## Distribution and functional role of $IP_3R$ receptors in mouse sino-atrial node

*Y.K. Ju, Department of Physiology (F13), University of Sydney, NSW 2006, Australia.*

Inositol 1,4,5-trisphosphate receptors ($IP_3Rs$) have been implicated in the generation of cardiac arrhythmias, although the mechanism involved is unclear. In mammalian sinoatrial node (SAN), where the heart beat originates, the expression and functional role of $IP_3Rs$ have not been investigated.

In order to determine whether SAN cells express $IP_3Rs$ and their functional role in cardiac pacemaking, we used a range of techniques to study mRNA and protein expression and distribution. We first examined mRNA expression of *$Ip_3rs$*, *Hcn4*, *Ryr2* and *Stim1* genes across different regions of the mouse heart, including central SAN, peripheral SAN, AV node, atria and ventricle. We found that all three *$Ip_3r$* isoforms were expressed in the SAN and other regions of the heart. In contrast, *Hcn4* expression was highest in the central SAN and showed progressive reduction in peripheral SAN, AV node, atria and ventricle.

Whole mount SANs were co-labelled with Cx43 antibody and either $IP_3R1$ or $IP_3R2$ antibody. Cx43 antibody was used to distinguish central SAN from peripheral SAN. We found 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. The results suggested that while the SAN expressed three $IP_3Rs$ isoforms, $IP_3R2$ was the only protein isoform detected in the central SAN and isolated single pacemaker cells. We also found that $Ca^{2+}$ sparks induced by membrane-permeable $IP_3$ ($IP_3$-BM) were predominately located near the sarcolemma (within 1.5 μm). The $IP_3R$ agonist endothelin-1 (ET-1) induced sinoatrial arrhythmias as revealed by SAN electrical mapping. ET-1 and $IP_3$-BM increased intracellular $Ca^{2+}$ and pacemaker firing rate whereas the $IP_3R$ antagonist, 2-aminoethoxydiphenyl borate (2-APB), decreased $Ca^{2+}$ and firing rate. Both of these effects were absent in SANs from $IP_3R2$ knockout mice. We estimate that the contribution of $Ca^{2+}$ release from $IP_3Rs$ to normal heart rate could be up to 14 % based on the spontaneous firing rate of isolated SANs from WT v. IP3R2 KO mice. The results provided clear evidence that the heart rate modulated by ET-1 and 2-APB was *via* their interaction with $IP_3Rs$.

All animal experiments were approved by animal ethics committees of all listed research institutes. $IP_3R2$ knock out mice are gift from Dr. Ju Chen. The details of gene targeting and generation of $IP_3R2$-deficient mice were published previously (Li *et al.*, 2005).

Li X, Zima AV, Sheikh F, Blatter LA, Chen J. 2005) Endothelin-1-induced arrhythmogenic $Ca^{2+}$ signaling is abolished in atrial myocytes of inositol-1,4,5-trisphosphate(IP3)-receptor type 2-deficient mice. *Circulation Research* **96(12):** 1274-81.

---

This study was supported National Health and Medical Research Council of Australia (program grant 354400 and project grant 570926) and the Health Research Council of New Zealand.