Acute application of n-3 polyunsaturated fatty acids modify calcium sparks in permeabilised rat cardiac myocytes

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Animal studies have demonstrated that acute administration of n-3 polyunsaturated fatty acids (PUFAs) prevent ischemia-induced arrhythmias (Billman, *et al.*, 1997). During and following ischemia, the sarcoplasmic reticulum (SR) becomes overloaded with Ca^{2+} and spontaneous release events occur (Daniels *et al.*, 1991). The subsequent rise in cytosolic [Ca^{2+}] activates sarcolemmal current which can in turn produce after-depolarisations and arrhythmias. PUFAs can reduce the ionic currents responsible for the cardiac action potential and this is believed to be the mechanism for their cardio-protective effects (Xiao *et al.*, 1997).

Studies on the effects of PUFAs on Ca^{2+} handling have shown that 10 μmol/l of eicosapentaenoic acid (EPA) resulted in a 15% reduction in the amplitude of spontaneous Ca^{2+} waves (Negretti *et al.*, 2000). Also 15 μmol/l EPA was found to reduce both the width and duration of Ca^{2+} sparks by ~25% (Honen *et al.*, 2003). When PUFAs were applied directly to the SR Ca^{2+} release channel (Ryanodine receptor, RyR) in artificial bilayers, 30-50 μmol/l caused a 50-80% decrease channel activity. It is not clear if the action of PUFA's on cell Ca^{2+} handing is mediated primarily by the sarcolemma or SR.

This study aimed to determine if PUFAs could directly affect the Ca^{2+} release properties of the intact SR. This was done by measuring the properties of Ca^{2+} sparks in permeabilised cardiac myocytes in which sarcolemmal ion currents did not contribute to Ca^{2+} release within the cell.

Sprague-Dawley rats were anesthetized with sodium pentobarbitone (1ml/kg), the hearts were removed and the cardiac ventricular myocytes were isolated by enzymatic digestion. Following isolation, the myocytes were treated with saponin to permeablise the sarcolemma. Ca^{2+} sparks were viewed using confocal microscopy in line scan mode using the Ca^{2+} indicator fluo-3. Fatty acids tested were oleic acid (OA), arachidonic acid (AA), EPA and docosahexaenoic acid (DHA). Images of Ca^{2+} sparks were collected prior to the addition of fatty acids and at 2 min and 5 min following their addition. Sham experiments were performed to ensure SR Ca^{2+} rundown did not occur.

Spark properties did not vary during experiments in both sham and OA (mono unsaturated fatty acid) treated cells indicating that rundown did not occur. However, PUFA's did affect some spark properties. AA at 50 μmol/l, significantly reduced (10%) spark width within 2 min of exposure. EPA at 50 μmol/l significantly reduced spark intensity (21%) within 5 min. Exposure to 50 μmol/l DHA for 2 min reduced intensity by ~25% and spark mean rate of rise by ~20%. Following exposure for 5 min, spark frequency reduced by ~30% and spark width reduced by ~7%. Even at 30 μmol/l DHA was observed to significantly alter spark properties within 2 min.

The actions of fatty acids on Ca^{2+} sparks in this study were similar to those seen on the open probability of RyRs (Honen *et al.*, 2003). During ischemia, fatty acids are released within the cell by PLA_2. Fatty acid concentrations (all species) up to 0.73 mmol/l have been measured in rat aortic plasma during transient ischemia (Chen *et al.*, 2001). Previously we have shown that 10-20% of membrane fatty acids can release n-3 PUFAs and so it is quite possible for free PUFA levels to reach 70 μmol/l. Therefore under physiological ischemic conditions it is likely that PUFAs could play an important role in protecting myocardium from ischemia by modulating Ca^{2+} handling.

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