J.Cell.Mol.Med. Vol 5, No 4, 2001 pp. 355-366

Invited Review

Gap junction remodeling and cardiac arrhythmogenesis: cause or coincidence?

Nicholas J. Severs *

National Heart and Lung Institute, Faculty of Medicine, Imperial College,
Royal Brompton Hospital,
London, UK

Received: November 14, 2001; Accepted: November 30, 2001

Abstract

Gap junctions, clusters of transmembrane channels that link adjoining cells, mediate myocyte-to-myocyte electrical coupling and communication. The component proteins of gap junction channels are termed connexins and, in *in vitro* expression systems, gap-junctional channels composed of different connexin types exhibit different biophysical properties. In common with other tissues, the heart expresses multiple connexin isoforms. Spatially defined patterns of expression of three connexin isoforms - connexin43, connexin40 and connexin45 - form the cell-to-cell conduction pathways responsible for the orderly spread of current flow that governs the normal cardiac rhythm. Remodeling of gap junction organization and connexin expression is a common feature of human heart disease conditions in which there is an arrhythmic tendency. This remodeling may take the form of disturbances in the distribution of gap junctions and/or quantitative alterations in connexin expression, notably reduced ventricular connexin43 levels. The idea that such changes may contribute to the development of a pro-arrhythmic substrate in the diseased heart has gained ground over the last decade. Recent studies using transgenic mice models have raised new opportunities to explore the significance of gap junction remodeling in the diseased heart.

Keywords: cardiomyocyte - gap junctions - connexins - intercellular communication

*Correspondence to: Professor N.J. SEVERS,
Cardiac Medicine, National Heart and Lung Institute
(Imperial College), Royal Brompton Hospital,
Sydney Street, London SW3 6NP, UK
Fax: + 44 20 7351 8476, Telephone: + 44 20 7351 8140
E-mail: n.severs@ic.ac.uk

Introduction

Activation of cardiac contraction requires orderly spread of the wave of electrical excitation from one cardiomyocyte to the next, throughout the heart. The subcellular structures responsible for this cell-to-cell flow of current are specialized cell junctions termed *gap junctions*. Gap junctions comprise closely apposed domains of the plasma membranes of neighbouring cells, packed with clusters of transmembrane channels. The channels connect the adjacent cytoplasmic compartments, forming sites of low resistance electrical coupling and conduits for the direct exchange of small molecules and ions. Gap junctions thus act as pathways for direct cell-to-cell signaling and co-ordination of cellular activities in tissue systems in general; if gap-junctional communication is compromised, normal tissue function cannot be maintained and disease may ensue [for review see [1]. Aberrant gap-junctional communication may in theory arise either from primary defects (i.e., those due to mutations) or from abnormalities that develop later in life. Mutations in genes that encode gap-junctional proteins are known to cause a number of human diseases [e.g., [2-4], and in the cardiovascular system have been linked to some forms of congenital malformation of the heart [5]. Quite distinct from these primary defects are alterations in gap junction organization and expression that may occur later in life, in association with adult heart disease. Gap junction remodeling of this last type has been discussed extensively as a factor that may potentially contribute to the development of arrhythmia, a major cause of death and disability in heart disease [reviews, [6-11]. However, recent computer modeling studies have questioned the extent to which gap junction changes of the magnitude observed would be likely to impact on altered conduction velocity and arrhythmogenesis [12]. This short review will briefly discuss selected aspects of our current understanding of disease-related gap-junction remodeling in the human heart, set in the context of recent findings in experimental animal models.

Gap junction structure and composition

Figure 1 is a reminder of the characteristic structure of the gap junction, as seen by electron microscopy. In thin sections, the gap junction is recognized by zones of near contact between adjacent plasma membranes; the two membrane are separated by a gap of ~ 2-3 nm, giving rise to a typical pentalaminar or septilaminar appearance (Fig. 1a). Freeze-fracture electron microscopy, which splits membranes enabling them to be viewed *en face*, reveals gap junctions as prominent clusters of particles and their imprints (Fig. 1b). Each particle within these clusters represents a protein hemi-channel penetrating through the membrane; the imprints are formed when hemichannels are plucked out of the half-membrane sheet viewed, remaining attached to the half-membrane that is fractured away. The component proteins of the gap-junctional channel, connexins, are assembled into hexamers termed connexons. Each connexon is equivalent to a single hemi-channel. Pairs of connexons from apposing plasma membranes join to form complete channels spanning both membranes and the extracellular gap. Twenty different connexin genes have now been identified in the mouse and the human [13]. Many tissues, including those of the cardiovascular system, express more than one connexin isoform in a cell type or tissue-related manner. Four main isoforms– connexin43, connexin40, connexin45 and connexin37 – are expressed in cardiovascular cells [reviews, [10, 11, 14], and further isoforms such as connexin46 [15] and connexin57 [16] may also be present in trace amounts. Connexins 43, 40 and 45 are expressed in cardiomyocytes; connexins 37, 40 and 43 in endothelial cells. Experiments using *in vitro* expression systems demonstrate that channels composed of different connexin isoforms exhibit distinctive biophysical properties; the precise functional properties of gap junctions *in vivo* were thus predicted to depend, at least in part, on the specific connexins from which they are constructed [review, [17]. Recent studies using transgenic mice have emphasized that while there is a capacity for functional compensation of one connexin isotype by another, individual connexins do indeed also appear to have unique roles *in vivo* [18-21].

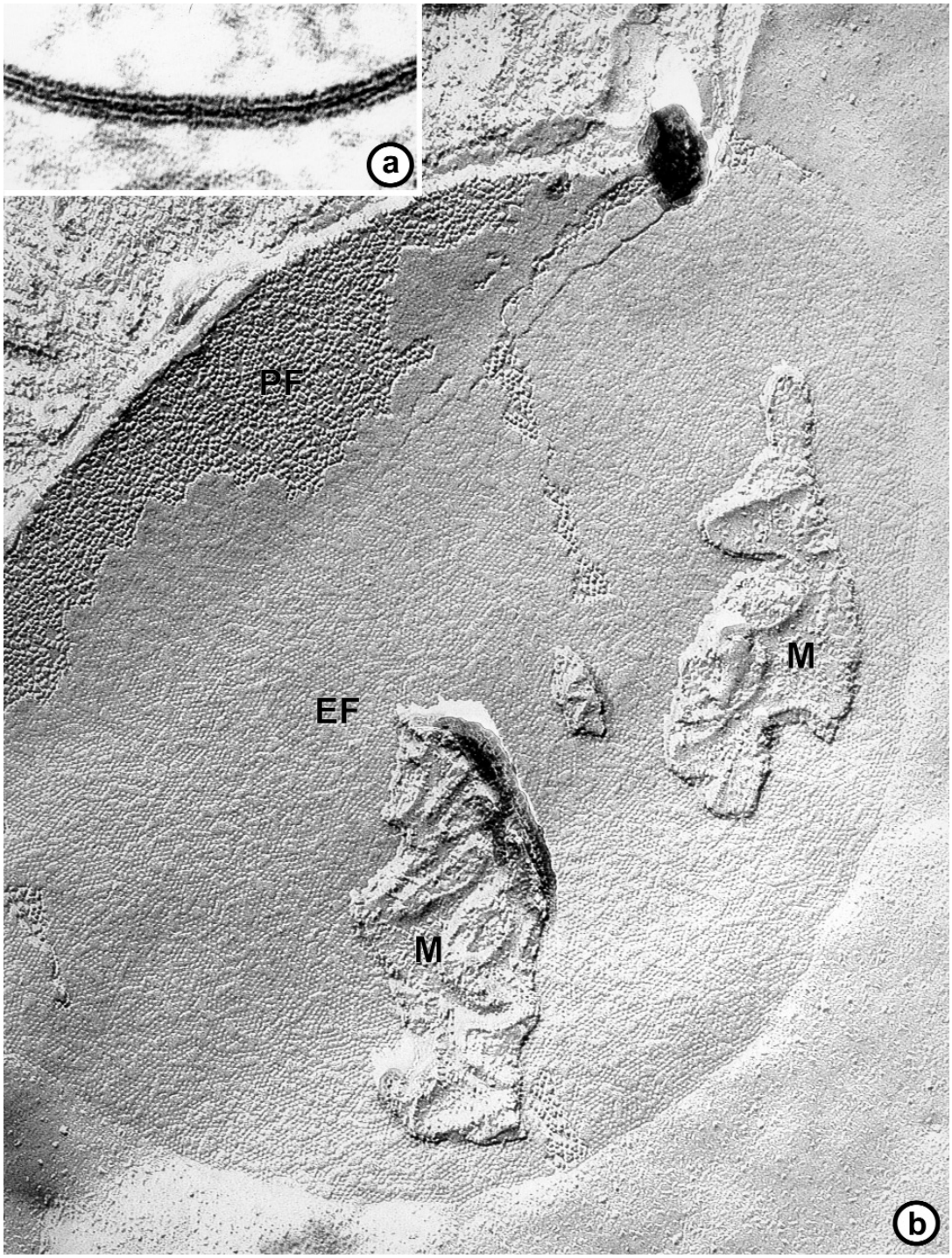

Fig. 1 Principal ultrastructural features of the cardiac gap junction. (a) High magnification thin-section electron micrograph illustrating the pentalaminar structure of the gap junction resulting from the two, closely apposed unit membranes. (b) Structure of gap junction as revealed by freeze-fracture electron microscopy. This technique splits biological membranes along their hydrophobic core, displaying details of membrane structure in *en face* view. The gap junction is seen as a cluster of particles on the protoplasmic fracture face (PF) of the lower half-membrane leaflet, and as imprints of particles on the exoplasmic face (EF) of the upper half-membrane leaflet. Each particle represents a connexon hemi-channel, and each imprint a pit from which a connexon has been fractured out onto the apposing half-membrane leaflet. (a) x 270,000; (b) x 114,000. (b) from Severs, N.J. Histol.Histopath. *10*:481-501 (1995) with permission.

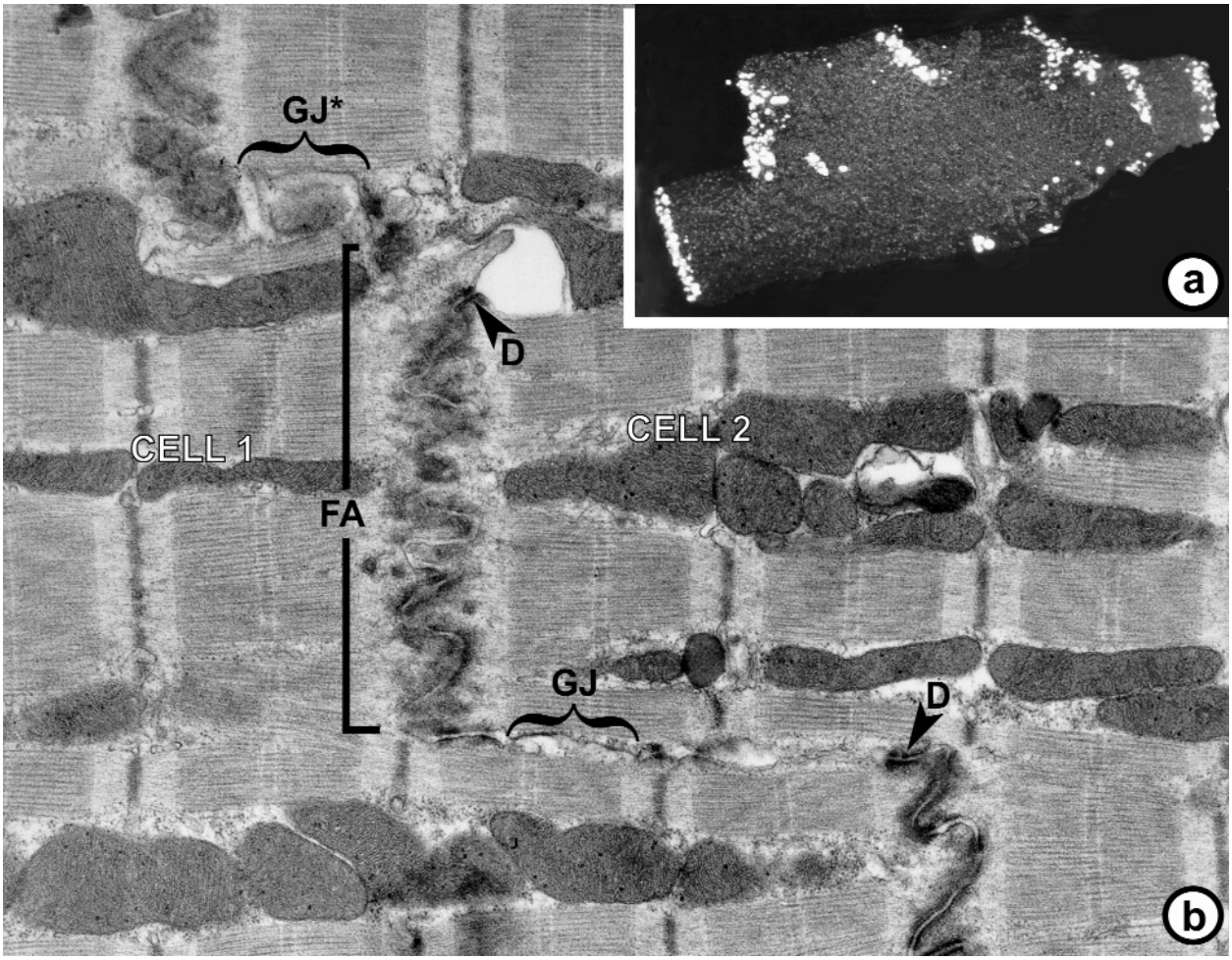

Fig. 2 (a) Localization of connexin43 by immunoconfocal microscopy of a dissociated ventricular myocyte. Note bright staining of the clusters of connexin43 gap junctions at the intercalated disks. These junctions face sideways (laterally) at the disks (see Figs. 2b and 4). For corresponding immunoconfocal micrograph of cardiomyocytes in a tissue section, see Figure 5a. (b) Thin-section electron micrograph illustrating the appearance of the intercalated disk between two cardiomyocytes. *Fasciae adherentes* junctions (FA) occupy the vertical segments of membrane, gap junctions (GJ) predominantly the lateral segments of membrane, and desmosomes (D) both regions. (GJ* indicates a gap junction that has been tangentially sectioned). (a) x 650; (b) x 16,000.

Connexin expression in cardiomyocytes of the normal heart

The predominant connexin of the heart, found in abundance in adult working ventricular and atrial cardiomyocytes of all mammalian species, is connexin43 [22]; review, [23]. Connexin40 and/or connexin45 may also be expressed in a cardiomyocyte subtype and chamber-specific manner [24-26]. Co-expression of two or all three connexins may thus occur, according to cardiomyocyte type. In addition to distinctive connexin expression profiles, differences in overall size, distribution and abundance of gap junctions characterize the various types of cardiomyocyte found in different regions of the heart [10, 11, 27, 28].

The familiar cardiomyocytes of the working ventricles are elongated, branching cells, extensively interconnected by clusters of connexin43-containing gap junctions organized in intercalated disks (Fig. 2). The intercalated disks also contain two types of anchoring junction, the *fascia adherens* and the desmosome, which mediate cell-to-cell linkage of the

contractile filaments and intermediate filament cytoskeleton, respectively (Fig. 3). These anchoring junctions act in concert with the gap junctions to integrate cardiac electromechanical function. In the intercalated disks of working ventricular myocardium, the gap junctions occupy laterally-facing segments of membrane (Figs. 2 & 4), with a population of especially large gap junctions circumscribing the disk periphery [29]. This and other features of gap junction organization, together with features of tissue architecture such as the size and shape of the cells, combine to encourage preferential propagation of the impulse in the longitudinal axis, thereby contributing to the normal pattern of anisotropic spread of the impulse of healthy contractile myocardium.

Atrial cardiomyocytes are slender cells compared with their ventricular counterparts; though intercalated disks are readily identified in atrial myocytes, their gap junctions are also frequently disposed along the lateral borders. Working ventricular myocytes normally lack connexin40, though this connexin is abundant in the atrial myocytes of many mammalian species, including humans [26, 30], co-organized with connexin43 in the same junctional plaques [11]. In both ventricular and atrial working myocardium, connexin45 is present only in very low quantities, though slightly higher levels are present in the atria than the ventricles [25, 26].

Further subtypes of myocyte, of distinctive morphologies, comprise the impulse generation and conduction system [27]. The myocytes of the sino-atrial and atrioventricular nodes are typically small, with haphazardly orientated poorly-developed contractile elements and small, sparse, dispersed gap junctions containing connexin45 [31-33], a connexin that forms low conductance channels *in vitro* [34]. These gap junction features correlate with poor coupling which, in the sino-atrial node, may contribute to the ability to drive the large mass of surrounding atrial tissue while remaining protected from its hyperpolarizing influence and, in the atrioventricular node, to the slowing of conduction which ensures sequential contraction of atria and ventricles. In the rabbit sino-atrial node, the connexin43 negative node is delineated from the surrounding atrial myocardium by a layer of extracellular matrix,

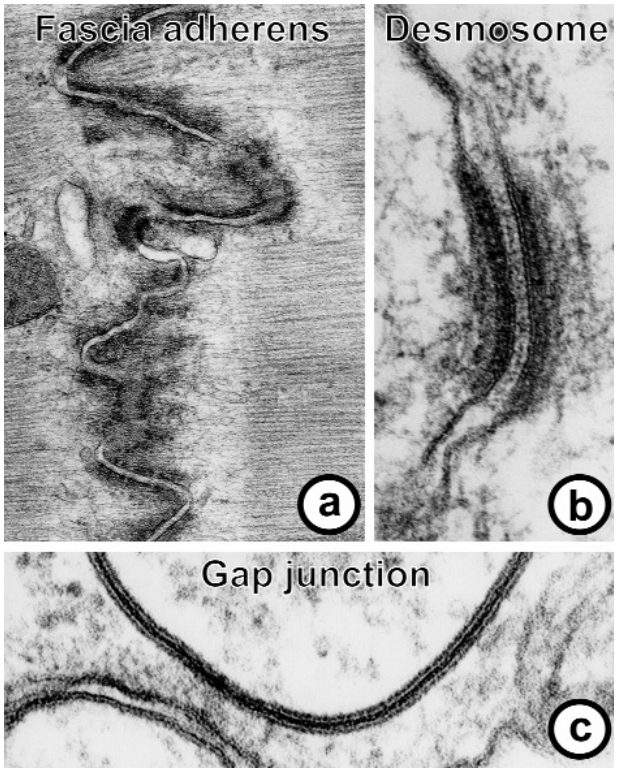

Fig. 3 Higher magnification thin-section views comparing the structure of the three junction types. (a) x 22,000; (b) and (c) x 100,000.

except for a restricted zone of connexin45/connexin43 co-expression at the nodal/crista terminalis border. This zone of co-expression has been hypothesized as a candidate pathway for directed exit of the impulse from the node into the atrial tissue [32]. In the rodent, the spatial pattern of expression of connexin45 reveals that the atrioventricular node and His bundle form part of an elaborately extended central conduction system circumscribing the atrioventricular and outflow junctional regions [31]. Whether these features are also present in the human impulse generation and conduction system is yet to be established.

In addition to connexin45, cardiomyocytes of the His-Purkinje conduction system in most mammals (including man) express connexin40 [11, 24, 25, 35, 36]. The ability of the bundle branches and Purkinje fiber system to distribute the impulse rapidly throughout the working ventricular myocardium correlates with large, abundant gap junctions which have high levels of

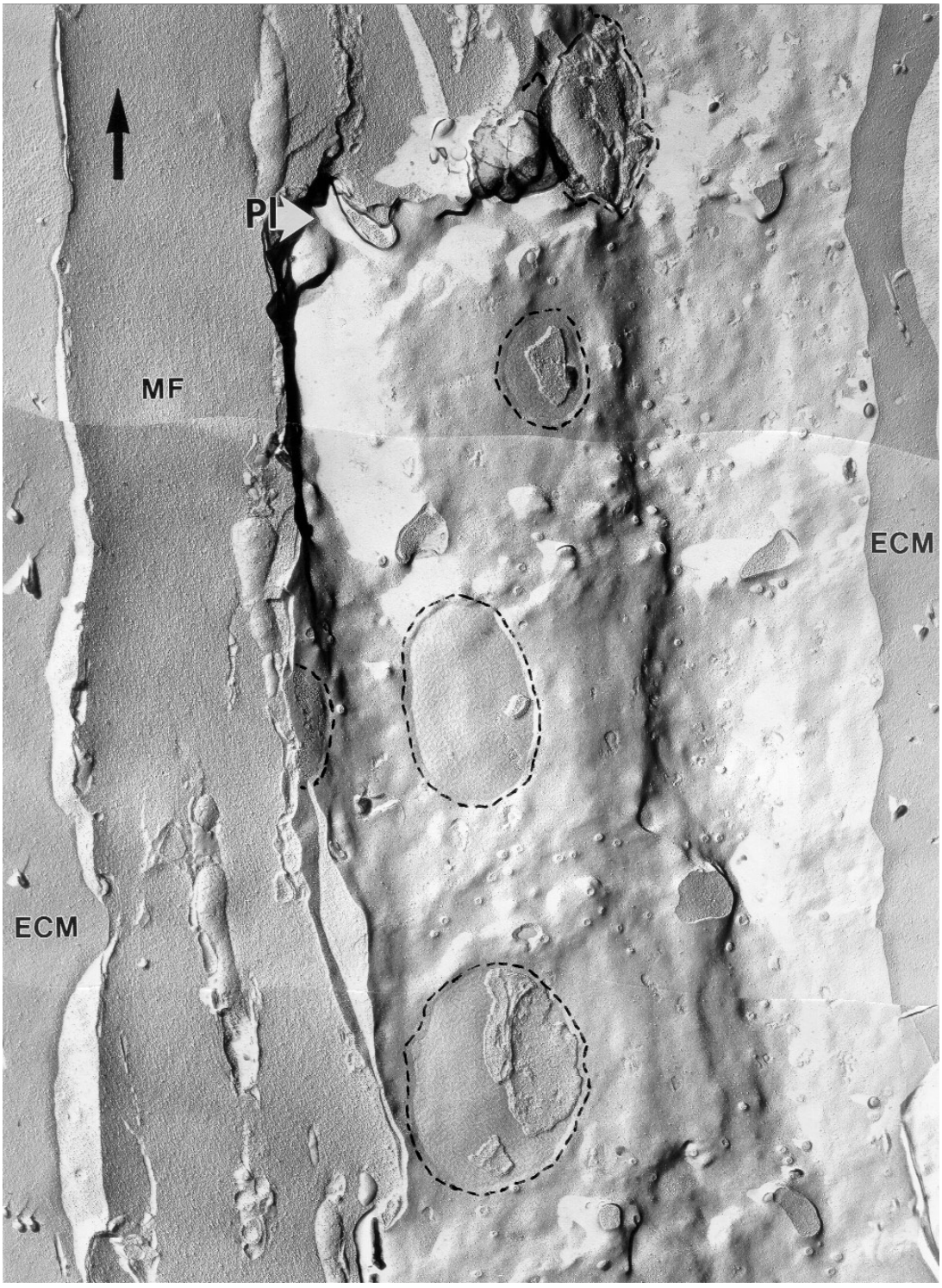

Fig. 4 Freeze-fracture electron micrograph showing arrangement of gap junctions within the lateral-facing segments of the intercalated disk membranes. Four gap junctions (encircled by dashes) are displayed *en face*; each of these would appear as in Fig. 1b if shown at higher magnification. To the left of the field, the fracture plane has cross-fractured into the cytoplasm, exposing the myofibrils (MF). ECM, extracellular matrix; arrow, direction of long axis of the cell. x 28,000. From Severs, N.J. Histol.Histopath. *10*:481-501 (1995) with permission.

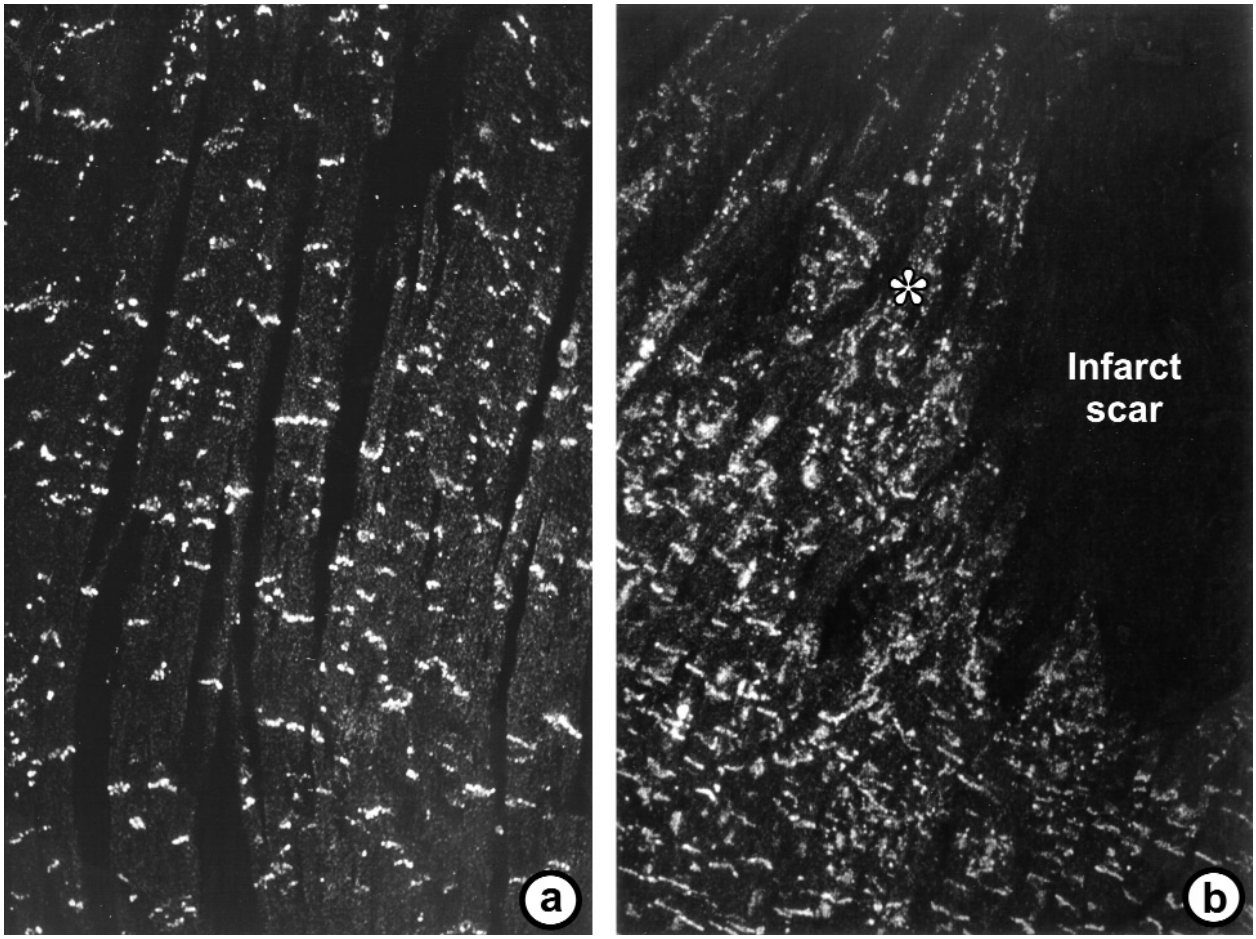

Fig. 5 (a) Distribution of connexin43-containing gap junctions in normal, healthy longitudinally-sectioned ventricular myocardium, as revealed by immunofluorescence confocal microscopy. Note rows of spots at intervals, corresponding to gap junction clusters at the intercalated disk. (b) Remodeling of connexin43 gap junctions in myocardium bordering infarct scar tissue. At the lower part of this immunoconfocal image, lines of immunolabeling are seen at intervals, representing linear rows of gap junctions organized in intercalated disks as in image (a). This normal ordered arrangement is severely disrupted in myocardium close to the infarct scar (asterisk). (a) x 250; (b) x 150. From reference [7] with permission.

connexin40 [24, 35, 37], a connexin associated with high conductance channels [38]. Connexin45 shows overlapping expression with connexin40 in the bundle branches and Purkinje fibers but in contrast to connexin40 is distributed throughout the entire conduction system [31]. In genetically engineered mice, deficiency of connexin40 leads to reduced conduction velocity through the conduction system and right bundle branch block [18, 21, 39-41]. Residual ability to support conduction, albeit with abnormal characteristics, is attributed to the presence of connexin45 [31].

Altered gap junction and connexin expression in human heart disease

From the established role of gap junctions as pathways for impulse propagation in the healthy heart, we first hypothesized, from observations made in the early 1990s, that alterations in gap junction organization and expression might potentially contribute to abnormal conduction and arrhythmogenesis in the diseased human heart [6, 42]. Such a concept does not attribute arrhythmia exclusively to gap junction-related alterations;

computer and cell culture models emphasize that arrhythmogenesis is multifactorial in origin, involving interplay between gap-junctional coupling, membrane excitability and cell and tissue architecture [43-45]. Within this perspective, an increasing number of studies over the last decade has focused on investigating the nature of cardiac disease-related gap junction and connexin remodeling in animal models and human tissue. As an aid to discussion, it is convenient to consider such remodeling under two inter-related headings; 1) structural remodeling, and 2) remodeling of connexin expression.

Structural remodeling involves major alteration in the arrangement and organization of gap junctions. A notable example is the loss of the normal ordered distribution of connexin43 gap junctions that occurs in the myocardial zone bordering infarct scar tissue in the ventricles of patients with ischemic heart disease [42]. Connexin43 immunolabeling in these zones is scattered extensively over the cells, while that at more distant sites remains largely confined to clearly ordered intercalated disk arrays (Fig. 5). Both laterally-disposed gap junctions that maintain contact between cells, and internalized, non-functional gap-junctional membrane, appear to contribute to the dispersed connexin43 labeling patterns seen on confocal microscopy [42]. Such abnormalities in gap junction disposition are not solely late changes associated with fibrosis, but have been shown in experimental animals to occur rapidly after myocardial infarction [46]. Somewhat similar alterations in gap junction distribution are found in ventricular hypertrophy in the rat [47,48] and, in one of these models, correlates with reduced longitudinal conduction velocity [47]. Disordered arrangements of ventricular connexin43 gap junctions are also prominent in human hypertrophic cardiomyopathy [42]

Another form of structural remodeling is apparent in human hibernating myocardium. The term "hibernating myocardium" refers to regions of myocardium that fail to contract properly in patients afflicted with ischemic heart disease, but which recover contractile function after coronary by-pass operation. In hibernating myocardium, the large connexin43 gap junctions at the periphery of the intercalated disk become markedly reduced in size compared with those of reversibly ischemic and normally perfused (and contracting) regions within the same diseased heart [49].

Structural remodeling inevitably results in disorganization of the normal ordered pattern of the microconduction pathways. Heterogeneity of distribution, involving focal reduction of connexin43 gap junctions, as observed in ischemic and hibernating hearts, could in theory contribute to localized conduction defects and contraction abnormalities [42, 49, 50]. Experimental evidence that it actually does so in practice comes from optical mapping and echocardiographic studies on chimeric mice created from connexin43-deficient embryonic stem cells and blastocysts of different strains [51]. The hearts of these chimeric mice, designed specifically to achieve heterogeneous expression of connexin43, exhibit abnormal myocardial excitation and contractile dysfunction [51].

With regard to remodeling of the amount of connexin expression, the most prominent alteration in human heart disease also involves connexin43. Quantitative northern and western blot analyses demonstrate markedly decreased levels of connexin43 transcript and protein in the left ventricle of transplant patients with end-stage congestive heart failure whether due to ischemic heart disease or idiopathic dilated cardiomyopathy [50]. As a reminder at the outset, measures of total connexin levels do not provide information on the quantity of functional (open) channels; hence, a reduction of connexin43 may not, *per se*, be detrimental. Overall levels of connexins are, perhaps, best thought of as indicators of the potential capacity for cell-to-cell communication. The idea that reduced levels of connexin43 may contribute to a pro-arrhythmic substrate has been questioned from computer modeling studies predicting that reductions of up to 40% in connexin43 levels would not, in fact, have a major effect on conduction velocity and altered anisotropy ratio [12]. In view of the complex relationship between passive and active membrane properties [43, 52, 53], the precise consequences of reduced connexin43 levels are difficult to predict *in vivo*. In the intact isolated heart, the incidence, frequency and duration of ventricular tachycardias after coronary occlusion is reportedly increased in a transgenic mouse model in which the normal connexin43 level is reduced by 50% [54]. Furthermore, transgenic mice generated to give

cardiac specific loss of connexin43 (86-95% reduction at 4 weeks) develop sudden cardiac death due to arrhythmia by 2 months of age [55]. Bearing in mind that the extent of connexin43 reduction in the diseased human ventricle varies considerably from one patient to the next, in some regions of some diseased hearts reaching a reduction of >90% of control values, and that the reduction observed is often superimposed on heterogeneity of connexin43 distribution [50], it would seem premature to discount a contribution from gap junction and connexin remodeling to the development of arrhythmia in at least some patients.

Apart from the decline in connexin43, the connexin40 level is increased in the ventricles of patients with congestive heart failure due to ischemic heart disease but not that due to idiopathic dilated cardiomyopathy [50]. Immunoconfocal microscopy localizes this increased connexin40 expression to a band of myocytes at the endocardial surface associated with and adjacent to the conduction system. The significance of this expanded zone of connexin40 expression is unclear; one speculation is that it could conceivably represent some form of compensatory response that might improve the spread of depolarization from the conduction tissues in the face of declining connexin43 levels in the ischemic ventricle. Recent evidence also suggests that connexin40 may play a role in an atrial arrhythmia, post-operative atrial fibrillation. A higher than average pre-existing level of atrial connexin40 correlates with increased incidence of atrial fibrillation after coronary artery by-pass grafting [30].

Concluding comment

In view of the role of gap junctions in mediating the patterns of current flow responsible for normal heart function, gap junction/connexin remodeling in heart disease was proposed as a candidate contributor to cardiac arrhythmogenesis. From where we now stand, remodeling of gap junctions – in particular, disorganization in the distribution pattern of connexin43 gap junctions and reduced levels of connexin43 in the ventricle – has been established to occur in defined categories of human heart disease and has been correlated with electrophysiologically identified pro-arrhythmic changes and overt arrhythmia in animal models. While such correlations do not prove cause and effect, and other factors (membrane excitability, cell size etc) certainly come into play, recent work on genetically engineered mouse models [18-21, 39-41, 51, 54, 55] has opened exciting opportunities for new insights into the significance of gap junction and connexin remodeling in cardiac arrhythmogenesis and contractile dysfunction.

Acknowledgments

Work in the author's laboratory is currently supported by the European Commission (QLRT-1999-00516) and British Heart Foundation (FS/99089, PG/2001047). I thank all members of my group who, over the last 10 years, have contributed to the work discussed in this review.

References

1. **Cardew G.**. Ed. Gap Junction-Mediated Intercellular Signalling in Health and Disease. John Wiley & Son Ltd., New York, 1999
2. **Scherer S.S., Bone, L.J., Deschenes, S.M., Abel, A., Balice-Gordon, R.J., Fischbeck, K.H.**. The role of the gap junction protein connexin32 in the pathogenesis of X-linked Charcot-Marie-Tooth disease. *Novartis Found Symp,* **219**:175-185, 1999
3. **Cohn E.S., Kelley, P.M.**. Clinical phenotype and mutations in connexin 26 (DFNB1/GJB2), the most common cause of childhood hearing loss. *Am. J. Med. Genet.,* **89**:130-136, 1999
4. **Mackay D., Ionides, A., Kibar, Z., Rouleau, G., Berry, V., Moore, A., Shiels, A., Bhattacharya, S.**. Connexin46 mutations in autosomal dominant congenital cataract. *Am. J. Hum. Genet.,* **64**:1357-1364, 1999
5. **Dasgupta C., Escobar-Poni, B., Shah, M., Duncan, J., Fletcher, W.H.**. Misregulation of connexin43 gap junction channels and congenital heart defects. In: Cardew G., ed., Gap Junction-Mediated Intercellular Signalling in Health and Disease. John Wiley & Sons Ltd., New York, 1999, pp. 212-221
6. **Green C.R., Severs, N.J.**. Distribution and role of gap junctions in normal myocardium and human ischaemic heart disease. *Histochemistry,* **99**:105-120, 1993

7. **Severs N.J., Gourdie, R.G., Harfst, E., Peters, N.S., Green, C.R.**. Review. Intercellular junctions and the application of microscopical techniques: the cardiac gap junction as a case model. *J. Microsc.,* **169**:299-328, 1993
8. **Severs N.J., Dupont, E., Kaprielian, R.R., Yeh, H.-I., Rothery, S.**. Gap junctions and connexins in the cardiovascular system. In: Yacoub M.H., Carpentier, A., Pepper, J., Fabiani, J.-N., eds., Annual of Cardiac Surgery 1996: 9th edition. Current Science, London, 1996, pp. 31-44
9. **Severs N.J.**. Gap junctions and coronary heart disease. In: De Mello W.C., Janse, M.J., eds., Heart Cell Communication in Health and Disease. Kluwer, Boston, 1998, pp. 175-194
10. **Severs N.J.**. Cardiovascular disease. In: Cardew G., ed., Gap Junction-Mediated Intercellular Signalling in Health and Disease. John Wiley & Sons Ltd., New York, 1999, pp. 188-206
11. **Severs N.J., Rothery, S., Dupont, E., Coppen, S.R., Yeh, H.-I., Ko, Y.-S., Matsushita, T., Kaba, R., Halliday, D.**. Immunocytochemical analysis of connexin expression in the healthy and diseased cardiovascular system. *Microsc Res Tech,* **52**:301-322, 2001
12. **Jongsma H.J., Wilders, R.**. Gap junctions in cardiovascular disease. *Circ Res,* **86**:1193-1197, 2000
13. **Willecke K., J. Eiberger, J. Degen, D. Eckardt, A. Romualdi, M. Gueldenagel, U. Deutsch and G. Soehl**. Structural and functional diversity of connexin genes in the mouse and human genome. Biol.Chem. *in press*: 2002.
14. **Beyer E., Seul, K.H., Larson, D.M.**. Cardiovascular gap junction proteins: molecular characterization and biochemical regulation. In: De Mello W.C., Janse, M.J., Norwell, M.A., eds., Heart Cell Communication in Health and Disease. Klewer Academic Publications, New York, 1997, pp. 45-51
15. **Paul D.L., Ebihara, L., Takemoto, L.J., Swenson, K.I., Goodenough, D.A.**. Connexin46, a novel lens gap junction protein, induces voltage- gated currents in nonjunctional plasma membrane of *Xenopus* oocytes. *J. Cell Biol.,* **115**:1077-1089, 1991
16. **Manthey D., Bukauskas, F., Lee, C.G., Kozak, C.A., Willecke, K.**. Molecular cloning and functional expression of the mouse gap junction gene connexin-57 in human HeLa cells. *J. Biol. Chem.,* **274**:14716-14723, 1999
17. **Bruzzone R., White, T.W., Paul, D.L.**. Connections with connexins: The molecular basis of direct intercellular signaling. *Eur. J. Biochem.,* **238**:1-27, 1996
18. **Tamaddon H.S., Vaidya, D., Simon, A.M., Paul, D.L., Jalife, J., Morley, G.E.**. High-resolution optical mapping of the right bundle branch in connexin40 knockout mice reveals slow conduction in the specialized conduction system. *Circ Res,* **87**:929-936, 2000
19. **Krüger O., Plum, A., Kim, J.-S., Winterhager, E., Maxeiner, S., Hallas, G., Kirchhoff, S., Traub, O., Lamers, W.H., Willecke, K.**. Defective vascular development in connexin 45-deficient mice. *Development,* **127**:4179-4193, 2000
20. **Plum A., Hallas, G., Magin, T., Dombrowski, F., Hagendorff, A., Schumacher, B., Wolpert, C., Kim, J.-S., Lamers, W.H., Evert, M., Meda, P., Traub, O., Willecke, K.**. Unique and shared functions of different connexins in mice. *Curr. Biol.,* **10**:1083-1091, 2000
21. **van Rijen H.V.M., Van Veen, T.A.B., Van Kempen, M.J.A., Wilms-Schopman, F.J.G., Poste, M., Krueger, O., Willecke, K., Opthof, T., Jongsma, H.J., de Bakker, J.M.T.**. Impaired conduction in the bundle branches of mouse hearts lacking the gap junction protein connexin40. *Circulation,* **103**:1591-1598, 2001
22. **Beyer E.C., Kistler, J., Paul, D.L., Goodenough, D.A.**. Antisera directed against connexin43 peptides react with a 43-kd protein localized to gap junctions in myocardium and other tissues. *J. Cell Biol.,* **108**:595-605, 1989
23. **Severs N.J.**. The cardiac muscle cell. *BioEssays,* **22**:188-199, 2000
24. **Gourdie R.G., Severs, N.J., Green, C.R., Rothery, S., Germroth, P., Thompson, R.P.**. The spatial distribution and relative abundance of gap-junctional connexin40 and connexin43 correlate to functional properties of the cardiac atrioventricular conduction system. *J. Cell Sci.,* **105**:985-991, 1993
25. **Coppen S.R., Dupont, E., Rothery, S., Severs, N.J.**. Connexin45 expression is preferentially associated with the ventricular conduction system in mouse and rat heart. *Circ. Res.,* **82**:232-243, 1998
26. **Vozzi C., Dupont, E., Coppen, S.R., Yeh, H.-I., Severs, N.J.**. Chamber-related differences in connexin expression in the human heart. *J. Mol. Cell. Cardiol.,* **31**:991-1003, 1999
27. **Severs N.J.**. Constituent cells of the heart and isolated cell models in cardiovascular research. In: Piper H.M., Isenberg, G., eds., Isolated Adult Cardiomyocytes. volume 1. CRC Press Inc., Boca Raton, 1989, pp. 3-41
28. **Saffitz J.E., Beyer, E.C., Darrow, B.J., Guerrero, P.A., Beardslee, M.A., Dodge, S.M.**. Gap junction structure, conduction, and arrhhythmogenesis: direction for future research. In: Spooner P.M., Joyner, R.W., Jalife, J., eds., Discontinuous Conduction in the Heart. Futura Publishing Company, New York, 1997, pp. 89-105

29. **Gourdie R.G., Green, C.R., Severs, N.J.**. Gap junction distribution in adult mammalian myocardium revealed by an antipeptide antibody and laser scanning confocal microscopy. *J. Cell Sci.,* **99**:41-55, 1991
30. **Dupont E., Ko, Y.S., Rothery, S., Coppen, S.R., Baghai, M., Haw, M., Severs, N.J.**. The gap-junctional protein, connexin40, is elevated in patients susceptible to post-operative atrial fibrillation. *Circulation,* **103**:842-849, 2001
31. **Coppen S.R., Severs, N.J., Gourdie, R.G.**. Connexin45 (α6) expression delineates an extended conduction system in the embryonic and mature rodent heart. *Dev. Genet.,* **24**:82-90, 1999
32. **Coppen S.R., Kodama, I., Boyett, M.R., Dobrzynski, H., Takagishi, Y., Honjo, H., Yeh, H.-I., Severs, N.J.**. Connexin45, a major connexin of the rabbit sinoatrial node, is co-expressed with connexin43 in a restricted zone at the nodal-crista terminalis border. *J. Histochem. Cytochem.,* **47**:907-918, 1999
33. **Honjo H., Boyett, M.R., Coppen, S.R., Takagishi, Y., Severs, N.J., Kodama, I.**. Heterogeneous expression of connexins in rabbit sinoatrial node cells: correlation between connexin isoform and cell size. *Cardiovasc. Res.,* in press:2002
34. **Moreno A.L., Laing, J.G., Beyer, E.C., Spray, D.C.**. Properties of gap junction channels formed of connexin 45 endogenously expressed in human hepatoma (SKHep1) cells. *Am. J. Physiol.,* **268**:C356-C365, 1995
35. **Gros D., Jarry-Guichard, T., ten Velde, I., De Mazière, A.M.G.L., Van Kempen, M.J.A., Davoust, J., Briand, J.P., Moorman, A.F.M., Jongsma, H.J.**. Restricted distribution of connexin40, a gap junctional protein, in mammalian heart. *Circ. Res.,* **74**:839-851, 1994
36. **Coppen S.R., Gourdie, R.G., Severs, N.J.**. Connexin45 is the first connexin to be expressed in the central conduction system of the mouse heart. *Exp. Clin. Cardiol.,* **6**:17-23, 2001
37. **Bastide B., Neyses, L., Ganten, D., Paul, M., Willecke, K., Traub, O.**. Gap junction protein connexin40 is preferentially expressed in vascular endothelium and conductive bundles of rat myocardium and is increased under hypertensive conditions. *Circ. Res.,* **73**:1138-1149, 1993
38. **Bukauskas F.F., 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.,* **68**:2289-2298, 1995
39. **Kirchhoff S., Nelles, E., Hagendorff, A., Krüger, O., Traub, O., Willecke, K.**. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice. *Curr. Biol.,* **8**:299-302, 1998
40. **Simon A.M., Goodenough, D.A., Paul, D.L.**. Mice lacking connexin40 have cardiac conduction abnormalities characteristic of atrioventricular block and bundle branch block. *Curr. Biol.,* **8**:295-298, 1998
41. **Hagendorff A., Schumacher, B., Kirchhoff, S., Lüderitz, B., Willecke, K.**. Conduction disturbances and increased atrial vulnerability in connexin40-defficient mice analyzed by transesophageal stimilation. *Circulation,* **99**:1508-1515, 1999
42. **Smith J.H., Green, C.R., Peters, N.S., Rothery, S., Severs, N.J.**. Altered patterns of gap junction distribution in ischemic heart disease. An immunohistochemical study of human myocardium using laser scanning confocal microscopy. *Am. J. Pathol.,* **139**:801-821, 1991
43. **Shaw R.M., Rudy, Y.**. Ionic mechanisms of propagation in cardiac tissue - Roles of the sodium and L-type calcium currents during reduced excitability and decreased gap junction coupling. *Circ. Res.,* **81**:727-741, 1997
44. **Rohr S., Kucera, J.P., Fast, V.G., Kleber, A.G.**. Paradoxical improvement of impulse conduction in cardiac tissue by partial cellular uncoupling. *Science,* **275**:841-844, 1997
45. **Spach M.S., J.F. Heidlage, P.C. Dolber and R.C. Barr**. 2000. Electrophysiological effects of remodelling cardiac gap junctions and cell size. Circ.Res. *86*:302-311.(Abstract)
46. **Matsushita T., Oyamada, M., Fujimoto, K., Yasuda, Y., Masuda, S., Wada, Y., Oka, T., Takamatsu, T.**. Remodelling of cell-cell and cell-extracellular matrix interactions at the border zone of rat myocardial infarcts. *Circ. Res.,* **85**:1046-1055, 1999
47. **Uzzaman M., Honjo, H., Takagishi, Y., Emdad, L., Magee, A.I., Severs, N.J., Kodama, I.**. Remodeling of gap-junctional coupling in hypertrophied right ventricles of rats with monocrotaline-induced pulmonary hypertension. *Circ. Res.,* **86**:871-878, 2000
48. **Emdad L., Uzzaman, M., Takagishi, Y., Honjo, H., Uchida, T., Severs, N.J., Kodama, I., Murata, Y.**. Gap junction remodelling in hypertrophied left ventricles of aortic-banded rats: prevention by angiotensin II type1 receptor blockade. *J. Mol. Cell. Cardiol.,* **33**:219-231, 2001
49. **Kaprielian R.R., Gunning, M., Dupont, E., Sheppard, M.N., Rothery, S.M., Underwood, R., Pennell, D.J., Fox, K., Pepper, J., Poole-Wilson, P.A., Severs, N.J.**. Down-regulation of immunodetectable connexin43 and decreased gap junction size in the pathogenesis of chronic hibernation in the human left ventricle. *Circulation,* **97**:651-660, 1998

50. **Dupont E., Matsushita, T., Kaba, R., Vozzi, C., Coppen, S.R., Khan, N., Kaprielian, R., Yacoub, M.H., Severs, N.J.**. Altered connexin expression in human congestive heart failure. *J Mol Cell Cardiol,* **33**:359-371, 2001
51. **Gutstein D.E., Morley, G.E., Vaidya, D., Liu, F., Chen, F.L., Stuhlmann, H., Fishman, G.I.**. Heterogeneous expression of gap junction channels in the heart leads to conduction defects and ventricular dysfunction. *Circulation,* **104**:1194-1199, 2001
52. **Rudy Y., Shaw, R.M.**. Cardiac excitation: an interactive process of ion channels and gap junctions. *Adv. Exp. Med. Biol.,* **430**:269-279, 1997
53. **Viswanathan P.C., Shaw, R.M., Rudy, Y.**. Effects of IKr and IKs heterogeneity on action potential duration and its rate dependence: a simulation study. *Circulation,* **99**:2466-2474, 1999
54. **Lerner D.L., Yamada, K.A., Schuessler, R.B., Saffitz, J.E.**. Accelerated onset and increased incidence of ventricular arrhythmias induced by ischaemia in Cx43-deficient mice. *Circulation,* **101**:547-552, 2000
55. **Gutstein D.E., Morley, G.E., Tamaddon, H., Vaidya, D., Schneider, M.D., Chen, J., Chien, K.R., Stuhlmann, H., Fishman, G.I.**. Conduction slowing and sudden arrhythmic death in mice with cardiac-restricted inactivation of connexin43. *Circ. Res.,* **88**:333-339, 2001