Confocal tiling images of inositol 1,4,5-trisphosphate receptors in intact mouse sinoatrial node

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It has been found that inositol 1,4,5-trisphosphate receptors (IP_3Rs), which function as inositol 1,4,5-trisphosphate (IP_3)-gated Ca^{2+} channels, are expressed in working cardiac myocytes. In atrial cells, accumulated evidence shows that IP_3R signalling is involved in arrhythmic activity. However, there is little direct evidence whether functional IP_3Rs exist in adult mammalian sino-atrial node (SAN), the origin site of generating rhythm in the heart.

To determine the distribution of IP_3R protein isoforms in mouse SAN, mice were deeply anesthetized with intra peritoneal pentobarbitone. The intact SANs were isolated from the right atria and whole mount mouse SANs preparations were used for immunohistochemical study as described previously (Ju *et al.*, 2007). The antibodies against two IP_3R isoforms, (IP_3R1, IP_3R2, 1:200, 1:100, Affinity Bioreagents), a gap junction protein connexin-43 (Cx43, 1 :100, Chemicon), the hyperpolarization-activated, cyclic nucleotide-gate cation channel (HCN4 1:1000, Abcam), anti-mouse or rat and anti-rabbit secondary antibodies (Alex-488 and Alex-594, Molecular Probes) were used, respectively. A Zeiss confocal microscope (LSM 510) with a motorized XY scanning stage was used to perform a tile or mosaic scan in order to image the entire intact SAN region at high resolution.

We found a very weak labelling of IP_3R1 in the central SAN, identified by absence of Cx43. IP_3R1 labelling appeared in the peripheral SAN, especially in the interatrial septum, which also showed strong expression of Cx43. In contrast, the entire SAN, including the central and peripheral SAN and the surrounding atrial tissue was uniformly labelled with IP_3R2 antibody. To distinguish the pacemaker cells from other cell types, such as endothelial cells and fibroblasts in SAN, we used HCN4 antibody as a protein marker for pacemaker cells. The fine structure of the central SAN labelled with HCN4 antibody was revealed by a confocal immunofluorescence tiling image (8×8) using ×63 oil lens. Sinoatrial arteries crossing through the SAN showed with a positive staining of IP_3R2 only. The co-localization of IP_3R2 and HCN4 (yellow in colour) in central SAN region suggests that the pacemaker cells express IP_3R2. This study provides new evidence that IP_3R2s are expressed in mouse sinoatrial node and could serve as an additional Ca^{2+} dependent mechanism in modulating cardiac pacemaker activity as well as other Ca^{2+}-dependent processes (Ju *et al.*, 2011)

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