

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 connexin43 (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, connexin43, 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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Table of Contents The Mammalian Myocardium A Wiley Biomedical Health Publication

1. Understanding the eBook The Mammalian Myocardium A Wiley Biomedical Health Publication
 - The Rise of Digital Reading The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Advantages of eBooks Over Traditional Books
2. Identifying The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an The Mammalian Myocardium A Wiley Biomedical Health Publication
 - User-Friendly Interface

4. Exploring eBook Recommendations from The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Personalized Recommendations
 - The Mammalian Myocardium A Wiley Biomedical Health Publication User Reviews and Ratings
 - The Mammalian Myocardium A Wiley Biomedical Health Publication and Bestseller Lists
5. Accessing The Mammalian Myocardium A Wiley Biomedical Health Publication Free and Paid eBooks
 - The Mammalian Myocardium A Wiley Biomedical Health Publication Public Domain eBooks
 - The Mammalian Myocardium A Wiley Biomedical Health Publication eBook Subscription Services
 - The Mammalian Myocardium A Wiley Biomedical Health Publication Budget-Friendly Options
6. Navigating The Mammalian Myocardium A Wiley Biomedical Health Publication eBook Formats
 - ePub, PDF, MOBI, and More
 - The Mammalian Myocardium A Wiley Biomedical Health Publication Compatibility with Devices
 - The Mammalian Myocardium A Wiley Biomedical Health Publication Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Highlighting and Note-Taking The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Interactive Elements The Mammalian Myocardium A Wiley Biomedical Health Publication
8. Staying Engaged with The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Joining Online Reading Communities
 - Participating in Virtual Book Clubs
 - Following Authors and Publishers The Mammalian Myocardium A Wiley Biomedical Health Publication
9. Balancing eBooks and Physical Books The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection The Mammalian Myocardium A Wiley Biomedical Health Publication
10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
11. Cultivating a Reading Routine The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Setting Reading Goals The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Carving Out Dedicated Reading Time

12. Sourcing Reliable Information of The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Fact-Checking eBook Content of The Mammalian Myocardium A Wiley Biomedical Health Publication
 - Distinguishing Credible Sources
13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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