Inhibitory effect of phenytoin on cardiac RYR2

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Heart failure affects approximately 1-2% of the Australian population (Sahle *et al.*, 2016). Heart failure is associated with changes in Ca^{2+} homeostasis in heart (Bers, 2006) including excessive diastolic leakage of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR2) into the cytoplasm. The Ca^{2+} leakage leads to a depletion of the SR and diminution of Ca^{2+} release during contraction (systole). In failing heart, the diastolic leakage can be prevented by the RyR2 inhibitor, dantrolene without inhibiting Ca^{2+} release during systole or affecting Ca^{2+} release in normal healthy hearts (Oo *et al.*, 2015; Maxwell *et al.*, 2012). Unfortunately, dantrolene is hepatotoxic (Muehlschlegel & Sims, 2009) and unsuitable for therapeutic use. Both phenytoin and dantrolene belong to the hydantoin class of compounds. The aim of this study was to investigate properties of phenytoin as an alternative RyR2 inhibitor.

Phenytoin (Dilantin^{TM}) is used to treat epilepsy (Gallop, 2010). It is known to modulate Na^+, K^+ and Ca^{2+} channels (Gallop, 2010). It decreases hyperexcitability through blocking the Na^+ current at serum concentrations between 40 and 80 μmol/l (10 to 20 μg/ml) with an IC_{50} of approximately 58 μmol/l (Lang *et al.*, 1993), without causing sedation or interfering with normal central function (Lang, 1993).

RyR2 channels were isolated from sheep heart and incorporated into artificial lipid bilayers. Single channel RyR2 activity was measured in the presence of cytoplasmic solutions containing 0.1 mmol/l Ca^{2+} and 2 mmol/l ATP (vehicle). The cytoplasmic bath was cycled between 1-minute periods of vehicle and solutions containing different concentrations of phenytoin (10, 20, 50, 100, 200 and 500 μmol/l). These measurements were also carried out with Calmodulin (CaM, 100 nmol/l) since it is known that the dantrolene effect required the presence of CaM (Oo *et al.*, 2015).

The IC_{50} values of phenytoin at -40mV were 19 ± 3 μmol/l and 10 ± 2 μmol/l (mean ± SEM) in the presence and absence of CaM, respectively. At +40mV the corresponding IC_{50} values were 16 ± 3 μmol/l and 15 ± 2 μmol/l. The Hill coefficients (H) of phenytoin inhibition at -40mV in the presence and absence of CaM were 1.0 ± 0.5 and 1.5 ± 1.0, respectively. At +40mV these values were H= 1.1 ± 0.6 and 1.8 ± 0. 8 in the presence and absence of CaM, respectively. These values are consistent with a single phenytoin-binding site for inhibition.

Our data provide the first indication that phenytoin could effectively inhibit RyR2 mediated release of Ca^{2+} in a manner paralleling that of dantrolene. This is an exciting finding as phenytoin has long been used as a therapeutic agent for epilepsy and hence, unlike chronic use of dantrolene, is human safe. Moreover, we showed that the IC_{50} of phenytoin in RyR2 is 10-20 μmol/l, which is ˜ 1/3 its IC_{50} for the Na^+ channels. Thus, for RyR2 inhibitory purposes, it is likely that it can be applied at lower concentrations than used therapeutically to inhibit Na^+ channels.

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