Gap Junctional Conductance in Cardiomyopathic Hamsters

The Role of c-Src

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Ap junctions, which are small hydrophilic channels connecting apposing cardiac cells, play an important role on the electrical and mechanical synchronization in heart muscle.1 Gap junctional proteins are arranged in a hexagonal array,2,3 and each protein consists of a pair of hemichannels (connexons), one from each cell.4 The junctional conductance is modulated by several factors including Ca2+, and second messengers such as cAMP and cGMP.5–7 Second messengers influence the junctional conductance through the phosphorylation of junctional proteins.7,8 The activation of cAMP-dependent protein kinase (PKA), for instance, increases junctional conductance in cardiac muscle, an effect suppressed by intracellular dialysis of a PKA inhibitor.9 The role of gap junctions in heart failure merits close scrutiny, given the high prevalence of conduction disorders such as intraventricular conduction delays in patients with cardiomyopathy. The complexity and diversity of human heart failure, along with the obvious limitations of work with human subjects, motivate the investigation of the molecular basis of conduction abnormalities in animal models of heart failure.

In cardiomyopathic hamsters, which mirror many aspects of cardiomyopathy and heart failure in humans,10 interstitial fibrosis, necrosis, and calcification are extensively distributed throughout the ventricle, particularly at an advanced stage of the disease.11 The distribution of connexin 43, the most abundant connexin in cardiac muscle,4 is diffuse, and the structure of the intercalated disks is irregular.12 According to some authors,12 the expression of connexin 43 is not changed in cardiomyopathic hamsters.

Recent studies performed in isolated ventricular cell pairs from cardiomyopathic hamsters (BIO T02) indicated that the junctional conductance is appreciably reduced in 11-month-old animals reaching, in some areas, values (0.8 to 2.5 nS) that are incompatible with the propagation of the action potential.13

The study of Toyofuku et al14 in this issue of Circulation Research provides evidence that increased levels of tyrosine phosphorylation of connexin 43 occur at a late stage of the disease when overt heart failure is present. Indeed, it is known that there is a high level of tyrosine phosphorylation in the intercalated disks15 and that tyrosine phosphorylation of gap junction proteins correlates with inhibition of cell communication.16 Moreover, the oncogene v-src appears to cause closure of gap junction channels. The residue Tyr265 in the COOH terminal tail of connexin 43 has been implicated as a potential target for v-src, despite the fact that v-src action is associated with serine phosphorylation.17,18 Furthermore, connexin 43 is a substrate for mitogen-activated protein kinase (MAPK), and phosphorylation of Ser255, Ser279, and Ser282 seems to initiate the downregulation of junctional communication.18

A dissection of the molecular basis of pp60 (v-src)–induced gating of connexin 43 channels surprisingly indicated that v-src-mediated gating does not require the tyrosine site but depends on the presence of two SH3 domains and MAPK phosphorylation sites.17 In neonatal rat cardiac cells, for instance, serine phosphorylation has been associated with reduction of junctional conductance.19 What Toyofuku et al14 propose is that the tyrosine phosphorylation state of connexin 43 is an important regulator of junctional conductance in cardiomyopathic hamsters at an advanced stage of the disease and that the enhanced tyrosine-phosphorylation is correlated with c-Src activity. It is then important to investigate whether similar levels of tyrosine phosphorylation occur in myopathic cells at earlier stages of the disease when junctional conductance is already reduced but no signs of heart failure are evident (W.C. De Mello, unpublished data, 1998).

The functional consequences of tyrosine phosphorylation of connexin 43 were also investigated by Toyofuku et al14 using transfected cells (HEK293) expressing constitutively active c-Src. Although the findings indicated a reduction of Ca2+ wave propagation, caution must be exercised when extrapolating these findings to adult cardiac cells because propagation of Ca2+ waves in heart muscle is still a controversial issue. Indeed, studies of Lamont et al20 indicated no propagation of Ca2+ waves in rat cardiac muscle.

Because the average value of junctional conductance in cardiac myocytes transfected with c-Src (Y527F) was 28.5 ± 16.3 nS compared with 126.6 ± 55.8 found in the controls,14 it is reasonable to conjecture that impulse conduction might be impaired by activation of c-Src in cardiomyopathy. Indeed, conduction velocity is known to be appreciably reduced in the ventricular muscle of T0-2 cardiomyopathic hamsters at 11 months of age, when overt heart failure is found.21 The decrease in junctional conductance in the failing heart, however, is probably related to multiple factors. An alteration in the arrangement of junctional proteins induced by the

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pathologic process is a feasible hypothesis. Previous studies\textsuperscript{22} indicated that isoproterenol or forskolin are not able to increase the junctional conductance in cardiomyopathic hamsters at an advanced stage of the disease. This finding is related to downregulation of β-adrenergic receptors and alteration of adenyl cyclase.

The question remains whether the impaired regulation of junctional conductance by the β-adrenergic receptor/adenyl cyclase pathway influences the depressant effect of MAPK and c-Src on cell-cell communication in cardiomyopathic hamsters. Toyofuku et al\textsuperscript{14} speculate that humoral factors such as α-adrenergic agonists, angiotensin II, and endothelin-1 might influence cell-cell coupling and contractility through c-Src-mediated tyrosine phosphorylation of connexin 43. Although it is known that both phenylephrine, an α-adrenergic agonist, and angiotensin II decrease the junctional conductance in adult rat heart,\textsuperscript{23,24} no information is available as to whether angiotensin II leads to tyrosine phosphorylation of junctional proteins. The study of Toyofuku et al\textsuperscript{14} certainly reopens an interest in the role of different kinases in the regulation of junctional communication, particularly in the failing heart.

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References


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