Ageing related change in store-operated $Ca2^+$ influx and TRPCs expression of sinoatrial node

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The sinoatrial node (SAN) is the primary pacemaker of the mammalian heart. Dysfunction of the SAN increases exponentially with age. The age-related remodelling of pacemaker currents and pacemaker action potential may contribute to age-related slowing of the heart rate and reduction in conduction velocity which result in arrhythmias (Kistler *et al*., 2004). We recently discovered that store-operated Ca^{2+} channel (SOCC) activity is present in the mouse sinoatrial node leading to Ca^{2+} entry (Ju *et al.,* 2007). In the present study we tested the hypothesis that changes in SOCC activity might play a significant role in ageing related SAN dysfunction.

The experiments were carried out in intact mouse SAN preparations from either adult (2 months) or old (> 18 months) mice. Intracellular Ca^{2+} was detected with the fluorescent Ca^{2+} indicator indo-1. The SOCC activity was determined from the influx of Ca^{2+} following depletion of the sarcoplasmic reticulum Ca^{2+} store. We found that the Ca^{2+} influx was reduced in old mice compare to adult mice (2 month) ($p < 0.05$, $n = 4$).

It is widely believed that the transient receptor potential cannonical (TRPC) gene family are candidates for encoding SOCC. Recently we have developed one-step quantitative PCR to study gene expression in mouse SAN. This method allows us to study expression levels of up to 8 genes from one mouse SAN. Central or peripheral SAN samples were isolated from mouse hearts and stored in RNAlater solution (Ambion Inc.). One-step real-time PCRs were performed using the Cells Direct kit (Invitrogen, Australia). We tested 4 housekeeping genes including 18s rRNA, β-actin, HPRT1, B2m as reference genes, to normalise the changes mRNA expression of TRPCs or other genes of interest. We also used TRPC1 DNA as sample input reference. HCN4 used as a positive control for the central SAN region. We quantified the mRNA expression of TRPCs (TRPC1, 3, and 4) and stromal interacting molecule 1 (STIM1), an endoplasmic reticulum-Ca^{2+} sensor protein thought to associate with TRPCs. We found a reduction of the TRPC3 mRNA expression in the SANs from old mice (20-24 months) compared to young mice (1 month). Our preliminary data suggests that changes in SOCC activity and expression of TRPC genes might play a role in SAN dysfunction, specifically in the ageing heart.

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