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Topical Review

The Antiarrhythmic and Anticonvulsant Effects of Dietary N-3 Fatty Acids

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Abstract. It has been shown in animals and probably in humans, that n-3 polyunsaturated fatty acids (PUFAs) are antiarrhythmic. We report recent studies on the antiarrhythmic actions of PUFAs. The PUFAs stabilize the electrical activity of isolated cardiac myocytes by modulating sarcolemmal ion channels, so that a stronger electrical stimulus is required to elicit an action potential and the refractory period is markedly prolonged. Inhibition of voltage-dependent sodium currents, which initiate action potentials in excitable tissues, and of the L-type calcium currents, which initiate release of sarcoplasmic calcium stores that increase cytosolic free calcium concentrations and activate the contractile proteins in myocytes, appear at present to be the probable major antiarrhythmic mechanism of the PUFAs.

Key words: Omega-3 — n-3 — Omega-6 — n-6 — Eicosapentaenoic acid — Docosahexaenoic acid — Arrhythmias — Seizures

Introduction

In the early 1960s there was considerable discussion regarding a possible role the free fatty acids released from the ischemic myocardium might play in the subsequent cardiac dysfunction observed following experimental coronary occlusion in animals and in humans after acute myocardial infarctions. Oliver et al. [41] reported high levels of free fatty acids, which attained millimolar concentrations in the venous blood of dogs, after experimental occlusion of a coronary artery. They attributed [42] the cardiac arrhythmias after acute myocardial infarction to the effects of the elevated free fatty acid levels on the functions of the heart. Opie [43] confirmed the elevation of the free fatty acids occurring shortly following the ischemic insult to the myocardium with associated arrhythmias. But later reported failure of infused triglycerides with heparin to cause serious cardiac arrhythmias in anesthetized, open-chest dogs despite levels of free fatty acids attaining millimolar concentrations in the coronary sinus blood [18]. Murnaghan reported [40], that while saturated fatty acids added to the perfusate of isolated rabbit hearts in vitro lowered the arrhythmia threshold, the addition of polyunsaturated α-linolenic acid (*cis*-9,12-octadienoic acid) raised that threshold. Gudbjarnasson [17] suggested that fish oil might prevent fatal cardiac arrhythmias from his observations in humans, but it was the studies of McLennan et al. [38], which showed that feeding rats fish oil for several months prevented ischemia-induced fatal ventricular arrhythmias. They confirmed their essential findings in marmosets [36] and others have repeated their findings in rats [3, 22, 31, 55]. McLennan reported [38] that feeding rats a diet in which the fat content was largely saturated or monounsaturated fats, resulted in a high incidence of irreversible ventricular fibrillation when their coronary arteries were subsequently experimentally ligated. When vegetable oils were the major source of the dietary fat, there was a reduction in arrhythmic mortality by some 70%. With tuna fish oil, however, McLennan

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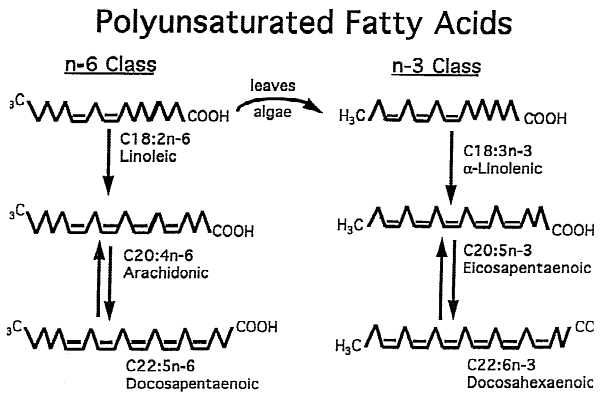

Fig. 1. Schematic representation of the two classes of the major dietary, essential, polyunsaturated fatty acids described in the text.

reported [39] irreversible ventricular arrhythmias to be completely prevented with or without reflow to the ischemic myocardium. These striking observations led us to pursue the possible mechanism(s) for such an antiarrhythmic action of the fish oil.

Figure 1 shows the polyunsaturated fatty acids (PUFAs) we will be discussing. The n-6 (or ω-6) class is present in our diets in large amounts in vegetable oils; the n-3 class comes primarily from fish oils in today's diets. Both are essential fatty acids since they are necessary for optimal health and cannot be formed in our bodies. They must be obtained from our diets. Linoleic acid (C18:2n-6, LA), the parent PUFA of the n-6 class, currently is abundantly present in the US diet. It can be elongated and desaturated in our bodies to form arachidonic acid (C20:4n-6, AA). In the choloroplasts of green leaves, algae and phytoplankton linoleic acid can be further desaturated to yield α-linolenic acid (C18:3n-3, LNA), the parent of the n-3 class of PUFAs. It is largely through ingestion of marine phytoplankton by krill and then fish that the n-3 fatty acids enter the food chain and become abundant in marine foods. A few vegetable oils, notably canola and flax seed oil contain α-linolenic acid which our bodies can also elongate and desaturate further to form eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA); the latter to a limited extent. In this elongation and desaturation the LNA uses and competes with the n-6 PUFAs for the same enzymes. Although the essential status of polyunsaturated fatty acids is generally recognized, the different physiologic and biochemical effects of the two classes are often not appreciated, so they unfortunately get lumped together under the rubric of "polyunsaturated fatty acids" in dietary recommendations. Both classes of PUFAs are needed for health.

Prevention of Ischemia-induced Sudden Cardiac Death by n-3 PUFAs in Dogs

We were interested in what physiologic and biochemical mechanisms could result in such antiarrhythmic effects.

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

n-3 PUFAs	Number of dogs tested		
	Total	Protected	*P*
Fish oil concentrate[a]	13	10	<0.005
EPA[b]	7	5	<0.02
DHA[c]	8	6	<0.004

[a] 72% n-3 PUFA with free EPA, 33.9% and DHA 25% (Pronova Biocare as. Lysaker, Norway; EPAX 6000FA)
[b] 98.4% free EPA; 1.1% free EPA (Pronova-Biocare a.s.)
[c] 90.8% free DHA; 0.9% free EPA (Pronova-Biocare a.s.)
The Table summarizes the effectiveness of free fish oil fatty acids and of pure n-3 fatty acids to prevent ischemia-induced fatal ventricular fibrillation in a dog model of sudden cardiac death [8, 9].

But first we wanted to see if we could confirm the findings of McLennan and Charnock. We studied a canine model of sudden cardiac death [5]. A surgically induced myocardial infarction was produced by ligating the left main coronary artery and an inflatable cuff was placed around the left circumflex artery. The dogs were allowed about a month to recover from the surgery and their myocardial infarction during which they were trained to run on a treadmill. The animals were then screened for susceptibility to fatal ventricular arrhythmias (VF) when their left circumflex artery was occluded while they were running on a treadmill. Some 60% of animals were found susceptible and these were the dogs studied. Once an animal is "susceptible" it remains susceptible on further exercise-ischemia trials [6]. The Table summarizes our results. In 10 of the 13 such dogs intravenous infusion of an emulsion of a concentrate of the fish oil free fatty acids (PUFAs) just prior to the exercise-ischemia test prevented the fatal VF ($P < 0.005$) [7]. In the control exercise-ischemia tests one week prior to the test with the infusion of the (PUFAs) and one week following that test all animals developed ischemia-induced VF requiring prompt defibrillation. In additional studies [8] we have found that pure eicosapentaenoic acid (C20:5n-3, EPA), docosahexaenoic acid (C22:6n-3, DHA), or α-linolenic acid (C18:3n-3, LNA) delivered on serum albumin are antiarrhythmic in this dog preparation. The number of animals tested were too few to determine relative efficacy of the three pure n-3PUFAs. We purposely infused the n-3 fatty acids rather than fed the dogs fish oil to be certain exactly what ingredient of the fish oil prevented the fatal VF. In dietary studies invariably several things must change, which may confound the study. But when the free fatty acids were infused intravenously just prior to producing the ischemia and the fatal VF is prevented, then, we think, we can feel confident the effect results from what has just been infused.

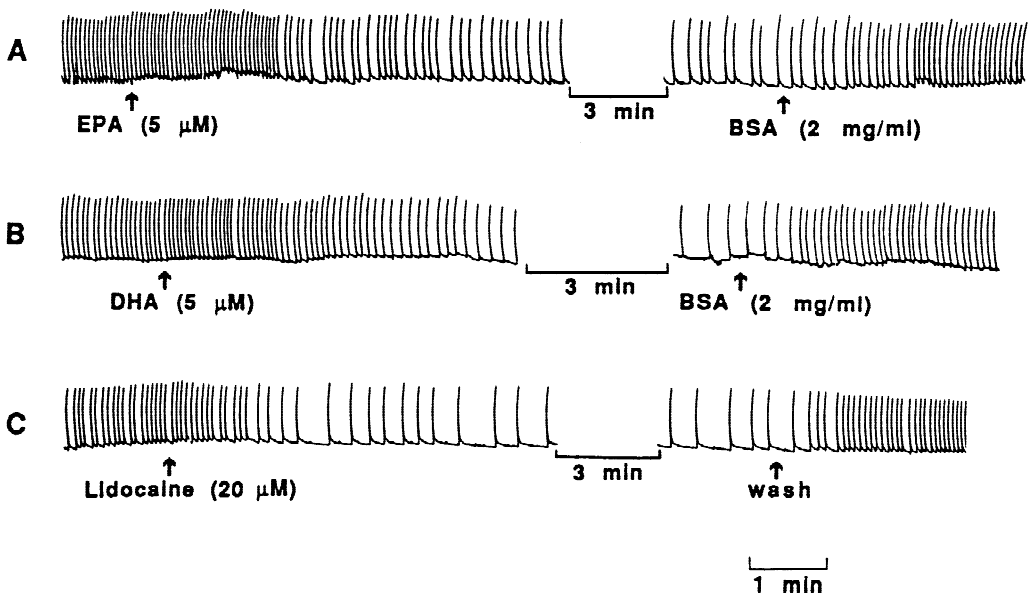

Fig. 2. A simple tracing of the rate and amplitude of contraction of a single cultured neonatal heart cell within a clump of cardiac myocytes adherent to a microscope coverslip in a perfusion chamber as recorded with an inverted microscope, a video camera and an edge monitor [24]. The cells were beating regularly when low micromolar concentrations of EPA or DHA were added to the superfusate. This slowed the beating rate of the myocyte. However, this effect was reversible as shown by the return to the control beating rate when the free fatty acids were extracted from the cell by delipidated bovine serum albumin (2 mg/ml) added to the perfusate. The bottom tracing shows that the antiarrhythmic local anesthetic lidocaine had similar effects in this preparation.

Effects of PUFAs on the Contractility of Cultured Neonatal Rat Cardiomyocytes

Having thus confirmed the findings of the earlier workers, we set about to determine the mechanism by which the n-3 polyunsaturated fatty acids (PUFA) produced their antiarrhythmic effect. To have a simple, available model to study in which we could visualize the production of arrhythmias and possible prevention of the arrhythmias by the PUFA, we studied cultured neonatal rat cardiac myocytes [24]. Hearts are quickly removed from one to two day old decapitated rat pups. The cardiac cells are separated with trypsin and collagenase digestion and the cells are plated on microscope coverslips. By the second day of culture one sees clumps of growing myocytes of a few to several hundred cells adherent to the coverslips. Each group of cells is contracting spontaneously, synchronously, and rhythmically. With a microscope, a video camera, and an edge monitor we can focus on a single myocyte in a clump of cells to see and record the rate and amplitude of contractions, as shown in Fig. 2. With this in vitro model we produced arrhythmias with a number of chemicals known to produce fatal VF in humans: elevated extracellular [Ca^{2+}], toxic levels of the cardiac glycoside ouabain [24], excessive β-adrenergic agonist isoproterenol [25], lysophosphatidyl choline, acyl or palmitoylcarnitine and even the calcium inophore A23187 [27] thromboxane [34]. With each agent a tachyrhythmia was induced. If the PUFAs were added to the fluid perfusing the isolated myocytes before the arrhythmogenic toxins were administered, they would in every instance prevent the expected arrhythmia. If the arrhythmia was first induced by the toxin and the PUFAs added to the superfusate in the continued presence of the toxin within a few minutes the arrhythmia would be terminated and the cells would resume beating regularly. Then in the continued presence of the toxin, the PUFA can be extracted from the cells with delipidated bovine serum albumin and the arrhythmia promptly resumes [24]. Figure 3 shows one such experiment which was particularly informative to us. The results indicated that it was only the free PUFAs partitioning (intercalating) into the membrane phospholipids that prevented the arrhythmias. If the fatty acid had been covalently bound to any constituent in the membrane, we would not have been able to extract it from the membrane. When the ethyl ester or the triglyceride of the PUFAs were tested, they were not antiarrhythmic in this model; the free carboxylic acid group is essential for this prompt antiarrhythmic action.

We then tested which PUFAs were antiarrhythmic [24]. Both the n-3 and n-6 classes of PUFAs are antiarrhythmic, whereas monunsaturated oleic acid and the saturated fatty acids, stearic, palmitic and lauric, were not. But arachidonic acid (C20:n-6, AA) was anomalous. As the free fatty acid in the presence of indomethacin, AA was antiarrhythmic just like the n-3 PUFAs. But cyclooxygenase metabolites of AA (except protacy-

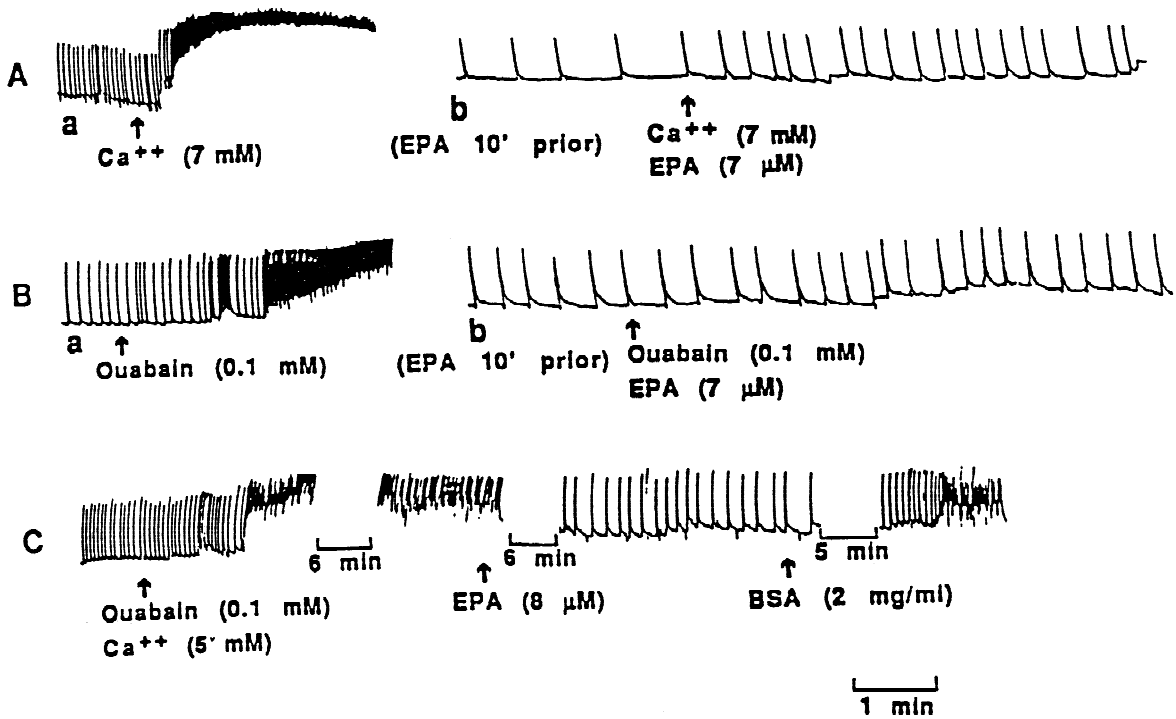

Fig. 3. The effects of n-3 PUFAs on the arrhythmic actions of $[Ca^{2+}]_e$ (5 or 7 mM) and the cardiac glycoside ouabain (0.1 mM) on cultured neonatal rat cardiomyocytes [24]. Both elevated Ca^{2+} (*A*) and ouabain (*B*) caused contracture and fibrillation of the myocytes. But when the EPA was added prior to the calcium or ouabain it slowed the beating rate and prevented the fibrillation. (*C*) When both ouabain and calcium were added to the superfusate they caused a violent arrhythmia, which was terminated by adding EPA to the same superfusate. The cells resumed a fairly regular rhythm, but when the free fatty acid was extracted from the myocytes by delipidated bovine serum albumin, still in the presence of the ouabain and elevated Ca^{2+}, the violent arrhythmia promptly resumed.

clin) cause arrhythmias, whereas cyclooxygenase metabolites of n-3 EPA do not [34]. This would account for the remaining fatal arrhythmias observed in the study by McLennan [39] when he fed his rats a vegetable oil, safflower oil, which is rich in n-6 polyunsaturated fatty acids. For this reason we have recommended that only the n-3 PUFAs should be tested in clinical trials as antiarrhythmic agents, as we have never observed them to induce arrhythmias.

The structural requirements for an antiarrhythmic compound that acts in the manner of these PUFA are a long acyl chain or hydrocarbon with two or more C=C unsaturated bonds and a free carboxyl group at one end. With this guideline we found all-*trans*-retinoic acid also to be specifically antiarrhythmic, whereas retinal and retinol were not [28].

Electrophysiologic Effects of PUFAs

The antiarrhythmic action of the PUFAs results from their effects on the electrophysiology of cardiac myocytes [28]. They cause slight hyperpolarization of the resting or diastolic membrane potential and the threshold voltage for the opening of the Na^+ channel becomes more positive. This results in an increased depolarizing stimulus of about 40 to 50% required to induce an action potential. In addition, the refractory period, phase 4 of the cardiac cycle, is prolonged by some 3-fold. These two effects on every myocyte in the heart would account for the increased electrical stability and resistance of the heart to lethal arrhythmias.

This electrical stabilizing effect of the n-3 PUFAs on every cardiomyocyte can be readily demonstrated in vitro [27]. Figure 4 shows the tracing of the rate and amplitude of contractions of a single cardiomyocyte in a clump of cells growing on a microscope coverslip. The cell was visualized with an inverted microscope. A video camera photographed the contractions that were traced and recorded with an edge monitor. When two platinum electrodes were placed across the microscope coverslip in a perfusion chamber and connected to an external voltage source, the regular beating rate could easily be doubled by stimulating the myocyte by the external field of 15 volts. When the external voltage source was turned off the myocyte regained its prior beating rate. When the same cell was exposed to n-3 EPA (15 μM) added to the superfusate, the beating rate began to slow down—a highly reproducible effect of the PUFAs on the neonatal rat cardiomyocytes—and now the myocyte paid no attention to the stimuli from the

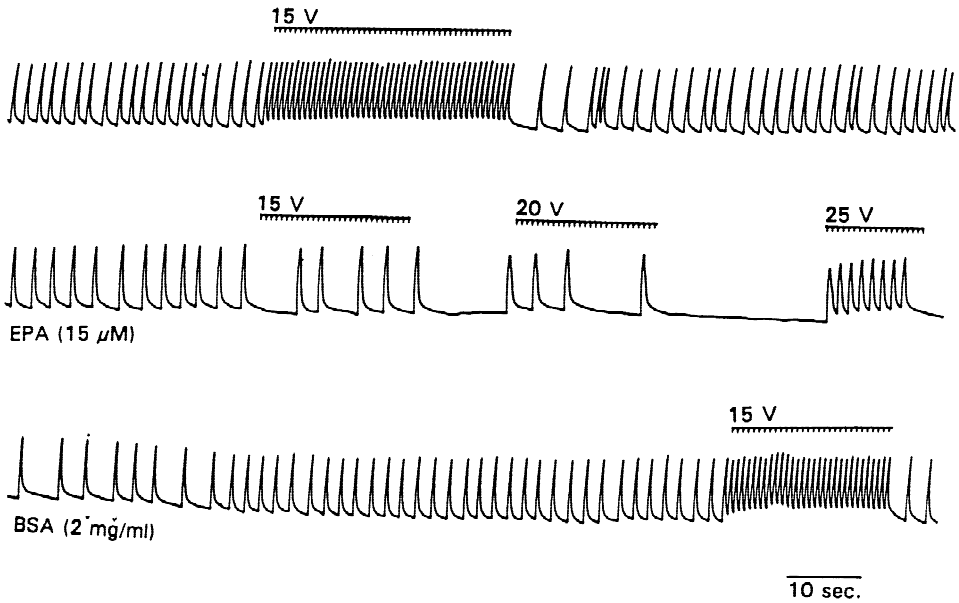

Fig. 4. The effect of EPA on the response of the cultured neonatal rat cardiomyocytes to electrical stimuli delivered from an external applied electrical voltage source [27]. The three strips are continuous tracings of the contraction rate and amplitude of a single myocyte within a clump of myocytes. The spontaneous beating rate and amplitude of contraction is apparent in the top tracing. An external electric field of 15 volts delivered stimuli at a rate that readily doubled the beating rate. The second tracing shows that with EPA (15 μM) added to the superfusate the beating rate slowed, but when an external electrical field of 15 V 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 delipidated bovine serum albumin to the superfusate the free EPA was extracted from the cardiomyocyte, the contractions returned to the control rate, and now the cells doubled their beating rate in response to stimuli delivered at 15 V, just as they had initially.

external voltage source at 15 or at 20 volts. External stimuli delivered at 25 volts succeeded in eliciting myocyte contractions but only in response to every other electrical stimulus. When delipidated bovine serum albumin (2 mg/ml) was added to the superfusate of the same coverslip to extract the free fatty acid from the cardiomyocytes, the beating rate returned to its control frequency and now the myocytes responded to the external electrical stimuli delivered at 15 volts, just as they had initially. When one considers that this electrical stabilization is an effect of the PUFAs directly on every cardiac myocyte, both atrial and ventricular, in the absence of neural or humoral effects, one can sense what a potent antiarrhythmic action these n-3 PUFAs may exert. Furthermore, the antiarrhythmic action should be independent of the pathologic condition causing the arrhythmias.

Effects of PUFAs on Membrane Ionic Currents and How These Effects Prevent Cardiac Arrhythmias

These effects in turn result from an action of the PUFAs to modulate the conductance of ion channels in the plasma membranes of the heart cells. The voltage-gated sodium current, I_{Na}, initiates and propagates action potentials in most cardiac myocytes. Our finding that the PUFAs increased the magnitude of a depolarizing stimulus required to elicit an action potential made it likely that the PUFAs were affecting the I_{Na}. Thus our exploration of the effects of the PUFAs on membrane ion currents and channels began with I_{Na} [52]. The PUFAs inhibited the I_{Na} in a concentration-dependent manner, with an IC_{50} of 4.8 μM in neonatal rat cardiomyocytes [52] but only 0.51 ± 0.06 μM in a human embryonic kidney cell line, HEK293t, transiently expressing human myocardial sodium α-subunits, hH1α [54]. Inhibition occurred within seconds of application of the PUFAs to the myocytes. It was voltage dependent, but not use dependent, consistent with the lipophilic nature of the PUFAs [21]. In both preparations, I_{Na} in the rat cardiomyocyte and $I_{Na\alpha}$ in the human myocardial α-subunit transiently expressed in HEK293t cells, the PUFAs caused a large voltage-dependent shift of the steady-state inactivation potential to more hyperpolarized values; the shift at $V_{1/2} = -19$ mV with 10 μM EPA in the neonatal rat cardiomyocyte (Fig. 5) and a further −27.8 mV with 5 μM EPA in the hH1α [54]. There was no effect of the PUFAs on the activation of the Na^+ channels, only on the inactivated channel (Fig. 5). The transition from the resting state to the inactivated state was accelerated in the presence of 5 μM EPA (the time constant for the development of inactivation in the control was shortened from 26.2 ± 0.78 msec, $n = 6$ to 3.67 ± 0.22 msec, $n = 6$, $P < 0.01$ with 5 μM EPA). Recovery of $I_{Na\alpha}$ from

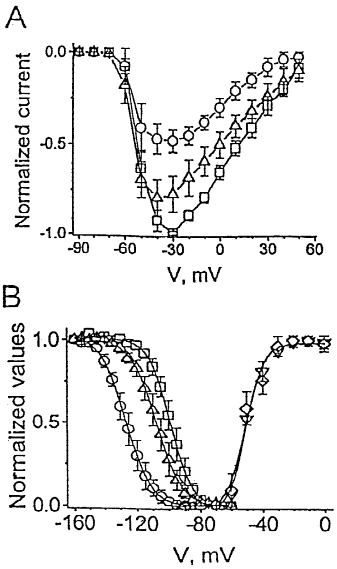

Fig. 5. The activation and inactivation of $I_{Na\alpha}$ of human cardiac Na^+ channel α-subunits, $hH1_\alpha$, expressed in human embryonic kidney cells, HEK293t, in the absence (□), presence (○), and washout (△) of EPA (5 μM) [54]. (*A*) Averaged and normalized current-voltage relationships ($n = 6$) of $I_{Na\alpha}$ are plotted, showing the inhibition of the peak Na^+ current in the presence of EPA and partial recovery following washout of EPA. (*B*) The averaged relative activation of $I_{Na\alpha}$ (*right*) was unaffected by EPA and the three curves—control, EPA and washout—of normalized activation were superimposable. By contrast (*left*), EPA produced an impressive shift of the steady-state inactivation to more hyperpolarized potentials and this was largely reversible on washout of the EPA. The same unchanged activation curves were also found for the complete $hH1_{\alpha\beta}$ sodium channel with both α and β1 units coexpressed, and for the neonatal rat cardiac myocyte. The shift of the steady-state inactivation potential to more negative potentials also occurred with $hH1_{\alpha\beta}$ and for the rat myocyte. The shifts were similar for both but not as large as seen in $hH1_\alpha$.

inactivation was markedly delayed; $t_{1/2}$ in the presence of 5 μM EPA was 34.8 ± 2.1 msec compared with 2.2 ± 0.1 msec before EPA was present ($n = 5$, $P < 0.001$). Thus, PUFAs prolonged the inactivated state of the hH1α channels by speeding the transition from the active to the inactivated state and retarding the slow inactivation phase of the channel. In more recent studies (Y-F. Xiao et al., *unpublished data*) the β1 subunit has been transiently coexpressed with the α-subunit in HEK293t cells and this shifted the steady-state inactivation potential to the right (to more depolarized potentials) returning the electrophysiology of the hH1αβ channels back almost to identity with what we had observed for the neonatal rat cardiomyocytes. EPA was found to have no effect on the activation but only on the inactivation of $I_{Na,\alpha\beta}$, $I_{Na,\alpha}$ and $I_{Na,rat}$. Consistent with the effects of these fatty acids solely on the inactivated state of the Na^+ channel, is the finding that the "binding" or interaction of these fatty acids to the inactivated state of the Na^+ channels displayed a 265-fold higher "affinity" for 5 μM EPA than channels in the closed resting, but activatable, state of $hH1_{\alpha\beta}$.

These effects of the n-3 PUFAs (and DHA and LNA do the same as EPA) we think are pertinent to the antiarrhythmic actions of these fatty acids. Our current hypothesis is that this voltage-dependent shift of the steady-state inactivation potential to more negative, hyperpolarizing voltages is important to the demonstrated antiarrythmic action of the PUFAs in ischemia-induced fatal arrhythmias. With a coronary thrombosis there occurs a gradient of depolarizations of cardiomyocytes within the ischemic tissue. Cells in the central core of the ischemic tissue quickly depolarize and die due to lack of oxygen and metabolic substrates. Depolarization results from the dysfunctional state of Na,K-ATPase and the rise of interstitial K^+ concentrations in the ischemic tissue. But at the periphery of the ischemic zone myocytes may be only partially depolarized. They become hyperexcitable since their resting membrane potentials become more positive, approaching the threshold for the gating of the fast Na^+ channel. Thus, any further small depolarizing stimulus may elicit an action potential, which, if it occurs out of phase with the electrical cycle of the heart, may initiate an arrhythmia. In the presence of the n-3 PUFAs, however, a voltage-dependent shift of the steady-state inactivation potential to more hyperpolarized resting potentials occurs. The consequence of this voltage-dependent, hyperpolarizing shift is that the negative membrane potential necessary to return these Na^+ channels from an inactive state to a closed resting, but activatable state, requires a physiologically unobtainable hyperpolarized resting membrane potential. Also these partially depolarized cells have Na^+ channels which within milliseconds can slip into "resting inactivation" from the closed resting state without eliciting an action potential [54]. The result of these two effects of the n-3 PUFAs is that these partially depolarized myocytes are quickly eliminated from functioning, and their potential arrhythmic mischief is aborted. By contrast, myocytes in the nonischemic myocardium, with normal resting membrane potential, will not be so drastically affected by this voltage-dependent action of the PUFAs and continue to function normally, as expected with an ideal antiarrhythmic agent.

But 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 occur in patients with 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. 2 [24] are examples of arrhythmias induced by excessive cytosolic Ca^{2+} fluctuations. Figure 6 is another example in which the cytosolic free Ca^{2+}

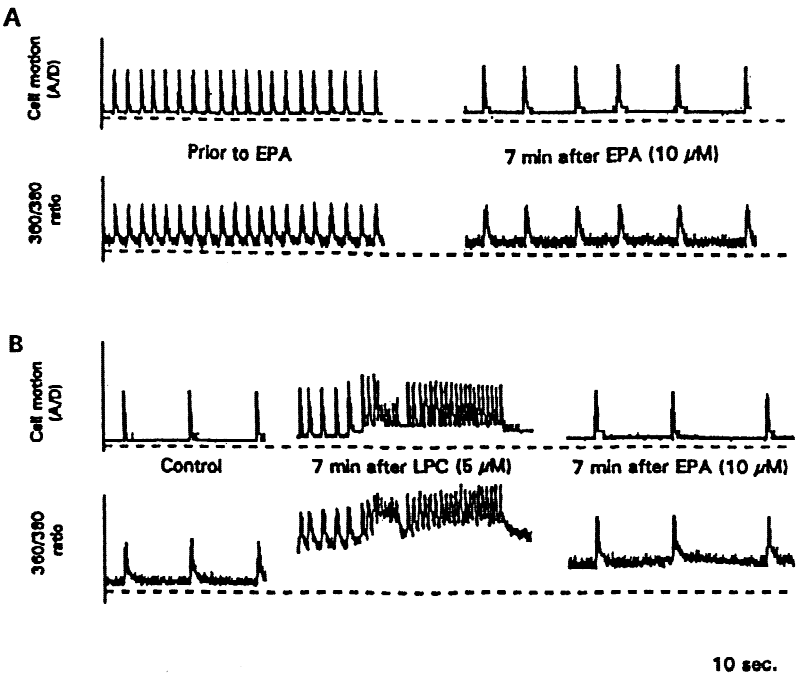

Fig. 6. Simultaneous measurements of $[Ca^{2+}]_i$ (as indicated by 360/380 fluoresence ratio of Fura 2) and cell contractions showing the effects of EPA and arrhythmogenic lysophosphatidylcholine in cultured neonatal rat cardiomyocytes [27]. (*A*) A representative recording illustrates the $[Ca^{2+}]_i$ transients (lower trace) and cell contractions (upper trace) before and after perfusion of 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 $[Ca^{2+}]_i$ levels with chaotic transients as cell contracture or tachyarrhythmias occur. Addition of EPA (10 μM) results in return to the initial slow control beating rate and $[Ca^{2+}]_i$ transients with the basal level reduced, but not to normal.

fluctuations were recorded simultaneously with the contractile activity of the neonatal cardiomyocytes [27]. In this experiment lysophophatidylcholine (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. [12, 15]). Perfusion of the isolated heart or myocyte with LPC had been shown to produce arrhythmias and contractures associated with cytosolic free Ca^{2+} overload. It produces a number of arrhythmogenic electrophysiological derangements including depolarization of the resting membrane potential [4, 14], increases in automaticity and occurrence of delayed after-depolarizations with triggered activity [44]. Furthermore, a correlation has been established between the elevated levels of LPC and the production of arrhythmias in the ischemic heart [12, 30]. In Fig. 6*A* [27] 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 cytoslic free Ca^{2+} which precedes the contraction spike by some 50 msec. 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 [27]. On another myocyte, which had a slow endogenous beating rate, Fig. 6*B* shows the effect of LPC (5 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.

Such excessive cytosolic free Ca^{2+} fluctuations, as shown in Fig. 6*B* after LPC, can induce delayed afterpotentials, which may trigger fatal arrhythmias if the after-potential occurs at a vulnerable moment in the electrical cycle of the heart. Because both $I_{Ca,L}$ and sarcoplasmic reticulum Ca^{2+}-release underlie many cardiac arrhythmias, together with Drs. A.M. Gomez and W.J. Lederer, the effects of the PUFAs on $I_{Ca,L}$ and Ca^{2+} sparks were examined [53]. Whole-cell voltage clamp techniques and confocal Ca^{2+} imaging were used to determine the effects of PUFAs on the voltage-gated L-type Ca^{2+} current ($I_{Ca,L}$), elementary sarcoplasmic reticulum Ca^{2+}-release events (Ca^{2+}-sparks), and $[Ca^{2+}]_i$ transients in isolated adult rat ventricular myocytes. Extracellular application of eicosapentaenoic acid 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. 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 voltage and time dependent but not use dependent. This is consistent with the lipophilic nature of these fatty acids which allow

them to enter the hospitable lipophilic environment of the membrane phospholipid bilayer and diffuse laterally to their site of interaction with the transmembrane ion channel protein. This is in contrast to hydrophilic agents which can only enter the ion channel to reach their active sites via the aqueous pore when the channel is in its open state and is, therefore, "use-dependent." The more action potentials, the more total time the channels are in the open configuration permeable to hydrophilic agents [21]. Thus the effects of the PUFAs on $I_{Ca,L}$ resemble their effects on I_{Na}, except that the steady state inactivation potentials for $I_{Ca,L}$ were shifted to the left to a much lesser degree.

When heart cells become "overloaded" with Ca^{2+}, they become arrhythmogenic [24, 27] and produce arrhythmogenic I_{TI} currents and waves of elevated $[Ca^{2+}]_i$ that propagate within the heart cell. Also during the Ca^{2+} overload the ryanodine receptors (RyRs) become more sensitive to the triggering process, produce an increased number of spontaneous Ca^{2+} sparks, and produce propagating waves of elevated Ca^{2+}, all of which can be viewed with the confocal microscope while measuring membrane current [53]. This allows examination of the subcellular links between I_{CaL}, the SR, and cellular Ca^{2+} signaling. There was no reduction or change in the individual sparks; the time-constant of decay of the calcium sparks were unchanged by the presence of EPA, nor were there any effects on the spatial spread of the Ca^{2+} sparks. These findings favored an efficient and unchanged coupling by EPA between the *L*-type channels in the sarcolemma and the RyRs in the SR. And when the number of Ca^{2+} sparks per unit of $I_{Ca,L}$ was determined before and after addition of EPA, the ratio of sparks to $I_{Ca,L}$ was unchanged [52]. This suggests that PUFAs reduce SR Ca^{2+} release only by decreasing the $I_{Ca,L}$ that triggers the SR Ca^{2+} release. Thus it seems our finding that the n-3 PUFAs are potent inhibitors of $I_{Ca,L}$ and that this prevents the cytosolic Ca^{2+} overload [24, 27] appears to be the mechanism by which this cause of triggered arrhythmias evoked by ischemia or cardiac toxins are prevented by the PUFAs.

It has been reported that fish oil feeding inhibits the SR Ca^{2+}-ATPase [49]. Another report, indicates that dietary EPA stimulates enhanced microsomal Ca^{2+}-ATPase activity [31]. In this study the activity of NADH-dependent cytochrome C reductase, a marker of SR was the same in the EPA and control groups. Both the purported reduction in SR Ca^{2+}-ATPase activity and the increased sarcolemmal Ca^{2+}-ATPase activity would tend to reduce cytosolic Ca^{2+} fluctuations and constitute additional actions of the PUFAs to influence the SR Ca^{2+} release. Our study, however, did not reveal such additional effects of the n-3 PUFAs.

Although at present we think that inhibitory effects of the PUFAs on I_{Na} and I_{CaL} 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 we and others [9, 23, 36, Y-F. Xiao, *unpublished results*] have found that the PUFAs 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} [Y-F. Xiao, *unpublished data*]. However, these effects on the important repolarizing K^+ currents would have the effect to prolong the action potential duration, whereas the PUFAs, if anything, slightly shorten the action potential duration [29]. Also 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. But Xiao has found other cardiac transmembrane ion currents to be also affected by the PUFAs. In fact, all ion currents that he has examined in cardiac myocytes (except I_{K1}) have been found inhibited by the same PUFAs. Y-F. Xiao (*unpublished data*) has found the cardiac chloride current and the ligand-activated acetylcholine potassium current also to be inhibited by the PUFAs.

Possible Sites of Primary Action of the PUFAs

An important question, that we have addressed, but not answered, is, "What is the primary site of interaction of the PUFAs with the cardiac myocytes, which results in their antiarrhythmic effects?" There seems to be at least three possibilities: (i) the PUFAs may interact or bind directly to the channel proteins; (ii) they may interact with the phospholipid sarcolemmal membrane changing its physical state so as secondarily to modulate the transmembrane ion channels; and (iii) they may interact or bind to some other protein in the myocyte (e.g., membrane anchoring proteins) which can affect the phospholipid sarcolemma so as to change its physical state and in turn affect conductance of the transmembrane ion channel proteins.

Our attempts to date to demonstrate direct binding of the PUFAs to the sodium channel have not been successful. We had devised a method using radiolabeled ^3H-retinoic acid which can act as a surrogate PUFA [28] and has a favorable UV absorption with a peak at 360 nm so that it will photoaffinity label only proteins to which it binds, as we found with serum albumin. Using this method we were not able to photoaffinity label a cardiomyocyte membrane protein of the size of the α-subunit of the Na^+ channel which could be identified by Western blot or immunoprecipitation as being the Na^+ channel protein (J.X. Kang, X. Li, A. Leaf, *unpublished data*). This attempt is obviously not definitive: The amount of

Na^+ channel protein extracted from the cardiomyocyte cell membranes may have been insufficient for detection. The duration of binding of the ^3H retinoic acid to a Na^+ channel protein at room temperature might have been of too short duration for it to be caught in the bound state during the time of the UV exposure, etc.

In another attempt to find direct binding of the PUFAs to the Na^+ channels we found that only the antiarrhythmic PUFAs noncompetitively displace ^3H-batrachotoxinin-20-α-benzoate bound to the sodium channel pore protein [26]. This is similar to our finding that PUFAs noncompetitively displaced ^3H-nitrendipine (a specific L-type calcium channel antagonist) from its binding site at the external pore of the calcium channel protein [19]. The displacement in each case, though specific, was noncompetitive. Thus, we cannot yet resolve the urgent question of whether the PUFAs bind specifically and primarily to ion channel proteins directly or are affected secondarily by changes in the phospholipids of the cell membranes to allosterically change the conformation of transmembrane protein channels.

The latter, second possibility that PUFAs have their primary effect on the physical state of the sarcolemmal phospholipid bilayer gains credence from the diversity of the ion channel proteins affected by the PUFAs. It was shown by Klausner et al. [32] that, to the extent they were tested, these same PUFAs affect the physical state, the packing of phospholipids in the membrane phospholipid bilayer. They, however, applied concentrations of the PUFAs that were considerably higher than those we have shown suppress conductance through the membrane ion channels. Also, we have found that the ethyl or methyl esters of the PUFAs have no antiarrhythmic effects [24], although their effects on the membrane phospholipid packing might be expected to be similar to that of the free fatty acids. Cholesterol, which compresses phospholipid membranes, or parinaric acid, which expands them, added to the myocyte perfusate, also had no effect on the action of the PUFAs. Eicosatetraynoic acid, the acetylenic analogue of arachidonic acid with 4 triple rather than double carbon-carbon bonds is purported to be a rigid rod, yet it is antiarrhythmic [24]. For these reasons we have been skeptical regarding a possible primary action of the n-3 PUFAs on the physical state of the sarcolemma, although we have not rigorously addressed this possibility and there may be cooperative effects of the PUFAs on the packing of membrane phospholipids in limited domains of the plasmalemma and on ionic currents.

The third possibility that the PUFAs may act primarily on a protein that could secondarily change the physical state of the plasma membrane needs to be studied. Since it has been demonstrated that proteins—actin [11], ankyrin and spectrin [47]—that purportedly anchor the plasma membrane to the cytoskeleton can modulate Na^+ currents, this possibility exists.

Clinical Indications of an Antiarrhythmic Effect of the PUFAs

At present we cannot say for sure whether the PUFAs will prevent lethal arrhythmias in patients, but three secondary prevention trials, which showed prevention of ischemia-induced sudden cardiac death, are encouraging. One dietary study [16] was a prospective, randomized, single-blinded, secondary prevention trial, which compared the effect of a Mediterranean α-linolenic acid-rich diet to the usual post-infarct prudent diet. The subjects on the more fat restricted experimental diet receiving the α-linolenic acid (C:18n-3) showed a remarkable reduction in mortality and morbidity of some 70%, including prevention of sudden death. Another study [10] was also a randomized, prospective, secondary prevention trial in which advice to eat oily fish two or three times weekly was compared with no such advice. This study did not record arrhythmic deaths. It, however, found no reduction in new events but a 29% reduction in mortality suggesting a reduction in sudden deaths, which comprise 50 to 60% of the acute mortality from heart attacks [2]. In both studies the survival curves showed a very early beneficial separation of the experimental *vs.* control groups, quite unlike the two years required in the cholesterol-lowering trial [35], before the lower mortality was significant. And in neither study were the beneficial effects associated with significant reductions of plasma cholesterol levels. The third study was also a secondary prospective double blinded clinical trial which reported a reduction in acute arrhythmic deaths in the cohort receiving the fish oil supplement [45]. A case control study [46] reported an inverse relationship between fish consumption and sudden cardiac death, suggesting an antiarrhythmic effect from ingestion of fish. An epidemiologic review based on the Physicians' Health Study reported a 52% reduction in the risk of sudden cardiac death in subjects who ate at least one fish meal per week [1].

Effects of the PUFAs on the Brain

Perhaps a bonus for pursuing the mechanisms by which n-3 PUFAs prevent fatal cardiac arrhythmias was our resulting expectations that these fatty acids would have important effects on the brain, as well. Once we knew that these fatty acids were affecting the ion currents in one excitable tissue, the heart, we could predict that they would have effects on other excitable tissues, muscle and brain—and they do. All excitable tissues utilize the same ionic currents for cell signaling and the transmembrane ion channel proteins are highly homologous. Whole cell voltage-clamp studies on hippocampal CA1 neurons showed that the same PUFAs, which are antiarrhythmic, inhibit the sodium and calcium currents in the

brain in a manner quite similar to their effects in the heart [51]. To determine a possible functional consequence of this electrical effect on the brain, we tested the possible anticonvulsant action of the PUFAs in rats using the cortical stimulation model of seizures. We found that the PUFAs infused on albumin over 30 minutes via a tail vein in prepared rats promptly, but modestly, raised the electrical threshold for seizure activity significantly for most of a day [50]. Whether this will translate into a useful medication for epilepsy, we do not now know. But it indicates a clear action of these interesting fatty acids on brain cells. An effect on muscle cells has also been shown.

Conclusions

It is apparent that there exists a basic control of cardiac and neural function by common dietary fatty acids which has been largely overlooked. The n-3 PUFA have been part of the human diet for some 2–4 million years during which time our genes were being adapted to our diet as hunter-gatherers [33] and they are safe. With some 250,000 sudden cardiac deaths annually, largely due to ventricular fibrillation, in the USA alone [2] there may be a potential large public health benefit from the practical application of this recent understanding. Initial reports suggest that the n-3 PUFAs are producing beneficial effects in the treatment of depression [20], bipolar and other behavioral diseases [48]. The knowledge that these fatty acids have direct physical effects on the fundamental property of the nervous system, namely its electrical activity, should encourage further exploration of potential beneficial effects on brain functions both normal and pathological. It seems likely that we are just scratching the surface of the potential health effects of these interesting dietary polyunsaturated fatty acids.

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