Cardiac Connexin-43

Cardiac connexin-43 (Cx43) creates gap junction channels (GJCs) at intercellular contacts and hemichannels (HCs) at the peri-junctional plasma membrane and sarcolemmal caveolae/rafts compartments.

1. Introduction

Cx43 is the most expressed cardiac protein in the heart out of all the 20 rodents and 21 human connexin isoforms. As a typical integral membrane protein, Cx43 is synthesized in the rough endoplasmic reticulum and translocated into the Golgi compartment (Figure 1a) for folding and oligomerization into connexons. These are termed hemichannels (HCs) and they then follow the secretory pathway to reach their final destination at the plasma membrane.

Figure 1. Electron microscopic images of the adult rat heart cardiomyocytes showing structures hosting Cx43. (a)—Golgi compartment in which Cx43 is oligomerized to HCs; (b-d)—post-Golgi HCs underwent targeted delivery to the particular membrane-subdomains, i.e., caveolae/rafts and gap junctions, close apposition of neighboring cardiomyocytes, which consist of paired HCs forming gap junction channels (GJCs). Gap junction plaques (arrows) are prevalent at the intercalated disc (b) and scarce at the lateral sides of the cardiomyocytes (c), while caveolae/rafts (brackets) are abundant at the lateral membranes (d). Peri-junctional plasma membranes (boxes in (e,f)) contain undocked HCs and Panx1 channels. The internalization of gap junctions into annular profiles (arrowheads in g-j) precedes degradation of HCs via proteasomal and lysosomal pathways. Abbreviations: G—Golgi compartment, N—nucleus, D—adhesive junction, desmosome, FA—fascia adherent junction, M—mitochondria, esc—extracellular space, scale bar—0.1 micrometer. The diagram demonstrates the putative topology of GJCs at the gap junction plaque,
HCs and Panx1 and also the Na\textsubscript{v}1.5 channels at the peri-junctional membranes together with transmitted ions and molecules; ICS—intracellular space, ECS—extracellular space. Tribulova, unpublished representative electron micrographs.

The mechanism of how newly translated oligomerized proteins arrive at their proper location is still being explored\textsuperscript{[1]–[3]}. Docking two HCs from neighboring cardiomyocytes into GJCs and assembly into gap junction plaques is quite prevalent at the intercalated discs, but rare at the lateral plasma membrane of cardiomyocytes in the healthy heart (Figure 1b,c). Connexin is characterized by four transmembrane segments with \( \alpha \)-helical conformation and N- and C-terminal tails projecting into the cytoplasm. Two extracellular loops (E1 and E2), containing about 31 and 34 amino acids, respectively, are highly conserved and covalently connected by three invariant disulphide bonds. The extracellular domains E1 and E2 of the Cx43 have a critical function in the formation of the complete GJCs. Early phosphorylation and the presence of adhesion molecules appears essential prerequisites for HCs to GJCs assembly\textsuperscript{[1]–[3]}. Some HCs in peri-junctional membranes (peri-nexus) do not undergo docking, and exert specific non-canonical functions beyond GJCs\textsuperscript{[3]–[4]}. However, their mutual proximity implies integrated HCs and GJCs interaction and cooperation (Figure 1e,f). In addition, the peri-junctional membranes contain clusters of Na\textsubscript{v} 1.5 channels that influence cardiac conduction\textsuperscript{[5]–[6]}, and these are combined with the large non-selective conductance channel constituted by pannexin1 (Panx1) \textsuperscript{[7]–[8]}. There is also evidence for an ephaptic contribution to cardiac conduction which influences synchronization and timing of action potential firing \textsuperscript{[9]–[10]}. The ephaptic conduction of electrical impulses occurs through transients in extracellular ions at the intercalated disc, and this prompts the activation of adjacent myocytes. This mechanism, however, appears to be localized in the peri-junctional region, and it implicates HCs and Panx1 channel activation.

Pannexins form channels at the plasma membrane surface that establish a pathway for communication between the cytosol of individual cells and their extracellular environment. Pannexins do not form intercellular GJCs, but only membrane channels. The pannexin family consists of 3 members, namely Panx1, Panx2, and Panx3, whereby Panx1 is the most expressed in the heart \textsuperscript{[11]–[12]}. Panx1 was found to bind directly to actin, and this suggest that microfilaments act to stabilize Panx1 cell surface distribution or even participate in Panx1 delivery or internalization\textsuperscript{[13]}. The current destination of HCs can also be determined by specific lipid rafts or membrane micro-domains associated with caveolae at the sarcolemma (Figure 1d). These are most important in compartmentalizing a variety of signaling activities\textsuperscript{[14]}. Both HC and GJC are recycled via a post-endocytic event and this is consistent with rapid myocardial Cx43 turnover and very short half-life of 1–2 h \textsuperscript{[15]–[16]}. GJCs from the middle part of the gap junction plaque internalize to form a circular profile as “annular gap junctions” (Figure 1g). These undergo degradation by proteasomes and lysosomes\textsuperscript{[17]–[18]}. However, the considerably rapid HCs/GJCs turnover is most important because it illustrates the dynamic nature of intercellular communication and Cx43 mediated signaling \textsuperscript{[19]–[20]}. In contrast to the Cx43 protein, the Panx1 channels are composed of long-lived pannexin proteins which suggests a slower life cycle where internalized pannexins are degraded by lysosomes\textsuperscript{[17]}.

In addition to evidence that GJCs and HCs promote opposite responses in cellular signaling events, molecular studies indicate differences in GJCs’ and HCs’ dependence on intramolecular loop/tail interactions for their activity \textsuperscript{[21]–[22]}. Monoclonal antibodies to the extracellular loop E2 enable HCs visualization of cell membranes without GJCs visualization because E2 epitope is not free in dodecameric GJCs \textsuperscript{[23]–[24]}. These antibodies could aid HCs topology study through the changes in their myocardial distribution in response to acute and chronic heart injury associated with arrhythmias.

As illustrated in the diagram in Figure 1, GJCs, HCs and the Panx1 channels create a unique and dynamic signaling network in the heart and vascular system to ensure tissue homeostasis and proper function.
This network coordinates myocardial contraction. It is also the most important in heart adaptation, and especially for the response to stressful or pathophysiological conditions by propagating both cell survival and apoptosis signals \[^{33,34,35,16,17,25,30,36}\]. It is a fundamental and permanent task to control this network when trying to prevent or treat heart and life-threatening arrhythmias. Evidence suggests that pharmacological HCs and Panx1 channel blocking prevents their undesirable action following stress or injury, and also that the protective GJCs function could provide new treatment opportunities.

2. Implication of HCs and Panx1 Channels in Disturbances of Ionic and Redox Homeostasis as Well as Pro-Inflammatory and Pro-Fibrotic Signaling

Compared to GJCs, the HCs and Panx1 channels operate out of cardiomyocyte membrane gap junctions. Therefore, dominant myocardial HCs and Panx1 channel topology appears restricted to the lipid rafts/caveolae on lateral cardiomyocyte plasma membrane (Figure 1d). In addition, they also act at the peri-nexus \[^{34}\], i.e., at the peri-gap junctional membrane (Figure 1e,f). HCs also perform the “non-canonical” functions of Cx43, beyond those of the GJCs and independent of gap-junction formation \[^{16,37,38}\].

Findings from various non-cardiac tissues that might be relevant for cardiomyocytes indicate that HCs channels remain closed in physiological resting conditions, but change to open conformation as the extracellular \(\text{Ca}^{2+}\) concentration decreases or intracellular \(\text{Ca}^{2+}\) concentration increases, which open Panx1 channels as well \[^{39,40,41,42,43}\]. However, HCs and Panx1 channels do not have the exact same regulation. HCs tend to be activated by strong depolarization, while Panx1 channels activity may be induced at the resting membrane potential \[^{44}\]. The pore diameter increases from 1.8 to 2.5 nm and this enables transmission of molecules up to 1 kDa. HCs opening enables typical single-channel conductance of approximately 220 pS \[^{45}\] and Panx1 channels have a large unitary conductance of around 500 pS. In addition, hydrophobic extracellular domains are also crucial in regulating \(\text{Ca}^{2+}\)-dependent conformational changes \[^{33}\].

The HCs and Panx1 channels can open following different extracellular stimuli. This is established in various non-cardiac tissues \[^{32,40,41,42,43,44,45,46,47}\], but highly relevant to the heart. The HCs and Panx1 channels can therefore be activated by membrane depolarization, metabolic inhibition, and stresses including shear mechanical membrane forces and ionic and ischemic stress \[^{31,17,48,49}\]. The HCs, and most likely also the Panx1 channels, are influenced by intra- and extra-cellular pH and phosphorylation and redox status \[^{34,33,16,40,49,50,51,52}\]. All these events during heart disease development can activate both HCs and Panx1 channels and promote arrhythmias through bi-directional ion passage and small metabolic or signaling molecules below 1–2 kDa. Thus, HCs and/or Panx1 channels can mediate \(\text{Na}^{+}\), \(\text{K}^{+}\), \(\text{Ca}^{2+}\), \(\text{cAMP}\), \(\text{ATP}\), \(\text{NAD}^{+}\) influx as well as the transmission of glutamate, glutathione, prostaglandin-E2 and epoxyeicosatrienoic acid \[^{3,14,53}\]. Panx-1 channels are known mostly as ATP release channels \[^{42}\]. Intracellular localized Panx1 has also been proposed as a regulator of sarcoplasmic reticulum-based \(\text{Ca}^{2+}\) homeostasis \[^{54}\].

HCs and Panx1 channel opening can profoundly affect intracellular \(\text{Ca}^{2+}\) homeostasis, contribute to \(\text{Na}^{+}\) and \(\text{Ca}^{2+}\) loading and \(\text{K}^{+}\) loss, alter redox status, and facilitate pro-inflammatory and pro-fibrotic conditions \[^{36,39,40,53,56}\]. These events impair GJCs function and produce myocardial electrical instability, thereby promoting malignant arrhythmias \[^{57,11,48,58,59}\]. It was also previously noted that the activation of peri-junctional Panx1 channels \[^{16}\] and \(\text{Na}_{\text{v}}\) 1.5 channels can influence cardiac conduction. Abnormal \(\text{Na}_{\text{v}}\) 1.5 channel function along with autoantibodies against Cx43 and activation of Panx1 channels may underlie heritable arrhythmic syndromes \[^{60,61,62}\].

HCs and Panx1 channel mediated ATP release is fundamental in the purinergic signaling which is so important in promoting inflammation in vascular function/dysfunction \[^{44,63,48,64,65,68}\]. The inflammation is known to impair GJCs function and significantly contribute to arrhythmogenesis. The Panx1 channels present at internal membranes may be implicated in \(\text{K}^{+}\) influx to mitochondria and
intracellular Ca\textsuperscript{2+} leak/release from the endoplasmic reticulum\textsuperscript{[27][52][67]}. Moreover, ATP binding to the P2Y receptors increases inositol 1,4,5-triphosphate which then releases Ca\textsuperscript{2+} from the ER and enhances ATP signaling to neighboring cells\textsuperscript{[1]}. Disturbances in intracellular ionic homeostasis and Ca\textsuperscript{2+} overload promote triggered “post-depolarization” and GJCs uncoupling which render the heart prone to malignant arrhythmia development; and especially VF\textsuperscript{[69][69][70][71][72]}. This triggered Cx activity and GJCs disordered expression and dysfunction are also implicated in AF development\textsuperscript{[70][73]}.

Cellular stress, including myocardial ischemia, enhances cardiomyocyte ATP release through the Panx1 channels that facilitate early fibroblast activation\textsuperscript{[42]}. This suggests an early paracrine event leading to pro-fibrotic responses which could be involved in arrhythmogenic substrate development. In contrast, HCs inhibition by TAT-Gap19 alleviated tissue fibrosis and introduced the possibility of preventing arrhythmias\textsuperscript{[74]}. Fibrosis precedes inflammation, and cardiac fibroblasts contribute to the inflammatory milieu through increased secretion of pro-inflammatory cytokines and chemokines released by the HCs and Panx1 channels\textsuperscript{[75][76]}. Here, the Panx1 channels are interesting because of their implication in a variety of cellular responses\textsuperscript{[1][12]} including apoptosis\textsuperscript{[27]}, inflammation\textsuperscript{[78]}, and innate immune processes\textsuperscript{[79][80]}. Inflammation is also a prominent feature of arrhythmogenic cardiomyopathy\textsuperscript{[81]}. Therefore, targeting inflammatory pathways could be an effective new mechanism-based therapy for familial non-ischemic heart muscle disease which causes sudden death in the young, and especially in athletes.

Figure 2 illustrates that functional HCs and Panx1 channels provide paracrine and autocrine communication pathways for ion and small molecule passage across the plasma membrane\textsuperscript{[1][16][17][48][64]}. HCs and Panx1 channels may regulate many cellular processes but little information on their mechanisms is available. Extended information on HCs and Panx1 channel function from in vitro sources indicates their implication in diverse physiological and pathological responses relevant to the cardiovascular system\textsuperscript{[33][1]}. However, the cardiac muscle remains ‘terra incognita’ and this provides challenges. This is especially important, in conjunction with rafts/caveolae related signal-transducing molecules, in the development of heart disease and life-threatening arrhythmias. The compartmentalized signal transduction appears to be an attractive area of research.
as a paracrine and autocrine communication pathway. HCs mediated ATP release is important in the purinergic signaling which is so important in cardiac pathophysiology. It may also be implicated in arrhythmogenesis (see text for details). Abbreviations: ICS—intracellular space, ECS—extracellular space, gcx—glycocalyx.

It is important that the caveolae and lipid rafts of cholesterol and sphingomyelin enriched membrane micro-domains are considered in HCs and Panx1 channels. These are all involved in the regulation of cell signaling pathways and in GJCs homeostasis. Caveolin-3 is the major caveolin isoform in cardiomyocytes, and this has been implicated in 17β-estradiol-elicited, rapid signaling to regulate Cx43 phosphorylation during ischemia. Moreover, the caveolae/lipid rafts have been involved in reactive oxygen species production, redox signaling and K⁺, K_ATP, Na⁺, and Ca²⁺ ion channel functions.

Cardiomyocytes and endothelial capillary cells are rich in caveolae which invaginate the plasma membrane, and this suggests the implication of these compartments in extensive HCs and Panx1 channel-mediated paracrine and autocrine signaling. The interaction of these channels and caveolins implies compartmentalized signaling, and this could be most important in the pathophysiological development of heart dysfunction and arrhythmias. Investigation of this issue presents future challenges.

### 3. Impact of HCs and Panx1 Channels Activity on Development of Cardiac Arrhythmias

There is still a lack of information on HCs and Panx1 channel roles in cardiac muscle. Nevertheless, it has been shown that both HCs and Panx1 channels are the potential route for Na⁺ influx and K⁺ efflux during ischemia in isolated ventricular cardiomyocytes. This can induce electrical disturbances. The opening of HCs and Panx1 channels during ischemia has also contributed to re-perfusion injury following brief cardiomyocyte ischemia. The activation of HCs and Panx1 channels during ischemia/hypoxia, combined with other metabolic inhibition and post-ischemic reperfusion mechanisms may compromise cardiomyocyte ability to maintain ionic homeostasis. This is an essential step in promoting both arrhythmias and apoptosis. Importantly, HCs contribute to cytoplasmic Ca²⁺ oscillations by providing bimodal Ca²⁺ dependent Ca²⁺ entry pathway. Moreover, ischemia and/or hypoxia are associated with increased production of NO and S-nitrosylation which is important in regulating HCs and Panx1 channel permeability. Opening of connexin 43 hemichannels is increased by lowering intracellular redox potential. The NADPH oxidase inhibitor apocynin prevented HCs activity by reducing nitroso-redox stress. All these factors strongly suggest that HCs are implicated in arrhythmogenesis.

The release of ATP via HCs or Panx1 channels in pathological conditions could induce post-depolarization triggered activity. This would generate ventricular tachycardia or Vf. Moreover, purinergic receptor activation by ATP induces ventricular tachycardia through membrane depolarization and Ca²⁺ homeostatic disorders. Resultant sporadic Panx1 channel opening then triggered action potentials and promoted arrhythmogenic activity.

In addition, extracellular ATP induced shortened action potential duration in tissue preparations of atrial and ventricular myocardium and in the myocardial sleeves of pulmonary veins. These events are known to precede AF. The implication of ATP or adenosine in AF development, and its post-ablation recurrence, have been reported. This AF was associated with a strong increase in atrial adenosine.

In addition, non-myocytes in scar tissue can be electrically coupled to cardiomyocytes through GJCs, HCs, and Panx1 channels and contribute to electrotonic conduction across scar tissue. Cx43 is...
increased in activated fibroblasts during fibrogenesis\textsuperscript{[26]}\textsuperscript{[123]}\textsuperscript{[124]} and it enhances the possibility of electrically coupled cardiomyocytes and fibroblasts \textsuperscript{[123]}\textsuperscript{[124]}\textsuperscript{[125]}. This hetero-cellular electrotonic coupling may facilitate conduction disturbances and arrhythmias \textsuperscript{[126]}. Fibroblasts and myofibroblasts can alter cardiomyocyte excitation-contraction coupling through paracrine mechanisms\textsuperscript{[127]}, and this suggests the paracrine modulation of myocardial contractility and synchronized contraction. Fibrotic tissue, however, is an inefficient electrical signal conductor and cannot rhythmically contract, thereby jeopardizing critical myocardial functions. This combination provides heterogeneous cardiac tissue, and this is a well-known predictor of arrhythmia risk. Although HCs and Panx1 channels present an attractive therapeutic target, their inhibition requires the development of more specific inhibitory agents.

References


113. Pustovit, K.B.; Potekhina, V.M.; Ivanova, A.D.; Petrov, A.M.; Abramochkin, D.V.; Kuzmin, V.S. Extracellular ATP and β-


Keywords
cardiac arrhythmias; gap junction channels; Cx43 hemichannels; Panx1 channels

Retrieved from https://encyclopedia.pub/7383