Inhibitory effect of dantrolene on skeletal muscle RYR in the presence of calmodulin

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Dantrolene is a muscle relaxant that has been used clinically as the treatment for malignant hyperthermia (MH) response to volatile anaesthetics such as halothane. Our group recently reported that dantrolene inhibition of RyR1 and RyR2 required calmodulin (CaM) which is usually absent in single channel recording experiments because it readily dissociates from RyRs (Oo *et al.*, 2015). However, nothing more is known about the properties of dantrolene inhibition of single RyR1 channels. Therefore, this study aimed to characterize dantrolene inhibition on RyR1 in the presence of exogenous CaM. CaM has a biphasic regulation on RyR1: activation at resting cytoplasmic Ca^{2+} (100 nmol/l) or inhibition at higher [Ca^{2+}] (Tripathy *et al.*, 1995). Therefore, we measured the effects of dantrolene on RyR1 differentially regulated by CaM at various cytosolic Ca^{2+} concentrations. Moreover, RyR1 can be both physiologically and pathologically activated by substances such as ATP, halothane and the interdomain domain destablising peptide, DP4, that mimics the effects of inherited mutations underlying MH susceptibility. Therefore, we tested if dantrolene can restore the channel activity affected by these activators.

RyR1 was isolated from rabbit skeletal muscle and incorporated into artificial lipid bilayers and their open probability P_o was measured using single channel recording. Control RyR1 activity was measured for periods of 1 min, and then during 1 min exposure to dantrolene and then again after washout, all done in the presence of exogenous 100 nmol/l CaM.

Dantrolene caused inhibition of RyR1 and RyR2, but only when CaM was presence in the cytoplasmic bath. In the presence of cytoplasmic [Ca^{2+}] (100 nmol/l) and 2 mmol/l ATP, dantrolene inhibited both RyR1 and RyR2 with identical hyperbolic dose-responses with IC_{50} of 0.16 ± 0.03 μmol/l and with a saturating P_o of 52 ± 4 % of control. In the absence of dantrolene, RyR1 has a bell-shaped cytoplasmic Ca^{2+} activation curve with half activation at 1.75 ± 0.62 μmol/l and half inhibition at 0.21 ± 0.02 mmol/l and a peak P_o = 0.94 ± 0.06. Dantrolene reduced P_o in sub-activating and inhibiting [Ca^{2+}] but failed to reduce peak P_o. Dantrolene caused 50-60% reduction in P_o of RyR1 activated by cytoplasmic Ca^{2+} alone (100 nmol/l) or in conjunction with halothane (5 mmol/l) or DP4 (10 μmol/l). To conclude, dantrolene inhibits RyR1 activity by: 1) decreasing RyR1 sensitivity to Ca^{2+} activation; 2) increasing sensitivity to Ca^{2+} inhibition; and 3) decreasing channel activation by halothane or by 4) reducing the effect of inter-domain disruption in the RyR1 protein.

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