n-3 PUFAs and Reperfusion Injury in Isolated Cardiomyocytes

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Abstract

Previous laboratory-based studies with experimental animals have indicated a protective effect of dietary fish oil (enriched with n-3 polyunsaturated fatty acids (PUFAs)) on reperfusion injury, which is believed to be induced by reactive oxygen species (ROS) and cellular Ca^{2+} overload. The broad aims of this thesis were to develop a cellular model for studying reperfusion injury, in order to investigate the reported protective effects of n-3 PUFAs, and to examine the underlying mechanisms associated with such protection. Cardiomyocytes were isolated from adult rat hearts and plated onto laminin-coated glass coverslips. Cells contracting under electrical field stimulation were induced to develop arrhythmic i.e. asynchronous contractile activity by the addition of ROS. Neither acute addition or overnight incubation of n-3 PUFAs (as the free fatty acid) conferred protection from ROS-induced asynchronous contractile activity in these cells. However, in cells isolated from rats supplemented with dietary fish oil (FO) for 3 weeks, a protective effect was observed in comparison to a saturated fat fed group. Dietary FO supplementation resulted in a significant increase in the proportion of n-3 PUFAs incorporated into myocardial membrane phospholipids. Basal membrane fluidity of cardiomyocyte membranes was not affected by diet. Similarly, under un-challenged conditions, diastolic and systolic $[Ca^{2+}]_i$ were not significantly different between cells isolated from rats fed FO versus saturated fat. However, following ROS addition, both diastolic and systolic $[Ca^{2+}]_i$ were significantly lowered in the FO group and the rate of rise in intracellular Ca^{2+} during contractions was lowered. There was no protective effect of dietary fish oil on reoxygenation injury in cardiomyocytes or reperfusion injury in isolated hearts. The mRNA levels of manganese-containing superoxide dismutase were significantly increased in the myocardium of rats fed FO compared with saturated fat and there was also a trend towards up-regulation of glutathione peroxidase and catalase. These results demonstrate the pleiotropic nature of the actions of fish oil fatty acids and indicate that for protection from reperfusion injury, incorporation of the fatty acids into the membrane phospholipids is required and this is associated with the enhancement of the antioxidant system and attenuation of the rise in intracellular Ca^{2+} during ROS exposure.