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COMMENTARY

Role of redox state in modulation of ion channel function by fatty acids and phospholipids

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There is a growing body of evidence that long-chain ω-3 polyunsaturated fatty acids (ω-3PUFAs), particularly docosahexaenoic acid (DHA), an essential fatty acid derived from fish and fish oils, possess an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer. DHA protection against atherosclerotic heart disease and sudden cardiac death has been reported from experimental studies, clinical trial and epidemiological analyses (Mori & Beilin, 2001). Several mechanisms may explain the cardioprotective effect of DHA: antiarrhythmic, hypolipidemic, and antithrombotic roles. Considering that at least half of the deaths from coronary artery disease are sudden cardiac deaths with fatal arrhythmia because of ventricular fibrillation, the major mechanism for the reduction of sudden cardiac death with treatment of DHA is likely because of its antiarrhythmic action. Indeed, cellular electrophysiologic studies have demonstrated that DHA slows the heart rate, shortens the action potential duration (APD), and prolongs relative refractory period (Billman *et al*., 1997), which stabilizes the electrical activity of cardiac myocytes.

The regulation of cardiac electrical activity by DHA has been ascribed to its ability to modulate sarcolemmal ion channels. DHA inhibits a variety of ion currents/channels, including Na^+ current (I_{Na}) and the cloned human cardiac Na^+ channel (Xiao *et al*., 1995), the L-type Ca^{2+} current (I_{CaL}) (Xiao *et al*., 1997), the Ca^{2+}-independent 4-aminopyridine-sensitive transient outward K^+ current (I_{to}) (Pound *et al*., 2001), the rat ventricular delayed rectifier K^+ current (Pound *et al*., 2001), the cloned voltage-gated K^+ channels Kv1.5 (Honoré *et al*., 1994) and Kv4.3 (Singleton *et al*., 1999). A recent study demonstrated that DHA augments the cardiac slowly activating delayed rectifier K^+ current (I_{Ks}) generated by coexpression of KvLQT1 and minK (Doolan *et al*., 2002). The modulation of these ion currents/channels is indeed in accordance with APD shortening and relative refractory period lengthening induced by DHA. However, the study reported by Jude *et al*. in this issue of *British Journal of Pharmacology* challenges the belief of direct modulation of ion channels being the mechanism for DHA's antiarrhythmic efficacy. Their data convincingly show that the peroxidation products of DHA, rather than DHA itself, block I_{to} and activate a delayed rectifier outward current (I_{ss}). The potential implication of these data is that the antiarrhythmic efficacy of DHA may not be owing to its ion channel regulating effects because, *in vivo*, peroxidation of DHA is unlikely to occur because of high levels of antioxidant in the circulating system.

One interesting question raised from the work reported by Jude *et al*. is whether the modulation of other ion currents/channels (e.g. I_{Na}, I_{CaL}, I_{Ks}, Kv1.5, Kv4.3, etc.) besides I_{to} and I_{ss}, by DHA, is also mediated by its peroxidation products? As mentioned above, DHA affects many ion currents/channels in a nonspecific manner and it is likely the summation of modulations of multiple ion currents/channels that confers the antiarrhythmic efficacy of DHA. Yet, it is unknown which form of DHA, unperoxidized molecules or peroxidized products, acts on these currents/channels. Before we could clarify that other ion currents/channels in addition to I_{to} and I_{ss} are also affected only by peroxidized DHA, it is too early to abandon the belief that the antiarrhythmic efficacy of DHA is a direct consequence of ion channel modulation. Nonetheless, the data presented by Jude *et al*. indeed provide a valid explanation for the previous observation that blockade of I_{Na} by DHA occurs only when the free acid form of DHA with a free carboxyl group is used, but not when an ethyl ester form which is much less sensitive to peroxidation than the free acid form is used (Xiao *et al*., 1995).

One important issue that needs to be clarified is what roles the peroxidation products of DHA play in arrhythmias. The suggestion made by Jude *et al*. that the antiarrhythmic effects of DHA are accounted for by the unperoxidized form of DHA because in *in vivo* conditions DHA is unlikely peroxidized, needs some discussion. It is true that under normal physiological situations, cellular antioxidants may be sufficient to prevent peroxidation of DHA, but under pathological conditions when oxidative stress increases and antioxidant capacity decreases, and when lethal arrhythmias occur as in the case of myocardial ischemia reperfusion injury and heart failure, lipid peroxidation may well occur. Indeed, it has been found that the myocardial level of DHA is significantly elevated by brief ischemia. In addition, the level of peroxidized polyunsaturated fatty acids in myocardial phospholipid was significantly increased (by two fold) (Starkopf *et al*., 1998). The increased peroxidation of DHA now can, according to Jude *et al*., block I_{to} and I_{ss} (and perhaps also other ion currents/channels) and produce antiarrhythmic effects. The finding that the combination of fish oil with vitamin E did not increase the benefit to the prevention of sudden cardiac death compared with fish oil alone (GISSI-prevenzione Investigators, 1999) support this

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notion. This implies that the peroxidation products of DHA more likely accounts for the antiarrhythmic efficacy of DHA. This is further evidenced by the earlier study by Weylandt *et al*. (1996) that DHA, in its storage form in membrane phospholipids, is not antiarrhythmic, and only when it is liberated from its storage in its free form which is more sensitive to oxidative stress, as in ischemia, can DHA prevent the arrhythmias. Moreover, DHA may act as an antioxidant and when being peroxidized prevents oxidation of endogenous molecules such as ion channel proteins so as to produce antiarrhythmic effects.

It is also important to know if other PUFAs (i.e. EPA, linoleic acid, α-linolenic acid, etc.) produce their cellular effects in their unperoxidized or peroxidized forms. These PUFAs are all sensitive to oxidative damage because of their high degrees of unsaturation, like DHA, and some of these PUFAs have also been shown to modulate ion currents/channels and posses antiarrhythmic efficacy against ventricular fibrillation and atrial fibrillation (Xiao *et al*., 1997; 1998; Billman *et al*., 1999; Jahangiri *et al*., 2000; Doolan *et al*., 2002; Lanzmann-Petithory, 2001). Intriguingly, like DHA, EPA inhibits I_{Na} only in its free form, but not in its ethyl ester form, which is less sensitive to oxidation (Xiao *et al*., 1998), indicating the role of peroxidized EPA in I_{Na} modulation.

In addition to fatty acids, other lipids such as phospholipids (and their metabolites) also can modulate cardiac ion channels. In contrast to PUFAs, phospholipid metabolites such as lysophosphatidylcholine (LPC) are proarrhythmic; they rapidly accumulate during ischemia and act as biochemical triggers of ischemic arrhythmias. For example, LPC can enhance human *ether-a-go-go*-related gene (HERG) K^+ channel function and this effect might contribute to extracellular K^+ accumulation and action potential shortening in the early phase of ischemia (Wang *et al*., 2001). In fact, LPC appears to produce dual effects on HERG; it enhances HERG by direct interactions with the channel proteins and depresses HERG *via* generation of reactive oxygen species (ROS), particularly superoxide anion, with the enhancing effect overweighing the depressing effect (unpublished observations). Antioxidants such as vitamin E or superoxide dismutase mimetics prevent HERG depression by LPC. Lysophosphatidylglycerol (LPG) produces similar effects to LPC (unpublished observations). In addition, ceramide, a sphingolipid metabolite overproduced during prolonged ischemia, markedly impairs HERG K^+ channel function *via* the stimulation ROS overproduction because the effect is abrogated by antioxidants (unpublished observations). Ceramide substantially increased production of ROS in treated cells. It appears that the redox potential is an important mediator and/or modulator of the effects of fatty acids and other lipids on cardiac ion currents/channels.

Moreover, redox state *per se* is also an important modulator of ion currents/channels. For example, Ward & Giles (1997) reported that hydrogen peroxide (H_2O_2) augmented I_{Na} in rat ventricular myocytes. H_2O_2 slows the inactivation of rabbit cardiac I_{to} (Wang *et al*., 1999). In human endothelial cells, H_2O_2 inhibits the inward-rectifying K^+ current and increases the Ca^{2+}-dependent K^+ current (Bychkov *et al*., 1999). Redox potentials also affect HERG K^+ channel function; however, the effects depend on the species of ROS. H_2O_2 or $FeSO_4$/ascorbic acid (an oxidative stimulus analogous to H_2O_2), which generates highly reactive hydroxyl group (OH^-) increases HERG current at negative potentials by shifting the HERG activation to more negative voltages (Taglialatela *et al*., 1997; Bérubé *et al*., 2001). In sharp contrast, superoxide anion stimulated by hyperglycemia or generated by xanthine/xanthase system suppresses HERG function (Zhang *et al*., 2003).

The findings documented by Jude *et al*. in this issue of *British Journal of Pharmacology* provide us new insights into the mechanisms of ion channel modulation by DHA. Their work, together with previous studies, addresses the importance of redox potential as a mediator and/or modulator of the effects of PUFAs and of other lipids. Their study also urges us to discriminate the forms (peroxidized or unperoxidized) when studying the effects of fatty acids or other lipids and lipid metabolites, and be cautious when extrapolating results from *in vitro* experiments, in which PUFAs may well be subject to peroxidation, to *in vivo* conditions and clinical settings. This is essential to better understand the mechanisms of PUFAs' effects and to properly benefit from these fatty acids for the secondary prevention of coronary heart disease, as well as other diseases. This has been largely overlooked in the past.

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