

Gap junction distribution in adult mammalian myocardium revealed by an anti-peptide antibody and laser scanning confocal microscopy

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Summary

A polyclonal antiserum, raised against a synthetic peptide matching part of the sequence of connexin-43 (a rat cardiac gap-junctional protein), was used in combination with laser scanning confocal microscopy to investigate gap junction distribution in cardiac tissues from a range of mammalian species. Comparison of the localised punctate staining patterns obtained in ventricular tissue with the distribution of intercalated disks as viewed by conventional light microscopy and electron microscopy, and with the staining observed by standard light-microscope immunofluorescence using the same antiserum, demonstrated highly specific labelling of clearly resolved individual gap junctions. Laser scanning confocal microscopy of ventricular myocardium showed the immunostained gap junctions to be confined to well-defined intercalated disks bisecting the long axis of the muscle fibre, whereas in the atrial myocardium, gap junctions were commonly distributed widely over the lateral surfaces of the myocyte body. Rat atrial gap junctions were significantly larger (as measured by the longest axial lengths of fluorescent spots), and showed a narrower spread of sizes, than their counterparts in the ventricle. Ventricular myocardium from six mammalian species including man gave similar immunostaining patterns, indicating conservation both of the epitope(s) detected by the antiserum, and of the general organisation of the cell-to-cell pathways for

electrical propagation, in the mammalian heart. Optical section series obtained by laser scanning confocal microscopy permitted the quantification and mapping of the three-dimensional distribution of gap junctions in ventricular intercalated disks with high clarity over substantial specimen depths. A consistent feature of gap junction organisation within disks of ventricular myocardium in all species studied was the presence of a conspicuous ring of large gap junctions around the periphery of the disk. Immunostained gap junctions lying within the interior zone delineated by the peripheral junctions generally occurred at lower numerical densities and were significantly smaller. In all species, less than 3% of all immunolabelled gap junctions measured were $>2\text{ }\mu\text{m}$ in maximal length, though a small proportion (0.06%) exceeded $4\text{ }\mu\text{m}$. The numerical density of immunolabelled gap junctions in the disk was similar between species; however, within species there was a significant decrease in numerical density with increasing disk size. The new features of intercalated disk structure revealed in this study may have an important part to play in the intercellular communication and electrical propagation properties of the mammalian heart.

Key words: gap junction, connexin-43, heart, intercalated disk, laser scanning confocal microscopy.

Introduction

Gap junctions are aggregates of intercellular channels that in the heart facilitate the transmission of electrical current between myocytes (for a review, see Page and Manjunath, 1986). By means of these channels, low-resistance pathways are formed, linking all the myocytes within the myocardium (Weidmann, 1952, 1966, 1970; Barr *et al.* 1965; Sommer and Johnson, 1979; De Mello, 1982; Page and Manjunath, 1986). The electrical impulses borne rapidly and repeatedly along these pathways stimulate the contraction of individual myocytes in

synchrony, which in turn provides the motive force for the pumping action of the heart.

In the ventricle of the mammalian heart, the principal sites of cell-to-cell plasma membrane interaction occur at the intercalated disk. Models for the organisation and distribution of gap junctions within the intercalated disk have been derived almost exclusively from electron-microscopic studies (McNutt, 1970; Page and McCallister, 1973; Forbes and Sperelakis, 1985; Severs, 1985, 1990). From longitudinal thin sections of myocytes, the intercalated disk is commonly envisaged as a series of membrane steps following the ends and sides of the myofibril

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