Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids1–3

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ABSTRACT In animal feeding studies, and probably in humans, n–3 polyunsaturated fatty acids (PUFAs) prevent fatal ischemia-induced cardiac arrhythmias. We showed that n–3 PUFAs also prevented such arrhythmias in surgically prepared, conscious, exercising dogs. The mechanism of the antiarrhythmic action of n–3 PUFAs has been studied in spontaneously contracting cultured cardiac myocytes of neonatal rats. Adding arrhythmogenic toxins (eg, ouabain, high Ca2+, lysophosphatidylcholine, β-adrenergic agonist, acylcarnitine, and the Ca2+ ionophore) to the myocyte perfusate caused tachycardia, contracture, and fibrillation of the cultured myocytes. Adding eicosapentaenoic acid (EPA; 5–15 μmol/L) to the superfusate before adding the toxins prevented the expected tachyarrhythmias. If the arrhythmias were first induced, adding the EPA to the superfusate terminated the arrhythmias. This antiarrhythmic action occurred with dietary n–3 and n–6 PUFAs; saturated fatty acids and the monounsaturated oleic acid induced no such action. Arachidonic acid (AA; 20:4n–6) is anomalous because in one-third of the tests it provoked severe arrhythmias, which were found to result from cyclooxygenase metabolites of AA. When cyclooxygenase inhibitors were added with the AA, the antiarrhythmic effect was like those of EPA and DHA. The action of the n–3 and n–6 PUFAs is to stabilize electrically every myocyte in the heart by increasing the electrostimulus required to elicit an action potential by ≈50% and prolonging the relative refractory time by ≈150%. These electrophysiologic effects result from an action of the free PUFAs to modulate sodium and calcium currents in the myocytes. The PUFAs also modulate sodium and calcium channels and have anticonvulsant activity in brain cells. Am J Clin Nutr 2000;71(suppl):202S–7S.

KEY WORDS n–3 Fatty acids, n–6 fatty acids, ventricular fibrillation, sudden cardiac death, cardiac arrhythmias

INTRODUCTION Interest in the possible antiatherosclerotic action of n–3 polyunsaturated fatty acids (PUFAs) has flourished since Bang et al (1) in 1976 attributed the low mortality of Greenland Eskimos from ischemic heart disease to the high content of n–3 PUFAs in their diets, despite their high total fat content by weight. Meanwhile, following other reports (2, 3), McLennan et al (4) fed rats high-fish-oil diets and reported that these prevented sustained ventricular fibrillation when the coronary arteries of the rats, after some weeks, were ligated. In another study, McLennan (5) reported that ≈40% of rats fed diets high in saturated fats or olive oil had sustained ventricular fibrillation that was reduced by 70% when vegetable oil was the major dietary source of fat, and was essentially prevented when tuna oil was the dietary fat. These interesting results stimulated us to pursue the possible mechanisms for the protective effects of the PUFAs against fatal arrhythmias.

EFFECTS OF n–3 FATTY ACIDS ON SURGICALLY PREPARED EXERCISING DOGS

First, to see if we could confirm McLennan et al’s results, we collaborated with GE Billman of The State University of Ohio in Columbus, who has studied potential antiarrhythmic agents in conscious exercising dogs for 2 decades. In his studies, the dogs were prepared surgically by ligating the left anterior coronary artery to produce a large myocardial infarction and placing a hydraulic cuff around the left circumflex artery that could be occluded later at will. The animals recovered for 1 mo, during which time they were trained to run on a treadmill. Billman found that occluding the left circumflex artery while the dogs were running on the treadmill to a set heart rate invariably caused sustained ventricular flutter or ventricular fibrillation < 2 min after occlusion in ≈60% of the prepared dogs. It was in susceptible animals such as these that we did our studies (6).

We tested 14 dogs and found that an intravenous infusion of 2–5 g concentrated n–3 PUFAs from fish oils (BioCare 6000FA; Pronova AS, Oslo) administered to the exercising dogs over 50–60 min just before occluding the left circumflex artery prevented the fatal arrhythmias in 11 animals (P < 0.001; 7). The same animals tested identically the week before and the week after the test with the n–3 PUFAs had the expected fatal arrhythmias,

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from which they were defibrillated. The free fatty acids were administered intravenously so that we could be certain that, if protection occurred, we could safely attribute the effect to the n–3 fatty acids infused. Another set of experiments was performed with use of pure free eicosapentaenoic acid (EPA; 20:5n–3), docosahexaenoic acid (DHA; 22:6n–3) and α-linolenic acid (18:3n–3). Each provided equal protection (8).

EFFECTS OF n–3 FATTY ACIDS ON SPONTANEOUSLY CONTRACTING CULTURED NEONATAL RAT HEART CELLS

To determine the mechanism of the antiarrhythmic action of n–3 PUFAs, we used a cultured heart-cell preparation to examine the effects of the PUFAs on cardiac myocytes free of hormonal or neural influences. We chose cultured contracting neonatal rat heart cells so that we could observe the effects of additives on the contractile behavior of the myocytes. Enzymatically separated heart cells grown on a microscope cover slip were placed in a perfusion chamber on the stage of an inverted microscope that was fitted with a video camera and an edge monitor. This enabled us to record the amplitude and rate of contractions of a single cardiomyocyte within a syncytium of myocytes (9) and observe the effect of arrhythmogenic agents added to the bathing medium of the cells. A short strip of the control, spontaneous, regular contractions of such a myocyte is shown in Figure 1A. When calcium concentrations in the bathing medium were increased to toxic levels, the contractions altered abruptly as the cell went into contractures with a rapid beating rate and finally, instead of the regular, rhythmic, synchronized, contractions; each cell in the syncytium contracted irregularly and asynchronously; this was the in vitro equivalent of fibrillation in vivo. However, when 10 μmol EPA/L was first added to the superfusate, the beating rate of the myocytes slowed and when the calcium concentration was increased to a toxic level, no arrhythmia developed. A similar situation with toxic concentrations of another arrhythmogenic agent, the cardiac glycoside ouabain, is shown in Figure 1B. When toxic concentrations of both Ca2+ and ouabain were added to the superfusate, the cell went into a violent arrhythmic state (Figure 1C). When the EPA was added, the arrhythmia was terminated and the cell resumed a regular beating rate despite the continued presence of the toxic concentrations of Ca2+ and ouabain in the bathing medium. Finally, when delipidated bovine serum albumin (BSA) was added to the perfusate, thus withdrawing the EPA from the cardiomyocytes, the arrhythmia recurred.

Albumin has several binding sites for fatty acids and is the normal means by which the hydrophobic fatty acids are transported through the hydrophilic aqueous plasma to the cells of the body. Three of these sites have high affinity for free fatty acids (10) and when albumin is delipidated these binding sites can reverse the usual traffic and withdraw the fatty acids from the cell membranes.

Results from experiments like that shown in Figure 1 brought to light some important and surprising facts (8). First, if we added the ethyl ester or triacylglycerol of the PUFA, no protection from the arrhythmia occurred. The free carboxyl group of the fatty acids is essential for producing the prompt prevention or termination of arrhythmias. Second, the fact that the delipidated BSA was able to reverse the effects of the PUFA indicates that the fatty acids do not need to be incorporated into the phospholipids of the cell membranes to express their antiarrhythmic actions. In
fact, they do not bind covalently with any constituent of the membrane or cells to produce their effect; if they had, the BSA could not have removed them from the cell membranes. This provides to us the startling fact that all that is needed is for the fatty acids to partition (ie, dissolve) into the hospitable environment of the lipophilic hydrocarbon chains of the acyl groups in the phospholipids of the myocyte cell membranes to exert their immediate antiarrhythmic actions.

Using this simple system we then determined which fatty acids were antiarrhythmic (8). The major dietary n–3 PUFAs, α-linolenic acid (ALA; 18:3n–3), EPA, and DHA, are antiarrhythmic, as are the n–6 PUFAs linoleic acid (LA; 18:2n–6) and arachidonic acid (AA; 20:4n–6). The latter, however, was anomalous because in ≈60% of tests it either had no antiarrhythmic action or induced violent arrhythmias. This arrhythmic action proved to be caused by several cyclooxygenase metabolites of AA (11) and not by the free AA itself. But because of this potential arrhythmic action, we focused our attention on the n–3 PUFAs, which are dependably only antiarrhythmic, for future clinical studies. Monounsaturated oleic acid and saturated fatty acids have no antiarrhythmic effects.

From these studies it was apparent that the structural requirements for an antiarrhythmic agent that acted in the manner of these PUFAs was a molecule with a long acyl or hydrocarbon tail, ≥2 unsaturated carbon-carbon double bonds, and a free carboxyl group at one end. Guided by these requirements we found that all-trans-retinoic acid was also specifically antiarrhythmic (12).

We subsequently induced arrhythmias by using this preparation with the following arrhythmogenic agents in addition to high calcium and ouabain: lysophosphatidyl choline and acylcarnitine (13); isoproterenol and cAMP (14); thromboxane, prostaglandin E₂, and other cyclooxygenase metabolites of AA (11); and even the calcium ionophore (A23187; 13). In every instance, if the n–3 PUFA was added to the bathing medium of the myocytes before addition of the toxin, arrhythmia was prevented and, if the arrhythmia was already induced, addition of the PUFA promptly terminated it. Thus, we realized that the effect of the PUFAs must be on the final common pathway of arrhythmogenesis, namely the excitability of the myocytes. On this basis, we sought to determine the effects of the PUFAs on the electrophysiology of single isolated ventricular myocytes from neonatal rats.

The antiarrhythmic PUFAs exerted definite changes in the electrophysiology of the myocytes (15). They slightly, but significantly, hyperpolarized the resting (diastolic) membrane potential of the myocyte and raised the voltage threshold for gating (ie, opening) the fast sodium channel. These 2 effects required a 50% increase in the strength of an electrical stimulus to elicit an action potential in the presence of the n–3 PUFAs and prolonged markedly the relative refractory period of the myocytes. Taken together, the antiarrhythmic action of n–3 PUFAs, we think, is to electrically stabilize the heart cells.

To illustrate this stabilizing effect of the PUFAs, the simple experiment shown in Figure 2 was performed (13). A tracing from a single cell in a clump of myocytes growing on a microscope cover slip showed the cell to be contracting regularly. With 2 platinum electrodes placed at opposite sides of the cover slip we were able to use an external electrical field to stimulate the cells to contract. When the voltage was turned on, the myocyte readily doubled its beating rate in response to the external electrical stimulus. When the external stimulus was stopped, the cell reverted to its endogenous, spontaneous beating rate. The second strip shows the same cell after EPA was added to the superfusate. The beating rate slowed, as expected, but when the external electrical stimulus was turned on, the myocyte did not respond to it.

**FIGURE 2.** Reduced electrical excitability of cultured neonatal cardiac myocytes in the presence of eicosapentaenoic acid (EPA) as measured by an edge monitor. The 3 tracings were all continuous recordings from the same cell within a group of cells. The top tracing shows that the cells doubled their beating rate in response to stimuli imposed from an external field of 15 V. After addition of EPA to the superfusate, the spontaneous beating rate slowed and the cells were unresponsive to stimuli imposed by fields of 15 or 20 V, but at 25 V every other stimulus captured a contraction. Addition of bovine serum albumin (BSA) and removal of EPA from the superfusate increased the beating rate and again the cells responded to the stimuli delivered at 15 V external field strength. Reprinted with permission from reference 12.
POLYUNSATURATED FATTY ACIDS AND HEART DISEASE

EFFECTS OF n–3 FATTY ACIDS ON OTHER EXCITABLE TISSUES

Once we found that the fatty acids affected the ion channels in an excitable tissue, the heart, we were able to predict that they must affect the other excitable tissues, namely muscle and nervous systems, as well. All excitable tissues use the same electrical signaling system generated by ion currents through specific membrane ion channels. A few years ago, before we were aware of any of the above effects of the PUFAs, we reported a direct relaxing effect on vascular smooth muscle in deepithelialized aortic rings of rats fed fish oil but not of those fed lard or corn oil as the major source of dietary fat (23). We now might attribute this to the blocking effect of the n–3 PUFAs on calcium channels. But it was the central nervous system that primarily interested us. E Furshpan of Harvard Medical School kindly tested the effect of EPA on cultured rat CA1 hippocampal neurons (E Furshpan, J Kang, A Leaf, unpublished observations, 1995). Primary cultures of these neurons were maintained chronically in the presence of kynurenic acid, which blocked their excitatory glutamate receptors, enabling them to exhibit little spontaneous electrical activity and survive. When the kynurenic acid was washed out, the neurons went into a potentially lethal storm of electrical activity, so the kynurenic acid was quickly replaced in the bathing medium to quiet the cells and prevent them from dying. Then, with EPA added to the washout fluid, the kynurenic acid was again washed out from the neurons. The EPA did not prevent the initial burst of electrical hyperactivity, but the activity quickly subsided to a nonlethal level. This sequence was sufficiently reproducible (though more studies are needed to define the experimental conditions) to suggest strongly that, as in the heart, this fatty acid could prevent hyperactivity in the cells from which

at 15 or 20 V. At 25 V the external stimuli began to capture contractions of the myocyte, which responded, however, only to every other stimulus. The third strip shows the same cell but with delipidated BSA added to the superfusate to withdraw the EPA from the cardiomyocytes. When the external electrical stimuli were started, the myocyte responded as it had initially to every stimulus delivered at 15 V. Considering that PUFAs have this stabilizing effect on every myocyte in the heart, one can sense the antiarrhythmic potential of these fatty acids. Additionally, this tells us that the antiarrhythmic action of PUFAs is independent of the exciting noxious stimuli, whether ischemia or toxins.

To pursue the cause of the electrophysiologic effects induced by the PUFAs, we, along with YF Xiao, used the whole-cell patch-clamp technique to determine the effects of the PUFAs on the individual membrane ionic currents responsible for all electrical activity of cells. The fast, voltage-dependent Na+ current was found to be inhibited strongly by the PUFAs as determined in the rat neonatal cardiomyocytes (16) and more recently in a stable human embryonic kidney cell line (HEK293), which has no intrinsic voltage-dependent Na+ current until transfected with the α-subunit of the human myocardial Na+ channel protein, hH1 (17). Both EPA and DHA produced a 50% inhibition (IC50) at 4.8 µmol/L, and at 40 µmol/L the sodium current (Ina) of the neonatal rat cardiomyocyte was blocked completely. The IC50 for the HEK293 cells expressing the human myocardial α-subunit was 0.51 µmol/L. But the effects on the L-type calcium current, (ICaL), were also potent (18). Inhibition was noted at a concentration of 10 nmol DHA/L (IC50: 0.8 µmol/L) and ICaL was blocked completely at a concentration of 5 µmol/L. The effects came on within seconds of exposure to the PUFAs; this is consistent with the known rapidity of movement of a free fatty acid from albumin back and forth across a phospholipid cell membrane (19). The effect of these PUFAs on the sodium and calcium channels is to prolong the duration of their inactivated state in addition to inhibiting the conductance of these channels (16, 17). We also found that the same antiarrhythmic PUFAs also inhibit the 2 repolarizing potassium currents—IK, the initial outward K+ current, and IKr, the delayed rectifier K+ current—but not IK1, the inward rectifier K+ current (Y-F Xiao, LP Morgan, A Leaf, unpublished observations, 1997), as others have also observed (20–22). However, these effects are probably not important to the antiarrhythmic action of the n–3 PUFAs because they would prolong the duration of the action potential, which does not occur in our experience, and the concentration of the fatty acids necessary to induce these effects on the K+ currents is higher than that needed to affect the changes we observed in the Na+ and Ca2+ currents.

Our current hypothesis is that the antiarrhythmic effect of these PUFAs results largely from their effects on the Na+ and Ca2+ currents. Ischemia of the myocardium, as occurs with an acute myocardial infarction or angina pectoris, rapidly results in partial depolarization of the resting membrane potential of the cardiac myocytes in the ischemic areas. This results from dysfunction of the Na+-K+-exchanging ATPase and the accumulation of K+ in the interstitial fluid. The partially depolarized cells are hyperexcitable because their resting membrane potentials approach the threshold potential for the gating of the inward Na+ current, which initiates the action potential. In this state the cells are vulnerable to induce an action potential as a result of any small further depolarizing stimulus, eg, injury currents. Should such an action potential occur during a vulnerable instant in the cardiac electrical cycle, an arrhythmia may be induced. Because the shift of the steady state inactivation potential by the n–3 PUFAs to more (negative) hyperpolarized membrane potentials is highly voltage dependent (16, 17), myocytes that are partially depolarized after an action potential are unable to resume a resting membrane potential sufficiently negative to return the prolonged inactivated state of the channel, caused by the n–3 PUFAs, back to a closed, but activatable state. These hyperexcitable cells are thus eliminated from producing further action potentials, whereas cells in the remaining nonischemic myocardium will continue to function normally in the presence of the n–3 PUFAs. This may be the major contribution to the preventive effects of these fatty acids against ischemia-induced fatal ventricular fibrillation. However, other potentially fatal arrhythmias can be induced by excessively increased cytosolic Ca2+ fluctuations, resulting in delayed afterpotentials, which can also initiate arrhythmias. These are believed to be the cause of cardiac arrhythmias induced by elevated plasma Ca2+ concentrations, toxic concentrations of cardiac glycosides, or excessive catecholamine concentrations, all of which may result clinically in fatal arrhythmias. The potent inhibition of the L-type Ca2+ current by the PUFAs, described above, should prevent such excessive cytosolic Ca2+ fluctuations and the likelihood of fatal outcomes. It is thought that with ischemia, both partial depolarization of myocardial cells and elevations in their cytosolic free Ca2+ concentration can occur, so generally it may be the combined effects on these 2 ion channels by the antiarrhythmic PUFAs that account for the protective effects. But this hypothesis should not be regarded as more than speculation until it is rigorously tested experimentally and clinically.
temporal lobe epilepsy originates. Subsequently, colleagues at the University of Leiden, Netherlands, showed that in their rat model, in which the electrical threshold for generalized seizure could be carefully monitored, the intravenous infusion of EPA raised the electrical threshold for seizure activity (24). Furthermore, colleagues at the University of Amsterdam found that in rat CA1 hippocampal neurons, the n–3 PUFAs affect the sodium and calcium channel currents as in cardiomyocytes, but the major effect was to prolong the inactivated state of these channels (25). These findings seem a bonus for having looked for a mechanism to provide a rational basis for the antiarrhythmic action of these PUFAs. Apparently, these n–3 PUFAs have antiarrhythmic as well as antiarrhythmic actions. Now that at least one physical basis for the effects of these fatty acids on the nervous system has been found, we hope that many more studies will explore how these fatty acids may modulate other important functions of the brain, for which we now have mostly only descriptive explanations. As with the heart, in which all cardiomyocytes are quickly exposed to these fatty acids, in the brain they cross the blood-brain barrier rapidly and should reach all cells promptly (26).

CONCLUSION

We do not yet know whether the antiarrhythmic actions described herein actually prevent fatal arrhythmias, which are the main cause of ≈250 000 sudden cardiac deaths annually in the United States alone (27). Two familiar secondary prevention trials that inadvertently found suppression of sudden cardiac death in experimental subjects who consumed n–3 fatty acids are encouraging (28, 29), as is the one cohort-control study by Siscovick et al (30). Three other, more recent clinical studies support antiarrhythmic actions of these n–3 PUFAs (31–33). The time has come to conduct some careful, prospective, randomized, placebo-controlled clinical trials to learn whether these PUFAs will prove to benefit the public health and to pursue the many remaining questions regarding the mechanism of n–3 PUFA actions in the heart, brain, and other excitable tissues.

REFERENCES


