Inhibitory effect of dantrolene on cardiac RyR2 in the presence of calmodulin

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Dantrolene is a well-known muscle relaxant that has been used clinically as the treatment for malignant hyperthermia (MH), a common anaesthetic emergency. MH is an inherited disorder in which mutations in skeletal ryanodine receptor (RyR1), corresponding to the catecholaminergic polymorphic ventricular tachycardia (CPVT) mutations in RyR2, lead to aberrant Ca^{2+} leak from the sarcoplasmic reticulum (SR) upon exposure of volatile anaesthetics. Dantrolene acts on skeletal and cardiac muscle by inhibiting Ca^{2+} release from the SR (Kobayashi *et al.*, 2005; Uchinoumi *et al.*, 2010). The precise mechanism for dantrolene inhibition of SR Ca^{2+} release is not known. Assays of Ca^{2+} release in intact myocytes and cell homogenates containing SR vesicles indicate that dantrolene inhibits the SR Ca^{2+} release channel (RyR) (Fruen *et al.*, 1997). Moreover, the dantrolene binding site on RyR1 had been identified (Parness & Palnitkar, 1995). However, single channel studies (the most powerful method for determining the functional mechanism in RyRs) do not find any effect of dantrolene on RyRs (Szentesi *et al.*, 2001).

Calmodulin (CaM) is known to regulate RyR2 directly by binding to residues 3583-3603 of RyR2 (Huang *et al.*, 2013). The dantrolene effect on RyR2 may involve CaM binding to RyR2 because the reduced CaM binding either in heart failure (Ono *et al.*, 2010) or due to CPVT mutations (Xu *et al.*, 2010) can be restored by dantrolene. CaM can dissociate from RyR2 within ~1 min so when RyR2 are isolated form cells and examined in artificial lipid bilayers, CaM is not usually present. This may provide an explanation as to why the dantrolene effect on RyR1 and RyR2 has not been observed in single channel recording experiments. Thus, we hypothesized that CaM is the missing protein required for dantrolene inhibition of RyR2.

To test our hypothesis, RyR2 was isolated form sheep heart and incorporated into artificial lipid bilayers and their activity was measured using single channel recording. Control RyR2 activity was measured for periods of 1 min, and then during 1 min exposure to dantrolene and then again after washout. This was repeated in the absence and presence of exogenous 100 nmol/l CaM. Solution changes were done by local perfusion.

In the presence of diastolic cytoplasmic [Ca^{2+}] (100 nmol/l), dantrolene (50 µmol/l) reduced the mean open probability of RyR2 to 45 ± 6% in the presence of CaM (n = 7, $P < 0.05$, student paired *t-test*) and had no significant effect when CaM was absent (95 ± 9 %, n=20, $P = 0.24$). The dantrolene exhibited a hyperbolic dose-response in the presence of CaM with IC_{50} of 0.16 ± 0.03 µmol/l and with a saturating relative P_o (E_{max}) of 52 ± 4 %. E_{max} increased to one as cytoplasmic Ca^{2+} was increased to levels above 1 µmol/l. Finally we examined the CaM-dependence of dantrolene in intact SR by measuring the frequency and amplitudes of Ca^{2+} waves in saponin permeablised cardiomyocytes. Dantrolene reduced the frequency and amplitude of Ca^{2+} waves when CaM was present but it had no effect on Ca^{2+} waves in the absence of CaM. To conclude, CaM is essential for the inhibitory effect of dantrolene on RyR2.

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