The effect of polyunsaturated fatty acids on cardiac ryanodine and inositol triphosphate receptors

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It is well recognised that the consumption of fish correlates with a reduction in mortality due to cardiovascular disease (Burr *et al.*, 1989). Whole heart studies have identified that dietary fish oil confers protection from cardiac arrhythmias (McLennan, 1993). Many studies have shown that the acute application of the polyunsaturated fatty acids present in fish oil, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) to cardiac myocytes significantly reduce the amplitude of the various sarcolemmal ion currents responsible for the cardiac action potential (Xiao *et al.*, 1995; Xiao *et al.*, 1997; Bogdanov *et al.*, 1998). It is believed that this reduction in electrical excitability is the mechanism by which fish oil confers protection from cardiac arrhythmias. However some arrhythmias also arise from abnormal calcium handling by internal stores. Thus it has been suggested that the anti-arrhythmic effects of long chain polyunsaturated fatty acids (PUFAs) may be related to their ability to alter calcium handling in cardiac myocytes (Honen & Saint 2002, O'Neill *et al.*, 2002). Therefore we investigated the effects of EPA and DHA on the kinetics of the cardiac calcium release channels (*i.e.* the ryanodine receptor (RyR) and the inositol triphosphate receptor (IP_3R)).

RyRs and IP_3R isolated from sheep hearts and were incorporated into artificial bilayers formed from a solution of phosphatidylethanolamine and phosphatidylcholine dissolved in either n-decane or n-tetradecane using standard techniques (O'Neill *et al.*, 2003). Cytoplasmic solutions contained 250 mM Cs^+ (230 mM $CsCH_3O_3S$, 20 mM CsCl), 10 mM TES at pH 7.4. Luminal solution contained 50 mM Cs^+ (30 mM $CsCH_3O_3S$, 20 mM CsCl), 10 mM TES and 1 mM $CaCl_2$, pH 7.4.

Concentrations of EPA ranging between 10 and 50 μM, when applied to either the cytosolic or luminal side of the RyR, produced a dose dependent inhibition of RyR open probability with K_I =32 μM and Hill coefficient, n_I = 3.8. This inhibition typically occurred within 30 seconds of application. Inhibition was independent of the n-alkane solvent and whether RyRs were activated by ATP or Ca^{2+}. Like EPA, the cytosolic application of 50 μM DHA also resulted in a reduction in channel open probability.

Like with RyR, the open probability of the IP_3R fell upon the application of 50 μM EPA. IP_3Rs were identified by their activation by IP_3 and inhibition by 10 μM heparin, a reversible IP_3R blocker.

The results suggest that both EPA and DHA affect calcium handling by directly inhibiting RyRs at micromolar concentrations**.** The actions of both EPA and DHA may be mediated via the membrane or by binding to a hydrophobic site on the channel itself. This provides a potential avenue by which PUFAs confer protection from cardiac arrhythmias.

Bers, D.M., Bridge, J.H. & Spitzer, K.W. (1989) *Journal of Physiology,* 417: 537-553.

Bogdanov, K.Y., Spurgeon, H.A., Vinogradova, T.M. & Lakatta, E.G. (1998) *American Journal of Physiology,* 274:H571-579.

Honen, B.N. & Saint, D.A. (2002) *Journal of Nutritional Biochemistry,* 13:322-329.

McLennan, P.L. (1993) *American Journal of Clinical Nutrition,* 57:207-212.

O'Neill, S.C., Perez, M.R., Hammond, K.E., Sheader, E.A. & Negretti, N. (2002) *Journal of Physiology,* 538(1): 179-184.

O'Neill E.R., Sakowska, M.M., Laver D.R. (2003) *Biophysical Journal,* 84: 1-16.

Xiao, Y.F., Kang, J.X., Morgan, J.P. & Leaf, A. (1995) *Proceedings of the National Academy of Sciences of the United States of America,* 92(24):11000-11004.

Xiao, Y.F., Gomez, A.M., Morgan, J.P., Lederer, W.J. & Leaf, A. (1997) *Proceedings of the National Academy of Sciences of the United States of America,* 94(8):4182-4187.