Review

Cardiac gap junction channels: modulation of expression and channel properties

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Abstract

In the heart, intercellular gap junction channels constructed from connexin molecules are crucial for conduction of the electric impulse. Cardiomyocytes can be interconnected by channels composed of three types of connexin proteins: Cx40, Cx43 or Cx45. In mammalian hearts, these three isoforms are regionally differently expressed and even between the species differences exist. Each of these channel-types possesses specific properties and are susceptible to modulation by various mechanisms. In this paper we compare the differences in properties of these channels as deduced from studies on transfected cells and isolated cardiomyocytes and discuss the factors involved in modulation of channel properties. Next, we evaluate the consequences of alterations in expression and modulation of channel properties for cardiac function. Therefore, we have compared reports on genetically engineered animals and discuss this information in relation to various pathophysiological disorders. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Normal growth, development and appropriate functioning of many tissues and organs depend on maintenance and regulation of intercellular communication. This can be mediated by extracellular neurohumoral factors, but most mammalian cells also directly connect by gap junctions. Gap junctions are agglomerates (plaques) of multiple intercellular channels which connect the cytoplasm of adjacent cells. Thereby they provide both electrical and metabolic coupling. Electrical coupling fulfills a key role in excitable tissues (e.g. smooth and cardiac muscle) where

propagation of the electric impulse is mediated by passage of ions through gap junction channels. Metabolic coupling, i.e. the transport of small metabolites, nucleotides and second messengers with a molecular weights up to 1.2 kD [1–3], might also play an important role in differentiated tissues (e.g. in electrical synapses of neurons) but certainly has a critical function in embryo- and organogenesis.

As depicted in Fig. 1A, each gap junction channel is composed of 12 connexin (Cx) molecules, assembled from two hexameric hemichannels (connexons), one of each delivered by both participating cells. The individual connexins form a multigene family of highly related but not identical transmembrane proteins (reviewed in [4]). In mammals, at least 15 members, named after their theoretical molecular mass (in kD), have been cloned to date (for review see [5–7]). The overall gene and protein structure is largely conserved among the various isoforms. Fig. 1B shows that all connexin proteins consist of four transmembrane segments (M1–M4), two extracellular loops (E1, E2), one intracellular loop (CL) and a cytoplasmic amino and carboxy terminus (for review see [4]). Strong

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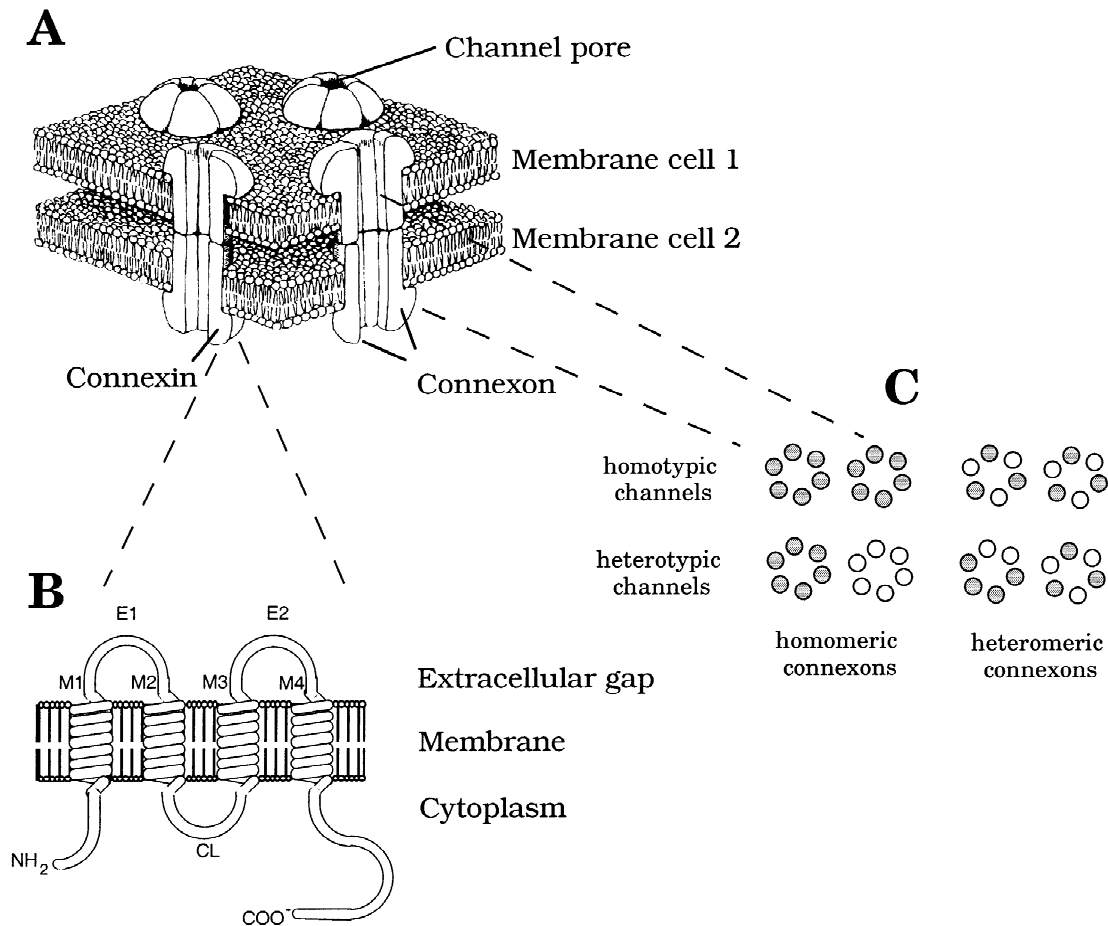**Guest Editor: J.E. Saffitz. Time for primary review 40 days.**

Fig. 1. Schematic illustration of gap junction structure. (A) Part of a gap junction plaque showing several channels interconnecting two cells and the composition of an individual channel from two half-channels (connexons) which are composed of connexin proteins. (B) Secondary structure of a single connexin protein. (C) Scheme explaining the composition of homotypic and heterotypic channels from homomeric and heteromeric connexons.

conservation of the amino acid sequence is mainly found in both extracellular loops, the amino terminus and the four transmembrane segments. Three of these transmembrane segments (1, 2 and 4) consist predominantly of hydrophobic amino acids while the third segment has a more amphiphatic character suggestive of a role in the inner lining of the aqueous pore [8]. The two extracellular loops (El and E2) each contain three conserved cysteines that likely participate in the process of docking of the two hemichannels [9–11]. Disulfide bonds in between the cysteines within El and E2 but also crossing the space between El and E2 create the β-sheet conformation required for interaction between the two opposing connexons (see [4]). After translation of the mRNA, connexins are inserted in the membranes of the endoplasmic reticulum [12], followed by oligomerization into connexons in the Golgi complex [13]. Once in the sarcolemma, a functional channel is created by head-to-head docking of hemichannels at spots where both plasma membranes are in close apposition, thereby creating an aqueous pore between both cells.

When both connexons are constructed from identical connexins (Fig. 1C), a homotypic channel is formed. The

degree of conservation (among different connexin isoforms) of the second extracellular loop [10], provides the opportunity for connexons built from one particular type of connexin (homomeric), to dock with a connexon composed of another isoform thereby creating a heterotypic channel. Additionally, even within one hemichannel, different connexins can be integrated (heteromeric connexon) which can result in both homotypic- and heterotypic channel formation.

The biochemical and electrical characteristics of gap junction channels depend on their constituent connexins. Moreover, the properties of gap junction channels can be modulated by various mechanisms like (de)phosphorylation of the individual connexin proteins, the transjunctional voltage gradient, intracellular hydrogen and free calcium concentration, and extracellular fatty acid composition. Alterations in the amount of channels can be induced by changes in expression level of the connexin proteins, turnover rate, and distribution (presumably affected by the phosphorylation state of the channels [14]). In this review we will focus on modulating factors affecting expression and characteristics of gap junctions in the mammalian heart.

2. Expression of gap junction proteins in the heart

Propagation of the action potential from cell to cell is mediated by current flow through gap junction channels, primarily located in the intercalated disc (ID) at the end-to-end intercellular connections. Large and small IDs are found parallel to the long axis of the cell (interplicate) while only small IDs can be found in regions at right angles to the long axis (plicate) [15].

In mammalian hearts, cardiomyocytes most prominently express gap junctions built of connexin40 (Cx40), connexin43 (Cx43) and connexin45 (Cx45). Therefore, we will restrict this review to these three isoforms. Another three isoforms have been reported; in several species, connexin37 is expressed in the endothelial cells of the endocardium, aorta and coronary vessels [16–18] where it is co-expressed with Cx40 [19]. Connexin46 protein has in addition to other isoforms been demonstrated in the rabbit sino-atrial node [20]. Connexin50 protein has been detected in the atrio–ventricular valves of rat heart [21].

The expression patterns of Cx40, Cx43 and Cx45 are subject to remarkable spatio-temporal differences [17,22–24]. In adult hearts Cx43 is the major expressed isoform. Fig. 2 depicts the generalized adult expression patterns of Cx40, Cx43 and Cx45. In mammalian hearts, Cx43 is expressed in virtually all myocytes of the working atrial- and ventricular mammalian myocardium regardless the stage of development. No Cx43 is found in the nodal cells of the SA and AV node. In the ventricular conduction system of mouse and rat, Cx43 is absent in the common His-bundle and proximal parts of both bundle branches, but it is expressed in more distal parts of the bundle branches. In larger species as cow, dog, pig and human, Cx43 is expressed in all parts of the ventricular conduction system.

Cx45 is expressed in the rabbit SA node [25]. In rat and mouse hearts, Cx45 is found in the AV node and complete ventricular conduction system but only at very low levels in the surrounding working myocardium of atria and ventricles [26,27].

The third connexin, Cx40, is strongly co-expressed with Cx43 in the working myocardium of the atria (except in rat). During fetal and neonatal development, Cx40 is still present in the ventricles where the intensity of expression gradually declines from endo- to epicard [28]. However, within days of birth, expression levels decrease and in adult stages Cx40 is absent in the ventricular working myocardium. Cx40 remains strongly expressed in the ventricular conduction system where it colocalizes with Cx45. Finally, low levels of Cx40 are present in the SA node and AV node of dog and cow [29], and the rabbit SA node [20,25]. Differences in expression patterns between the hearts of different species have been described in detail by Gros and Jongsma [30] and quoted references.

3. Modulation of gap junction channels built of Cx40, Cx43 and Cx45 in a reduced cell system

Cardiac gap junction channels have been intensively studied in cultured cardiac myocytes. Although ventricular cardiomyocytes probably exclusively express Cx43, other cardiomyocytes can express more than one type of connexin. The expression of more than one connexin isoform might give rise to heterotypic/heteromeric channel formation, thereby creating a plethora of different channel types
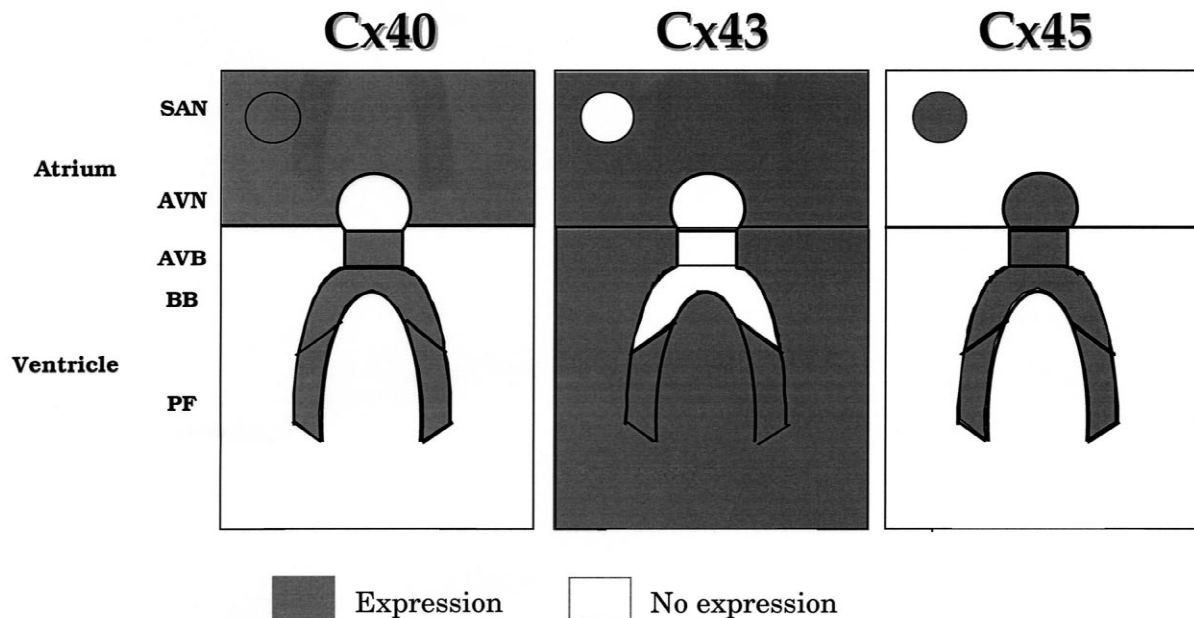

Fig. 2. Generalized expression pattern of Cx40, Cx43 and Cx45 in the different regions of the mammalian heart. SAN, sinoatrial node; AVN, atrioventricular node; AVB, atrioventricular bundle or His-bundle; BB, bundle branches; PF, Purkinje fibers.

with a high level of complexity. Appropriate assessment of the electrical properties of the individual gap junction isoforms requires a simplified cell system expressing only one isoform.

The genes encoding for the different cardiac connexins have been characterized, cloned and introduced in non-communicating tumor cells or Xenopus oocytes in order to create a reduced cell system overexpressing (exogenous) gap junction channels built of one type of connexin only. Such a simplified system allows for evaluation of single channel properties and some of the modulating factors affecting these properties. We will focus our attention on the genes cloned from humans, mice and rats.

The isoform- but also the species-specific characteristics are particularly determined by variations in the amino acid sequence of the cytoplasmic loop (between transmembrane segments 2 and 3) and the C-terminus where multiple amino acid motifs are found to be susceptible to phosphorylation by different intracellular protein kinases [6,31–33]. Phosphorylation occurs predominantly on serine residues, but also on threonine and tyrosine residues. Interestingly, cyclic nucleotides which activate protein kinases, are also able (in nanomolar concentrations) to modulate channel properties directly [34]. Together with more conserved domains of the protein, the cytoplasmic loop and carboxy-terminus might be involved in different modes of regulation. The C terminus, N terminus, first transmembrane segment, first extracellular loop and a conserved proline residue in the second transmembrane segment, are reported to participate in the voltage gating properties of the channels [35,36], possibly similar to pH gating, according to a ball-and-chain model [37]. In addition, voltage gating might be influenced by the interaction of a connexon with its opposing connexon [38]. Protonation of three conserved histidine residues in the cytoplasmic loop might be required in the process of pH gating [39–41]. In Cx43 channels, these histidines may serve as a receptor for as-yet unidentified parts of the carboxy-terminus in order to facilitate pH gating according to the ball-and-chain

model [42,43]. A similar model was postulated for Cx40 but not for Cx45 [44]. A cooperative interaction has been described for Cx40 and Cx43 which increases the pH sensitivity of those channels [45]. Within the physiologically relevant range of intracellular Ca^{2+} and H^+ concentrations, it is unlikely that Ca^{2+} or H^+ independently strongly affect the conductive properties of Cx43 channels [41,46]. Yet, there might be a (not completely understood) synergistic mechanism between Ca^{2+} and H^+ that potentiates their effect on Cx43 channels and could be of importance under conditions of cardiac ischemia when intracellular Ca^{2+} and H^+ are elevated [47,48].

Intercellular coupling concerns macroscopic (electrical) conductance (g_j) and metabolic coupling. Both are determined by the number of expressed channels (N), the single channel conductance (γ_j) or permeability and the open probability of a single channel (P_o). Single channel properties can be affected by modulation of γ_j but also by changes in P_o of the channels. Electrical coupling of cells can be studied using the double-voltage-clamp technique [49,50]. Metabolic coupling is evaluated by the passage of small fluorescent (dye) metabolites [1]. Most commonly used tracers are Lucifer yellow (LY, 443 Da), 2′7′-dichlorofluorescein (DCF, 401 Da) and 6-carboxyfluorescein (6CF, 376 Da). The following sections summarize the characteristics of homotypic channels built of Cx40, Cx43 and Cx45 as deduced from studies on transfected cells (see also Table 1).

3.1. Cx40 channel properties

The Cx40 gene has been characterized and cloned from several species (see [6]). The coding region exists of 358 amino acids in human Cx40 (hCx40) and mouse Cx40 (mCx40) and of 356 in rat Cx40 (rCx40). On Western blot, the protein can be separated in two bands of 40- and 42 kD [51,52]. To study the single channel characteristics, the gene has been introduced into several communication deficient cell-lines.

Table 1
General properties of Cx40, Cx43 and Cx45 from human, mouse and rat

	Cx40			Cx43			Cx45		
	Human	Rat	Mouse	Human	Rat	Mouse	Human	Rat	Mouse
Coding region (aa)	358	356	358	382	382	382	396	396	395
Motifs for PKC	7	12	15	10	14	14	15	17	17
PKA	2	1	1	4	3	3	1	1	1
PKG	1	3	3	3	4	4	0	0	0
MAP kinase	0	0	0	2	3	3	0	0	0
Dye permeability	+LY [52]	+DCF, +6CF [54]	+LY [9]	+LY [65]	+LY [59]	+LY [9]	−DCF, −LY [59] −6CF, +DCF [80]	N.D.	+LY [9]
Single channel conductance (pS)	30, 80, 120 [52]	158, 180 [54]	121, 153 [51] 36, 198 [56]	30, 60, 90 [59] 20, 40, 70 [65]	30, 60, 90 [59] 20, 40, 70 [65]	40, 60 [51]	22,36 [59] 28 [80]	N.D.	22, 39 [51]
V_0	N.D.	+/−50 mV [54]	N.D.	+/−60 mV [60]	N.D.	N.D.	+/−20 mV [78]	N.D.	N.D.

aa, Amino acids; pS, pico-Siemens; for others see abbreviation list.

Cx40 channels are mildly sensitive to the transjunctional voltage gradient (V_j) with a half-maximal inactivation at $+/-50$ mV [53,54]. Depending on the composition of the pipette solution [55], single channel recordings in transfected cell-lines show a variety of large conductance states of 120–200 p5 and less frequently, a much smaller substate of -30–40 pS [51,52,54,56]. Although rCx40 transfected in N2A cells appears cation selective, this was not found for mCx40 [54,57]. Both hCx40 and mCx40 are permeant to LY [9,52], while rCx40 is permeant to DCF and 6CF [54]. Sequence analysis of the cytoplasmic loop and the carboxy-terminus has revealed multiple consensus motifs for phosphorylation by several intracellular protein kinases [31,32]. Western analysis showed that Cx40 indeed exists in a phosphorylated and non-phosphorylated configuration [51,52]. The sequences for hCx40, rCx40 and mCx40 differ significantly. The hCx40 protein contains seven putative sites for modulation by protein kinase C (PKC), but rCx40 and mCx40 contain 12 and 15 sites, respectively. The number of putative sites for modulation by PKA and PKG are two and one in human but one and three in rat and mouse. Consensus sites were not found for modulation by MAP-kinase in the three species.

In Hela cells transfected with mCx40, direct activation of both PKC and PKA increased phosphorylation of Cx40 (measured by incorporation of ^{32}P), but did not alter electrophoretic mobility of the 40 kD native protein on Western blot [51]. In contrast, in hCx40 transfected cells, a shift from 40 to 42 kD was found after stimulation of PKA [52]. In addition g_j, γ_j and dye permeability increased significantly [52]. Up till now, no modulatory role for activated PKG has been reported.

3.2. Cx43 channel properties

Characteristics of Cx43 channels, the main cardiac connexin, have been studied extensively (see [6]). The coding region exists of 382 amino acids in human, mouse and rat.

Like Cx40 channels, Cx43 channels are not very sensitive to V_j. Conductance starts to decrease when ΔV_j exceeds $+/-40$ mV and half-maximal inactivation occurs at $+/-60$ mV [58]. Cx43 channels are not ion selective [57]. rCx43 and hCx43 channels exhibit three γ_j values; a major state at 40–60 pS, and two states of minor occurrence of 20–30 pS and 70–100 pS. In mCx43 only the two largest are detected [51,59,60]. Dye permeability has been shown for LY [9,51,61,62].

hCx43 contains ten putative phosphorylation sites [31,32] for PKC, four for PKA, three for PKG and two for MAP kinase. In rCx43 and mCx43, 14 sites for PKC, three for PKA, four for PKG and three for MAP kinase can be identified (see Table 1). In most studies, the protein presents on Western blot as three bands with molecular weights ranging from 41 to 46 kD. They represent the non-phosphorylated state (NP), a phosphorylated state (P1)

and a highly phosphorylated state (P2) of gradually larger molecular size [60,63–65]. Phosphorylation of Cx43 has been described mainly on serine but also on tyrosine residues and seems to be involved in trafficking and insertion into the membrane [14]. Direct stimulation of PKC with TPA (a phorbol ester) rapidly (hyper)-phosphorylates Cx43 on Ser368 [66]. After prolonged stimulation, down-regulation of Cx43 and trafficking failure are observed (see [4]). Additionally, phosphorylation of rCx43 on Ser255 accelerates internalization and degradation [67].

Serine phosphorylation, mediated by PKA, PKC, PKG and MAP kinase, merely stimulates the prevalence of the smallest γ_j. In contrast, treatment of cells with agents promoting dephosphorylation, shifts γ_j to the largest state [60]. Permeation to dye molecules seems closely related to the size of γ_j. However, changes in γ_j appear not exclusively related to changes in g_j because phosphorylation might also affect P_o. Stimulation of PKG (in rCx43 transfected cells) and PKC (in rCx43 and hCx43 transfected cells), reduces both dye permeability and γ_j [59,65,68]. Surprisingly, PKG stimulation causes a reduction in g_j, whereas stimulation of PKC increases γ_j although both decrease γ_j. This can only be explained by a different effect on P_o assuming that time limits a substantial change in the number of channels (the effect of phosphorylation occurs within minutes while half-life of Cx43 is about 1.5 h [69]). hCx43 channels are insensitive to modulation by PKG [65]. The substitution of Ala at position 257 in hCx43 instead of Ser in rCx43 explains why rCx43 is susceptible to phosphorylation by PKG and hCx43 is not. PKA activation using cAMP and forskolin reduces γ_j in hCx43 channels [68], but has no effect on rCx43 channels [59].

Finally, tyrosine phosphorylation reduces g_j which can be induced by inhibition of tyrosine-phosphatase, or by activation of viral tyrosine kinases as p130*gag-fps* and v-Src [70,71]. This is illustrated by a reduction of g_j when Cx43 is phosphorylated by v-Src on tyr265 [72]. Besides the tyrosine kinase activity of v-Src, downstream activation of MAP-kinase induces phosphorylation on serine residues [73]. In rat liver epithelial cells, activation of MAP kinase by epidermal growth factor and of PKC and MAP kinase by platelet-derived growth factor, rapidly decreases g_j by phosphorylation of Cx43 on Ser255, 279 and 282 [74–76].

3.3. Cx45 channel properties

Cx45 is the least extensively studied of the three connexins expressed in cardiomyocytes. The gene has been cloned from mouse and human (see [6]), and also from rat (personal communication, Dr. M.F.A. Bierhuizen). The coding region of the gene consists of 396 amino acids in human and rat, and 395 in mouse. Most investigations concern hCx45 endogenously expressed in SkHepl cells [41,77,78] or mCx45 transfected in Hela cells [9,79]. Cx45 channels are selective for cations [80]. They are steeply

voltage dependent compared to Cx40 and Cx43, with half maximal activation at $+/-20$ mV [56,78]. Cx45 channels are more sensitive to changes in intracellular pH than Cx43 channels, with a complete block of junctional communication at pH 6.3 [41]. Unitary conductances are small with values of about 20 and 40 pS [59,79,80]. mCx45 but not hCx45 channels appear moderately permeable for LY [9,59,62]. Veenstra et al. showed that hCx45 channels are permeant to DCF but not to 6CF which depends on the charge of the molecules [80].

There are 15 putative phosphorylation sites [31,32] for PKC in hCx45 and 17 in rCx45 and mCx45. In all three species one site is found for modulation by PKA but no consensus sites were found for modulation by PKG or MAP-kinase. As with Cx40 and Cx43, Cx45 is phosphorylated mainly on serine, but also on tyrosine residues [77,79,81,82]. Phosphorylation of serine residues in the carboxy-tail of mCx45 stabilizes the protein [83]. There is debate about the electrophoretic mobility of the protein. Some groups have separated Cx45 as a single band of 45 kD [26,83], or of 48 kD [78], while others claim to have detected two bands of 46 kD and 48 kD of which the 46-kD band was proposed to be either a proteolytic product [77], or the unphosphorylated state of the native protein [79]. Kwak et al. have reported that γ_j of hCx45 channels increases upon stimulation of PKC, but not of PKA and PKG. Phosphorylation by PKC elicits a third conductance state of 16 pS [59]. γ_j of mCx45 channels is not affected by stimulation of PKC, PKA, PKG nor by tyrosine phosphatase inhibition mediated by pervanadate. However, γ_j decreases on stimulation of PKA and inhibition of tyrosine phosphatase, but increases on stimulation of PKC. Changes in g_j on stimulation of PKA and phosphatase inhibition were accompanied by an increase in phosphorylation as shown on Western blot [79].

3.4. From transfected cells back to the heart

Even in a simplified cell system there is complexity in regulation, because of differences in properties of homotypic channels of Cx40, Cx43 and Cx45 with their differential modulation by protein kinases. Additionally, at least in transfected cells, heterotypic channels exist for Cx40/Cx45 and Cx43/Cx45 [9,84]. Recently, two studies suggested the formation of both heterotypic and heteromeric Cx40/Cx43 channels [85,86] which is in contrast with previous data [11,53,87]. Heterotypic- and heteromeric channel properties differ from those of the two participating connexins when expressed as a homotypic channel [85,86,88]. In transfected cells the connexins are under the control of an artificial promoter. Therefore, modulation of properties is restricted to short-term processes. In cardiomyocytes prolonged stimulation of protein kinases and exposure to signaling molecules may provoke additional effects on expression and distribution of the channels. In Section 4 we will discuss the regulation of channel properties in cardiomyocytes.

4. Modulatory mechanisms of gap junction channels in cardiac myocytes

In cardiomyocytes, most studies regarding short-term regulation of gap junction channels (affecting single channel properties) are performed using cultured neonatal myocytes or pairs/clusters of freshly isolated adult myocytes derived from incomplete dissociation of cardiac tissue. Studies on changes in expression/distribution of connexins induced by prolonged stimulation of protein kinases or by application of growth factors, are generally performed using cultured neonatal (1–2 days old) rat ventricular myocytes. These cells are relatively easy to isolate in large amounts and can be cultured in monolayers for more than a week while retaining their electrical activity. In contrast, adult myocytes lose their expression of connexins very rapidly on isolation. Also, they do not attach in confluent monolayers.

Cultured neonatal ventricular myocytes express Cx40 and Cx43 and a small amount of Cx45 [28,89]. In culture, similar to the intact neonatal ventricle, the heterogenic expression of Cx40 declines in time and is susceptible to culture conditions [28]. It is unknown whether the heterogeneity of Cx40 expression (including regulation) in culture is a reflection of the original expression in the heart.

In neonatal cardiomyocytes, gap junctional coupling is characterized by channels with a main γ_j of 40–45 pS, but also a substate of 20 pS (using K-gluconate as charge carrier) [90–92]. Dephosphorylation of Cx43 channels increases γ_j to 70 pS [91]. Dye permeability seems related to the size of γ_j. Although channels of other connexins might be present, the measured γ_j values closely resemble those described for Cx43 channels in transfected cells.

In adult myocytes, γ_j values have been measured both in ventricular and atrial cells. Both guinea-pig and rabbit ventricular cells express channels insensitive to V_j with a main γ_j value of ~100 pS (using CsCl as charge carrier), presumably reflecting Cx43 channels [18,93]. In rabbit atrial cells, next to the 100 pS conductance a larger conductance of 185 pS was measured which likely represents the gating of Cx40 channels [18].

Several studies on modulation of channel properties show that especially under conditions of ischemia, g_j might be synergistically modulated by H^+, Ca^{2+} and amphiphatic lipid metabolites [47,48,94–96]. The effect of phosphorylation of connexin molecules on modulation of channel properties can be studied using membrane permeant drugs which directly activate the protein kinases. In addition, application of several extracellular factors has been used to study receptor mediated activation of kinases (see Fig. 3).

Acute stimulation of PKG, as described for rCx43 channels in transfected cells, reduces g_j, γ_j and dye permeability [90,97]. In many (but not all [90]) reports an increase of γ_j is found upon application of cAMP [97–99], isoproterenol [99,100], forskolin [99], norephinephrine
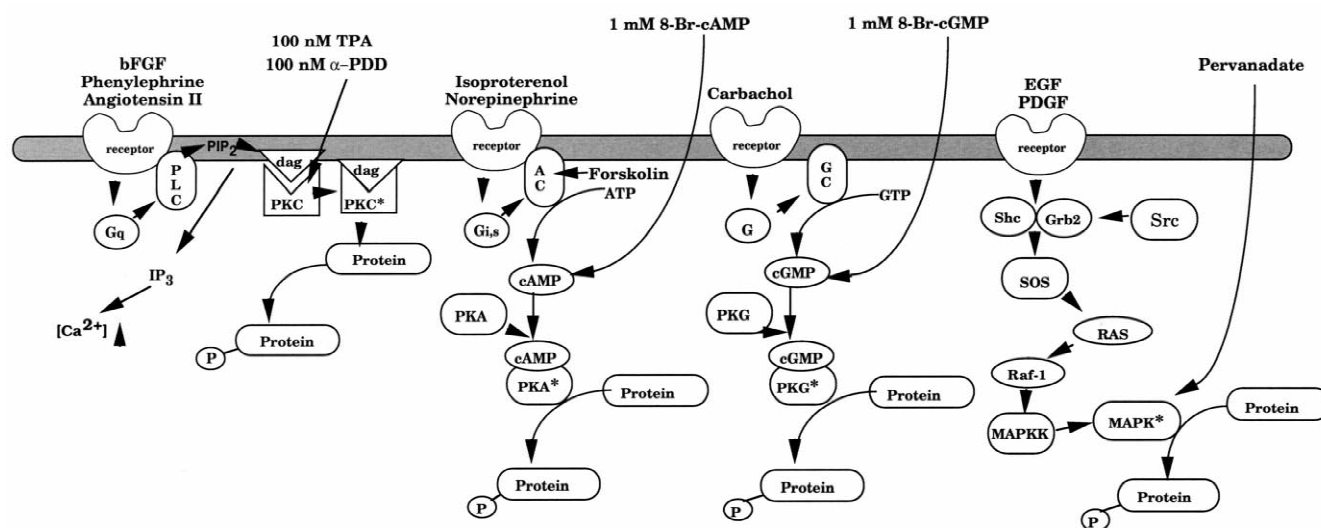
Fig. 3. Simplified scheme showing signal transduction pathways initiated by receptor-binding of extracellular neuro-humoral factors (or synthetic analogues) or application of membrane-permeant drugs, finally resulting in the intracellular activation of protein kinases. Abbreviations: PLC, phospholipase C; IP_3, inositol triphosphate; DAG, diacylglycerol; AC, adenylate cyclase; GC, guanylate cyclase. For more abbreviations see list, and regarding MAP kinase signaling in review [159].

[101], or by dialyzing the catalytic subunit of PKA into the cells [102]. The modulating role of PKC is debated. Stimulation of PKC increased phosphorylation of Cx43 on seryl residues [103]. Reports describing modulation mediated by PKC show a decrease of γ_j and dye coupling but an overall increase in g_j [90], or a decrease in g_j without changes in γ_j [104].

Cardiomyocytes possesses a local renin–angiotensin system in which angiotensin-I (AngI) is converted by angiotensin converting enzyme (ACE) to angiotensin-II (AngII). AngII, the effective metabolite of the system, exerts its action through binding at AT-1- and AT-2 receptors, thereby activating PKC and MAP kinases Erk1 and Erk2, respectively. De Mello and co-workers have shown that administration of renin, Ang-I or Ang-II to adult rat myocytes rapidly reduces g_j due to activation of PKC [105–108]. In contrast, inhibition of ACE increases γ_j [107]. Phenylephrine binding to α_1-adrenergic receptors also activates PKC and, similarly to Ang-II, decreases g_j [109]. Additionally, basic FGF (bFGF) reduced metabolic coupling as assessed by dye-transfer while phosphorylation of Cx43 was strongly increased mainly on serine residues but also on tyrosine residues [110].

These short-term effects (within 5–10 min) on gap junctional conductance are probably caused by connexin phosphorylation because within this limited period no changes in expression or translocation are expected. When the stimulation period is prolonged to 6–24 h, additional changes have been reported in expression level of the connexin proteins in rat neonatal cardiomyocytes.

Myotrophin, strongly increased the amount of Cx43 mRNA [111] while cAMP significantly increased conduction velocity in strands of cultured myocytes [82]. This effect was accompanied by an increased expression (gap junctions increased in number and size) of Cx43 and Cx45

which resulted from an increase in Cx43 mRNA and an enhanced translation of Cx45 mRNA. A similar increase in Cx43 expression, functionally shown by more and larger gap junctions, was observed in an AT-1 receptor mediated response after stimulating cells with Ang-II [112]. Wnt-1 stimulation of neonatal rat cardiomyocytes increased Cx43 mRNA and protein which resulted in an enhanced dye permeability and propagation of calcium [113]. In non-myocyte cardiac cells like fibroblasts, bFGF increased both Cx43 mRNA and protein thereby increasing dye transfer [114]. Additionally, potentially interesting factors as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor β (TGFβ), tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β), affect Cx43 channel properties in non-cardiac cells [75,76,115–117]. Finally, mechanical stretch which activates multiple signaling pathways has been reported to strongly increase expression of Cx43 but not of Cx40 in rat neonatal cardiomyocytes [118,119]. Increased expression of Cx43 resulted both from an increase in mRNA and an decrease in turnover of the protein [119].

5. Implications of gap junction modulation for cardiac function

Results from studies on transfected cells and cardiomyocytes are reasonably comparable. The question remains to what extent modulation of intercellular coupling affects cardiac performance. In only one study on patients suffering from complex congenital hearts defects, a Ser364Pro mutation was detected which in transfected cells appeared to induce abnormal behavior of Cx43 channels [120] but this was not confirmed by others [121]. The spatial differences in expression of Cx40, Cx43 and

Cx45 channels (Section 2) combined with differences in connexin properties and modulation (Sections 3 and 4), appear to be reflected into specific conductive properties in different regions of the heart. The most direct approach to unraveling the specific role of a given connexin is targeted deletion of the gene involved. Using this strategy, Cx40, Cx43 and Cx45 knock-out (KO) mice have been generated.

Another approach is to study the function of gap junction channels in multiple forms of cardiac disease where changes in expression, distribution and phosphorylation during the progression of the underlying pathophysiology have been reported.

5.1. Connexin knock-out mice

Cx43 KO mice die perinatally due to an obstruction of the right outflow tract caused by cardiac malformations [122,123]. Isolated and perfused neonatal Cx43 KO hearts were completely unexcitable [124]. In contrast, heterozygous mice are viable and reproductive. The heterozygous hearts have a normal morphology and show a 50% reduction in expression of Cx43 mRNA and protein [125]. The consequences of this severe reduction in Cx43 protein for ventricular conduction are debated. Initial reports mention a 30–40% reduced ventricular conduction velocity and a prolongation of the QRS complex [125,126]. However, detailed optical mapping did not reveal differences [127]. During ischemia an increased susceptibility to ventricular arrhythmias was found [128]. A similar 50% reduction in Cx43 expression was found in the atria but this did not affect conduction velocity [126]. Transgenic mice overexpressing a dominant-negative form of Cx43 (leading to non-functional channels) are phenotypically similar to Cx43 KO mice [129].

Electrophysiological characteristics of mice heterozygous for Cx40 deletion are comparable to wild-type mice [130], but Cx40 KO mice show multiple aberrations: prolongation of PQ interval [130,131], reduced atrial conduction velocity with a prolonged P-wave, prolonged sinus-node-recovery time, prolonged Wenckebach period, atrial tachyarrhythmias following burst pacing, and reduced A–V conduction; [132,133], impaired left bundle branch conduction and right bundle branch block [134]. No differences were found between WT and KO mice regarding ventricular conductive properties which is explained by the absence of Cx40 in the ventricle. A prolongation of QRS duration was described [130,133], but not when the ventricle was paced from the apex [133] which indicates that the prolongation is due to impairment of the conduction system [134].

Two groups have reported the generation of a Cx45 KO mouse [135,136]. Heterozygous mice are comparable with WT animals as with heterozygous Cx40 KO mice. However, Cx45 KO mice die in utero on day 10.5 pc. Embryonic hearts were dilated due to defective vascular development [136]. Interestingly, within 24 h of the first contractions of these developing hearts in utero, severe impairment of atrial contraction develops and atrio–ventricular conduction block appears [135].

Obviously, gap junctions play an important role in growth and development. Thus, deletion of connexin genes not only affects their specific cardiac function but may also induce developmental alterations. To date, inducible KO mice are constructed in which the connexin gene is surrounded by loxP sites. With this approach, an inducible system activates a recombinase protein (Cre) which connects both loxP sites by deletion of the DNA (connexin gene) in between [137]. In this way, deletion of the genes can be induced at any stage of development which may result in clearer distinction between the role of gap junctions in development and cardiac function.

5.2. Ischemic heart disease and cardiac hypertrophy

Most myocardial diseases are marked by alterations at the expression level of the connexins and/or the anisotropic distribution of gap junction plaques [138–140]. A decrease in expression level of ventricular Cx43 is often detected by Western (protein)- or Northern (mRNA) analysis [141,142]. Obviously, changes in cell and tissue homeostasis and alterations in (circulating) levels of second messengers and growth factors (see Section 4) have an impact on the alteration in expression pattern. In hearts of hypertensive rats (spontaneous or renin transgenic) where the renin–angiotensin signaling is forced, a threefold increase in Cx40 expression and a threefold decrease in Cx43 expression was reported [143]. Regardless the nature of cardiac disease, one of the most commonly found alterations is a shift in distribution of gap junction plaques from the end-to-end IDs to lateral cell borders (see [144]). In healthy myocardium, the anisotropic distribution of gap junctions contributes to differences between conduction velocity in longitudinal and transverse directions. A redistribution of Cx43 gap junctions has been reported in the borderzone of infarcts [142] where it was associated with the localization of reentrant circuits [145], but also in hibernating myocardium [146], in hypertrophied hearts [139], in ischemic hearts [141] and in hypertrophic cardiomyopathy [138]. Often, the redistribution is accompanied by a decrease in size of the gap junction plaques [146] or in the total amount of plaques per ID [139]. In the study of Uzzaman et al. this change was associated with a 30% reduction in longitudinal conduction velocity [139].

Despite the fact that in most cardiomyopathies an increased propensity to arrhythmias has been reported, the functional contribution of both type of alterations, i.e. change in density and distribution, are not completely understood. The significance of redistribution to lateral cell-borders is debatable because lateral gap junctions may prove non-functional if located in intracellular invagina-

tions of the sarcolemma [147]. However, both redistribution and a 20–40% reduction in gap junctions per se will decrease the anisotropy in the tissue which is often considered as antiarrhythmic [148], but by some as proarrhythmic because it decreases the 'wavelength' of the myocardium [149]. Despite these significant alterations, Jongsma and Wilders computed only small to moderate changes in conduction velocity and anisotropy ratio [150]. Both and Jongsma and Wilders and Spach et al. showed that the contribution of changes in cytoplasmic resistivity (cellular geometry) might be equally important especially for longitudinal conduction [150,151]. In addition, Spach et al. showed that discontinuity in anisotropy, which results in nonlinear conduction, increases the propensity for arrhythmias far more than uniform changes in anisotropy ratio in a continuous system. This implies that conduction block in a discontinuous system can be induced by minor alterations in intercellular coupling compared to the extensive uncoupling required in a continuous system [152]. Modulation by phosphorylation of the connexin proteins has been introduced as an important factor for remodeling of gap junctions in diseased hearts. In normal hearts, Cx43 exists in a phosphorylated state. Beardslee et al. suggested that unphosphorylated Cx43 is principally degraded by the proteosomal pathway and phosphorylated Cx43 by the lysosomal pathway [69]. Recently, the same group showed in a model of acute ischemia that uncoupling of cardiac tissue is associated with dephosphorylation of Cx43 and accumulation of unphosphorylated Cx43 in the gap junction plaques [153]. In hereditary cardiomyopathic hamsters, endstage congestive heart failure correlates with a c-Src mediated increase in phosphorylation of Cx43 on tyrosine residues. As a consequence, g_j between pairs of myocytes decreased [154]. These studies suggest that in the intact heart phosphorylation serves as an important modulator of cardiac conduction.

Studies on remodeling of gap junctions in atrium are scarce. In dog atrium, atrial fibrillation induced by pacing increased the expression of Cx43. In chronic atrial fibrillation in the goat, Cx40 expression is reduced. Moreover, this reduction is heterogeneous. In contrast to the dog, in the goat atrium no changes in the amount of Cx43 mRNA and protein have been detected, but the prevalence of unphosphorylated Cx43 seems increased [140,155,156].

6. Concluding remarks

A vast amount of information regarding the specific properties of different gap junction channels, their expression in the heart and the mechanisms to modulate their properties, has been acquired during the past decade. Extrapolation of this knowledge obtained from experiments with transfected cells or isolated and cultured cardiomyocytes to the intact heart is a challenge for the near future. Deletion of connexin genes affects cardiac

conduction only to a moderate extent. This is illustrated by heterozygous Cx40- and Cx43 KO mice in which conduction properties are comparable to WT hearts. These observations were supported by a theoretical study [150]. In cardiomyopathic hearts, alterations in gap junctions expression/distribution may be involved in the increased propensity to arrhythmogenesis, but changes in cellular dimensions following the pathophysiological process, may be relevant as well [151]. Inducible KO mice will be an adequate tool in the near future. Generation of mice with (inducible) forced expression, or targeted deletion of potentially involved genes, allows to focus the attention on specific factors in the variety of involved signaling processes. For example, Nguyen-Tran et al. showed that mice deficient in HF-1b, a transcription factor preferentially expressed in the cardiac conduction system, display sudden cardiac death. This was associated with a reduced expression, and redistribution of Cx40 in the cardiac conduction system [157]. Furthermore, in another mouse model, a constitutive active retinoic acid receptor provokes cardiac hypertrophy accompanied with a heterogeneous reduction of Cx43 expression in the ventricles [158]. These are examples of new models that in the near future may advance our knowledge on the relevance of gap junctions in cardiovascular disease.

Acknowledgements

References

[1] Simpson I, Rose B, Loewenstein WR. Size limit of molecules permeating the junctional membrane channels. Science 1977;195:294–296.

[2] Bevans CO, Kordel M, Rhee SK, Harris AL. Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. J Biol Chem 1998;273:2808–2816.

[3] Goldberg GS, Lampe PD, Nicholson BJ. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. Nat Cell Biol 1999;1:457–459.

[4] Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annu Rev Biochem 1996;65:475–502.

[5] Kumar R, Wilders R, Joyner RW et al. An experimental model for an ectopic focus coupled to ventricular cells. Circulation 1996;94:833–841.

[6] Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. Eur J Biochem 1996;238:1–27.

[7] White TW, Paul DL. Genetic diseases and gene knockouts reveal diverse connexin functions. Annu Rev Physiol 1999;61:283–310.

[8] Zhou XW, Pfahnl A, Werner R et al. Identification of a pore lining segment in gap junction hemichannels. Biophys J 1997;72:1946–1953.

[9] Elfgang C, Eckert R, Lichtenberg-Fraté H et al. Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells. J Cell Biol 1995;129:805–817.

[10] White TW, Bruzzone R, Wolfram S, Paul DL, Goodenough DA. Selective interactions among the multiple connexin proteins expressed in the vertebrate lens: the second extracellular domain is a determinant of compatibility between connexins. J Cell Biol 1994;125:879–892.

[11] White TW, Paul DL, Goodenough DA, Bruzzone R. Functional analysis of selective interactions among rodent connexins. Mol Biol Cell 1995;6:459–470.

[12] Falk MM, Kumar NM, Gilula NB. Membrane insertion of gap junction connexins: polytopic channel forming membrane proteins. J Cell Biol 1994;127:343–355.

[13] Musil LS, Goodenough DA. Multisubunit assembly of an integral plasma membrane channel protein, gap junction connexin43, occurs after exit from the ER. Cell 1993;74:1065–1077.

[14] Musil LS, Goodenough DA. Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques. J Cell Biol 1991;115:1357–1374.

[15] Severs NJ. The cardiac gap junction and intercalated disc. Int J Cardiol 1990;26:137–173.

[16] Reed KB, Westphale EM, Larson DM et al. Molecular cloning and functional expression of human connexin37, an endothelial cell gap junction protein. J Clin Invest 1993;91:997–1004.

[17] Delorme B, Dahl B, Jarry-Guichard T et al. Developmental regulation of Connexin40 gene expression in mouse heart correlates with the differentiation of the conduction system. Dev Dyn 1995;204:358–371.

[18] Verheule S, van Kempen MJA, Te Welscher PHJA, Kwak BR, Jongsma HJ. Characterization of gap junction channels in adult rabbit atrial and ventricular myocardium. Circ Res 1997;80:673–681.

[19] van Kempen MJA, Ten Velde I, Wessels A et al. Differential connexin expression accommodates cardiac function in different species. Micros Res Tech 1995;31:420–436.

[20] Verheule S, van Kempen MJA, Postma S, Rook MB, Jongsma HJ. Gap junctions in the rabbit sinoatrial node. Am J Physiol 2001; in press.

[21] Gourdie RO, Green CR, Severs NJ, Thompson RP. Immunolabelling patterns of gap junction connexins in the developing and mature rat heart. Anat Embryol 1992;185:363–378.

[22] van Kempen MJA, Vermeulen JLM, Moorman AFM et al. Developmental changes of connexin40 and connexin43 mRNA distribution patterns in the rat heart. Cardiovasc Res 1996;32:886–900.

[23] Delorme B, Dahl E, Jarry-Guichard T et al. Expression pattern of connexin gene products at the early developmental stages of the mouse cardiovascular system. Circ Res 1997;81:423–437.

[24] Alcoléa S, Théveniau-Ruissy M, Jarry-Guichard T et al. Downregulation of Connexin45 gene products during mouse heart development. Circ Res 1999;84:1365–1379.

[25] Coppen SR, Kodama I, Boyett MR et al. Connexin45, a major connexin of the rabbit sinoatrial node, is co-expressed with connexin43 in a restricted zone at the nodal-crista terndnalis border. J Histochem Cytochem 1999;47:907–918.

[26] Coppen SR, Dupont E, Rothery S, Severs NJ. Connexin45 expression is preferentially associated with the ventricular conduction system in mouse and rat heart. Circ Res 1998;82:232–243.

[27] Coppen SR, Severs NJ, Gourdie RO. Connexin45 (α6) expression delineates an extended conduction system in the embryonic and mature rodent heart. Dev Genet 1999;24:82–90.

[28] Kwak BR, van Kempen MJA, Theveniau-Ruissy M, Gros DB, Jongsma HJ. Connexin expression in cultured neonatal rat cardiomyocytes reflects the pattern of the intact ventricle. Cardiovasc Res 1999;44:370–380.

[29] Kwong KF, Schuessler RB, Green KG et al. Differential expression of gap junction proteins in the canine sinus node. Circ Res 1998;82:604–612.

[30] Gros D, Jongsma HJ. Connexins in mammalian heart function. BioEssays 1996;18:719–730.

[31] Kemp BE, Pearson RB. Protein kinase recognition sequence motifs. Trends Biochem Sci 1990;15:342–346.

[32] Kennely PJ, Krebs EG. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. J Biol Chem 1991;266:15555–15558.

[33] Pinna LA, Ruzzene M. How do protein kinases recognize their substrates? Biochim Biophys Acta 1996;1314:191–225.

[34] Bevans CG, Harris AL. Direct high affinity modulation of connexin channel activity by cyclic nucleotides. J Biol Chem 1999;274:3720–3725.

[35] Suchyna TM, Xu LX, Gao F, Fourtner CR, Nicholson BJ. Identification of a proline residue as a transduction element involved in voltage gating of gap junctions. Nature 1993;365:847–849.

[36] Verselis VK, Ginter CS, Bargiello TA. Opposite voltage gating polarities of two closely related connexins. Nature 1994;368:348–351.

[37] Revilla A, Castro C, Barrio LC. Molecular dissection of transjunctional voltage dependence in the connexin-32 and connexin-43 junctions. Biophys J 1999;77:1374–1383.

[38] White TW, Bruzzone R, Goodenough DA, Paul DL. Voltage gating of connexins [letter]. Nature 1994;371:208–209.

[39] Spray DC, Burt JM. Structure–activity relations of the cardiac gap junction channel. Am J Physiol 1990;258:C195–C205.

[40] Ek JF, Delmar M, Perzova R, Taffet SM. Role of histidine 95 on pH gating of the cardiac gap junction protein connexin43. Circ Res 1994;74:1058–1064.

[41] Herrnans MMP, Kortekaas P, Jongsma HJ, Rook MB. pH sensitivity of the cardiac gap junction proteins, connexin45 and 43. Pflfgers Arch 1995;431:138–140.

[42] Morley GE, Taffet SM, Delmar M. Intramolecular interactions mediate pH regulation of connexin43 channels. Biophys J 1996;70:1294–1302.

[43] Morley GE, Ek-Vitorin JF, Taffet SM, Delmar M. Structure of connexin43 and its regulation by pH_i. J Cardiovasc Electrophysiol 1997;8:939–951.

[44] Stergiopoulos K, Alvarado JL, Mastroianni M et al. Hetero-domain interactions as a mechanism for the regulation of connexin channels. Circ Res 1999;84:1144–1155.

[45] Gu H, Ek-Vitorin JF, Taffet SM, Delmar M. Coexpression of connexins 40 and 43 enhances the pH sensitivity of gap junctions: a model for synergistic interactions among connexins. Circ Res 2000;86:E98–E103.

[46] Firek L. Modification of gap junction conductance by divalent cations and protons in neonatal rat heart cells. J Mol Cell Cardiol 1995;27:1633–1643.

[47] Burt JM. Block of intracellular communication: interaction of intercellular H^+ and Ca^{2+}. Am J Physiol 1987;253:C607–C612.

[48] White RL, Doeller JE, Verselis VK, Wittenberg BA. Gap junctional conductance between pairs of ventricular myocytes is modulated synergistically by H^+ and Ca^{2+}. J Gen Physiol 1990;95:1061–1075.

[49] Neyton J, Trautmann A. Single-channel currents of an intercellular junction. Nature 1985;317:331–335.

[50] White RL, Spray DC, Campos de Carvalho AC, Wittenberg BA, Bennett MYL. Some electrical and pharmacological properties of gap junctions between adult ventricular myocytes. Am J Physiol 1985;249:C447–C455.

[51] Traub O, Eckert R, Lichtenberg-Frate H et al. Immunochemical and electrophysiological characterization of murine connexin40 and -43 in mouse tissues and transfected human cells. Eur J Cell Biol 1994;64:101–112.

[52] van Rijen HVM, van Veen AAB, Hermans MMP, Jongsma HJ. Human connexin40 gap junction channels are modulated by cAMP. Cardiovasc Res 2000;45:941–951.

[53] Bruzzone R, Haefliger JA, Gimlich RL, Paul DL. Connexin40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins. Mol Biol Cell 1993;4:7–20.

[54] Beblo DA, Wang HZ, Beyer EC, Westphale EM, Veenstra RD. Unique conductance, gating, and selective permeability properties of gap junction channels formed by connexin40. Circ Res 1995;77:813–822.

[55] Valiunas V, Bukauskas FF, Weingart R. Conductances and selective permeability of connexin43 gap junction channels examined in neonatal rat heart cells. Circ Res 1997;80:708–719.

[56] Bukauskas FF, Elfgang C, Willecke K, Weingart R. Biophysical properties of gap junction channels formed by mouse Connexin40 in induced pairs of transfected human HeLa cells. Biophys J 1995;68:2289–2298.

[57] Veenstra PD, Wang HZ, Beblo DA et al. Selectivity of connexin-specific gap junctions does not correlate with channel conductance. Circ Res 1995;77:1156–1165.

[58] Moreno AP, Rook MB, Fishman GI, Spray DC. Gap junction channels, distinct voltage-sensitive and -insensitive conductance states. Biophys J 1994;67:113–119.

[59] Kwak BR, Hermans MMP, De Jonge HR et al. Differential regulation of distinct types of gap junction channels by similar phosphorylating conditions. Mol Biol Cell 1995;6:1707–1719.

[60] Moreno AP, Sáez JC, Fishman GI, Spray DC. Human connexin43 gap junction channels. Circ Res 1994;74:1050–1057.

[61] Fishman GI, Spray DC, Leinwand LA. Molecular characterization and functional expression of the human cardiac gap junction channel. J Cell Biol 1990;111:589–598.

[62] Steinberg TH, Civitelli R, Geist ST et al. Connexin43 and Connexin45 form gap junctions with different molecular permeabilities in osteoblastic cells. EMBO J 1994;13:744–750.

[63] Musil LS, Cunningham BA, Edelman GM, Goodenough DA. Differential phosphorylation of the gap junction protein connexin43 in junctional communication-competent and -deficient cell lines. J Cell Biol 1990;111:2077–2088.

[64] Laird DW, Puranam KL, Revel JP. Turnover and phosphorylation dynamics of connexin43 gap junction protein in cultured cardiac myocytes. Biophys J 1991;273:67–72.

[65] Kwak BR, Sáez JC, Wilders R et al. Effects of cGMP-dependent phosphorylation on rat and human Connexin43 gap junction channels. Pflfgers Arch 1995;430:770–778.

[66] Lampe PD, TenBroek EM, Burt JM et al. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. J Cell Biol 2000;149:1503–1512.

[67] Lampe PD, Kurata WE, Warn-Cramer BJ, Lau AF. Formation of a distinct connexin43 phosphoisoform in mitotic cells is dependent upon p34cdc2 kinase. J Cell Sci 1998;111:833–841.

[68] Moreno AP, Fishman GI, Spray DC. Phosphorylation shifts unitary conductance and modifies voltage dependent kinetics of human connexin43 gap junction channels. Biophys J 1992;62:51–53.

[69] Beardslee MA, Laing JG, Beyer EC, Saffitz JE. Rapid turnover of Connexin43 in the adult rat heart. Circ Res 1998;83:629–635.

[70] Kurata WE, Lau AF. p130gag-fps disrupts gap junctional communication and induces phosphorylation of connexin43 in a manner similar to that of pp60v-src. Oncogene 1994;9:329–335.

[71] Warn-Cramer BJ, Cottrell GT, Burt JM, Lau AF. Regulation of connexin43 gap junctional intercellular communication by mitogen activated protein kinase. J Biol Chem 1998;273:9188–9196.

[72] Swenson KI, Piwnica-Worms H, McNamee H, Paul DL. Tyrosine phosphorylation of the gap junction protein connexin43 is required for the pp6Ov-src-induced inhibition of communication. Cell Regul 1990;1:989–1002.

[73] Zhou L, Kasperek EM, Nicholson BJ. Dissection of the molecular basis of pp6O[v-src] induced gating of connexin 43 gap junction channels. J Cell Biol 1999;144:1033–1045.

[74] Lau AF, Kanemitsu MY, Kurata WE, Danesh S, Boynton AL. Epidermal growth factor disrupts gap-junctional communication and induces phosphorylation of connexin43 on serine. Mol Biol Cell 1992;3:865–874.

[75] Warn-Cramer BJ, Lampe PD, Kurata WE et al. Characterization of the MAPK phosphorylation sites in the Cx43 gap junction protein. J Biol Chem 1996;271:3779–3786.

[76] Hossain MZ, Ao P, Boynton AL. Platelet-derived growth factor-induced disruption of gap junctional communication and phosphorylation of connexin43 involves protein kinase C and mitogen-activated protein kinase. J Cell Physiol 1998;176:332–341.

[77] Laing JG, Westphale EM, Engelmann GL, Beyer BC. Characterization of the gap junction protein, Connexin45. J Membr Biol 1994;139:31–40.

[78] Moreno AP, Laing JG, Beyer BC, Spray DC. Properties of gap junction channels formed of connexin45 endogenously expressed in human hepatoma (SKHep1) cells. Am J Physiol 1995;268:C356–C365.

[79] van Veen TA, van Rijen HV, Jongsma HJ. Electrical conductance of mouse connexin45 gap junction channels is modulated by phosphorylation. Cardiovasc Res 2000;46:496–510.

[80] Veenstra ED, Wang HZ, Beyer EC, Brink PR. Selective dye and ionic permeability of gap junction channels formed by Connexin45. Circ Res 1994;75:483–490.

[81] Butterweck A, Gergs U, Willecke K, Traub O. Immunochemical characterization of the gap junction protein Connexin45 in mouse kidney and transfected human HeLa cells. J Membrane Biol 1994;141:247–256.

[82] Darrow BJ, Fast VG, Kléber AG, Beyer BC, Saffitz JE. Functional and structural assessment of intercellular communication: increased conduction velocity and enhanced connexin expression in dibutyryl cAMP-treated cultured cardiac myocytes. Circ Res 1996;79:174–183.

[83] Hertlein B, Butterweck A, Haubrich S, Willecke K, Traub O. Phosphorylated carboxy terminal serine residues stabilize the mouse gap junction protein Connexin45 against degradation. J Membrane Biol 1998;162:247–257.

[84] Moreno AP, Fishman GI, Beyer EC, Spray DC. Voltage dependent gating and single channel analysis of heterotypic gap junctions formed of Cx45 and Cx43. Prog Cell Res 1995;4:405–408.

[85] He DS, Jiang JX, Taffet SM, Burt JM. Formation of heteromeric gap junction channels by connexins 40 and 43 in vascular smooth muscle cells. Proc Natl Acad Sci USA 1999;96:6495–6500.

[86] Valiunas V, Weingart R, Brink PR. Formation of heterotypic gap junction channels by connexins 40 and 43. Circ Res 2000;86:E42–E49.

[87] Haubrich S, Schwarz HJ, Bukauskas F et al. Incompatibility of connexin 40 and 43 Hemichannels in gap junctions between mammalian cells is determined by intracellular domains. Mol Biol Cell 1996;7:1995–2006.

[88] Koval M, Geist ST, Westphale EM et al. Transfected Connexin45 alters gap junction permeability in cells expressing endogenous Connexin43. J Cell Biol 1995;130:987–995.

[89] Darrow BJ, Laing JG, Lanipe PD, Saffitz JB, Beyer EC. Expression of multiple connexins in cultured neonatal rat ventricular myocytes. Circ Res 1995;76:381–387.

[90] Kwak BR, Jongsma HJ. Regulation of cardiac gap junction channel permeability and conductance by several phosphorylating conditions. Mol Cell Biochem 1996;157:93–99.

[91] Kwak BR, van Veen TAB, Analbers LJS, Jongsma HJ. TPA increases conductance but decreases permeability in neonatal rat cardiomyocyte gap junction channels. Exp Cell Res 1995;220:456–463.

[92] Rook MB, van Ginneken ACG, De Jonge B et al. Differences in gap junction channels between cardiac myocytes, fibroblasts, and heterologous pairs. Am J Physiol 1992;263:C959–C977.

[93] Rüdisüli A, Weingart R. Electrical properties of gap junction channels in guinea-pig ventricular cell pairs revealed by exposure to heptanol. Pflfgers Arch 1989;415:12–21.

[94] Massey KD, Minnich BN, Burt JM. Arachidonic acid and lipoxygenase metabolites uncouple neonatal rat cardiac myocyte pairs. Am J Physiol 1992;263:C494–C501.

[95] Wu J, McHowat J, Saffitz JE, Yainada KA, Corr PB. Inhibition of gap junctional conductance by long chain acylcarnitines and their preferential accumulation in junctional sarcolemma during hypoxia. Circ Res 1993;72:879–889.

[96] Dekker LR, Fiolet JW, VanBavel E et al. Intracellular Ca^{2+}, intercellular electrical coupling, and mechanical activity in ischemic rabbit papillary muscle. Effects of preconditioning and metabolic blockade. Circ Res 1996;79:237–246.

[97] Burt JM, Spray DC. Intropic agents modulate gap junctional conductance between cardiac myocytes. Am J Physiol 1988;254:H1206–H1210.

[98] De Mello WC, van Loon P. Influence of cyclic nucleotides on junctional permeability in atrial muscle. J Mol Cell Cardiol 1987;19:83–94.

[99] De Mello WC. Impaired regulation of cell communication by βadrenergic receptor activation in the failing heart. Hypertension 1996;27:265–268.

[100] De Mello WC. Increase in junctional conductance caused by isoproterenol in heart cell pairs is suppressed by cAMP-dependent protein kinase inhibitor. Biochem Biophys Res Commun 1988;154:509–514.

[101] De Mello WC. Influence of α-adrenergic receptor activation on junctional conductance in heart cells: interaction with β-adrenergic adrenergic agonists. J Cardiovasc Pharmacol 1997;29:273–277.

[102] De Mello WC. Further studies on the influence of cAMP-dependent protein kinase on junctional conductance in isolated heart cell pairs. J Mol Cell Cardiol 1991;23:371–379.

[103] Saez JC, Nairn AC, Czemik AJ et al. Phosphorylation of connexin43 and the regulation of neonatal rat cardiac myocyte gap junctions. J Mol Cell Cardiol 1997;29:2131–2145.

[104] Mflnster PN, Weingart R. Effects of phorbol esters on gap junctions of neonatal rat heart cells. Pflfgers Arch 1993;423:181–188.

[105] Dc Mello WC. Influence of intracellular renin on heart cell communication. Hypertension 1995;25:1172–1177.

[106] De Mello WC. Is an intracellular renin–angiotensin system involved in control of cell communication in heart? J Cardiovasc Pharmacol 1994;23:640–646.

[107] De Mello W, Altieri P. The role of the renin–angiotensin system in the control of cell communication in the heart: effects of enalapril and angiotensin II. J Cardiovasc Pharmacol 1992;20:643–651.

[108] De Mello WC. Renin–angiotensin system and cell communication in the failing heart. Hypertension 1996;27:1267–1272.

[109] De Mello WC, Cherry RC, Manivannan S. Electrophysiologic and morphologic abnormalities in the failing heart: effect of enalapril on the electrical properties. J Card Fail 1997;3:53–61.

[110] Doble KS, Chen Y, Bose DG, Litchfield DW, Kardami B. FGF-2 decreases metabolic coupling and stimulates phosphorylation as well as masking of Cx43 epitopes in cardiac myocytes. Circ Res 1996;79:647–658.

[111] Mukheijee DP, McTiernan CF, Sen S. Myotrophin induces early response genes and enhances cardiac gene expression. Hypertension 1993;21:142–148.

[112] Dodge SM, Beardslee MA, Darrow BJ et al. Effects of angiotensin II on expression of the gap junction channel protein connexin43 in neonatal rat ventricular myocytes. J Am Coil Cardiol 1998;32:800–807.

[113] Ai Z, Fischer A, Spray DC, Brown AM, Fishman GI. Wnt-1 regulation of connexin43 in cardiac myocytes. J Clin Invest 2000;105:161–171.

[114] Doble BW, Kardant B. bFGF stimulates Cx43 expression and intercellular communication of cardiac fibroblasts. Mol Cell Biochem 1995;143:81–87.

[115] Maldonado PE, Rose B, Loewenstein WR. Growth factors modulate junctional cell-to-cell communication. J Membr Biol 1988;106:203–210.

[116] van Rijen HVM, van Kempen MJA, Postma S, Jongsma HJ. Tumour necrosis factor a alters the expression of Connexin43,

Connexin40, and Connexin37 in human umbilical vein endothelial cells. Cytokine 1998;10:258–264.

[117] Tonon R, D'Andrea P. Interleukin- iβ increases the functional expression of connexin 43 in atricular chondrocytes: evidence for a Ca^{2+}-dependent mechanism. J Bone Miner Res 2000;15:1669–1677.

[118] Zhuang J, Yamada KA, Saffitz JE, Kleber AG. Pulsatile stretch remodels cell-to-cell communication in cultured myocytes. Circ Res 2000;87:316–322.

[119] Wang TL, Tseng YZ, Chang H. Regulation of connexin 43 gene expression by cyclical mechanical stretch in neonatal rat cardiomyocytes. Biochem Biophys Res Commun 2000;267:551–557.

[120] Britz-Cunningham SH, Shah MM, Zuppan CW, Fletcher WH. Mutations in the Connexin43 Gap-Junction gene in patients with heart malformations and defects of laterality. New Engl J Med 1995;332:1323–1329.

[121] Gebbia M, Towbin JA, Casey B. Failure to detect connexin43 mutations in 38 cases of sporadic and familial heterotaxy. Circulation 1996;94:1909–1912.

[122] Reaume AG, de Sousa PA, Kulliarni S et al. Cardiac malformation in neonatal mice lacking connexin43. Science 1995;267:1831–1834.

[123] Ya J, Erdtsieck-Ernste EBHW, de Boer PAJ et al. Heart defects in Connexin43-deficient mice. Circ Res 1998;82:360–366.

[124] Johnson CM, Green KG, Kanter EM et al. Voltage-gated Na^+ channel activity and connexin expression in Cx43-deficient cardiac myocytes. J Cardiovasc Electrophysiol 1999;10:1390–1401.

[125] Guerrero PA, Schuessler RB, Davis LM et al. Slow ventricular conduction in mice heterozygous for a connexin43 null mutation. J Clin Invest 1997;99:1991–1998.

[126] Thomas SA, Schuessler RB, Berul CI et al. Disparate effects of deficient expression of connexin43 on atrial and ventricular conduction: evidence for chamber-specific molecular determinants of conduction. Circulation 1998;97:686–691.

[127] Morley GE, Vaidya D, Samie FH et al. Characterization of conduction in the ventricles of normal and heterozygous Cx43 knockout mice using optical mapping. J Cardiovasc Electrophysiol 1999;10:1361–1375.

[128] Lerner DL, Yamada KA, Schuessler RB, Saffitz JB. Accelerated onset and increased incidence of ventricular arrhythmias induced by ischemia in Cx43-deficient mice. Circulation 2000;101:547–552.

[129] Sullivan R, Huang GY, Meyer RA et al. Heart malformations in transgenic mice exhibiting dominant negative inhibition of gap junctional communication in neural crest cells. Dev Biol 1998;204:224–334.

[130] Kirchhoft S, Nelles B, Hagendorff A et al. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice. Curr Biol 1998;8:299–302.

[131] Simon AM, Goodenough DA, Paul DL. Mice lacking connexin40 have cardiac conduction abnormalities characteristic of atrioventricular block and bundle branch block. Cur Biol 1998;8:295–298.

[132] Hagendorff A, Schumacher B, Kirchhoff S, Lflderitz B, Willecke K. Conduction disturbances and increased atrial vulnerability in Connexin40 deficient mice analyzed by transesophagal stimulation. Circulation 1999;99:1508–1515.

[133] Verheule S, van Batenburg CA, Coenjaerts FE et al. Cardiac conduction abnormalities in mice lacking the gap junction protein connexin40. J Cardiovasc Electrophysiol 1999;10:1380–1389.

[134] van Rijen HVM, van Veen AAB, van Kempen MJA et al. Impaired conduction in the bundle branches of mouse hearts lacking the gap junction protein connexin40. Circulation 2001; in press.

[135] Kumai M, Nishii K, Nakamura K et al. Loss of connexin45 causes a cushion defect in early cardiogenesis. Development 2000;127:3501–3512.

[136] Kruger O, Plum A, Kim J et al. Defective vascular development in connexin 45-deficient mice. Development 2000;127:4179–4193.

[137] Kistner A, Gossen M, Zimmermann F et al. Doxycycline-mediated quantitative and tissue-specific control of gene expression in transgenic mice. Proc Natl Acad Sci USA 1996;93:10933–10938.

[138] Sepp R, Severs NJ, Gourdie RG. Altered patterns of intercellular junction distribution in human patients with hypertrophic cardiomyopathy. Heart 1996;76:412–417.

[139] Uzzaman M, Honjo H, Takagishi Y et al. Remodeling of gap junctional coupling in hypertrophied right ventricles of rats with monocrotaline-induced pulmonary hypertension. Circ Res 2000;86:871–878.

[140] van der Velden HMW, van Kempen MJA, Wijffels MCEF et al. Altered pattern of Connexin40 distribution in persistent atrial fibrillation in the goat. J Cardiovasc Electrophysiol 1998;9:596–607.

[141] Peters NS, Green CR, Poole-Wilson PA, Severs NJ. Reduced content of connexin43 gap junctions in ventricular myocardium from hypertrophied and ischemic human hearts. Circulation 1993;88:864–875.

[142] Smith JH, Green CR, Peters NS, Rothery S, Severs NJ. Altered patterns of gap junction distribution in ischemic heart disease. Am J Pathol 1991;139:801–821.

[143] Bastide B, Neyses L, Ganten D et al. Gap junction protein connexin40 is preferentially expressed in vascular endothelium and conductive bundles of rat myocardium and is increased under hypertensive conditions. Circ Res 1993;73:1138–1149.

[144] Severs NJ. The cardiac muscle cell. Bioessays 2000;22:188–199.

[145] Peters NS, Coromilas J, Severs NJ, Wit AL. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. Circulation 1997;95:988–996.

[146] Kaprielian RR, Gunning M, Dupont E et al. Downregulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle. Circulation 1998;97:651–660.

[147] Matsushita T, Oyaxnada M, Fujimoto K et al. Remodeling of cell-cell and cell–extracellular matrix interactions at the border zone of rat myocardial infarcts. Circ Res 1999;85:1046–1055.

[148] Spach MS. Changes in the topology of gap junctions as an adaptive structural response of the myocardium. Circulation 1994;90:1103–1106.

[149] Rensma PL, Allessie MA, Lammers WJ, Bonke FI, Schalij MJ. Length of excitation wave and susceptibility to reentrant atrial arrhythmias in normal conscious dogs. Circ Res 1988;62:395–410.

[150] Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. Circ Res 2000;86:1193–1197.

[151] Spach MS, Heidlage JF, Dolber PC, Barr RC. Electrophysiological effects of remodeling cardiac gap junctions and cell size. Experimental and model studies of normal cardiac growth. Cire Res 2000;86:302–311.

[152] Fast VG, Darrow BJ, Saffltz JE, Kleber AG. Anisotropic activation spread in heart cell monolayers assessed by high-resolution optical mapping. Role of tissue discontinuities. Circ Res 1996;79:115–127.

[153] Beardslee MA, Lerner DL, Tadros PN et al. Dephosphorylation and Intracellular Redistribution of Ventricular Connexin43 During Electrical Uncoupling Induced by Ischemia. Cire Res 2000;87:656–662.

[154] Toyofuku T, Yabuki M, Otsu K et al. Functional role of c-Src in gap junctions of the cardiomyopathic heart. Circ Res 1999;85:672–681.

[155] van der Velden HM, Ausma J, Rook MB et al. Gap junctional remodeling in relation to stabilization of atrial fibrillation in the goat. Cardiovasc Res 2000;46:476–486.

[156] Elvan A, Huang XD, Pressler ML, Zipes DP. Radiofrequency catheter ablation of the atria eliminates pacing-induced sustained atrial fibrillation and reduces connexin 43 in dogs. Circulation 1997;96:1675–1685.

[157] Nguyen-Than VT, Kubalak SW, Minamisawa S et al. A novel genetic pathway for sudden cardiac death via defects in the transition between ventricular and conduction system cell lineages. Cell 2000;102:671–682.

[158] Hall DG, Morley GE, Vaidya D et al. Early onset heart failure in transgenic mice with dilated cardiomyopathy. Pediatr Res 2000;48:36–42.

[159] Seger R, Krebs EG. The MAPK signaling cascade. FASEB J 1995;9:726–735.