RyR2 peptides mimic Ca^{2+} dysfunction associated with disease mutations and suggest greater susceptibility in atrial than ventricular myocytes

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Missense mutations in RyR2 underlie a number of cardiomyopathies including cathecholaminergic polymorphic ventricular tachycardia (CPVT) and type-2 arrhythmogenic right ventricular dysplasia (ARVD2). These conditions are associated with tachyarrhythmias during exercise or stress and affected patients are at increased risk of sudden cardiac death (Francis *et al.*, 2005). The mutations associated with CPVT or ARVD2 occur in 3 clusters, termed the N-terminal, central and the pore-forming regions of RyR2 (or regions 1, 2 & 3 respectively). This clustering of mutations and the qualitative similarity in phenotype led to the proposal of a common underlying molecular mechanism: Initially, it was proposed that the N-terminal and central regions represent interacting subdomains, which serve to stabilise the channel in the closed configuration (Yamamoto & Ikemoto, 2002). More recently, evidence has been presented that the large cluster of mutations in region 3 reflects the presence of a second pair of interacting subdomains, which comprise amino acid residues 3778-4220 and the cytosolic regions of the transmembrane domain located between residues 4498-4959 (Hamada *et al.*, 2007).

The interacting subdomain hypothesis suggests that it should be possible to mimic the effects of specific mutations by designing RyR2 peptides, which interact competitively to disrupt the subdomain interactions (Yamamoto & Ikemoto, 2002), *e.g.*, a peptide comprising a section of region 2, with the necessary amino acid sequence to bind to the corresponding recognition site on region 1, should interact competitively to weaken the subdomain interaction, thereby mimicking the disease state. However, a similar peptide containing a disease mutation should lack the structural characteristics necessary to interact competitively with the corresponding region 1 binding site, and have little or no effect on channel gating. In support of this hypothesis, we have shown that in permeabilized rat ventricular myocytes, a peptide corresponding to residues 2460-2495 of region 2 (DPc10) causes: i) a sustained increase the SR Ca^{2+} leak; ii) a transient increase in Ca^{2+} spark frequency; and iii) a decrease in the cytosolic [Ca^{2+}] threshold for spontaneous Ca^{2+} waves (Yang *et al.*, 2006). However, a similar peptide containing a disease mutation (A2474S) linked to CPVT had no effect on RyR2 function.

Here we report preliminary findings regarding the effects of DPc15 and DPc15-mut: novel region 3 peptides, corresponding to residues 4752-4773 of rabbit RyR2. DPc15mut contains the H4762P mutation of human RyR2, linked to CPVT. Experiments were done on saponin-permeabilized rat myocytes isolated from either the ventricle or the atrium. Rats (Wistar, 200g) were humanely killed in accordance with UK legislation. During and after permeabilization, cells were exposed to a mock intracellular solution containing (mM) ATP 5, phosphocreatine 10, HEPES 15 (free Ca^{2+} 200 nM, Mg^{2+} 1 mM. pH 7.1, 21^{o}C) Solutions also contained fluo-3 (10 μM), allowing changes in cytosolic [Ca^{2+}] to be detected using confocal microscopy. Under these conditions, addition of DPc15 to ventricular myocytes had no significant effect on: 1) Ca^{2+} sparks; 2) Ca^{2+} waves or the background fluorescence, an index of the RyR2 mediated Ca^{2+} leak (n = 6). However, in atrial myocytes, DPc15 caused: 1) a transient increase in spark frequency; 2) a sustained rise in background fluorescence and complex changes in the properties of spontaneous Ca^{2+} waves (n = 6). Consistent with the interacting subdomain hypothesis, DPc15mut had no significant effect on Ca^{2+} regulation in atrial or ventricular myocytes. Assuming the active peptide accurately mimics the CPVT mutation, these findings suggest that with less severe changes in RyR2 function, the atrium may be more susceptible than the ventricle.

Francis J, Sankar V, Nair VK & Priori SG. (2005) *Heart Rhythm*, **2:** 550-4.
Hamada T, Bannister ML & Ikemoto N. (2007) *Biochemistry*, **46:** 4272-9.
Yamamoto T & Ikemoto, N. (2002) *Biochemical and Biophysical Research Communications*, **291:** 1102-8.
Yang Z, Ikemoto N, Lamb GD & Steele DS. (2006) *Cardiovascular Research*, **70:** 475-85.