ELSEVIER

Membrane fluidity changes are associated with the antiarrhythmic effects of docosahexaenoic acid in adult rat cardiomyocytes

Wayne R. Leifert,*† Anisa Jahangiri,*† and Edward J. McMurchie†

**Department of Physiology, The University of Adelaide, and †CSIRO Health Sciences and Nutrition, Adelaide, Australia*

Previous studies using neonatal rat cardiomyocytes have reported antiarrhythmic effects of long-chain polyunsaturated fatty acids (PUFAs). In this study, we examined the effects of the n-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) on the spontaneous contractile activity and membrane fluidity of adult rat ventricular myocytes. Cardiomyocytes were induced to contract spontaneously by continuous superfusion of a solution containing the arrhythmogenic agents isoproterenol (a β-adrenergic receptor agonist) or lysophosphatidylcholine. The percentage of cardiomyocytes displaying spontaneous contractions induced by isoproterenol when pretreated with the saturated fatty acid docosanoic acid was 48.1 ± 7.7%; the percentage for cardiomyocytes pretreated with DHA was 7.1 ± 2.4% ($P < 0.01$). DHA significantly prevented lysophosphatidylcholine-induced spontaneous contractions (17.7 ± 6.5%) compared with treatment with the saturated fatty acid stearic acid (78.0 ± 7.3%, $P < 0.01$). The membrane fluidizing agent benzyl alcohol also significantly prevented spontaneous contractions in cardiomyocytes. Membrane fluidity was determined by steady-state fluorescence anisotropy (r_{ss}) using the fluorescent probe N-((4-(6-phenyl-1,3,5-hexatrienyl)phenyl)propyl) trimethyl-ammonium p-toluene-sulfonate (TMAP-DPH). DHA and benzyl alcohol dose-dependently decreased the r_{ss}; however, saturated fatty acids were without effect. These results suggest that the antiarrhythmic mechanisms of the n-3 PUFAs such as DHA may involve changes in membrane fluidity. (J. Nutr. Biochem. 11: 38–44, 2000)

Keywords: polyunsaturated fatty acids; arrhythmia; cardiomyocytes; docosahexaenoic acid

Introduction

Coronary heart disease (CHD) remains one of the leading causes of mortality in many industrialized countries, with factors such as the intake of saturated fat being strongly related to the development of CHD. Recent evidence suggests that dietary n-3 polyunsaturated fatty acids (PUFAs), in particular docosahexaenoic acid (DHA; 22:6, n-3), which is abundant in fish oil, can reduce both the incidence and mortality of CHD.[1] Prominent among these studies are those of Kromhout et al.,[2] Burr et al.,[3] and Siscovick et al.[1] In the latter study it was demonstrated that an intake of 5.5 g of n-3 fatty acids per month (equivalent to one fatty fish meal per week) was associated with a 50% reduction in the risk of primary cardiac arrest. Together the results of these studies suggest that dietary n-3 PUFAs both improve the chances of surviving an episode of myocardial ischemia and prevent primary cardiac arrest, and they may do so by their intrinsic antiarrhythmic activity.

The above epidemiologic studies augment experimental animal studies that have established that dietary supplementation with n-3 PUFAs in the form of fish oil alters the membrane phospholipid fatty acid composition of cardiomyocytes primarily by an increase in the proportion of n-3 PUFAs, particularly DHA.[4,5] Accompanying this, a decrease in the incidence of ischemia-induced and reperfusion-induced ventricular arrhythmias[5,6] and an increase in the ventricular fibrillation threshold in both rats and marmosets have been reported.[6,7]

Address correspondence to Dr. Ted McMurchie, CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide BC, South Australia, 5000, Australia.
Received June 2, 1999; accepted October 4, 1999.

655 Avenue of the Americas, New York, NY 10010
0955-2863/00/$–see front matter
PII S0955-2863(99)00069-8

The antiarrhythmic properties of the n-3 PUFAs have also been studied in vitro using isolated multicellular cardiomyocyte preparations from 2-day-old neonatal rats[8–11] and in electrically-field stimulated adult rat cardiomyocytes, which can be protected from developing asynchronous contractile activity by the acute addition of n-3 PUFAs.[12–14] The spontaneous contractions of neonatal syncytia induced into arrhythmias by a variety of arrhythmogenic stimuli could be prevented or terminated by addition of n-3 PUFAs and, to a lesser extent, n-6 PUFAs, but not saturated fatty acids.

The present study sought to determine whether the antiarrhythmic effects of the n-3 PUFA DHA were associated with changes in membrane fluidity using the adult rat ventricular myocyte as the experimental model. In contrast to our previous results using electrically-field stimulated adult rat cardiomyocytes,[12] these studies involved establishing a model of predictable spontaneous contractile activity induced by isoproterenol or lysophosphatidylcholine as arrhythmogenic agents in nonstimulated adult rat cardiomyocytes. Although this model approximates the neonatal cardiomyocyte model developed by Leaf and his colleagues,[8–11] more importantly, it allows us to determine whether similarity exists between the freshly isolated adult rat cardiomyocytes and the cultured neonatal rat heart myocytes with regard to the antiarrhythmic effects of the n-3 PUFAs, particularly DHA.

Materials and methods

Animals

Ethics approval was obtained from both the CSIRO and the University of Adelaide Animal Experimentation Ethics Committees. Male Sprague-Dawley rats, aged 12 weeks, were obtained from the University of Adelaide (Adelaide, SA, Australia). Animals were housed in groups of up to five per cage, with food and water provided ad libitum. Room temperature was maintained at 23°C with constant 55% humidity, and lights were maintained on a 12-hour light (8 AM to 8 PM)/dark cycle.

Perfusion and culture media

Calcium-free Tyrode perfusion media contained (in mM): 137.7 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11.0 glucose, and 10.0 (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) (HEPES), pH 7.4. $CaCl_2$ was added prior to use to give the appropriate concentrations indicated. Tyrode solution was prepared using ultra-pure (Milli-Q) water, filtered through a 0.22 μm Millipore filter (Millipore, Sydney, New South Wales, Australia) prior to use, and gassed with 100% oxygen (O_2). DMEM culture medium was supplemented with 1 mM Ca^{2+}, 10 mM HEPES, 25 mM $NaHCO_3$, 100 U/mL penicillin G, 100 mg/mL streptomycin, 2 mM carnitine, 5 mM creatine, 5 mM taurine, and 1 mg/mL bovine serum albumin (BSA).

Preparation of adult rat ventricular cardiomyocytes

Hearts were perfused in a retrograde, nonrecirculating Langendorff mode with Tyrode solution containing 1.5 mM Ca^{2+} for 4 minutes, followed by nominally Ca^{2+}-free Tyrode solution (nonrecirculating) for 2 minutes. The buffers were maintained at 37°C and gassed with 100% O_2 during perfusion. The heart was then perfused in a recirculating manner for 30 minutes with Tyrode solution supplemented with 20 μM Ca^{2+}, 300 U/mL collagenase (type 1A), and 0.1% (w/v) delipidated BSA (fraction V) at 37°C. Ventricles were teased apart and agitated in Tyrode solution containing 20 μM Ca^{2+}, 2% (w/v) BSA, and 30 mM 2,3-butanedione monoxime at 25°C. The suspension was filtered through a 250 μm nylon-mesh gauze. The concentration of Ca^{2+} was increased stepwise to 1 mM over 45 minutes. Aliquots of cardiomyocyte suspension were added to petri dishes containing 12 mm (diameter) glass coverslips coated with laminin (25 μg/mL). This procedure allowed rod-shaped cardiomyocytes to adhere to coverslips within 60 minutes at room temperature (>90% rod-shaped and viable as determined using trypan blue staining). The glass coverslips with viable, adhering cardiomyocytes were washed twice with DMEM culture medium containing 1 mM Ca^{2+}, pre-equilibrated with 5% carbon dioxide (CO_2):95% O_2. Cardiomyocytes were maintained in DMEM in a humidified incubator at 37°C and gassed with 5% CO_2 in air.

Measurement of cardiomyocyte contraction

A video camera mounted on an inverted Olympus microscope (Olympus, Melbourne, Victoria, Australia) housed in a perspex chamber maintained at 37°C transferred images to a computer connected between the camera and the monitor. An on-line, real-time computer program was used (LabVIEW, National Instruments, Victoria, Australia) that allowed for continuous observation of changes in cell length during contractile activity.

Spontaneous contractile activity

Cardiomyocytes were normally superfused with Tyrode solution containing 1 mM Ca^{2+}. Under these conditions and in the absence of electrical-field stimulation, quiescent cardiomyocytes, when treated with the arrhythmogenic agents isoproterenol (10 μM) or lysophosphatidylcholine (10 μM), can be induced to contract spontaneously. To test the effects of DHA or the membrane fluidizing agent benzyl alcohol on preventing spontaneous contractile activity, both agents were added to the superfusing solution 5 minutes prior to the addition of isoproterenol or lysophosphatidylcholine. The saturated fatty acids stearic acid (18:0) and docosanoic acid (22:0) were used as control fatty acids. The percentage of cardiomyocytes spontaneously contracting was determined by measuring 20 to 40 cells in duplicate for the number of heart preparations shown in the figure legends. In some experiments, asynchronous contractile activity was induced by superfusion with 10 μM lysophosphatidylcholine in the presence of electrical-field stimulation (25 V, pulse duration of 5 ms at 1 Hz) using two platinum wire electrodes located at either end of the superfusion chamber (Grass S4 stimulator, Grass, Quincy, MA USA). Asynchronously contracting cells (in the presence of electrical-field stimulation) were defined as cells that exhibited a contraction rate exceeding the rate of applied electrical stimulation (i.e., cells not contracting in synchrony with the applied electrical stimulus). Asynchronous contracting cells usually contracted at 2 to 4 contractions per second.

Effect of n-3 PUFAs on cardiomyocyte sarcolemmal membrane fluidity

Membrane fluidity was determined by measuring the steady-state fluorescence anisotropy (r_{ss}) of the probe N-((4-(6-phenyl-1,3,5-hexatrienyl)phenyl)propyl) trimethyl-ammonium p-toluene-sulfonate (TMAP-DPH) according to a modification of the method of de Jonge et al.,[15] which we have previously described.[12] Isolated ventricular myocytes attached to laminin-coated glass coverslips were washed in Tyrode buffer. Cardiomyocytes were then loaded with 1 μM TMAP-DPH for 15 minutes at 37°C. The coverslips

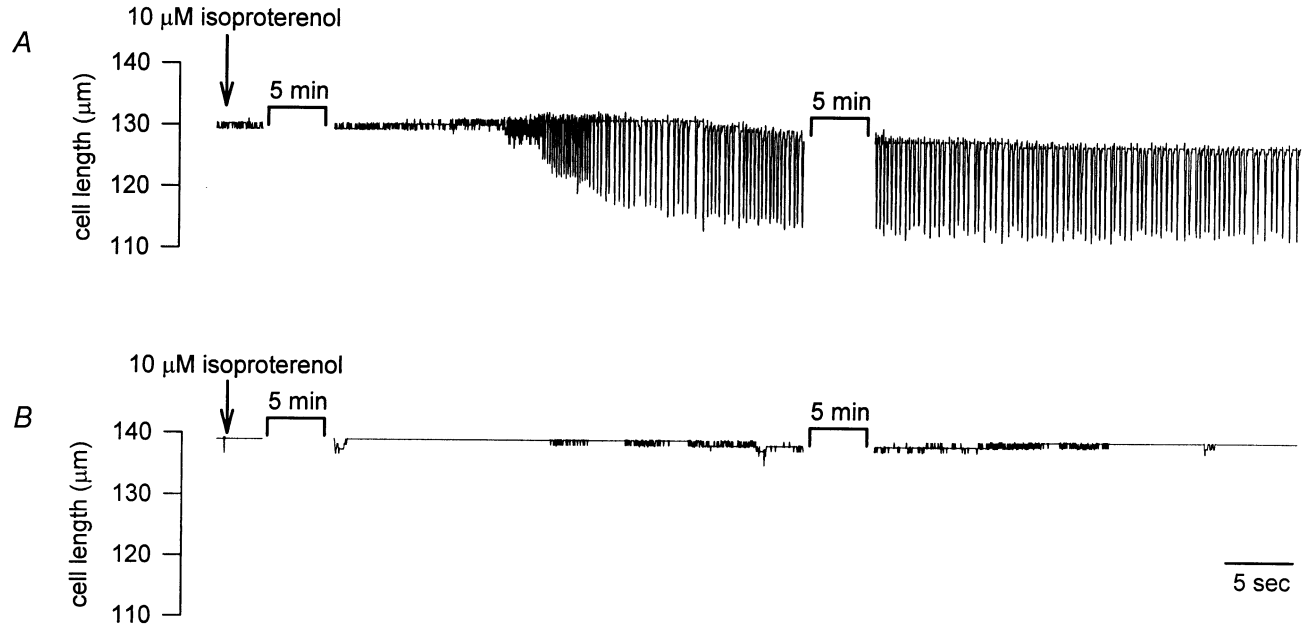

Figure 1 Change in cell length of a single cardiomyocyte in response to 10μM isoproterenol in adult rat ventricular myocytes in the presence of (*A*) 10μM docosanoic acid (saturated fatty acid, 22:0) or (*B*) 10 μM docosahexaenoic acid (DHA; 22:6 n-3) measured as described in Materials and methods. Cardiomyocytes were continuously superfused with isoproterenol (added as indicated by the arrow), which resulted in the development of rapid spontaneous contractions after approximately 5 minutes in the cell preincubated in docosanoic acid. However, DHA preincubation prevented isoproterenol-induced spontaneous contractions.

were placed in a spectrofluorimeter cuvette containing the following in Tyrode buffer: DHA (1–20 μM), benzyl alcohol (1–20 mM), or control (no additions) for 10 minutes and r_{ss} values were measured according to the formula described by Lentz[16]:

$$r_{ss} = \frac{(I_{VV} - GI_{VH})}{(I_{VV} + 2GI_{VH})}$$

where I_{VV} and I_{VH} represent the fluorescence intensity parallel and perpendicular to the excitation plane (when set vertically), respectively; and G is a correction factor for the difference in the transmission efficiency for vertically and horizontally polarized light and is calculated by I_{HV} divided by I_{HH}. Measurements were obtained using a spectrofluorimeter (Hitachi 650-10S, Tokyo, Japan), provided with vertical and horizontal polarization filters (Polaroid, Melbourne, Victoria, Australia). The excitation and emission monochromators were positioned at wavelengths of 350 nm and 430 nm, respectively, with slit width set to 10 nm for both excitation and emission modes. Readings were corrected for both background fluorescence of TMAP-DPH and light scatter by the cardiomyocyte preparation itself. Vertical polarization values (I_{VV}) were routinely 13 or more times greater than the signal generated by the background. At least three coverslips from each preparation were used for the above measurements. The number (n) refers to the number of rat hearts from which cardiomyocytes were prepared. All fatty acid treatments contained 0.04% v/v ethanol (final concentration). This concentration of ethanol did not significantly influence contractile activity or measurements of r_{ss}.

Chemicals

Fatty acids, DMEM culture medium, BSA (fraction V), carnitine, creatine, taurine, 2,3-butane-dione monoxime, collagenase type 1A, laminin, isoproterenol, and L-α-phosphatidylcholine were from Sigma Chemical Co. (Castle Hill, NSW, Australia). Stock solutions (50 mM) of fatty acids were prepared in ethanol containing 0.003% (w/v) 2[3]-t-butyl-4-hydroxyanisole and stored at −80°C under nitrogen. Solutions of isoproterenol (5 mM stock) were prepared daily in 10 mM ascorbic acid. Penicillin/streptomycin was from GIBCO-BRL (Melbourne, Australia). BSA was delipidated by washing in acetone, petroleum spirit, and diethyl ether. TMAP-DPH was from Molecular Probes (Eugene, OR USA). All other chemicals were of the highest grade available.

Statistics

Statistical analysis was performed using the computer software program Instat (GraphPad Software, San Diego, CA USA). Student's unpaired *t*-test, Welch's test, or one-way analysis of variance with Student-Newman-Keul's multiple comparison test (where required) was used to compare differences between means. For each comparison a *P*-value of less than 0.05 was considered significant. Data are expressed as mean ± SEM (n = the number of rat heart preparations).

Results

Following the above-described isolation procedure, more than 90% of the cardiomyocytes that adhered to the laminin-coated coverslips were quiescent and rod-shaped with clear cross-striations and no observable cell membrane blebbing.

Spontaneous contractile activity (in the absence of electrical field stimulation) was determined by comparing the contractility of cardiomyocytes before and after addition of 10 μM isoproterenol. *Figure 1A* demonstrates the generation of spontaneous contractility in a cardiomyocyte after 5 minutes of superfusion with Tyrode buffer containing isoproterenol. Under these conditions quiescent cardiomyocytes begin to progressively display rapid spontaneous

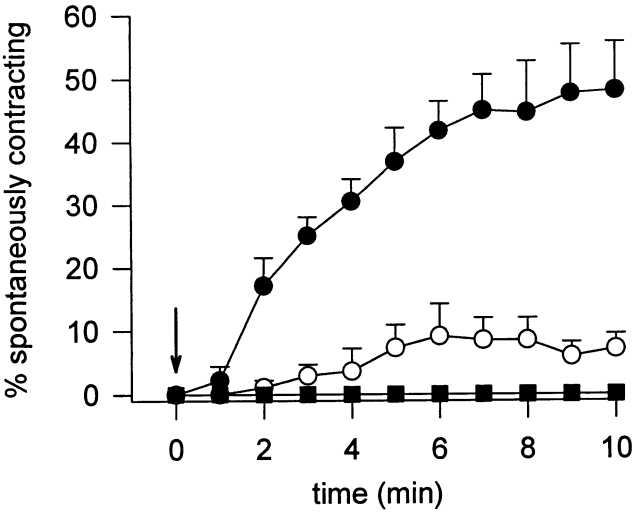

Figure 2 Time-course for the development of isoproterenol-induced spontaneous contractile activity in adult rat ventricular myocytes. Cardiomyocytes were preincubated for 5 minutes in either 10 μM docosanoic acid (●, $n = 5$), 10 μM docosahexaenoic acid (DHA; ○, $n = 5$), or 10 mM benzyl alcohol (■, $n = 3$). Following the addition of 10 μM isoproterenol to the superfusing medium (indicated by the arrow), the number of spontaneously contracting cardiomyocytes was determined over 10 minutes as the percentage of cells exhibiting rapid spontaneous contractions (usually at a rate of 1–4 spontaneous contractions per second). Each value represents the mean ± SEM.

contractions at a rate of usually one to four contractions per second. However, 5 minutes of preincubation with 10 μM DHA prevented the development of spontaneous contractions (*Figure 1B*). The time course for the percentage of cardiomyocytes spontaneously contracting is shown in *Figure 2*, which compares the effects of the saturated fatty acid docosanoic acid ($n = 5$), DHA ($n = 5$), and the membrane fluidizing agent benzyl alcohol ($n = 3$). The percentage of cardiomyocytes treated with the saturated fatty acid docosanoic acid increased to 48.1 ± 7.7% after 10 minutes of isoproterenol incubation. However, treatment with DHA (7.1 ± 2.4%, $P < 0.01$) or benzyl alcohol significantly prevented isoproterenol-induced spontaneous contractile activity (*Figure 2*). The effect of benzyl alcohol could be rapidly reversed by washout with Tyrode buffer.[12]

Following 5 minutes of preincubation of cardiomyocytes with lysophosphatidylcholine, the number of cardiomyocytes spontaneously contracting was also dependent on the nature of fatty acid pretreatment. *Figure 3* demonstrates that following DHA preincubation the number of cardiomyocytes spontaneously contracting was significantly lower compared with stearic acid (saturated fatty acid) preincubation (17.7 ± 6.5%, $n = 4$ and 78.0 ± 7.2%, $n = 4$, respectively; $P < 0.01$). Furthermore, the percentage of cardiomyocytes exhibiting asynchronous contractile activity (more than 1 contraction per second in the presence of electrical field stimulation applied at a stimulation frequency of 1 Hz at 25 V) following DHA preincubation was also significantly lower than that of stearic acid preincubation (32.7 ± 8.2 versus 84.5 ± 7.1; $P < 0.05$). Oleic acid (18:1, n-9) preincubation (data not shown) resulted in a similar percentage of cardiomyocytes spontaneously or asynchronously contracting compared with that obtained following stearic acid preincubation of cardiomyocytes.

To determine the effects of DHA and benzyl alcohol on sarcolemmal membrane fluidity, r_{ss} of TMAP-DPH was measured. *Figure 4* shows the r_{ss} values of cardiomyocytes following treatment with DHA or benzyl alcohol incubation at several concentrations. DHA significantly decreased the r_{ss} value of TMAP-DPH (i.e., increased membrane fluidity)

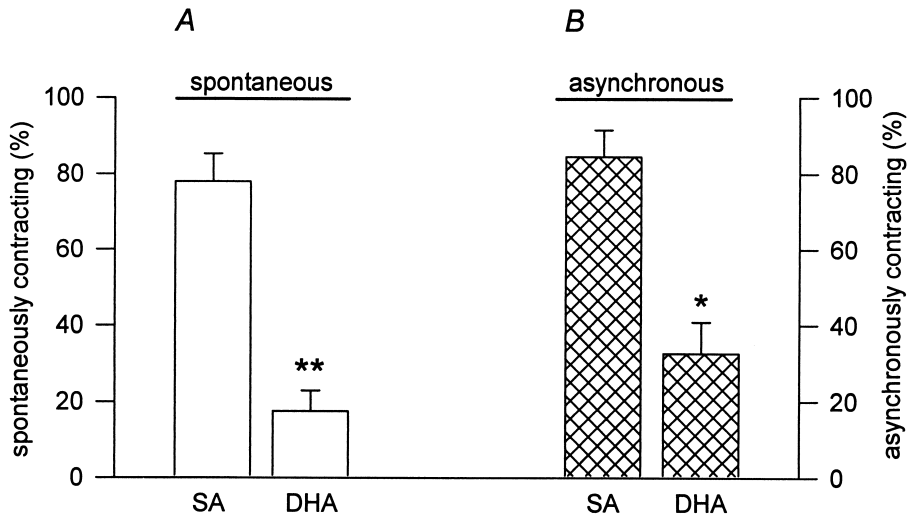

Figure 3 Effects of stearic acid (SA) and docosahexaenoic acid (DHA) on the percentage of cardiomyocytes exhibiting lysophosphatidylcholine-induced (*A*; open bars) spontaneous contractile activity or (*B*; hatched bars) asynchronous contractile activity in the presence of electrical field stimulation in adult rat ventricular myocytes. Cardiomyocytes were preincubated for 5 minutes in either 10 μM SA ($n = 4$) or 10 μM DHA ($n = 4$). Following 5 minutes of lysophosphatidylcholine (10 μM) superfusion, the percentage of spontaneously-contracting cardiomyocytes was determined as the number of cells exhibiting rapid spontaneous contractions (in the absence of electrical field stimulation; usually at a rate of 1–4 spontaneous contractions per second) or asynchronous contractions (contractions exceeding the rate of electrical-field stimulation of 1 Hz). Each value represents the mean ± SEM. *$P < 0.05$, **$P < 0.01$.

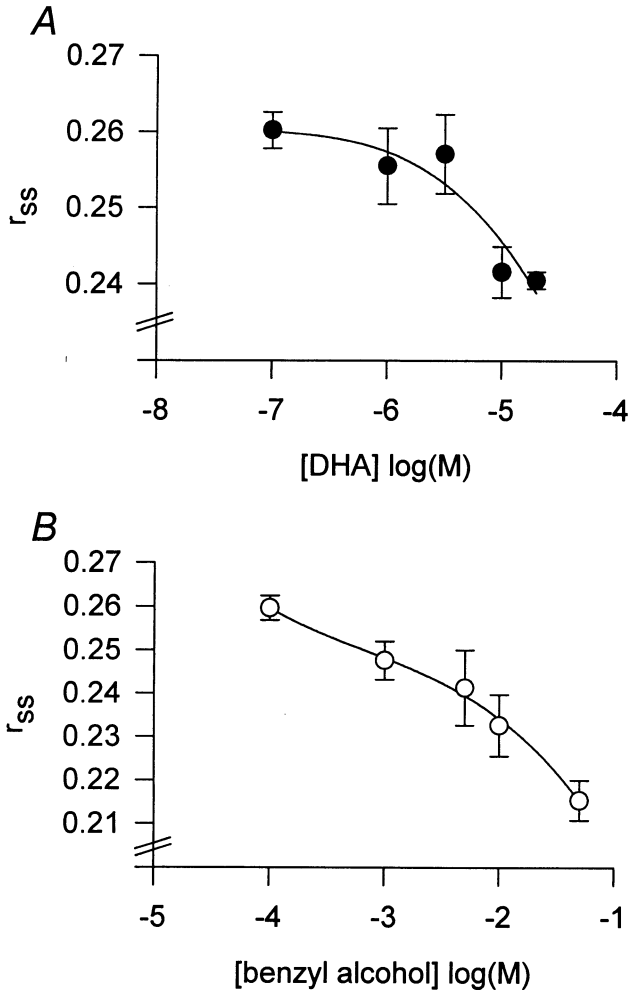

Figure 4 The effect of various agents on adult rat ventricular myocyte sarcolemmal membrane fluidity. Steady-state fluorescence anisotropy (r_{ss}) was determined as described in the methods using the probe N-((4-(6-phenyl-1,3,5-hexatrienyl)phenyl)propyl) trimethyl-ammonium p-toluene-sulfonate (TMAP-DPH) following 10 minutes of treatment in either (*A*) docosahexaenoic acid (DHA, $n = 6$) or (*B*) benzyl alcohol ($n = 6$). Each r_{ss} value represents the mean ± SEM.

in cardiomyocyte sarcolemmal membranes from 0.260 ± 0.002 (no additions, $n = 6$) to 0.241 ± 0.003 with 10 μM DHA ($n = 6$, $P < 0.01$). Other fatty acids tested by us previously,[12] including the DHA methyl ester and the saturated fatty acids docosanoic acid and stearic acid (data not shown), did not alter the membrane fluidity when compared with control (no additions). However, benzyl alcohol (at 10 mM) significantly decreased the r_{ss} value from 0.259 ± 0.003 (no additions, $n = 6$) to 0.232 ± 0.007 ($n = 6$, $P < 0.05$). The decreased value for r_{ss} would indicate that both DHA and benzyl alcohol increased the fluidity (decreased anisotropy) in adult rat cardiomyocyte sarcolemmal membranes.

Discussion

This study demonstrated that in Ca^{2+}-tolerant ventricular myocytes isolated from adult rats, spontaneous contractile activity elicited in normally quiescent cardiomyocytes by isoproterenol or lysophosphatidylcholine could be prevented by acute addition of the n-3 PUFA DHA. DHA also increased cardiomyocyte sarcolemmal membrane fluidity as measured by r_{ss} using the fluorescent probe TMAP-DPH. As such, a mechanism for this effect on cardiomyocyte contractility may involve effects of DHA on sarcolemmal membrane fluidity, a proposal supported by the similar effects observed using the membrane fluidizing agent benzyl alcohol.

The results of the present study are in general agreement with those reported by Leaf and colleagues using spontaneously contracting, cultured neonatal rat cardiomyocytes treated acutely with n-3 PUFAs.[8,9,11,17] Together with their results, our results show that cardiomyocytes isolated from adult rats respond in a manner similar to those from neonatal rat hearts with regard to the effects of acute addition of n-3 PUFAs. Kang and Leaf[18] reported that the acute administration of the n-3 PUFAs are likely to be exerting their effects in neonatal cardiomyocytes via changes in membrane ion channel activity, particularly those involving Ca^{2+} and Na^{+} currents, whereas others have reported effects of n-3 PUFAs on K^{+} currents.[19] Our results confirm our previous studies that electrically-field stimulated adult rat cardiomyocytes are protected from developing asynchronous contractile activity elicited by isoproterenol by acute addition of n-3 PUFAs,[12,13] and these effects may be due to changes in the excitability of the cells induced by changes in cell membrane fluidity. In this regard it would seem that freshly isolated cardiomyocytes from the adult rat behave in a similar manner to cultured cardiomyoctes from neonatal rats with respect to the effect of n-3 PUFAs. Considerable differences exist between adult and neonatal cardiomyocytes including changes in excitation-contraction coupling[20] and ionic currents, which are also subject to considerable modulation during development.[21] It may well have been expected that the influence of n-3 PUFAs on the above parameters may have been different between these cell types. Therefore, in light of these physiologic differences between the cell types studied, we considered it important to document the above findings.

Elevated $[Ca^{2+}]_i$ levels play a significant role in the progression of triggered cardiac arrhythmia following ischemia and reperfusion after exposure to catecholamines and digitalis.[22] Specifically, early afterdepolarizations elicited by β-adrenergic receptor stimulation are believed to be an underlying mechanism of ventricular tachycardia.[23] Lysophosphatidylcholine has been reported to accumulate in ischemic myocardium and exert deleterious effects on membrane integrity causing electrophysiologic alterations and mechanical dysfunction leading to contracture.[24,25] In addition, lysophosphatidylcholine induces Ca^{2+} overload in isolated cardiomyocytes[26] and may play an important role in ischemia-reperfusion injury. Many clinically encountered arrhythmias result from the phenomenon of reentry and this can arise in many areas of the heart.[22,27] Therefore, the normal propagation of the impulse conduction wave through areas of the functional syncytium can be perturbed in such areas due to damage arising from, for example, heart failure or the imposition of ischemia. Because the imposition of arrhythmic stimuli can induce normally quiescent

cells to beat spontaneously, this indicates that single cardiomyocytes have the potential to develop automaticity in the whole heart. This may give rise to regions of abnormal excitability and conductivity in the working myocardium, thus generating arrhythmias. Therefore, agents (such as n-3 PUFAs) that have been shown to protect isolated cells from displaying aberrant contractile behavior may, in the above situation, work to protect these cells from functioning abnormally in the working syncytia.

Acute addition of the n-3 PUFA DHA as the free acid but not as the methyl ester form (data not shown) was able to prevent spontaneous contractile activity from occurring. At the concentrations of n-3 PUFAs used in this study, rapid effects were observed, inferring that transfer of the free fatty acids from the superfusion medium to the cardiomyocyte sarcolemmal membrane also occurs in a very short time. Thus, it is unlikely that incorporation of free fatty acids into membrane phospholipids occurred within this time, a notion supported by the results of Weylandt et al.[10] Indeed, this is also supported by other studies demonstrating that acutely administered n-3 PUFAs do not appear to require esterification into membrane phospholipids for their antiarrhythmic actions to be apparent.[8–11] However, some form of specific membrane orientation, possibly involving anchoring of the charged end of the molecule at the membrane/water interface, may be required for these nonesterified fatty acids to elicit their effect. Furthermore, in this orientation, their effect appears to be one of reducing the excitability of the cardiomyocyte sarcolemmal membrane, preventing aberrant action potentials from occurring. In this and other studies, such effects were clearly evident by the need to increase the stimulating voltage following addition of the n-3 PUFAs.[12]

In addition to the effects of DHA on suppressing spontaneous contractile activity in adult rat cardiomyocytes, DHA also influenced cardiomyocyte sarcolemmal membrane fluidity measured by steady state fluorescence using the fluorescent probe TMAP-DPH. A mechanism for the antiarrhythmic effect of the n-3 PUFAs that may involve effects on sarcolemmal membrane fluidity also is supported by the effects of the membrane fluidizing agent benzyl alcohol. This effect on membrane fluidity may also induce a general effect on cell membrane potential and elicit more specific effects on voltage-sensitive Ca^{2+} and Na^{+} currents that have been reported recently.[28,29] In this context, ion channel activity is very much a membrane-associated, lipid-dependent process that may be modulated by changes in membrane fluidity.[30] In addition, we have also demonstrated inhibitory effects of benzyl alcohol and DHA on inward Na^{+} currents and single Na^{+} channels in adult rat cardiomyocytes (manuscript submitted).

The cell system utilized in this study using acutely applied n-3 PUFAs to mimic the action of antiarrhythmic agents contrasts with other studies in which dietary n-3 PUFAs, incorporated into cell membrane phospholipids, exhibit antiarrhythmic effects in experimental animal models.[5–7] However, the two systems are not mutually exclusive and the antiarrhythmic action of dietary n-3 PUFAs may proceed via similar mechanisms in both situations. As pointed out by Nair et al.,[31] incorporation of dietary n-3 PUFAs and their longer chain metabolites into cell membrane phospholipids may provide a pool of n-3 PUFAs that can be liberated by phospholipase action following the imposition of arrhythmogenic stimuli. The resulting nonesterified fatty acids could then exert antiarrhythmic effects in a manner similar to that described in this study and in the studies using neonatal rat cardiomyocytes.[8–11]

Conclusions

The results of the present study, which show a potent effect of the n-3 PUFAs on cardiac contractility at the cellular level possibly mediated by effects on membrane fluidity, provide the beginnings of a mechanistic explanation for the antiarrhythmic effects of n-3 PUFAs reported from various epidemiologic and clinical studies and studies with experimental animals.[1–3,32]

References

1 Siscovick, D.S., Raghunathan, T.E., King, I., Weinmann, S., Wicklund, K.G., Albright, J., Bovbjerg, V., Arbogast, P., Smith, H., Kushi, L.H., Cobb, L.A., Copass, M.K., Psaty, B.M., Lemaitre, R., Retzlaff, B., Childs, M., and Knopp, R.H. (1995). Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* **274,** 1363–1367

2 Kromhout, D., Bosschieter, E.B., and de Lezenne Coulander, C. (1985). The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *New Engl. J. Med.* **312,** 1205–1209

3 Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C., and Deadman, N.M. (1989). Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and reinfarction trial (DART). *Lancet* **334,** 757–761

4 Abeywardena, M.Y. and Charnock, J.S. (1995). Dietary lipid modification of myocardial eicosanoids following ischemia and reperfusion in the rat. *Lipids* **30,** 1151–1156

5 McLennan, P.L., Abeywardena, M.Y., and Charnock, J.S. (1988). Dietary fish oil prevents ventricular fibrillation following coronary artery occlusion and reperfusion. *Am. Heart. J.* **116,** 709–717

6 McLennan, P.L., Bridle, T.M., Abeywardena, M.Y., and Charnock, J.S. (1993). Comparative efficacy of n-3 and n-6 polyunsaturated fatty acids in modulating ventricular fibrillation threshold in marmoset monkeys. *Am. J. Clin. Nutr.* **58,** 666–669

7 Pepe, S. and McLennan, P.L. (1996). Dietary fish oil confers direct antiarrhythmic properties on the myocardium of rats. *J. Nutr.* **126,** 34–42

8 Leaf, A. and Kang, J.X. (1996). Prevention of cardiac sudden death by N-3 fatty acids: A review of the evidence. *J. Intern. Med.* **240,** 5–12

9 Kang, J.X. and Leaf, A. (1996). Protective effects of free polyunsaturated fatty acids on arrhythmias induced by lysophosphatidylcholine or palmitoylcarnitine in neonatal rat cardiac myocytes. *Eur. J. Pharmacol.* **297,** 97–106

10 Weylandt, K.H., Kang, J.X., and Leaf, A. (1996). Polyunsaturated fatty acids exert antiarrhythmic actions as free acids rather than in phospholipids. *Lipids* **31,** 977–982

11 Kang, J.X. and Leaf, A. (1995). Prevention and termination of beta-adrenergic agonist-induced arrhythmias by free polyunsaturated fatty acids in neonatal rat cardiac myocytes. *Biochem. Biophys. Res. Commun.* **208,** 629–636

12 McMurchie, E.J., Leifert, W.R., and Head, R.J. (1999). Antiarrhythmic properties of n-3 fatty acids in cardiomyocytes: A role for membrane fluidity? In *Essential Fatty Acids and Eicosanoids* (R.A. Riemersma, R.A. Armstrong, R.W. Kelly, and R. Wilson, eds.), pp. 284–289, AOCS Press, Champaign, IL, USA

13 Leifert, W.R., McMurchie, E.J., and Head, R.J. (1997). Prevention of asynchronous beating in adult rat cardiomyocytes by acute addition of n-3 polyunsaturated fatty acids. *J. Mol. Cell. Cardiol.* **29,** A323

14 Leifert, W.R., Jahangiri, A., and McMurchie, E.J. (1999). Antiarrhythmic fatty acids and antioxidants in animal and cell studies. *J. Nutr. Biochem.* **10,** 252–267

15 de Jonge, H.W., Dekkers, D.H., Bastiaanse, E.M., Bezstarosti, K., van der Laarse, A., and Lamers, J.M. (1996). Eicosapentaenoic acid incorporation in membrane phospholipids modulates receptor-mediated phospholipase C and membrane fluidity in rat ventricular myocytes in culture. *J. Mol. Cell. Cardiol.* **28,** 1097–1108

16 Lentz, B.R. (1989). Membrane "fluidity" as detected by diphenylhexatriene probes. *Chem. Phys. Lipids* **50,** 171–190

17 Kang, J.X. and Leaf, A. (1994). Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. *Proc. Natl. Acad. Sci. USA* **91,** 9886–9890

18 Kang, J.X. and Leaf, A. (1996). Antiarrhythmic effects of polyunsaturated fatty acids. Recent studies. *Circulation* **94,** 1774–1780

19 Bogdanov, K.Y., Spurgeon, H.A., Vinogradova, T.M., and Lakatta, E.G. (1998). Modulation of the transient outward current in adult rat ventricular myocytes by polyunsaturated fatty acids. *Am. J. Physiol.* **274,** H571–H579

20 Klitzner, T.S. (1991). Maturational changes in excitation-contraction coupling in mammalian myocardium. *J. Am. Coll. Cardiol.* **17,** 218–225

21 Wetzel, G.T. and Klitzner, T.S. (1996). Developmental cardiac electrophysiology recent advances in cellular physiology. *Cardiovasc. Res.* **31,** E52–E60

22 Katz, A.M. (1977). Ionic and pharmacological actions on cardiac rate and rhythm. In *Physiology of the Heart.* (P.J. Goodhart and H.L. Goodhart, eds.), pp. 229–256, Raven Press, New York, NY, USA

23 De Ferrari, G.M., Viola, M.C., D'Amato, E., Antolini, R., and Forti, S. (1995). Distinct patterns of calcium transients during early and delayed afterdepolarizations induced by isoproterenol in ventricular myocytes. *Circulation* **91,** 2510–2515

24 Sedlis, S.P., Hom, M., Sequeira, J.M., Tritel, M., Gindea, A., Ladenson, J.H., Jaffe, A.S., and Esposito, R. (1997). Time course of lysophosphatidylcholine release from ischemic human myocardium parallels the time course of early ischemic ventricular arrhythmia. *Coron. Artery Dis.* **8,** 19–27

25 Liu, S.Y., Yu, C.H., Hays, J.A., Panagia, V., and Dhalla, N.S. (1997). Modification of heart sarcolemmal phosphoinositide pathway by lysophosphatidylcholine. *Biochim. Biophys. Acta* **1349,** 264–274

26 Chen, M., Hashizume, H., and Abiko, Y. (1996). Effects of beta-adrenoceptor antagonists on Ca(2+)-overload induced by lysophosphatidylcholine in rat isolated cardiomyocytes. *Br. J. Pharmacol.* **118,** 865–870

27 Opie, L.H. (1998). Myocardial reperfusion: New ischaemic syndromes. In *The Heart. Physiology, from Cell to Circulation.* pp. 589–606, Lippincott-Raven, Philadelphia, PA, USA

28 Xiao, Y.F., Kang, J.X., Morgan, J.P., and Leaf, A. (1995). Blocking effects of polyunsaturated fatty acids on Na^+ channels of neonatal rat ventricular myocytes. *Proc. Natl. Acad. Sci. USA.* **92,** 11000–11004

29 Pepe, S., Bogdanov, K., Hallaq, H., Spurgeon, H., Leaf, A., and Lakatta, E. (1994). Omega 3 polyunsaturated fatty acid modulates dihydropyridine effects on L-type Ca^{2+} channels, cytosolic Ca^{2+}, and contraction in adult rat cardiac myocytes. *Proc. Natl. Acad. Sci. USA* **91,** 8832–8836

30 McMurchie, E.J. (1988). Dietary lipids and the regulation of membrane fluidity and function. In *Physiological Regulation of Membrane Fluidity* (R.C. Aloia, C.C. Curtain, and L.M. Gordon, eds.), pp. 189–237, Alan R. Liss Inc., New York, NY, USA

31 Nair, S.S., Leitch, J.W., Falconer, J., and Garg, M.L. (1997). Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. *J. Nutr.* **127,** 383–393

32 de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., Martin, J.L., Monjaud, I., Guidollet, J., Touboul, P., and Delaye, J. (1994). Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **343,** 1454–1459