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Connexins: the Basis of Functional Coupling of Myocytes

J. Alex, A. R. J. Cale, S. C. Griffin, M. E. Cowen, L. Guvendik

Efficient inter-myocyte communication is essential for synchronised myocardial contraction. Gap junctions are areas where adjacent cell membranes are more closely apposed to each other. Within these gap junctions are present communication ports called connexins. Connexin channels are composed of two grummet shaped hemi-channels called connexons. Each connexon in turn is a hexamer of 6 connexin protein molecules. Connexin channels are selectively permeable to certain ions and molecules less than 1kDa in weight and less than 2nm in diameter. There are three isotypes of connexins expressed by the human myocardium, connexin-40 (Cx40), connexin-43 (Cx43), and connexin-45 (Cx45). Each isotype has distinct unitary conductance, permeability, and gating properties. Cx40 are high conductance channels expressed by atrial myocytes and the conduction system. Cx43 is mainly expressed by ventricular and to a lesser extent by atrial myocytes. Cx45 are low conductance channels mainly expressed by myocytes in the border zone between the conduction system and working myocardium. Connexins regulation, expression, and turnover are actively modulated by myocytes in health and disease. There is increased expression and lateralisation of Cx40 in atrial fibrillation. Cx43 levels are reduced in ischaemic myocardium with lateralisation in the peri-infarct region. In end stage heart failure and dilated cardiomyopathy there is an increase in the expression of Cx40 with a concurrent reduction in Cx43 and Cx45 levels. It is becoming increasingly clear that connexins play an important role in cardiovascular homeostasis in the normal myocardium as well as in disease states. Future research could be directed at achieving ways of optimising and modulating connexin expression as a therapeutic tool. **J Clin Basic Cardiol 2005; 8: 19–22.**

Key words: gap junction, connexin, myocardial coupling

Electrical coupling of myocytes is essential for effective wave front propagation of cardiac action potential and myocardial contraction. Within the intercalated discs there are 3 types of intercellular junctions, the fascia adherens, desmosomes (macula adherens), and gap junctions. Connexins (Cx) are protein channels within gap junctions that form a communication port between the plasma membranes of adjacent cells. They enable electrical coupling of adjacent cells by allowing cell-cell ion transfer and propagation of action potential. They are dynamic carefully regulated channels that maintain cytoplasmic resistivity, cellular geometry, and the syncytial property of the myocardium. Direct cell-cell current transfer in the myocardium occurs only through connexin channels. They also play an important role in normal cardiogenesis. Cardiac myocytes actively adjust the level of coupling by changes in connexin expression, regulation of turnover, and modulation of channel properties.

Histology and Structure

Intercalated Discs

These are specialised transverse junctions between cardiac myocytes at sites where they meet end to end. They coincide with the Z-lines seen in light microscopy. Intercalated discs bind the myocytes, transmit force of contraction, and provide areas of low electrical resistance for rapid excitation through the myocardium. Structurally it is an interdigitating junction and consists of 3 types of membrane-to-membrane contact. The predominant contact is the fascia adherens, desmosomes occur less frequently and gap junctions are present mainly in the longitudinal portions of the interdigitations. Fascia adherens and desmosomes provide mechanical stability and help maintain cell structure by providing anchorage to actin, myosin, and intermediate filaments. Neither play any role in intercellular communication. Actin filaments anchor onto the fascia adherens at each end of the myocyte and thereby transmit contractile forces from cell to cell. Desmosomes tightly link adjacent cells by providing anchorage for the intermediate filaments of the cytoskeleton.

Gap Junctions (Communication Junctions)

These are broad patches where adjacent plasma membranes are more closely opposed leaving a narrow intervening gap (2–4 nm), hence the name gap junctions. Within these patches are hundreds of pores called connexins. They permit the intercellular passage of ions and other small molecules up to 1 kDa in weight and < 2 nm in diameter. Larger molecules and some ions are denied access. They serve as sites for exchange of metabolites, control of growth and development, cell recognition and differentiation. More importantly they facilitate electrical coupling of myocytes permitting synchronous contraction [1–9].

A connexin channel comprises a pair of grommet like hemi-channels (connexons) penetrating the plasma membranes of apposing cells. Each hemi-channel is an oligomer of 6 connexin phosphoprotein molecules hexagonally arranged to enclose an aqueous central pore of 1.5–2 nm diameter. 2 hemi-channels (connexons) dock to form one functional channel across a narrow gap. The phosphoproteins arranged in a slightly twisted conformation, highly susceptible to intracellular Ca^{++}, maintain tubal patency. Increase in intracellular Ca^{++} results in closure of the pores. The unitary conductance and voltage dependence of a channel is regulated by the carboxyl terminal of the connexin phosphoprotein [9–12]. A particle-receptor (or "ball and chain") model has been proposed to explain the mechanism of gating [9]. The carboxyl terminal domain acts as the gating particle that binds to a receptor affiliated with the pore. The carboxyl terminal domain of Cx40 plays an important role in pH gating. The half-life of connexin protein ranges from 60–90 minutes [13]. There are 3 different connexin isotypes in the human myocardium, Cx40, Cx43, Cx45 named numerically on the basis of their predicted mass in kilodaltons [13–16]. In principle 3 types of channels can be formed, homotypic, heterotypic, and heteromeric. When both hemi-channels are identical and composed of only one type of connexin molecule it is homotypic. Heterotypic channels consist of 2 different hemi-channels, each made up of a different type of connexin. If a hemi-channel is an oligomer of

more than one connexin isotype it is termed heteromeric. Cx43 and Cx40 form heterotypic channels with Cx45, while Cx43 does not form heterotypic channels with Cx40. Heterotypic and heteromeric channels are functionally distinct from either parent connexin channels.

Connexin Isotypes and Their Chamber Specific Distribution in the Heart

Cx40: Expressed by atrial myocytes and conduction system [1–8, 12–22]. High conductance channels that allow rapid propagation of action potential. There is a 10-fold quantitative increase in Cx40 between AV node and AV bundle and a further 10-fold increase between AV bundle and purkinje fibres. They also form hybrid channels with Cx45 but not with Cx43.

Cx43: Mainly expressed by ventricular and to a lesser extent by atrial myocytes [1–8, 12–22]. They facilitate coupling of purkinje fibres to surrounding working myocytes. They also form hybrid channels with Cx45 but not with Cx40.

Cx45: Expressed in SA node, AV node, and in border zones between conduction pathway and surrounding myocardium [1–8, 12–22]. They are voltage sensitive channels of low conductance that insulates the central conductive tissue from the working myocardium in the AV node and bundle of His. They also form hybrid channels with both Cx40 and Cx43. The presence of Cx45 in transitional zones between the impulse generation/conduction system and working myocardium is thus of interest because of the potential ability of this connexin to link otherwise incompatible Cx43 and Cx40 expressing zones.

Atria: Atrial myocytes are slender cells with less extensive intercalated disks and more lateral junctional contacts than ventricular myocytes. Gap junctions are larger than nodal junctions, with moderate amounts of Cx40, Cx43, and Cx45.

Ventricle: Mainly Cx43, characteristically lack Cx40 and have very low amounts of Cx45. In the ventricle the Cx45 is mainly concentrated in the conduction system.

Figure 1. Gap junction

SA and AV nodes: Nodal myocytes are small with a dearth of contractile elements and a small, sparse, dispersed gap junctions composed of Cx45 and small amount of Cx40, and almost no Cx43. These features correlate with poor coupling which, in the SA node, is linked to its ability to drive the large mass of surrounding atrial tissue while remaining protected from its hyperpolarising influence, and in the AV node to a slowing of conduction, which ensures sequential contraction of atria and ventricles. Cx45 is present continuously throughout the ventricular conduction system and explains how impulse conduction is maintained (albeit with conduction abnormalities) in the Cx40 knockout mouse.

Purkinje fibres: The ability of the Purkinje fibre system to distribute the impulse rapidly through the working ventricular myocardium correlates with the large abundant gap junctions that, in addition to Cx43 have high levels of Cx40, a connexin associated with high conductance channels.

The selective distribution of Cx40, 43, and 45 underlies the precisely orchestrated pattern of current flow governing normal cardiac rhythm. This compartmentalised expression of specific connexins allows for the orderly and sequential spread of impulses.

Connexins are from a gene family of at least 15 members. Thus there is potential for functional diversity in connexin expression and function. Connexin half-life varies from about 60–90 minutes. Each gap junction has specific properties regarding permeability, voltage gating, and electrical conductance. They are relatively non-selective in permeability. *In vitro*, homomeric gap junctions express distinct unitary conductances, gating properties, and permeability characteristics compared to heteromeric gap junctions, for example, heteromeric Cx40-Cx43 gap junctions are more sensitive to halothane induced closure than homomeric Cx40 gap junctions, they are also more pH sensitive.

Cx40, Cx43 and Cx45 are permeable to molecules $\leq$ 1kDa. However their conductance varies, Cx40 channels have the greatest unitary conductance (121 or 158 pS) and Cx45 channels have the smallest (20 pS). Cx43 channels exhibit multiple conductance (50 and 90 pS are the most frequently observed). Conductance is also modulated by transjunctional voltage, H^+, Ca^{++}, cAMP levels, phosphorylation state of connexins, and the composition and concentration of membrane lipids. Intracellular acidification leads to connexin closure, Cx45 is far more sensitive than Cx43 to pH. All connexin channels are voltage sensitive. Cx43 channels are relatively voltage insensitive and highly permeable to anions and cations. In contrast Cx40 and Cx45 channels are highly cation-selective. Cx45 gaiting is highly voltage dependent.
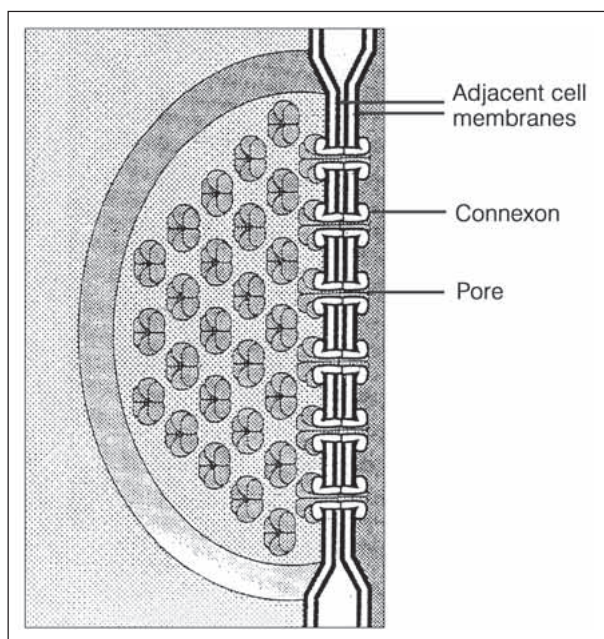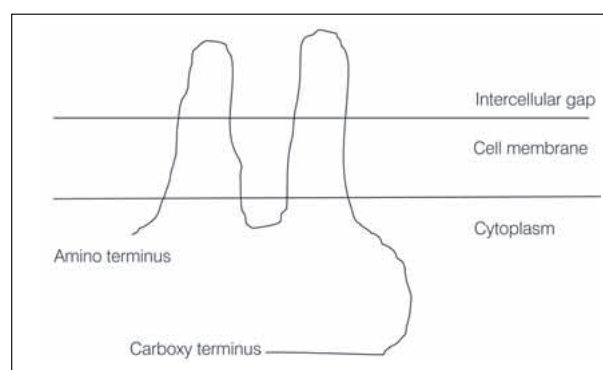

Figure 2. Connexin protein structure

Connexins and Heart Disease

In heart disease the amount and distribution of connexins are altered. In ischaemia there is increased myocardial resistivity and reduced conductivity by increasing cardiac contractile dysfunction and arrhythmia tendency. In diseased myocardium there is a 30–40 % reduction in connexin per intercalated disk. In postischaemic ventricles the reduction is limited to a few cell layers around the affected area, but it's more widespread in hypertrophic myocardium.

IHD [23–27]: The spatial distribution of connexins is altered in the infarct border zone. Distant from the infarct scar there is reduction in the quantity of immuno-detectable Cx43 in regions of normal gap junction distribution (47 % reduction in gap junctional surface area per unit cell volume and a 30 % reduction per cell), i. e., normal number of intercalated disks per myocyte and with normal junction sizes but a reduction in the number of gap junctions (connexins) per intercalated disk. In the infarction border zone there is redistribution of Cx43 and an increased susceptibility to ventricular arrhythmias. Myocytes immediately abutting healed infarcts have Cx43 gap junctions spread longitudinally over the cell surface and not in discrete transversely orientated intercalated disks as in normal myocardium. Such changes in gap junction organisation and orientation in myocardial ischaemia may be implicated in the elusive link between subcellular structure and contractile dysfunction in IHD. The consequent localised heterogeneous conduction and reduced conduction velocity provide an explanation for the genesis of re-entry arrhythmias and susceptibility to VF/VT.

Ventricular hypertrophy [28, 29]: During early stages there is an up-regulation of Cx43 mediated by cAMP and angiotensin II. In late stages of cardiac failure conduction velocity and connexin expression are down-regulated. The Cx43 gap junction expression per myocyte is not significantly different from normal but there is a 40 % reduction per unit volume of myocyte.

End stage heart failure and dilated cardiomyopathy [28, 29]: There is an increase in Cx40 mRNA in ischaemic heart failure. This increase is more pronounced in the left ventricular myocytes than in the right. There is a concurrent reduction in Cx43 and Cx45 mRNA and proteins. Thus in the failing myocardium the distribution of Cx43 becomes spatially heterogenous.

Conduction abnormalities and arrhythmias [30–39]: Right bundle branch block (RBBB), left bundle branch block (LBBB), impaired SA nodal, AV nodal, and interatrial conduction defects are noted with reduced expression of Cx40. Lateralisation and increased expression of Cx40 in atrial myocytes is associated with an increased risk of AF. In human atrium conduction velocity is inversely related to Cx40 levels.

Reduction in the number and density of Cx43 is associated with increased ventricular conduction velocity and vulnerability to arrhythmias. There is no proportionate relationship between the amount of reduction in Cx43 and the development of arrhythmias. Shift of Cx43 from terminal intercalated disks to the lateral borders is noted in virtually all forms of ventricular arrhythmias. The intercalated disk size was not changed. Lateralisation of connexins is a feature of diseased myocardium, however the intercalated disk size is not affected by the reduction in terminal Cx43 content.

Hibernating myocardium [23, 24, 29]: The Cx43 content of intercalated disks is reduced by 23 % and 33 % in reversibly ischaemic and hibernating myocardium, respectively, and average size of gap junction is reduced by 13 % and 30 %, respectively. There is a possibility that alteration in intercellular coupling at gap junctions could directly or indirectly contribute to the pathogenesis of hibernating myocardium. Also reduced gap junction coupling in areas of hibernating myocardium disrupt the wave front propagation of impulses and interfere with coordinated myocardial contraction.

Conclusion

The importance of connexins in the pathogenesis of cardiac disease lies in its central role as the communication port between myocytes. Changes in connexin turnover, distribution, and dynamics result in a loss of electrical synchronisation [37–39]. On the other hand the changes in connexin expression in heart disease serves to reduce the cell-to-cell transfer of cytotoxic metabolites, free radicals, and ions (especially released Ca^{++}) from dying myocytes to healthy ones. The net result is limitation of the disease process. Thus connexins play an important role in syncytial well being during health and limits the extent of myocardial damage during disease. More research is needed to find ways of optimising and modulating connexin expression in disease states.

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