Expression of TRPC4 and desmonplakin in mouse hearts

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The stretch-activated channels (SACs) are non-selective cation channels that respond to mechanical stress with an increase in open probability (Guharay & Sachs, 1984). Studies have shown that TRPC1 forms a stretch-activated cation channel in vertebrate cells (Maroto *et al.*, 2005). In our previous study, TRPC4 which is a closely related subgroup to TRPC1 (Schaefer, 2005), displayed a punctate labelling pattern in the plasma membranes and at cell termini (Ju *et al.*, 2007). Further Cx43-positive ($Cx43^+$) gap junctions were observed in the cardiomyocytes in mouse sinoatrial node (SAN) (Ju *et al.*, 2007). In this study, we further characterised the expression and distribution of TRPC4 and desmonplakin (DP), a necessary component of desmosomes in mouse hearts.

Single cardiomyocytes were enzymatically isolated from SAN region of mouse hearts. SANs were kept in KB solution for at least 4h at 4°C, before being triturated gently to dissociate the cells. The cells were then placed onto glass coverslips and maintained at room temperature for 1h to allow cells to adhere to the glass. Isolated single cardiomyocytes were then fixed in 4% paraformaldehyde for 1 min. They were permeabilised by incubating for 5 min in phosphate-buffered saline (PBS) containing 2% normal goat serum (NGS), 1% bovine serum albumin (BSA) and 0.1%Triton X-100. After blocking of nonspecific binding sites by incubation for 30 min with 0.01%BSA in PBS containing 10% NGS, single cardiomyocytes were exposed to primary antibodies. In order to determine the localisation of TRPC4 in cardiac myocytes, we double labelled for 1) TRPC4 and Cx43; and 2) TRPC4 and DP. In single cardiomyocytes, $Cx43^+$ gap junctions were detected in the plasma membrane and at cell termini, overlapping as well as non-overlapping but juxtaposed areas of Cx43 and TRPC4 were frequently seen, and suggesting Cx43 and TRPC4 were closely associated at the intercalated disc. DP^+ desmosomes were prominently detected at cell termini and in the plasma membrane. TRPC4 and DP were often juxtaposed at the intercalated discs.

Arrhymogenic right ventricular cardiomyopathy (ARVC) is a disease of desmosomes. Several studies have identified that mutations in genes that encode key components of the desmosome such as DP are responsible for ARVC (Dokuparti *et al.*, 2005; Sen-Chowdhry *et al.*, 2005). Our results have suggested that the specific localisation of TRPC4 imply that TRPC4 may form SACs in the region of cell connections in mouse hearts. We are currently working on different animal models to explore the possible implication of TRPC4 in cardiomyopathy.

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