Cx37, Cx45, and Cx40 have been found in specific regions of myocardium along with Cx43 (Beyer, 1993; Dermietzel and Spray, 1993).

THE ROLE OF GAP JUNCTIONS IN NEURONS

Unidirectional Action Potential Conduction

There are two basic ways in which gap junctions function within the milieu of neurons to create a unidirectional flow of information that, in essence, makes the gap junction functionally like a chemical synapse. They are electrotonic inhibition and modulation of electrotonic coupling (Jaslove and Brink, 1987). The former relies on the properties of nonjunctional membrane while the latter involves intrinsic properties of the junctional membrane itself. For electrotonic inhibition there two general cases to consider. The first is frequency dependence and the second is input impedance mismatches between two coupled neurons. The frequency-dependent inhibition effectively creates a low bandpass filter, where action potential amplitude is attenuated but long-duration membrane polarizations are not. This is a result of one cell (postsynaptic) having a high input impedance and thus a long membrane time constant. A further complicating factor is that often the membrane channels can dramatically affect the cellular resistance based on their voltage and time-dependent behaviors and in effect modulate the low bandpass properties. Impedance mismatch finds one cell of two coupled adjacent cells with a much lower impedance (higher conductance) than its partner. It is capable of generating sufficient current density to polarize the adjacent cell, but the other cell cannot reciprocate.

Both frequency dependence and impedance mismatch are reliant on nonjunctional membrane parameters (see Jaslove and Brink, 1987). The intrinsic properties of gap junctions which result in unidirectional flow of information (action potential conduction) were first illustrated by Fursphan and Potter (1957, 1959) using the rectifying electrotonic motor synapse of the crayfish. The time course of rectification was too fast to be accounted for by chemical synapses. The behavior of the rectifying synapse could be explained on the basis of geometry (one small high-resistance cell versus another large lower-resistance cell), but in the crayfish motor synapse this explanation is not relevant as it has been shown that the junction itself possesses intrinsic rectification properties (Jaslove and Brink, 1986). The rectification phenomenon can, in short, be explained by asymmetric voltage dependence of the junction, where the time course of voltage inactivation in one polarity is very rapid (Margiotta and Walcott, 1983; Jaslove and Brink, 1986, 1987). The asymmetry most likely means that the two hemichannels comprising one gap junction channel have different voltage-dependent processes. Homotypic gap junctions such as those composed of connexin43 show voltage dependence which is symmetric and the time constant(s) are many milliseconds to minutes in length (Wang *et al.*, 1992; Brink *et al.*, 1996). Verselis *et al.* (1994) and Bukasukus *et al.* (1995) used heterotypic gap junction channels composed of Cx26 and Cx32 and showed asymmetric voltage dependence. A similar result was reported by Moreno *et al.*, (1995) for Cx45 and Cx43. The time course of the voltage dependence in either case was still orders of magnitude too slow to explain the crayfish rectifier, but clearly heterotypic gap junction channel formation is one very plausible explanation. Another possible mechanism which could work on homotypic and heterotypic channels equally well is phosphorylation where the phosphorylation is asymmetric such that only one hemichannel is affected.

Bi-directional Conduction

The best examples of conduction are understood by studying large invertebrate axons which are septate. The septate axons are, in fact, individual axons which form end-to-end contacts, called septa, with adjacent axons. Both the crayfish lateral axons and earthworm medial and lateral axons are septate. The septal membranes contain gap junctions (Jaslove and Brink, 1987; Brink and Dewey, 1978, 1980). Unlike the motor synapse of the crayfish which rectifies and allows current flow in only one direction, the septal membranes are able to pass current in either direction. Furthermore, under appropriate experimental conditions an action potential can conduct across a septum in either direction (Jaslove and Brink, 1987). Interestingly, Eccles *et al.* (1933) were the first to show the bi-directional conduction of an action potential across the septal membranes of the medial septate axon of earthworm.

Under normal physiological conditions the septate axon conducts action potentials in one direction (normally anterior to posterior) based on sensory input which is from the anterior of the animal (Bullock, 1945). The conduction velocity for the median septate axon was measured and found to be between 17–25 m/s (Eccles *et al.*, 1933; Bullock, 1945). The individual axonal segments in these (septate) axons are about 1 mm in length and the septal membranes are thus spaced. If each septal membrane had the delay of a chemical synapse (0.5 ms), then the apparent conduction velocity in any one direction would be ~2 m/s.

Unfortunately, the connexin types are not known in the well-studied invertebrate systems and there are no counterparts for the rectifying electrical synapse of crayfish within the mammalian nervous system, where we know something about the connexin types (Dermietzel and Spray, 1993; Beyer, 1993).

THE ROLE OF GAP JUNCTIONS IN CARDIAC MUSCLE

Action potential conduction in working myocardium appears unidirectional because the action potentials originate from a pace maker, the sinoatrial node. Experiments by a number of investigators have shown that the intercalated disc allows current flow in either direction across the junction (Weidmann, 1966; Weingart, 1986). Likewise action potential conduction across intercalated discs can occur in either direction (Burt, 1991).

The question still remains: how do gap junctions participate in the conduction of an action potential? The discs lie perpendicular to the longitudinal plane for the intracellular current flow associated with conducting action potentials. Since there is ample data to show that gap junctions allow current flow between two adjacent myocardial cells, one need only disrupt or sufficiently inhibit the gap junction-mediated longitudinal action current and the result should be a reduced conduction velocity or block of conduction.

There are two related approaches used to address this question. One is to use agents which are known to close gap junction channels and observe the consequences. In fact, an excellent example of this was shown by Burt (1991) where 2 mM halothane was bubbled into a perfusate-blocked conduction of action potentials between cardiac myocytes. Halothane is known to alter gap junctional membrane conductance (Burt, 1991). This evidence would be very compelling, except for the observation that halothane (and for that matter, almost all the lipophilic-like agents known to affect junctional membrane conductance) is suspected to influence other membrane channels and cellular processes.

The other approach, which precedes the use of lipophilic agents or cytosolic acidity or raised intracellular Ca^{2+} levels (Lowenstein, 1981; Spray and Bennett, 1985; Burt, 1991), is the use of osmotic disruption of gap junctions. This method was first employed by Barr *et al.* (1965, 1968) and Berger and Barr (1969) to demonstrate that the gap junctions (nexus) between frog cardiac cells, and later mammalian cardiac cells and smooth muscle cells of the gastrointestinal tract, were vital to the conduction of action potentials from cell to cell. The method utilized a single sucrose gap which could render the extracellular space around the cells within such a gap ion free. The experimental design for the sucrose gap experiments has a strip of smooth muscle or cardiac muscle passing through three compartments where the center compartment contains a saline solution with sucrose as the major osmotic agent rather than the traditional cations and anions. The thickness of the compartment walls is very thin; they are usually made of rubber (Berger and Barr, 1969). This results in a high extracellular resistance within the gap. Thus, with single sucrose gap lengths equal to or greater than the space constant of the tissue (two cell lengths), any current generated by a stimulated or spontaneously generated action potential on one side of the gap could only flow through the gap via the intracellular spaces of cells connected by gap junctions. For the conduction/propagation of an action potential, the total extracellular current which flows must be equal to the total current in the intracellular domain ($I_i = I_o$). Thus, for the experiment to work, a shunt resistance has to be placed across the sucrose gap that had a resistance roughly equivalent to the collective longitudinal intracellular cellular resistance mediated from cell to cell by gap junctions. A simplified model can be envisioned as a line of cells linked in series by gap junctions. Barr *et al.* (1965) demonstrated the single sucrose gap recording method and further showed that the application of a threefold excess of hypertonic sucrose in the single gap results in the loss of a conducted action potential even with a shunt resistance 1000 times smaller than control. Further they showed that junctional membrane contact was greatly reduced or completely lost. If the sucrose gap was washed with an isosmotic sucrose, after the third exposure, full recovery was possible and the junc-

tional complexes could once again be visualized. These data stand as the most direct demonstration of one of the obvious roles gap junction channels play in a multicellular excitable tissue such as heart. The width of the sucrose gap was important to the experimental outcome. For example, if long gaps were used (mm lengths), even control data would not produce conducting action potentials. This is equivalent to having internodal lengths in myelinated axons which are too long to allow for saltatory conduction.

A third approach has been to use uncoupling agents to alter conduction of the action potentials and compare it to a discontinuous cable model which allows the number of functioning gap junction channels to be manipulated. Cole *et al.* (1988) performed such a study comparing action potential conduction in a model of 40 myocardial cells linked in series by gap junctions with experiments of myocardial cell bundles where octanol was used to reduce the number of gap junction channels functioning at any one time. The nonjunctional membrane conductances used in the model were those given by Beeler and Reuter (1977). The model used did not incorporate voltage dependence into the gap junction channels but rather treated them as resistive elements when open. Others have estimated the number of gap junctions "open" at any instant in time within an intercalated disc. That number ranges from 10^5–10^4 (Rudisuli and Weingart, 1991). The experimental and theoretical data presented by Cole *et al.* (1988) were quite similar. One of the striking aspects of the model was the illustration that only a few gap junction channels were required to permit action potential conduction/propagation from one cell to another. The calculated conduction velocity declined as the number of gap junction channels was decreased. Figure 1 shows the log of channel number on the X-axis and conduction velocity on the Y-axis. The data indicate that even changes in active channel number over two orders of magnitude are not able to dramatically affect conduction velocity. Reducing the channel number from 40,000 to 400 (1/100) reduced conduction velocity from 62.5 to 16.4, ~ a factor of 4. The reduction from 40,000 to 4,000 (1/10) or 62.5 cm/s to ~40 cm/s yields a factor of only 1.56.

These data and the modeling experiments clearly define a role for gap junctions in the conduction/propagation of action potentials from cell to cell. The K^+ within cells is the ion that is most likely to be responsible for carrying current both intracellularly and intercellularly via gap junctions to propagate action

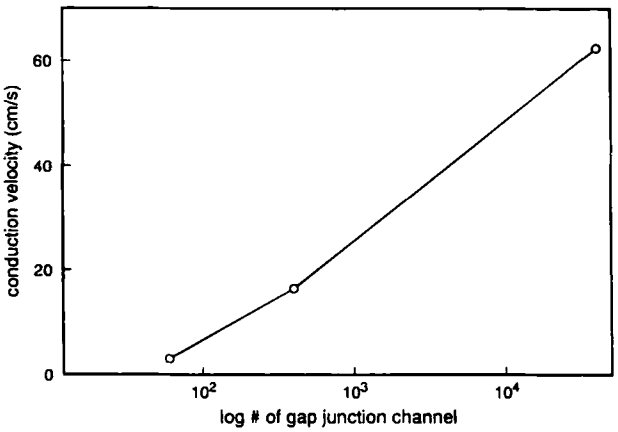

Fig. 1. Plot of conduction velocity in cm/sec versus the log of the number of gap junction channels within a disc. The data are taken from Cole *et al.*, (1988).

potentials. So why not simply have an elongated cation channel or K^+ channel connecting cells? The simplest answer is that other solutes which diffuse through gap junction channels might be able to affect conduction velocity. In fact, there are examples which illustrate the importance of the poor selectivity properties of gap junction channels in the heart as critical to normal function. The ability of cardiac gap junction channels in working myocardium (dominated by Cx43) to pass cAMP is not necessarily directly critical to the propagation of action potentials but its effects on nonjunctional channels could affect conduction velocity by alteration of membrane current densities associated with the action potential. Ca^{2+} channels are one such group (Mirro *et al.*, 1980). Furthermore, the effects on contractile strength are of clear physiological importance and, in fact, Tsien and Weingart (1976) showed that cAMP diffused through gap junctions in myocardium and enhanced contractile strength as more and more cells received cAMP via gap junction-mediated movement.

A MODEL CONNEXIN: ARE ITS PROPERTIES AN ASSET OR DEFICIT TO CONDUCTION?

In mammalian heart the most common connexin is Cx43. In working myocardium it is often the only connexin that is easily demonstrated in the intercalated disc region. Figure 2 shows typical rCx43 gap junction gating as observed in rat NRK cells. Unitary conductance is 120 pS in 165 mM CsCl pipette solu-

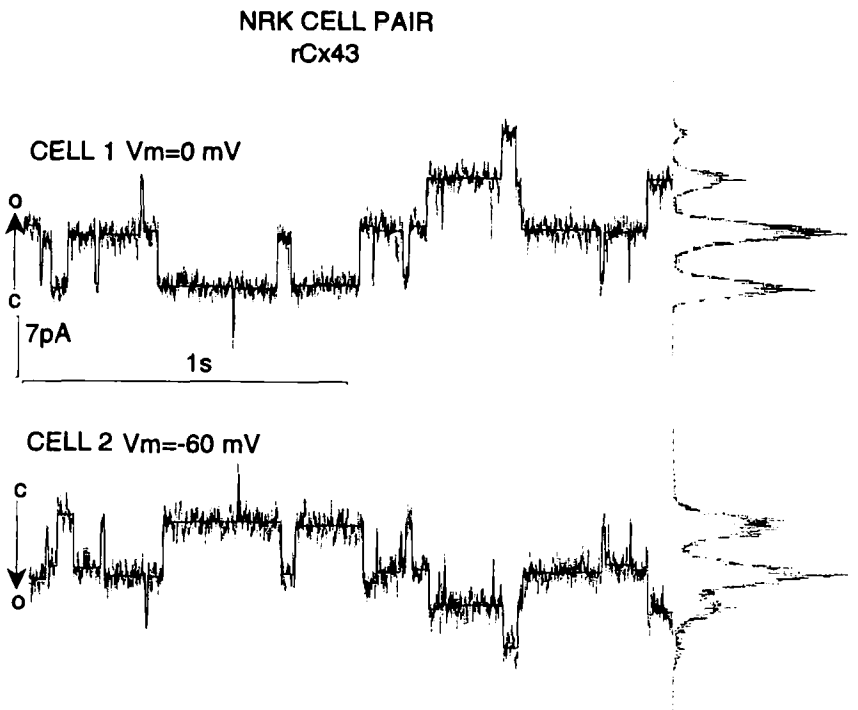

Fig. 2. Current tracings from a two whole-cell recording. Cell 1 was held at 0 mV while cell 2 was stepped to −60 mV. The two seconds of recording was taken 60 s after the onset of the step. Note that the channel activity in the two recordings is of equal amplitude but opposite polarity. The left-hand side of the figure shows the point-by-point amplitude histograms for the two tracings. Comparison also reveals the two to be almost identical. Vertical bar = 7 pA, horizontal bar = 1 s. The vertical arrows indicate the direction of (c) closed to (o) open for the channels. The external bathing solution was a CsCl saline and the pipette was also a CsCl solution (Brink *et al.*, 1996). The solid line in the two tracings is an idealization of the unitary activity.

tion (Brink *et al.*, 1996). The record represents a steady-state recording where a transjunctional voltage (V_j) of 60 mV was maintained for 2 min. The record shown is 2 s long but was taken 1 min after the onset of the voltage step. While the data here do not show how many channels were active at the onset of the step, other data (Wang *et al.*, 1992) show that Cx43 is relatively voltage insensitive with a V_o of ~80 mV (using step durations of 2 s). The steady-state gating shown in Fig. 2 belie the notion that dynamic alterations in gating might be important in the regulation of intercellular ion/solute movement mediated by gap junctions. The low bandpass filtration was 500 Hz and the multichannel record shows sojourns of many tens to hundreds of milliseconds. In recent studies on hCx43, mean open times were determined to fall between 0.42 to 4.2 s over a large V_j range. Closed time ranged from 0.21 to 1.42 s (Brink *et al.*, 1996). In addition, hCx43 is voltage dependent but also displays voltage-dependent mode shifting (Brink *et al.*, 1996). This results in some channels of an otherwise homogeneous population entering prolonged periods of silence (gated closed for the duration of a voltage step). If mode shifting can be shown to be ligand dependent (cAMP for example), then it could be a major dynamic modulator of junctional conductance (Brink *et al.*, 1996).

Could voltage dependence and/or mode shifting of gap junctions affect action potential conduction? A simple calculation can illustrate what the expected voltage drop across a gap junction channel might be during an action potential. Assume a model where the length (L) of each cell is 80 μm, the radius is 7.5 μm, the maximum rate of rise of the action potential is 100 V/s, and the conduction velocity (θ) is 50 cm/s (see Cole *et al.*, 1988). Furthermore, we will take the resistivity of the myoplasm to be 400 Ω-cm (Weidmann, 1966). The total longitudinal resistance for a cell is then 1.8 MΩ The voltage drop across the long axis

of the cell (V_c) is estimated by $V_c = \{[V/s]/\theta\}*L$ which equals 25 mV. The total longitudinal current is then 25 mV/1.8 MΩ = 14 nA. Assuming there are 4×10^4 channels (Cole *et al.*, 1988; Rudiusli and Weingart, 1991), then dividing the number of channels into the total longitudinal current yields 0.35 pA/channel or 3.5 mV/channel assuming its conductance to be 100 pS. If there are 4000 channels instead of 4×10^4, then the voltage drop equals 35 mV. For 4×10^5 channels the voltage drop is 0.35 mV per channel, and for a million or more channels the drop is less than 100 μV per channel (Brink, 1991) Another issue to consider is the time duration over which this voltage drop would be imposed during the conduction of an action potential. For a $\theta = 50$ cm/sec the time for the action potential to pass over a 1 mm length is about 2 ms. For Cx43 gap junctions, these parameters would not be meaningful in terms of affecting the number of viable channels as Cx43 voltage dependent kinetics, while multiple time constants of inactivation (Wang *et al.*, 1992) are too slow to be meaningfully affected by so short a time duration and one of so small an amplitude under normal conditions.

Cx43 is the most ubiquitous connexin and it can also co-exist with other connexins in the *in situ* circumstance. It is able to form heterotypic channels with other connexins as well (Cx43-Cx46; White *et al.*, 1995; Cx43-Cx45; Moreno *et al.*, 1995). Assuming most of the heterotypic forms do not generate silent gap junction channels, any form should be able to perform the duties required for the conduction of an action potential. K^+ or an equivalent is all that is necessary to ensure action-potential propagation. The possibility of different selective permeability properties for heterotypic channels does hold some regulatory promise though. The diffusion of cAMP, for example, from cell to cell could be different relative to homotypic channels.

Whether heteromeric formation of connexins occurs and has physiological effects distinct from that of homomeric forms remains to be determined. The most plausible difference which would impact on function is selective permeability rather than gating. This distinction arises from the notion that almost all gap junction channels sit in an electric field environment (no standing V_j normally) where the evidence strongly suggests they are open most of the time.

In the heterotypic case, unless the kinetics of voltage dependence is made more rapid by a factor of 2 or 3, it cannot be expected to affect the rapid conduction of an action potential. Even the well-documented Cx32/Cx26 heterotypic gap junction channel (Verselis *et al.*, 1994; Bukauskas *et al.*, 1995) shows kinetics too slow to impact on a conducting action potential. Heteromeric forms might possess intrinsic kinetics like those necessary for rectifying synapse; only more experimentation will tell.

OTHER CONNEXINS

Cx32 and Cx26 both have voltage-dependent kinetics (Verselis *et al.*, 1994) which are too slow to affect action potential conduction. Both are permeable to fluorescent dyes (Veenstra, this volume). The homotypic forms show symmetric voltage dependence with V_o values of ~50 to 90 mV respectively (Verselis *et al.*, 1994).

CONCLUSIONS

The single most important factor that is critical to the conduction of an action potential in multicellular tissues is the total number of channels in the junctional membrane connecting two cell interiors. The voltage-dependent kinetics of gap junctions is not a limiting factor under normal physiological conditions. The poor selectivity properties are also not a limiting factor for the propagation of action potentials in excitable cells. The selective permeability properties are of consequence with regard to the movement of specific molecules from cell to cell such as second messengers. These molecules could affect the conduction velocity of an action potential by altering the gating of channels on nonjunctional membranes.

ACKNOWLEDGMENTS

This work was in part supported by NIH grant HL31299.

REFERENCES

Barr, L., Dewey, M. M., and Berger, W. (1965). *J. Gen. Physiol.* **48**, 797–823.

Barr, L., Berger, W., and Dewey, M. M. (1968). *J. Gen. Physiol.* **51**, 346–368.

Beeler, G. W., and Reuter, H. (1977). *J. Physiol.* **268**, 177–210.

Benz, R. (1986). In *Ion Channel Reconstitution* (Miller, C., ed.), Plenum Press, New York, pp. 553–573.

Bennett, M. V. L., and Verselis, V. K. (1992). *Sem. Cell Biol.* **3**, 29–47.
Bennett, M. V. L., Zheng, X., and Sogin, M. L. (1995). *Prog. Cell Res.* **4**, 3–8.
Berger, W., and Barr, L. (1969). *J. Appl. Physiol.* **26**, 378–382.
Beyer, E. C. (1993). *Int. Rev. Cytol.* **137**, 1–37.
Blatz, A. L., and Magleby, K. L. (1984). *J. Gen. Physiol.* **84**, 1–23.
Brink, P. R., (1991). *J. Cardiovas. Electrophysiol.* **2**, 360–366.
Brink, P. R., and Dewey, M. M. (1978). *J. Gen. Physiol.* **72**, 67–86.
Brink, P. R., and Dewey, M. M. (1980). *Nature* **285**, 101–102.
Brink, P. R., and Fan, S. F. (1989). *Biophys. J.* **56**, 579–593.
Brink, P. R., and Ramanan, S. V. (1985). *Biophys. J.* **48**, 299–309.
Brink, P. R., Ramanan, S. V., and Christ, G. (1996). *Am. J. Physiol. Cell*, in press.
Bukauskas, F., Elfgand, C., Willecke, K., and Weingart, R. (1995). *Pflugers Arch.* **429**, 870–872.
Bullock, T. H. (1945). *J. Neurophysiol.* **8**, 55–71.
Burt, J. M. (1991). In *Biophysics of Gap Junction Channels* (Perrachia, C., ed.), CRC Press, Boca Raton, Florida, pp. 75–96.
Cole, W. C., Picone, J. B., and Sperelakis, N. (1988). *Biophys. J.* **53**, 809–818.
Dermietzel, R., and Spray, D. C. (1993). *Trends Neurosci.* **16**, 186–92.
Eccles, J., *et al.* (1933). *J. Physiol.* **77**, 23–25.
Fursphan, E. J., and Potter, D. D. (1957). *Nature* **180**, 342–343.
Fursphan, E. J., and Potter, D. D. (1959). *J. Physiol.* **145**, 289–325.
Fushiki, S., and Kinoshita, C. (1995). *Progr. Cell Res.* **4**, 239–244.
Goodenough, D. A. (1975). *Cold Spring Harbor Symp. Quant. Biol.* **40**, 37–48.
Jaslove, S. W., and Brink, P. R. (1986). *Nature* **323**, 63–65.
Jaslove, S. W., and Brink, P. R. (1987). In *Cell-to-Cell Communication* (DeMello, C., ed.), Plenum Press, New York.
Little, T. L., Xia, J., and Duling, B. R. (1995). *Circ. Res.* **76**, 498–504.
Lowenstein, W. R. (1981). *Physiol. Rev.* **61**, 829–913.
Makowski, L., Casper, D., Philips, W., Baker, T., *et al.* (1984). *Biophys. J.* **45**, 208–218.
Margiotta, J. F., and Walcott, B. (1983). *Nature* **305**, 52–55.
Mirro, J., Bailey, J. D., and Watnabe, A. M. (1980). In *The Slow Inward Current and Cardiac Arrythmias* (Zipes, D. P., Bailey, J. C., and Eiharrar, V., eds.), Martinus Nijhoff Publishers, pp. 111–127.
Moreno, A. P., Fishman, G. I., Beyer, E. C., and Spray, D. C. (1995). *Prog. Cell Res.* **4**, 405–408.
Neyton, J., and Trautmann, A. (1985). *Nature* **317**, 331–335.
Robinson, S. R., Hampson, E. C. G. M., Munro, M. N., and Vaney, D. I. (1993). *Science* **262**, 1072–1074.
Rudiusli, A., and Weingart, R. (1991). In *Biophysics of Gap Junction Channels* (Perrachia, C., ed.), CRC Press, Boca Raton, Florida, pp. 43–56.
Spray, D. C., and Bennett, M. V. L. (1985). *Ann. Rev. Physiol.* **47**, 281–303.
Tsien, R., and Weingart, R. (1976). *J. Physiol.* **260**, 117–141.
Veenstra, R. D., Wang, Z., Beyer, E. C., Ramanan, S. V., and Brink, P. R. (1994a). *Biophys. J.* **66**, 1915–1928.
Veenstra, R. D., Wang, Z., Beyer, E. C., and Brink, P. R. (1994b). *Circ. Res.* **268**, 706–712.
Veenstra, R. D., Wang, H. Z., Beblo, D. A., Cliton, M. G., Harris, A. L., Beyer, E. C., and Brink, P. R. (1995). *Cir. Res.* **77**, 1156–1165.
Verselis, V. K., Bargiello, T. A., Rubin, J. B., and Bennett, M. V. L. (1994). *Nature* **368**, 348–351.
Wang, H.-Z., Li, J., Lemanski, L. F., and Veenstra, R. D. (1992). *Biophys. J.* **63**, 139–151.
Weidmann, S. (1966). *J. Physiol.* **187**, 322–342.
Weingart, R. (1986). *J. Physiol.* **370**, 267–284.
White, T. W., Paul, D., Goodenough, D. A., and Bruzzone, R. (1995). *Mol. Biol. Cell* **6**, 459–470.