Editorial

Closing the gap in understanding the regulation of intercellular communication

Jeffrey E. Saffitz*, Kathryn A. Yamada

The Center for Cardiovascular Research, Washington University, St. Louis, MO 63110, USA

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See article by Van Rijen et al. [2] (pages 941–951) in this issue.

Of the various ways that cells ‘talk’ to one another, intercellular communication via gap junctions is certainly the most direct. Composed of arrays of densely packed channels that link the cytoplasmic compartments of neighboring cells, gap junctions provide a direct route by which cells can exchange ions and small molecules (up to ~1 kDa in mass) [1]. Different patterns and distributions of gap junctions between like and disparate cell types may create both preferential communication pathways and communication boundaries within a tissue or organ. In the heart, gap junctions are responsible for intercellular transfer of current and, thus, control the precise spatiotemporal pattern of electrical activation to coordinate the contractile activities of individual cells and ensure effective pump function.

Gap junctional coupling has traditionally been regarded as a ‘passive’ electrophysiological property of heart muscle which, like cell size and shape, functions as an important determinant of impulse propagation but one not subject to active regulation, at least not in the way that ion channels responsible for depolarization and repolarization undergo rapid transitions in functional state during the cardiac cycle. We now know that this outmoded view of gap junction channels is inaccurate. Rather than being fixed passive conduits, gap junction channels are, in fact, regulated by complex mechanisms that are only now being identified. A paper in the current issue of Cardiovascular Research by van Rijen et al. [2] adds new information to the increasingly complex picture of regulation of intercellular communication at gap junctions.

The contribution by van Rijen et al. [2] must be regarded in the context of the larger picture of gap junction channel diversity. At least 13 members of the connexin family of gap junction channel proteins have been cloned and sequenced [1]. As a general rule, each connexin is expressed in multiple tissues, and individual cells express more than one connexin. At least three connexins are expressed in the mammalian heart — connexin43 (Cx43), connexin45 (Cx45) and connexin40 (Cx40) — but in different amounts and combinations in different regions of the heart. Cx43 is expressed in many tissues and is present throughout the heart including atrial and ventricular muscle, and most if not all components of the conduction system. In contrast, expression of Cx40 in the adult mammalian heart is restricted to atrial muscle and the cardiac conduction system. Cx40 is also expressed in the lung and in vascular smooth muscle cells and endothelium. The expression of multiple connexins by individual cells in a given tissue raises the distinct possibility that single channels may be composed of more than one connexin. Because each cell’s contribution to a complete channel consists of a hemi-channel (referred to as a connexon) containing six connexin subunits, the potential number of unique ‘hybrid’ connexons and channels is truly vast [1]. Little is known about the natural occurrence or biological significance of hybrid channels in vivo, but a great deal of work involving in vitro expression systems has clearly demonstrated that single channels composed of more than one connexin subunit type can form and may exhibit distinct biophysical properties.

The potential diversity of gap junction channel types raises major questions about the biological purposes suberved by expression of multiple connexins in the heart. Insights have been gained recently through studies of mice in which the expression of one or more connexins has been manipulated genetically. Modest slowing of ventricular conduction velocity has been observed in adult mice that are heterozygous for a Cx43 null mutation (Cx43 +/− mice) [3]. Interestingly, no slowing of atrial conduction has
been observed in these mice, even though both atrial and ventricular muscle express Cx43 abundantly in roughly equal amounts and the levels of Cx43 are reduced by approximately 50% in both tissues [3]. These observations suggest that Cx40, present in the atria but not the ventricles of adult mice, is the major electrical coupling protein in the atria whereas Cx43 fulfills this role in the ventricle. This prediction has been substantiated by recent studies in Cx40 knockout mice which exhibit atrial and atrioventricular conduction disturbances [4]. These observations suggest that the functional role of an individual connexin in the heart may be determined more by its tissue distribution than the specific biophysical properties of its channels. While this conclusion may be simplistic, it is noteworthy that mice in which Cx43 has been totally replaced by Cx40 or by Cx32 (a gap junction channel protein that is normally expressed in the liver and other tissues but not in the heart) exhibit little or no overt phenotype [5]. Thus, one connexin may substitute for another as long as they both form channels with reasonably similar properties. When expression of more than one connexin is manipulated, however, the effects may be more dramatic. For example, the combination of partial deficiency in Cx43 expression (Cx43 +/-) and total deficiency in Cx40 expression (Cx40 --/-) leads to severe morphological and electrophysiological derangements [6].

Diverse connexin expression patterns and channel composition are not the only determinants of intercellular communication in the heart. Increasing evidence implicates changes in protein phosphorylation in the regulation of many aspects of connexin biology including intracellular trafficking and assembly of protein into channels at the cell surface, single channel properties including unitary conductances and channel open state probability, and connexin degradation and turnover kinetics [7]. Most of the work on phosphorylation of the cardiac connexins has centered on Cx43. Much of the Cx43 in cardiac myocytes consists of phosphorylated isoforms, suggesting that one or more constitutively active kinases promote Cx43 phosphorylation in the heart. The responsible enzyme(s) have not been identified or characterized. Changes in the basal levels of Cx43 phosphorylation affect many aspects of channel function but the consequences of different phosphorylating or dephosphorylating treatments on Cx43 channel function are variable and depend, in part, on the specific tissue or cell type in which the protein is being expressed. Much remains to be learned about the diversity of Cx43 phosphorylation isoforms under basal conditions and the signaling pathways mediating biologically relevant changes in Cx43 phosphorylation in response to physiological and pathophysiological stimuli. Even less is known about phosphorylated isoforms of the other cardiac connexins, Cx45 and especially Cx40. In this regard, the paper by van Rijen et al. [2] is of particular interest. van Rijen et al. [2] stably transfected a human Cx40 cDNA into a human hepatoma cell line (SKHep1 cells) which has been used in previous studies of connexin function because these cells are ‘communication deficient’. Most cells including those in established cultured cell lines express endogenous connexins. For this reason, studies of gap junction channel proteins have often been performed in selected cells that express little or no endogenous connexin and, hence, are ‘communication deficient’. SKHep1 cells are not completely communication deficient; they express low levels of Cx45 which can establish intercellular junctions. Nevertheless, expression of endogenous Cx45 in SKHep1 cells is sufficiently limited such that these cells provide an experimental system in which the biological properties of channels formed by the transfected sequences can be studied. van Rijen et al. [2] showed that the transfected SKHep1 cells expressed Cx40 abundantly which was detected by immunohistochemistry at sites of intercellular apposition. When the transfected cells were incubated with a membrane permeant analogue of cAMP (8-Br-cAMP), the mobility of Cx40 on Western blots was shifted, consistent with the protein being phosphorylated. This was associated with a 46% increase in macroscopic junctional conductance between cell pairs, and a change in the distribution of single channel conductances, including most notably a shift in conductance values from 30 to 46 pS and disappearance of an 80 pS conductance. Finally, exposure of transfected SKHep1 cells to 8-Br-cAMP caused a 58% increase in the number of cells into which the membrane-permeable fluorescent dye Lucifer yellow was passed from a single injected cell, presumably reflecting an increase in junctional coupling between these cells.

These results strongly implicate phosphorylation by cAMP-dependent pathways in the regulation of Cx40 channel function and thus, open a new avenue of inquiry in cell–cell communication in the cardiovascular system. There are several important caveats to bear in mind, however, when considering the findings of van Rijen et al. [2] with respect to the role of cAMP-mediated phosphorylation of Cx40 in cardiac electrophysiology. Activation of signaling pathways and the resultant biological effects of changes in protein phosphorylation may differ in diverse cell types. Phosphorylation of Cx40 by cAMP-dependent pathways and changes in channel properties observed in SKHep1 cells may not reflect closely what occurs in the heart. Activation of signaling pathways by cAMP may have multiple direct and indirect effects on downstream targets. van Rijen et al. [2] used a relatively specific inhibitor to show that changes in Cx40 electrophoretic mobility were likely mediated by protein kinase A, but other kinases and/or phosphatases may also play important roles in regulating Cx40 channel function. Shifts in mobility on polyacrylamide gels are relatively insensitive indicators of specific isoforms of phosphorylation and do not necessarily correlate with specific functional changes. Other indirect changes in Cx40 caused by cAMP could have led to functional alterations independent of changes in the electrophoretic mobility of the protein. For these
reasons, it is not yet possible to extrapolate the findings of van Rijen et al. [2] to the role of Cx40 phosphorylation in modulating electrical conduction in the heart or intercellular communication in the vasculature. Nevertheless, the results of this study are valuable and intriguing because they focus attention on the regulation of Cx40 channel function by cAMP-dependent pathways and raise the possibility that intercellular coupling and impulse propagation in the heart may be regulated by phosphorylation of Cx40 channels.

Much work remains to be done to characterize specific residues in the cardiac connexins that are targets of phosphorylation in response to cAMP and other protein phosphorylation and dephosphorylation pathways. A particularly exciting future approach involves characterization of phosphorylated connexin isoforms using tandem mass spectrometry. Biomedical mass spectrometry has been revolutionized by the recent advent of ionization methods such as electrospray ionization to introduce proteins into the mass spectrometer in liquid solution [8]. Peptides produced by digesting proteins with proteases of known specificity can be separated by liquid chromatography prior to ionization. Because the primary sequences of the cardiac connexins are known, the identity of phosphoamino acids can be determined definitively using this approach. The functional consequences of changes in specific phosphoamino acids in the cardiac connexins can be elucidated under conditions in which connexin channel assembly, function and turnover are perturbed pharmacologically or by pathophysiological stimuli, or in which specific residues are targeted by mutagenesis. With this approach, it should be possible to capitalize on the work of van Rijen et al. [2] and others to further close the gap in understanding intercellular communication in the cardiovascular system.

References