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Membrane Effects of the n-3 Fish Oil Fatty Acids, which Prevent Fatal Ventricular Arrhythmias

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Abstract. Fish oil fatty acids are known to exert beneficial effects on the heart and vascular systems. We have studied the membrane effects on ion channel conductance by the n-3 fish oil fatty acids that account for these beneficial effects. We have confirmed that these fatty acids prevent fatal cardiac arrhythmias in a reliable dog model of sudden cardiac death. This finding was followed by experiments indicating that the n-3 fatty acids electrically stabilize heart cells and do so largely through modulation of the fast voltage-dependent Na^+ currents and the L-type Ca^{2+} channels in a manner, which makes the heart cells resistant to arrhythmias. Others and we have demonstrated that these membrane effects on the heart can prevent fatal cardiac arrhythmias in humans.

Introduction

Coronary heart disease (CHD) is the leading cause of death in the United States and in industrialized countries. Many reports have appeared since the epidemiologic evidence of Bang et al. (Bang, Dyerberg & Horne, 1976) called attention to the low mortality from CHD among the Greenland Eskimos, which they attributed to potential antiatherosclerotic effects of the Eskimos' diet high in oil of marine vertebrates (Dyerberg et al., 1978). This hypothesis stimulated many investigators to seek antiatherosclerotic effects of n-3 fatty acids, which are abundant in marine vertebrates' oil. Although it has been shown that the n-3 fish oil fatty acids can cause many biochemical and physiologic effects (Hallaq & Leaf, 1993), which in humans would be expected to reduce atherosclerosis, the evidence from short-term clinical trials that they do so is still minimal.

While many other investigators were seeking the actions of fish oil on heart, actions they thought would prevent atherosclerosis, two Australian investigators, Peter McLennan and John Charnock, were the first to demonstrate that the fish oil fatty acids were antiarrhythmic in animals (McLennan, Abeywardena & Charnock, 1988). Their basic experiment was direct and clear. For 3 or 4 months they fed rats a diet in which they could control the major fat component. At the end of the dietary period, they ligated the coronary arteries of the rats and counted the number of animals that died of ventricular fibrillation (VF). In one report McLennan showed that slightly more than 40% of the rats fed a diet providing 12% of calories as saturated fat died of VF. This mortality rate was not significantly reduced by an olive oil (monounsaturated fat) diet. But the group fed a diet of tuna fish oil (rich in n-3 fish oil fatty acids, PUFA) had no arrhythmic deaths (McLennan, 1993). McLennan's group also reported a similar antiarrhythmic action of the n-3 PUFAs in nonhuman primates (McLennan et al., 1992). Others since then repeated their findings in rats. When we learned of their findings, we decided we should see if we could confirm their surprising results.

But first let us explain what the polyunsaturated fatty acids are, that we will be discussing; please *see* Figure 1. There are two classes of essential polyunsaturated fatty acids, the n-6 (ω6) and n-3 (ω3). Both classes are essential because we cannot make them in our body; they must come in our diet. Also they are essential for normal growth and development and for

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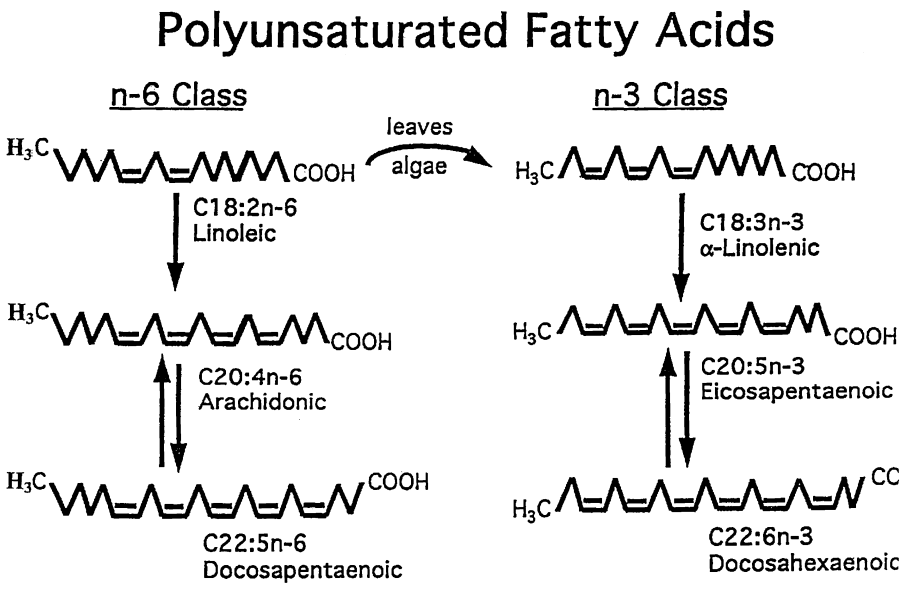

Fig. 1. Polyunsaturatedfatty acids.

optimal function of heart and brain, and probably of other systems. The parent fatty acid of the n-6 class, linoleic acid (C18:2n-6, LA) has 18 carbon atoms in its acyl chain and the first C=C double bond is 6 carbons back from the methyl end of the fatty acid, hence the "n-6" appellation. In the bodies of animals including humans, LA can be elongated and desaturated through a series of enzymatic steps to form arachidonic acid (C20:4n-6, AA). AA is the source of the n-6 eicosanoids resulting from oxygenation of AA by cyclooxygenase, lipoxygenase and epoxygenase enzymes, to form prostaglandins, leukotrienes, lipoxines and P-450 compounds, which in many instances are potent cell messengers. The source of the n-6 fatty acids in our diet is from the plant seed oils commonly used in cooking and in dressings; e.g., corn, soybean, sunflower, canola oils all contain some 70% n-6 fatty acids.

In the chloroplast of green plants, algae and phytoplankton, LA can be further desaturated in the n-3 position to yield α-linolenic acid (C18:3n-3, ALA), the 18-carbon parent fatty acid of the n-3 class. ALA can be further elongated and desaturated by the same enzymes which convert n-6 LA to AA to form the 20-carbon n-3 analog of AA, namely eicosapentaenoic acid (C20:5n-3, EPA). EPA in turn can compete with AA for the same cyclooxgygenase, lipoxygenase and epoxygenase enzymes to form a different class of eicosanoids, which in several important instances can oppose and counteract the action of the n-6 eicosanoids. The final elongation desaturation product of the n-3 class is docosahexaenoic acid (C22:6n-3, DHA), the longest and most unsaturated fatty acid normally encountered in our diets. EPA and DHA are physiologically the most important members of the n-3 class. Their source is largely from marine vertebrates and they are accumulated in the phospholipids in our cell membranes, especially in brain and heart.

The Effect of n-3 Fish Oil Fatty Acids in Preventing Arrhythmias in a Dog Model of Sudden Cardiac Death

To see if we could confirm the surprising findings of McLennan, we turned to a highly reliable dog model of sudden cardiac death (Billman, Hallaq & Leaf, 1994; Billman, Kang & Leaf, 1997, 1999). For this, dogs were prepared surgically by ligating the left descending coronary artery, producing a large anterior wall infarction, and in the same operation a hydraulic cuff was placed around the right circumflex coronary artery so that it could be compressed at will. The dogs were then trained to run on a treadmill during the month allowed for them to recover from the surgery and the myocardial infarction (MI). The result of the exercise stress combined with an additional ischemic stress resulted in some 60% of the dogs developing a fatal VF within 2 minutes of the occlusion of the left circumflex coronary artery. These responsive dogs provided a stable preparation in which to test potential antiarrhythmic agents and are the ones we studied.

Figure 2 illustrates the typical response of one of the susceptible dogs to the exercise-ischemia protocol (Billman et al., 1994). The top control No. 1 tracing is an electroventriculogram. Because the dog was running on the treadmill, its pulse rate was elevated. The additional ischemic stress from occlusion of the left circumflex artery for 2 minutes resulted in a ventricular tachyarrhythmia and the circulation failed. As soon as the dog lost consciousness the dog was defibrillated. The second tracing is of the same dog brought back into the laboratory one week la-

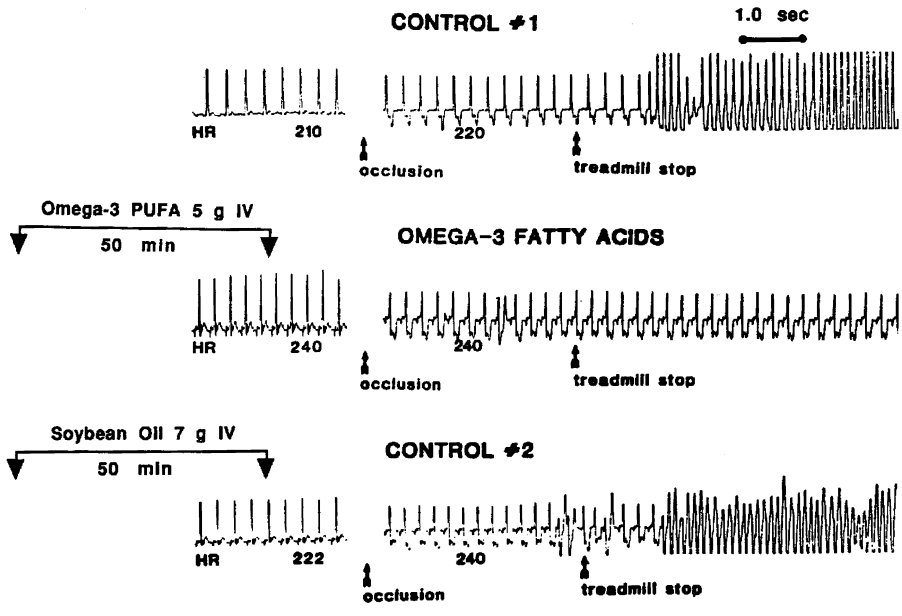

Fig. 2. Prevention of ischemia-induced sudden death in a prepared dog by intravenous Omega-3 PUFA. Representative ventricular electrogram from the same dog with and without intravenous infusion of n-3 fatty acids. *Top panel* (*Control 1*): Exercise-plus-ischemia test done 1 week before an exercise-plus-ischemia test (*middle panel*) immediately preceded by intravenous (i.v.) n-3 fatty acids (Omega-3 PUFA). *Bottom panel* (*Control 2*): Test repeated 1 week after the one-time 50-min infusion of n-3 fish oil fatty acids. This time a lipid emulsion derived from soybean oil (Intralipid®) lacking free n-3 fatty acids was infused. Reproduced, with permission, from Billmann et al., 1994.

Table 1. Prevention of ischemia-induced fatal ventricular arrhythmias by n-3 polyunsaturated fatty acids in a dog model of sudden cardiac death

	Number of dogs tested		
n-3 Polyunsaturated fatty acids	Total	Protected	*P*
Fish oil concentrate[1]	13	10	< 0.005
Eicosapentaeonic acid[2]	7	5	< 0.02
Docosahexaenoic acid[3]	8	6	< 0.004
α-Linolenic acid[4]	8	6	< 0.004

[1] 72% n-3 polyunsaturated fatty acids with 33.9% free eicosapentaenoic acid and 25% docosahexaenoic acid (Pronova Biocarc, Lysaker, Norway) (EPAX 6000FA)
[2] 98.4% free eicosapentaenoic acid, 1.1% free docosahexaenoic acid (Pronova Biocare)
[3] 90.8% free docosahexaenoic acid, 0.9% free eicosapentaenoic acid (Pronova Biocare)
[4] >99% free α-linolenicacid (Nu-Chek-Prep, Elysian, MN)
Reproduced, with permission from Lippincott, Williams & Wilkins, from Billmann, Kang & Leaf, 1999.

ter, when the same protocol was repeated. This time just before the left coronary artery was occluded a phospholipid emulsion containing free n-3 fatty acids was infused intravenously. Additional ischemic stress from occluding the left circumflex coronary artery failed to induce VF. One week later, control No. 2 was performed on the same dog, and an emulsion of soybean oil, which lacks any free n-3 fatty acids, was infused. Within 2 min of occluding the left circumflex artery, VF occurred. This is the protocol we used to test the antiarrhythmic effect of the n-3 fish oil fatty acids, with a control one week before and one week after the test with the intravenous infusion of the fish oil free fatty acids. Table 1 (Billman et al., 1999) summarizes our experiments on the dogs.

The prevention of fatal arrhythmias by the emulsion of fish oil concentrate ($P < 0.005$), confirms the studies of McLennan et al. (McLennan et al., 1988, 1992; McLennan, 1993). These authors used feeding experiments, which were criticized because of possible confounding factors occurring in long-term feeding studies in animals. We infused the fatty acids just before an ischemic stress in our prepared dogs. We believed that if the n-3 fatty acid infusion were promptly associated with an effect in the protocol we used, we could then feel confident the effect resulted from what had just been infused.

Because fish oil contains many ingredients, we wanted to know which ingredients were effective. When we tested pure EPA and DHA, each alone, carried on albumin, was highly protective (Billman et al., 1999). Even the parent fatty acid of the n-3 class of PUFAs, α-linolenic acid (C18:n-3, ALA), which is not present in fish oil, was protective (Billman et al., 1999), but it is largely metabolized for energy and not stored in the phospholipids of cell membranes. We were unable to test a sufficient number of dogs to

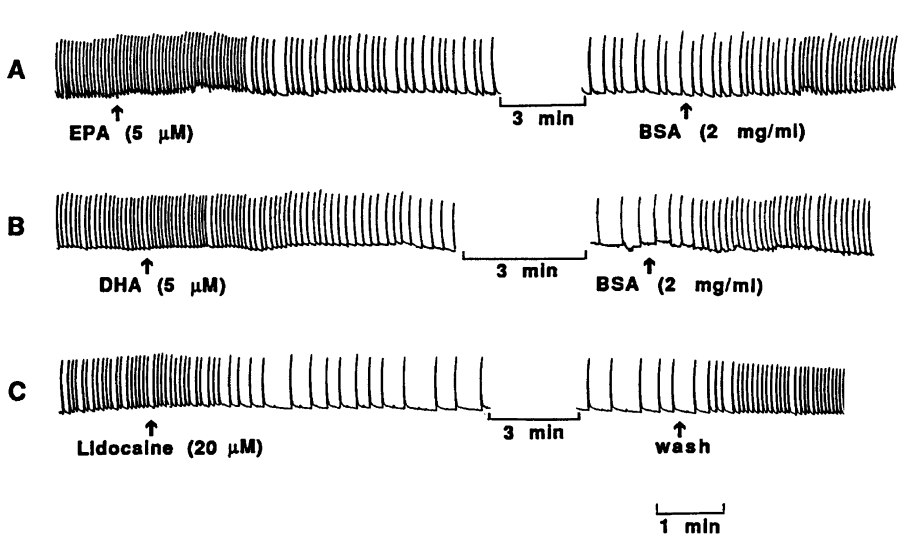

Fig. 3. Effects of EPA and DHA on spontaneous contraction rates of cultured neonatal rat cardiomyocytes. Perfusion of the myocytes with 5 μM EPA (*A*) or DHA (*B*) slowed the beating rate within 2 minutes. Addition of delipidated bovine serum albumin (2 mg/ml) to superfusate extracted the EPA or DHA and returned the contraction rate to control levels. Tracing *C* shows similar effects of lidocaine 20 μM on the spontaneous contraction rate. Reproduced, with permission, from Kang & Leaf, 1994a.

learn whether one fatty acid was more potent than the other.

Effects of n-3 PUFAs on Cultured Neonatal Rat Cardiomyocytes

To learn if there were any plausible biochemical or physiologic effects of these n-3 fatty acids, which could explain their antiarrhythmic action, the effects of the n-3 PUFAs on cultured neonatal cardiomyocytes were studied. One can quickly remove the hearts from several one- to two-day old rat pups and separate the individual myocytes enzymatically. The myocytes are then plated on microscope covers-slips and grown in an appropriate culture medium. By the second day in culture, the heart cells are adherent in monolayer clumps to the cover slip and each clump is beating spontaneously, rhythmically and simultaneously. A cover slip with cells is transferred to a temperature-controlled perfusion chamber on an inverted microscope. With a video camera, a monitor screen, and an edge-monitor one can focus on the contraction of a single myocyte in a clump of myocytes and with a recorder preserve a tracing of the contraction amplitude and rate of contractions (Kang & Leaf, 1994a).

Figure 3 shows the characteristic slowing of the beating rate of the myocytes when low micromolar concentrations of EPA or DHA were added to the medium bathing the isolated heart cells. When delipidated bovine serum albumin was added to the superfusate, the EPA or DHA was extracted from the heart cells and the beating rate returned to the control rates (Kang & Leaf, 1994a). So the slowing effect of the fatty acids on the beating rate is reversible. Toxic agents, known to produce fatal arrhythmias in humans, were added to the medium bathing the cultured cells and the effects of adding the n-3 fatty acids observed. In this way we tested increased extracellular Ca^{2+} (Kang & Leaf, 1994a), the cardiac glycoside ouabain (Kang & Leaf, 1994a), isoproterenol (Kang & Leaf, 1994b), lysophosphatidyl choline and acylcarnitine (Kang & Leaf, 1996), thromboxane (Li, Kang & Leaf, 1997), and even the Ca^{2+} ionophore A23187 (Kang & Leaf, 1996). All of these agents induced tachyarrhythmias in the isolated myoctes.

Figure 4 shows the effects of elevated perfusate Ca^{2+} and ouabain on the myoctes (Kang & Leaf, 1994). Both agents induced rapid contractions, contractures and fibrillation of the myocytes. When EPA was added to the superfusate, the beating rate slowed and when high Ca^{2+} or ouabain was added in the presence of EPA, no arrhythmia was induced. Furthermore, as shown in Fig. 4*C*, after a violent fibrillation was induced in the cells by both elevated calcium and ouabain, addition of EPA stopped the arrhythmias and the cells resumed their fairly regular contractions. Then, addition of the delipidated bovine serum albumin to remove the free fatty acid from the myocytes resulted in the recurrence of the arrhythmia.

This taught us two important facts. First, that the EPA could be extracted from the cells in the continued presence of the toxins and the arrhythmia returned, indicated that the fatty acids were acting without strong ionic or covalent binding to any constituent in the cell membrane. If they had, we would not have been able to extract the EPA from the cells with the albumin. It therefore seems the free fatty acids act directly on the heart cells and need only partition (dissolve) into the hospitable hydrophobic interior of phospholipids of the plasma membranes of myocytes to elicit their antiarrhythmic actions. Second, when we tested the ethyl ester of the EPA, it had no prompt antiarrhythmic action; only the free fatty acid with its negative carboxyl charge is antiarrhythmic (Kang & Leaf, 1994a).

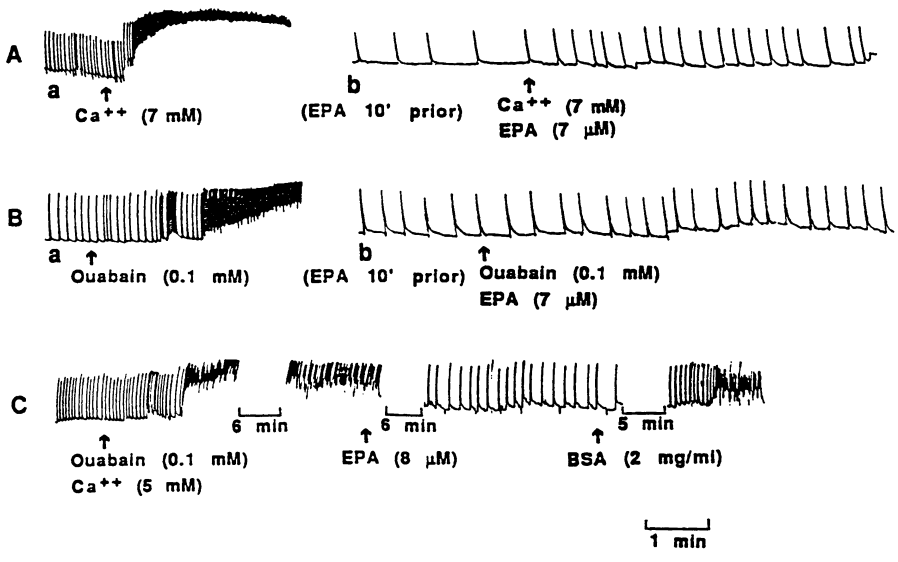

Fig. 4. Prevention and termination of arrhythmias by EPA. Perfusion of myocytes with medium containing 7 mM Ca^{2+} (*A,a*) or 0.1 mM ouabain (*B,a*) induced contracture and fibrillation of myocytes. Washing cells with medium containing 1.2 mM Ca^{2+} returned fibrillations to control beating rate (*not shown*). Myocytes were then perfused with 7 μM EPA. When beating rate had slowed, addition of 7 mM Ca^{2+} (*A,b*) or 0.1 mM oubain (*B,b*) failed to induce arrhyhthmias. *C*. Fibrillation was induced by ouabain (0.1 mM) plus Ca^{2+} (5 mM) in perfusion medium. Addition of EPA (8 μM) terminated fibrillation. Subsequent addition of delipidated BSA (2 mg/ml) still in the presence of ouabain and high Ca^{2+} concentrations, extracted free EPA and the fibrillation resumed. Reproduced, with permission, from Kang & Leaf, 1994a.

At this point we had found that the arrhythmias induced in the isolated neonatal rat cardiomyocytes could in every instance be prevented by the prior addition of the EPA or DHA to the superfusate bathing the cells. Adding the EPA or DHA after an arrhythmia was induced, would stop the arrhythmia. It was apparent that the n-3 PUFA were affecting the excitability/automaticity of the cardiomyocytes, so the effects of the n-3 PUFAs on the electrophysiololgy of the myocytes were examined (Kang, Xiao & Leaf, 1995).

Heart, brain and muscle are excitable tissues and their function is to generate electrical currents to signal their actions in the body. This they do by activating and then inactivating ion channels in their plasma membranes to allow specific ions to move through their plasma membranes, thus creating ionic currents. In heart cells these ionic currents create action potentials by the sequential opening and closing of fast voltage-dependent sodium channels in the cardiomyocytes — *see* Fig. 5. The fast movement of positive Na^+ ions into the myocyte depolarizes the resting membrane potential and initiates an action potential. This is followed by outwardly directed potassium currents: I_{to}, the initial outward current and I_k, the delayed rectifier current, which move positive K^+ ions out of the cells, repolarizing the myocytes back to their resting membrane potential. An inward calcium current, $I_{Ca,L}$ temporarily delays the repolarization of the membrane potential by bringing positive Ca^{2+} into the myocytes, producing the plateau of the action potential. The orderly,

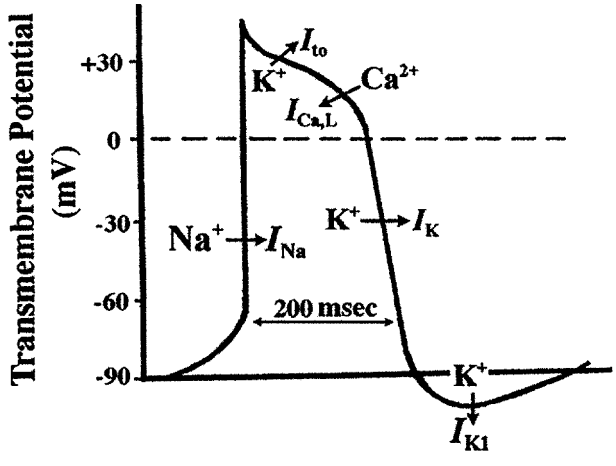

Fig. 5. Schematic depiction of normal cardiac action potential, as described in the text.

sequential occurrence of these currents creates the action potentials, which couple the electrical and mechanical functions of the heart, resulting in its rhythmic contractions. Fatal arrhythmias occur when the electrical signals become chaotic and the heart can no longer function as a pump.

The n-3 fatty acids modulate the ionic currents in the plasma membrane of heart cells (Xiao et al., 1995) and the human myocardial sodium channel expressed in HEK293 cells (Xiao et al., 1998, 2000). There is an effect of the n-3 PUFA on the sodium current, which contributes significantly to their antiarrhythmic action. These fatty acids shift the steady-state

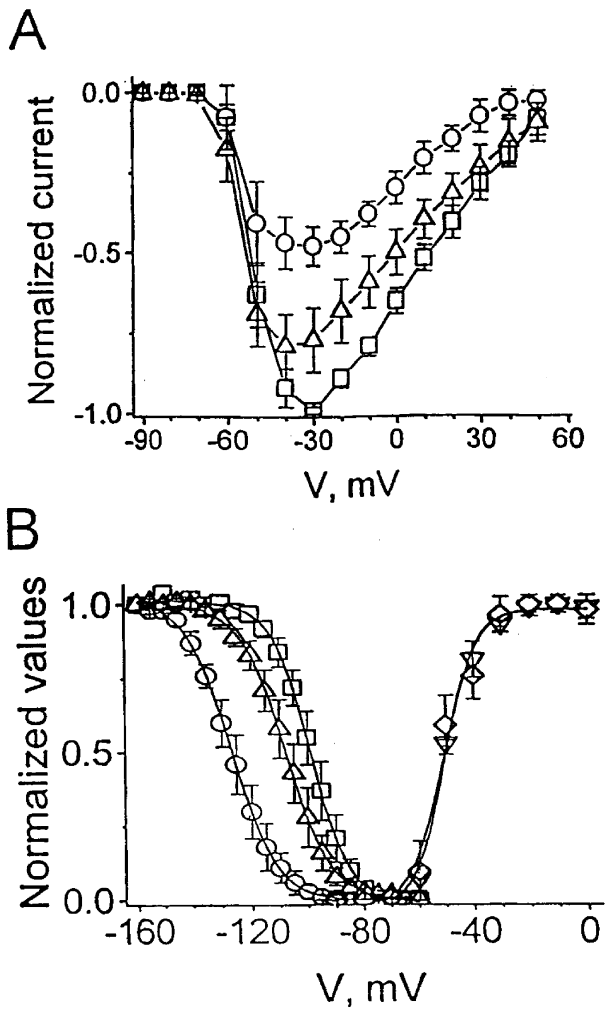

Fig. 6. Effects of EPA on activation and inactivation of the human myocardial Na^+ channel (α-plus β1-subunits) transiently expressed in HEK293t cells. (*A*) Normalized current-voltage relationship in absence (*circles*) and presence (*squares*) of 5 μM EPA ($n = 8$), and after washout of EPA with BSA. (*B*). Relative whole-cell activation conductance in absence and presence of 5 μM EPA. Normalized activation curves are superimposable, which indicates that EPA has no effect upon activation of Na^+ currents. Reproduced, with permission, from Xiao et al., 1998.

inactivation to hyperpolarized potentials (Xiao et al., 1995, 1998, 2000), *see* Fig. 6. When an ion channel opens, it is considered to be in its activated state. The subsequent closing of the ion channel occurs during its inactivated state. After an action potential, repolarization of the normal myocyte resting potential occurs promptly, but before most sodium channels have recovered to their closed state, from which they can respond again with an action potential to another depolarizing stimulus. They are still relatively refractory. But that refractory period can be markedly prolonged by the presence of the n-3 fatty acids (Xiao et al., 1998, 2000), which shift the steady-state inactivation to hyperpolarized potentials. This simply means that in the presence of the n-3 PUFA, a considerably longer time or a more negative membrane potential is required to return the sodium channels to their resting, closed but activatable state.

Our Current Hypothesis on how these Membrane Ionic Effects on Heart Cells Prevent Fatal Arrhythmias

Our current hypothesis regarding the mechanism of action of the n-3 polyunsaturated fatty acids in preventing fatal arrhythmias is based on their actions to inhibit the fast, voltage-dependent sodium current (Xiao et al., 1995, 1998, 2000) and the L-type calcium currents (Xiao et al., 1997). With a myocardial infarction there occurs a gradient of depolarization of cardiomyocytes (Pinto & Boyden, 1999). In the central core of the ischemic zone cells rapidly depolarize and die. The depolarization results from deficiency of ATP in the ischemic cells, causing a dysfunctional Na,K-ATPase and the rise of interstitial K^+ concentration in the ischemic zone. However, at the periphery of the ischemic zone in juxtaposition to the remaining normally perfused myocardium, myoctes may be only partially depolarized. They become hyperexcitable because their resting membrane potential has become more positive (Pinto & Boyden, 1999), approaching the threshold for generating action potentials (activating fast Na^+ channels). Thus, any further small depolarizing stimulus (e.g., current of injury) may elicit an action potential, which, if it occurs at a vulnerable moment during the cardiac electrical cycle, may initiate an arrhythmia. With non-homogeneous rates of conduction of the action potential in the ischemic tissue fatal reentry arrhythmias are likely. In the presence of the n-3 PUFAs, however, a voltage-dependent shift of the steady-state inactivation curve to more hyperpolarized potentials occurs (Xiao et al., 1995, 1998, 2000). The consequence of this hyperpolarizing shift is that sodium channel availability is decreased, and the potential necessary to return these Na^+ channels in partially depolarized myocytes to a closed but activatable state is physiologically unobtainable. Also, these partially depolarized cells have Na^+ channels, which in milliseconds can slip into "resting inactivation" in response to sub-threshold depolarizations without eliciting an action potential (Lawrence et al., 1991; Goldman, 1995) and do this even faster in the presence of the fish oil fatty acids (Xiao et al., 1998, 2000). The results of these effects of the n-3 PUFAs is that these partially depolarized myocytes are quickly made inexcitable and their potential arrhythmic mischief is aborted. Myocytes with normal membrane potentials in the nonischemic myocardium will not be so drastically affected by the PUFAs and will continue to function normally. This latter point we would like to clarify with the experiment shown in Fig. 7 (Xiao et al., 2000). This figure shows the effect

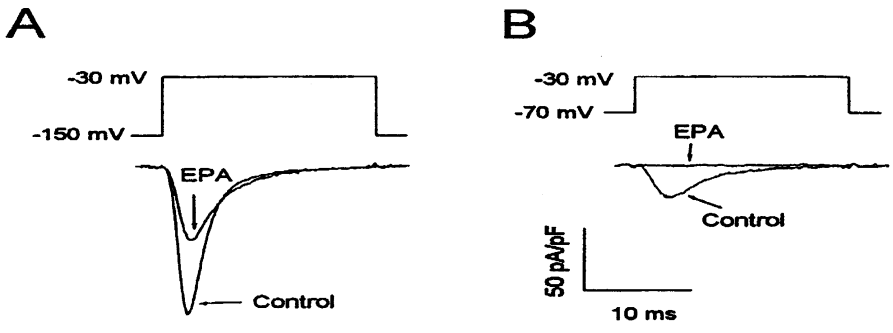

Fig. 7. Effects of EPA on resting and inactivated $hH1_{\alpha\beta}$ Na^+ channels. Current tracings were evoked by voltage steps from −150 mV to −30 mV (*A*) and from −70 to −30 mV (*B*) in the absence and presence of 5 μM EPA. Each value represents 6–15 cells (mean ± SE). Normalized current was calculated as $I_{Na\alpha\beta\ (EPA)}/I_{Na\alpha\beta\ (control)}$ from the same corresponding cell. Reproduced, with permission, from Xiao et al., 1998.

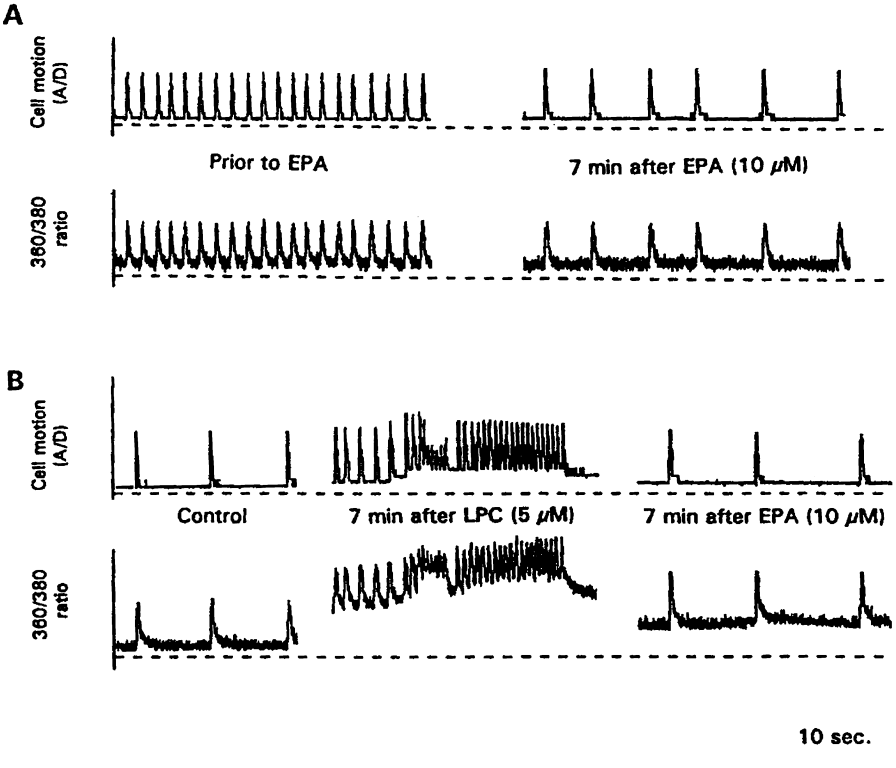

Fig. 8. Simultaneous measurements of $[Ca^{2+}]_i$ (as indicated by the 360/380 A fluorescence ratio of Fura 2) and cell contractions showing the effect of EPA and arrhythmogenic lysophosphatidylcholine (LPC) in cultured neonatal cardiomyocytes. (*A*) A representative recording of the $[Ca^{2+}]_i$ transients (*lower trace*) and cell contractions (*upper trace*) before and after perfusion with EPA (10 μM) in the absence of LPC ($n = 6$). (*B*) In another cell, tracings show that LPC (5 μM) induces an elevation of basal $[Ca^{2+}]_i$ with chaotic transients as cell contracture and tachyarrhythmias occur. Addition of EPA (10 μM) results in return to the spontaneous initial slow beating rate and $[Ca^{2+}]_i$ transients were reduced but not to normal. Reproduced, with permission, from Elsevier, from Kang & Leaf, 1996.

of EPA on the resting myocyte held at a potential of −150 mV (Fig. 7*A*) and an inactivated partially depolarized myocytes held at a potential of −70 mV (Fig. 7*B*) in $hH1_{\alpha\beta}$ (human myocardial Na^+ channels transiently expressing both the α- and β1-subunits in HEK293 cells). It can be seen that from a membrane potential held at −150 mV, even in the presence of 5 μM EPA, there is still a sufficiently robust I_{Na} to induce an action potential, which would propagate through the heart and cause a normal cardiac contraction. By contrast, in the partially depolarized cell with a membrane potential held at −70 mV, even the control current was much reduced. This current, however, would likely induce an aberrant action potential and, given the nonhomogeneous conduction rates of action potentials in the ischemic myocardium, cause a fatal, reentrant arrhythmia. But in the presence of the same 5 μM concentration of EPA, any I_{Na} would be eliminated. This is what we mean in saying that partially depolarized myocytes would be eliminated from any proarrhythmic effects in the presence of n-3 PUFAs.

The L-Type Ca^{2+} Current, $I_{Ca,L}$

Not all fatal cardiac arrhythmias are caused by dysfunction of the Na^+ channel. Many serious arrhythmias can be triggered by excessive cytosolic free Ca^{2+} fluctuations. In clinical practice these may be seen in patients with extensive bone metastases, hyperparathyroidism, immobilization of extremities (which have in common hypercalcemia) and cardiac glycoside toxicity. The effects of the n-3 PUFAs on arrhythmias induced by some cardiac toxins shown in Fig. 3 are examples of arrhythmias induced by

excessive cytosolic Ca^{2+} fluctuations. Figure 8 is another example in which the cytosolic free Ca^{2+} fluctuations were recorded simultaneously with the contractile activity of the neonatal cardiomyocytes (Kang & Leaf, 1996). In this experiment lysophosphatidylcholine (LPC), an amphiphile, was the toxic agent. It has been incriminated as one of the endogenous chemical mediators of ventricular arrhythmias in ischemic myocardium, which accumulates very early in the ischemic heart (for reviews, *see* refs. Corr, Gross & Sobel, 1984; Corr et al., 1987). In Fig. 8*A* (Kang & Leaf, 1996) are shown the simultaneous tracings of myocyte contraction (top) and cytosolic free Ca^{2+} levels as estimated by 360/380 nm fluorescence intensity ratio of Fura-2 (lower tracing) in a spontaneously contracting control myocyte before and after the addition of EPA (10 μM) to the superfusate. The contraction of the myocyte results from the spike in cytosolic free Ca^{2+} when the concentration of $[Ca^{2+}]_i$ is sufficient to interact with the contractile proteins in the heart cells. The peak of the free Ca^{2+} precedes the contraction spike by some 50 ms. The time-averaged cytosolic free Ca^{2+} levels remain very low, normally circa 100 nM. EPA reduced the beating rate without altering the amplitude of contractions, as reported (Kang & Leaf, 1996). On another myocyte, which had a slow endogenous beating rate, Fig. 8*B* shows the effect of LPC (15 μM) on increasing the cytosolic free Ca^{2+} concentrations and fluctuations and the resulting tachyarrhythmia. The presence of EPA (10 μM) added to the superfusate reduced the cytosolic $[Ca^{2+}]_i$, sufficiently to terminate the tachyarrhythmia, though not to normal concentrations in this experiment. That this beneficial effect of EPA, namely, termination of the arrhythmia, results from the action of PUFA to inhibit $I_{Ca,L}$, is shown in adult rat cardiomyocytes in Fig. 9 (Xiao et al., 1997).

Excessive cytosolic free Ca^{2+} fluctuations, such as those shown in Fig. 8*B* after LPC, can induce delayed after-potentials. If the after-potentials become of sufficient magnitude they may activate Na^+ channels to initiate aberrant action potentials. If these occur at a vulnerable moment in the electrical cycle of the heart, fatal arrhythmias may occur. Extracellular application of EPA and the other antiarrhythmic polyunsaturated fatty acids, but not saturated or monounsaturated fatty acids, produced a prompt and reversible concentration-dependent inhibition of $I_{Ca,L}$ The concentration of EPA to produce 50% inhibition of $I_{Ca,L}$ was 0.8 μM in neonatal rat heart cells and 2.1 μM in adult rat ventricular myocytes (Xiao et al., 1997). While the EPA-induced suppression of $I_{Ca,L}$ did not significantly alter the shape of the current-voltage relation, it did produce a small but significant, negative shift of the steady-state inactivation curve ($\Delta V_{1/2} = -3$ to -5 mV). The suppression of the $I_{Ca,L}$ by the PUFAs was

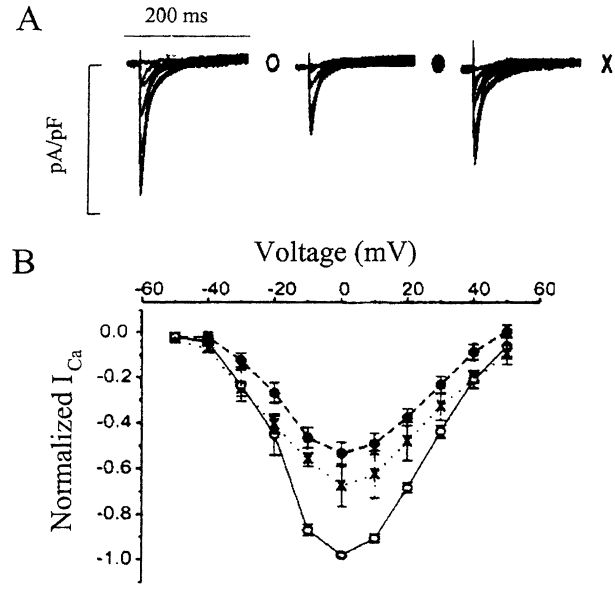

Fig. 9. Suppressant effects of EPA on the voltage-activated L-type Ca^{2+} current in adult rat ventricular myocytes. Superimposed current density traces were elicited by 200 ms pulses from a potential of −50 to 50 mV. From a holding potential of −80 mV, brief depolarizations were applied to maintain a constant intracellular Ca^{2+} load. To elicit a test pulse, a slow ramp from −80 to −60 mV was applied, and the membrane potential was held at −60 mV for 50 ms before the test depolarization was applied. $I_{Ca,L}$ records under control conditions (*empty circles*), 1.5 μM EPA (*filled circles*), and washout (*crosses*) are shown from a typical cell (*A*), while averaged normalized relationships are shown (*B*) for control. ($n = 11$), 1.5 μM EPA ($n = 11$), and washout ($n = 5$). The current density values were normalized to the level observed under control condition for each experiment. Reproduced, with permission, from Xiao et al., 1997.

voltage- and time-dependent, but not use-dependent. This is consistent with the lipophilic nature of these fatty acids (Hille, 1997). Thus the effects of the PUFAs on $I_{Ca,L}$ resemble their effects on I_{Na}, except that the steady-state inactivation potentials for $I_{Ca,L}$ in the rat cardiomyocytes were shifted to the left to a much lesser degree.

It is of interest to compare the actions of the n-3 fatty acids with that of two kinds of available pharmaceutical drugs, both of which inhibit the Na^+ and Ca^{2+} ion channels, such as the Class 1 antiarrhythmic Na^+-channel blockers or the L-type Ca^{2+}-channel blockers. There are several striking differences: a) The n-3 fatty acids have been part of the human diet for hundreds of thousands of years, during the time our genes were being adapted to our environment, including the diet of our hunter-gatherer forebears (Leaf & Weber, 1987), and they are safe. b) By contrast, the available antiarrhythmic pharmaceutical drugs are all potentially toxic (Cardiac Arrhythmia Suppression Trial, 1989). c) The n-3 EPA and DHA each modulate several ion currents, whereas pharmaceutical drugs tend to be specific for

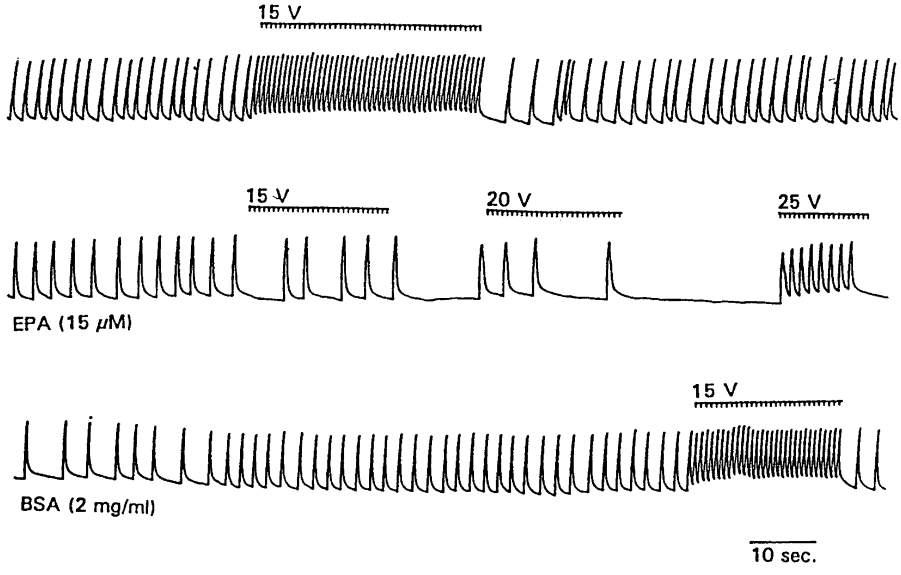

Fig. 10. The response of the cultured neonatal rat cardiomyocytes to electrical stimulii delivered from an external applied voltage source. The three strips are continuous tracings of the contraction rate and amplitude of a single myocyte within a clump of myocytes. In the top tracing an external field of 15 V delivered stimuli that readily doubled the beating rate. The second tracing shows that with EPA (15 μM) added to the superfusate, the beating rate began to slow, but when an external electrical field of 15V was applied, the cells paid no attention to the stimuli nor did they at 20 V. At 25 V they responded, but only to every other stimulus. Upon addition of the delipidated bovine serum albumin (BSA) to the superfusate, the free EPA was extracted from the myocyte and the contractions returned to the control rate. Now the cells doubled their beating rate in response to stimuli delivered at 15 V, just as they had before EPA application. Reproduced with permission, from Elsevier, from Kang & Leaf, 1996.

a single or a couple of cardiac ion channels so that it requires a number of drugs acting together to inhibit the same group of ion channels. d) The drugs act on heart cells to increase the number of ion channels per cardiomyocyte through an effect of the drug on sodium channel gene expression, whereas the n-3 fish oils do not induce such an action, as we have reported, comparing mexiletine and EPA on cultured rat heart cells (Kang, Li & Leaf, 1997). f) It seems appropriate with the drugs to refer to their physiological effects as blocking or inhibitory, since they are capable of eliminating physiologic effects, whereas the n-3 fatty acids modulate action in a more gentle manner, as they reduce but do not seem to eliminate physiologic or biochemical processes in our bodies (Force et al., 1991).

Although at present we think that the inhibitory effects of the PUFAs on I_{Na} and $I_{Ca,L}$ seem the major effects accounting for their antiarrhythmic actions, we are not unmindful that they affect other sarcolemmal ion currents as well. By whole-cell voltage-clamp measurements they have been reported (Honore et al., 1994; Bogdanov et al., 1995; Xiao, Morgan & Leaf, 2002) to also inhibit K^+ currents — the transient outward current, I_{to}, and the delayed rectifier current, I_K, but not the inward rectifying current, I_{K1} (Xiao et al., 2002). However, these effects on the important repolarizing K^+ currents would have the effect to prolong the action potential duration, whereas the PUFAs slightly but significantly, shorten the action potential duration (Kang, Xiao & Leaf, 1995). In addition, the concentrations of EPA required to affect the repolarizing K^+ currents were considerably larger than those required to affect the I_{Na} and the $I_{Ca,L}$, as described above. For these reasons we do not consider the modulating action of the PUFAs on polarizing potassium currents to be contributing to the antiarrhythmic effects of the n-3 fish oil fatty acids.

The Electrical Stabilizing Effect of n-3 Fatty Acids

This electrical stabilizing action of the n-3 fatty acids can be demonstrated by a simple experiment. Figure 10 (Kang & Leaf, 1996) shows a continuous tracing of the contraction of a single myocyte in a clump of myocytes on a microscope cover-slip. Initially the myocyte is contracting regularly. Two platinum electrodes were placed across the cover slip with their tips dipped into the fluid perfusing the heart cells and connected to a voltage source. With 15 V pulses it was possible to double or triple the spontaneous beating rate. When the external voltage source was turned off the cell resumed its control spontaneous beating rate. The middle trace is a continuation of the recording from the same cell. When EPA was added to the superfusate the beating

rate began to slow and now the myocyte paid no attention to stimuli at 15 V or at 20 V from the external source. At 25 V the cell responded but only to every other stimulus. When delipidated bovine serum albumin was added to the medium perfusing the myoytes the EPA was extracted from the myocytes and the beating returned to its control rate. Now the myocyte responded to external stimuli delivered at 15 V just as it had initially (Kang & Leaf, 1996). This action of the n-3 fatty acids occurring directly on every cardiomyocyte on the cover-slip in the absence of hormonal or neural control, indicates the potent electrical stabilizing action of these interesting n-3 fish oil fatty acids.

What Is the Mechanism of this Stabilizing Effect of the n-3 Fish Oil Fatty Acids?

This stabilizing action of the fish oil fatty acids we first encountered when we saw that 10 μM EPA in neonatal rat cardiomyocytes prevented a fixed depolarizing current from eliciting an action potential (Kang, Xiao & Leaf, 1995). A stronger depolarizing potential was needed to elicit the action potential. The stabilizing effect of the n-3 fatty acids results from the modulating effect of the n-3 PUFA on the conductance of membrane ionic currents. As shown in our hypothesis on the cause of fatal arrhythmias during a myocardial infarction, the inactivated state of the Na^+ channels of the partially depolarized cardiac myocytes is shifted by n-3 PUFAs to much more negative potentials for returning the channels to a resting state but one that is responsive again to another depolarizing stimulus. This, then, is the action of the n-3 fish oil fatty acids, which results in electrical stabilization of the cardiac myocytes.

This effect of the n-3 PUFAs on Na^+ channels, together with their inhibitory effect on L-type Ca^{2+} channels (Xiao et al., 1997), which in turn prevents triggered arrhythmic after-potential discharges caused by excessive cytosolic Ca^{2+} fluctuations, we currently think are the major mechanisms for the antiarrhythmic effects of these PUFAs.

Do these Membrane Ionic Conduction Effects of Fish Oil Fatty Acids Prevent Fatal Arrhythmias in Humans?

While we were studying the modulation of ion currents in the membranes of heart cells, others were testing the effects of the n-3 fish oil fatty acids on their ability to prevent arrhythmias in humans. There have been two clinical trials, which have demonstrated that these fish oil fatty acids do, in fact, prevent fatal arrhythmias in humans. The first of these was published in 1989 by Burr et al. (1989) found a 29% reduction in mortality among 1015 men, who had had a recent myocardial infarction, and were randomly assigned to eat at least two portions weekly of oily fish compared with an equal number who were not so advised. Later the mortality was correctly attributed to prevention of fatal arrhythmias by eating fish. More recently the GISSI-Prevenzione trial tested a supplement of a 1.0 g capsule containing 850 mg EPA + DHA daily among 11,324 patients with a recent MI (GISSI-Prevenzione Investigators 1999). The primary endpoint was a combination of death, nonfatal MI and stroke. At 3.5 years, the n-3 fatty acid supplement significantly reduced the primary endpoint compared with the control group. This benefit resulted largely from a 45% reduction in fatal arrhythmias (Marchioli et al., 2002)

Very recently my group of colleagues completed the Fatty Acid Antiarrhythmia Trial (FAAT) (Leaf et al., 2005), another clinical trial involving 402 patients with implanted cardioverter defibrillators (ICDs) because they had experienced a prior episode of cardiac arrest. Our subjects were randomized to either four capsules of fish oil containing EPA + DHS (total of 2.6 g daily) or four capsules of similar appearance but containing olive oil. We had considerable difficulty with non-compliance in our subjects. The percentage of subjects who discontinued their prescribed oil supplements before the end of their 12 months in the trial was 35%. Nevertheless in the 236 subject who completed the duration of our trial there was a relative risk of 0.62 (95% confidence limits of 0.39 to 0.97; $P = 0.037$) and corrected by a multivariate analysis the relative risk was 52 (95% confidence limits of 0.32 to 0.83; $P = 0.0060$). A relative risk of 0.62 means a 32% reduction of fatal arrhythmias occurred in those receiving the fish oil supplement compared with those receiving the placebo olive oil. Similarly, a relative risk of 52 means a 48% reduction in fatal arrhythmias in those receiving the fish oil supplement as compared with those receiving the placebo olive oil.

And that is the take-home message of this clinical trial.

I end my review of the antiarrhythmic actions of the n-3 fish oil fatty acids to indicate to readers that studying membrane biology is not an esoteric activity, but that such research can lead directly to understanding—an understanding that, in the case of the n-3 PUFAs, will provide a potential for preventing sudden cardiac death due to fatal arrhythmias. This is what the studies by others and what our own studies on the membrane effects on ion channel conductance by the n-3 fatty acids have taught us. I say this with urgency and confidence because today there are some 400,000 deaths annually caused by fatal arrhythmias and millions more worldwide, which have the potential to be prevented because we

and others before us have pursued the membrane biology of cardiac arrhythmias.

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