Ultrafast sodium channel block by dietary fish oil prevents dofetilide-induced ventricular arrhythmias in rabbit hearts

K. S. Dujardin,1 B. Dumotier,3 M. David,4* M. Guizy,4* C. Valenzuela,4 and L. M. Hondeghem2

1Heilig Hart Klinik, Division of Cardiovascular Diseases, Roeselare; 2Department of Pharmacology, Katholieke Universiteit Leuven, Leuven, Belgium; 3Novartis Pharma Aktiengesellschaft, Exploratory Development, Basel, Switzerland; 4Instituto de Investigaciones Biomédicas “Alberto Sols” Consejo Superior de Investigaciones Científicas/Universidad Autónoma de Madrid, Madrid, Spain

Submitted 21 October 2007; accepted in final form 28 July 2008

Dujardin KS, Dumotier B, David M, Guizy M, Valenzuela C, Hondeghem LM. Ultrafast sodium channel block by dietary fish oil prevents dofetilide-induced ventricular arrhythmias in rabbit hearts. Am J Physiol Heart Circ Physiol 295: H1414–H1421, 2008. First published August 1, 2008; doi:10.1152/ajpheart.01219.2007.—Several epidemiologic and clinical studies show that following myocardial infarction, dietary supplements of ω-3 polyunsaturated fatty acids (ω3FA) reduce sudden death. Animal data show that ω3FA have antiarrhythmic properties, but their mechanisms of action require further elucidation. The effects of ω3FA supplementation were studied in female rabbits to analyze whether their antiarrhythmic effects are due to a reduction of triangulation, reverse use-dependence, instability, and dispersion (TRIaD) of the cardiac action potential (TRIaD as a measure of proarrhythmic effects). In Langendorff-perfused hearts challenged by a selective rapidly activating delayed rectifier potassium current inhibitor that has been shown to exhibit proarrhythmic effects (dofetilide; 1 to 100 nM), ω3FA pretreatment (30 days; n = 6) prolonged the plateau phase of the monophasic action potential; did not slow the terminal fast repolarization; reduced the dofetilide-induced prolongation of the action potential duration; reduced dofetilide-induced triangulation; and reduced dofetilide-induced reverse use-dependence, instability of repolarization, and dispersion. Dofetilide reduced excitability in ω3FA-pretreated hearts but not in control hearts. Whereas torsades de pointes (Tdp) were observed in five out of six in control hearts, none were observed in ω3FA-pretreated hearts. Docosahexaenoic acid (DHA) inhibited the sodium current with ultrafast kinetics. Dietary ω3FA supplementation markedly reduced dofetilide-induced TRIaD and abolished dofetilide-induced Tdp. Ultrafast sodium channel block by DHA may account for the antiarrhythmic protection of the dietary supplements of ω3FA against dofetilide-induced proarrhythmia observed in this animal model.

antiarrhythmia agents; omega-3 fatty acids; ion channels; torsades de pointes

ORAL SUPPLEMENTATION WITH fish oils or ethyl esters of ω-3 polyunsaturated fatty acids (ω3FA) are associated in some large clinical studies with significant risk reductions of sudden cardiac death, which, although not directly compared in a head-to-head trial, appear larger than those observed with amiodarone in similar study populations, with less toxicities (1, 2, 9, 11, 14, 27). However, controversy exists about the pro- or antiarrhythmic effects of ω3FA (8, 35). Antiarrhythmic effects of ω3FA have been reported in animal (5, 33) and in cellular models (29). Similar to amiodarone, it has been reported that ω3FA block sodium (42), calcium (41), and potassium channels (17, 24, 26); likewise, they also exhibit antiadrenergic actions (36). In contrast with amiodarone, at therapeutic concentrations, ω3FA do not widen QRS or prolong the QT interval (13). Many class III antiarrhythmic drugs such as dofetilide, as well as noncardiac drugs, can be associated with a prolongation of the QTc interval of the electrocardiogram (ECG) and development of polymorphic ventricular arrhythmias torsades de pointes (TdP), an arrhythmia where the ECG exhibits characteristic twisting undulations of the cardiac activations. Therefore, QT prolongation itself is regarded by some as a proarrhythmic liability (37). Changes of the QT interval on the surface ECG generally reflect changes of the plateau and the repolarization phase of the action potential but can also be affected by changes in the conduction and dispersion of the APD. It thus follows that the QT interval can be lengthened by slowed repolarization and sometimes by slowed conduction and dispersion of the action potentials as well. Slowed repolarization gives the action potential a more triangular shape, referred to as triangulation (21). To be effective against tachyarrhythmias, drug-induced prolongation of action potential duration (APD) should be most marked during tachycardia; instead many drugs prolong the APD little during tachycardia, but primarily at slow heart rates. This is termed reverse use-dependence (21), which inherently leads to the instability of APD (38). Finally, all three mechanisms result in spatial and temporal dispersion. Triangulation, reverse use-dependence, instability, and dispersion are referred to as TRIaD. APD prolongation without TRIaD can be antiarrhythmic, whereas drugs inducing TRIaD are proarrhythmic, inducing preferentially TdP when APD is prolonged but ventricular fibrillation when APD is shortened (20, 21, 38).

Therefore, in the present study we investigated the effects of dietary supplements of esterified ω3FA: 1) how do they block sodium channels without widening the QRS, 2) what are their effects upon TRIaD, 3) can they antagonize the proarrhythmic effects of drugs that increase TRIaD (e.g., dofetilide), 4) by what mechanism(s) might they be considered a powerful antiarrhythmic alternative, and 5) under what conditions might they be less effective.

MATERIALS AND METHODS

The effects of ω3FA feeding as used in the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI Prevenzi-
one study) (14) were studied in 2.5-kg female rabbits. Rabbits were selected because, as in humans, the main repolarizing current in the ventricles is the rapidly activating delayed rectifier potassium current (\(I_{Kr}\)). Females were selected because, as in humans, they appear more sensitive to the development of TdP (30). Other species were deemed less appropriate because the rat has the transient outward potassium current (\(I_{to}\)) current as the primary repolarizing current, whereas dogs and guinea pigs have much slower delayed rectifier potassium current (\(I_{Ks}\)) in addition to \(I_{Kr}\), which may obscure any proarrhythmia; dog hearts are also too large for easy Langendorff perfusion. The rabbits were fed with standard Lapina (Quartes, Belgium) at libitum. Six hearts from animals fed with food enriched with 15 mg \(\omega-3\)FA-kg \(^{-1}\) day \(^{-1}\) for 30 days [mixing daily 55% eicosapentaenoic acid ester (EPA-EE) and 45% docosahexaenoic acid ethyl ester (DHA-EE) to their food as required; Omacor; Solvay] were compared with six control hearts. The investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and was approved by the Veterinary Department of the Belgian Government. Following stunning by the captivating bolt, the heart was quickly removed and perfused in the Langendorff mode. The His-bundle was sectioned, and the distal part was stimulated. Recording electrodes were placed under the left ventricular endocardium and on the epicardium. A grounded potassium-perfused electrode served as the reference. The heart was perfused at a constant pressure of 80 cmH\(_2\)O with a bicarbonate buffer containing (in mM) 118 NaCl, 4 KCl, 22 NaHCO\(_3\), 1.1 MgCl\(_2\), 0.4 NaH\(_2\)PO\(_4\), 1.8 CaCl\(_2\), 5 dextrose, 2 pyruvate, and 0.038 creatine. The perfusate was equilibrated with 95% O\(_2\)-5% CO\(_2\) adjusted to obtain a pH of 7.35 at 36° \(^\circ\)C. Stimulation occurred at 1.5 times the threshold stimulation current. More detailed descriptions of the experimental system were described previously (19).

Electrophysiological measurements. The experiment consisted of brief and long protocols. The brief protocols were applied every 10-100 nM. The low plasma concentrations in plasma. However, the plasma-free \(\omega-3\)FA are generally toxic to cells and are kept at low concentrations. The DMSO concentration remained 0.1% at all times.

Sodium current measurements. Human embryonic kidney (HEK)293 cells that do not express endogenously sodium channels or accessory subunits were cultured at 37° C in DMEM supplemented with penicillin-streptomycin (Sigma, London, UK), 10% bovine fetal serum, 1% antibiotic-antimycotic amino acids in a 5% CO\(_2\) atmosphere (15). Transfection of Nav1.5 channels (2 \(\mu\)g) and CD8 (1 \(\mu\)g) was performed using Lipofectamine2000 (10 \(\mu\)l). Before experimental use, the cells were incubated with polylysine microbeads precoated with anti-CD8 antibody (Dynabeads M450; Dynal Biotech, Oslo, Norway) as described (15).

The intracellular pipette filling solution contained (in mM) 5 NaCl, 5 KCl, 130 CsF, 1 MCl\(_2\), 4 Na\(_2\)ATP, 10 HEPES-K, and 5 EGTA and was adjusted to pH 7.2 with CsOH. The bath solution contained (in mM) 140 NaCl, 5 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 10 HEPES-Na, 10 glucose, 10 tetraethylammonium, and 2.8 Na-acetate and was adjusted to pH 7.4 with NaOH. DHA (Sigma, St. Louis, MO) was dissolved as previously described (17). Sodium currents were recorded at room temperature (21–23°C) using the whole cell patch-clamp technique with an Axopatch 1C patch-clamp amplifier (Axon Instruments, Foster City, CA). Micropipettes were pulled from borosilicate glass capillary tubes (GD-1; Narishige, Tokyo, Japan) on a programmable horizontal puller (P-87; Sutter Instrument, San Rafael, CA) and heat polished with a microforge (Narishige). To minimize voltage errors, micropipette resistance was 1 to 2 MΩ. Data analysis was performed using the CLAMPfit program of pCLAMP 9.0.1 and Origin 7.0.3 (Microcal Software, Northampton, MA). The curve-fitting procedure used a nonlinear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot.

Free \(\omega-3\)FA are generally toxic to cells and are kept at low micromolar concentrations in plasma. However, the plasma-free \(\omega-3\)FA concentration can vary greatly depending on the hormonal, metabolic, and nutritional state of the individual. About 99.9% of free \(\omega-3\)FA are bound to albumin in the plasma (10). The low plasma concentration of free \(\omega-3\)FA is maintained by a competition between binding sites on albumin and cell membrane phospholipids. The range of free DHA human plasma concentration is <2.8 \(\mu\)M (10). Since DHA and EPA can compete each other for binding to Nav1.5 channels, we chose to analyze only the effects produced by DHA on the current generated by their activation.

Statistical analysis. Data are expressed as means ± SE. Comparisons of two means were done using a Student’s \(t\)-test, and \(P < 0.05\) was considered significant. Instability and unexcitability were not normally distributed so that nonparametric tests were used instead. Goodness of fit was judged by the \(\chi^2\) criterion (17). Comparison of events was done with a Fisher exact test. Due to the small number of experiments (n = 6) and large variation of effects, only major drug effects can become significant.

RESULTS

Effects of dietary supplements of \(\omega-3\)FA. \(\omega-3\)FA significantly prolonged \(APD_{90}\), \(APD_{60}\), and \(APD_{30}\) (Table 1). \(APD_{30}\) was not
prolonged more than APD90 (Fig. 1), i.e., ω3FA prolonged the APD without triangulation. As expected, the prolongation of APD resulted in a significant prolongation of the effective refractory period (ERP), but this prolongation was more than twice as long as the prolongation of APD90 (Table 1). Thus additional electrophysiological change(s) must play an important role (discussed in Dofetilide leads to unexcitability in ω3FA-pretreated hearts).

At baseline (i.e., before the administration of dofetilide), ω3FA pretreatment had no significant effects on reverse use-dependence, instability, dispersion, or ectopic activity, and neither group exhibited TdP or ventricular tachycardia (VT). Threshold stimulation current and activation times were also not significantly changed (Table 1).

Dofetilide leads to unexcitability in ω3FA-pretreated hearts. All control hearts could fully execute the experiment (including 100 nM dofetilide), whereas none of the ω3FA hearts could; one heart became unexcitable at 10 nM dofetilide, and all others at 30 nM [no data at 100 nM in ω3FA-pretreated hearts (Fig. 2, right)].

Since dofetilide acts by a class III mechanism (40), pretreatment with ω3FA might emphasize the prolongation of the action potential of dofetilide and thereby lead to unexcitability. However, 10 nM dofetilide prolonged APD90 by 235 ± 75 ms in control but only by 193 ± 47 ms in ω3FA hearts (P = 0.64); 30 nM dofetilide prolonged APD90 by 402 ± 152 ms in control but only by 247 ± 107 ms in ω3FA hearts (P = 0.47). Furthermore, APD60 started to exceed 1,000 ms at 3 nM dofetilide in control hearts (Fig. 2) but only at 30 nM dofetilide in ω3FA-pretreated hearts. In control hearts there were 467 APD60 measurements exceeding 1,000 ms but only six in ω3FA hearts, whereas 26 APD60 measurements exceeded 1,500 ms in control hearts, and no such prolongations occurred in ω3FA hearts. Finally, APD60 > 1,000 ms occurred in four control hearts but was seen in only one ω3FA experiment. Thus ω3FA pretreatment clearly does not augment dofetilide-induced APD prolongation and cannot account for the unexcitability in ω3FA-pretreated hearts.

A block of sodium channels can also reduce excitability (34, 43). However, in control hearts dofetilide did not slow conduction, agreeing with reports that dofetilide does not block sodium channels. In control hearts, conduction slowed indirectly when the action potential impinged on the tail of the preceding action potential. However, in all ω3FA experiments, the slowing of conduction was already noted at short diastolic intervals, i.e., before impinging on the tail of the preceding action potential. In fact, no conduction was possible until ~30 ms following the end of the preceding action potential. Interestingly, action potentials triggered about 60 ms after the end of the action potential conducted at virtually normal velocity. Thus if this extension of refractoriness was due to block of sodium channels, then there had to be a marked block at short diastolic intervals but virtually no block at slightly longer intervals. Attempts to characterize such fast recovery appeared impossible with the MAP; the time interval over which the recovery occurred was so brief that only rarely could an intermediate conduction velocity be observed. Furthermore, in the presence of dofetilide, the variability of APD rendered it nearly impossible to hit this brief time period. Therefore, the effects of DHA on sodium current were studied with voltage clamp to be able to analyze this recovery process in a short period of time.

DHA (1 μM) reduced the sodium current only slightly when membrane potential was maintained (holding potential) at −120 mV (Fig. 3A). However, at −90 mV, it decreased significantly and at −80 mV the block became 42.0 ± 7.1% (n = 6; P < 0.05). The block increased sharply with increasing concentrations so that by 10 μM, the marked reduction of the current already developed even at −120 mV. Since we were primarily interested in therapeutic concentrations that do not reduce the current at well-polarized potentials (−120 mV), the kinetic studies were therefore done at 1 μM.

Following a 500-ms depolarizing pulse, a 10-ms test pulse was applied after various recovery times and at different holding potentials (Fig. 3B, inset). The Nav1.5 magnitude of the current recorded in the test pulse was plotted versus the time elapsed between the end of the 500-ms prepulse and the beginning of the test pulse. From these data, the recovery time constant (τre) for the sodium currents was extracted by least-square fitting of the data. At −120 mV, τre was 9.4 ± 2.3 ms and similar to that observed in control cells (9.4 ± 2.0 ms; n =

---

Table 1. Comparison of electrophysiological characteristics obtained in control and ω3FA-pretreated rabbit hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>ω3FAs</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action potential duration, ms</td>
<td>165±3.2</td>
<td>204±15.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Action potential duration, ms</td>
<td>217±5.5</td>
<td>248±10.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Action potential duration, ms</td>
<td>252±6.2</td>
<td>278±12.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Effective refractory period, ms</td>
<td>216±22.9</td>
<td>278±4.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Reverse use-dependence, ms</td>
<td>−2±0.8</td>
<td>−3±0.9</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Instability, ms</td>
<td>5±0.9</td>
<td>6±0.7</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Dispersion, ms</td>
<td>19±5.4</td>
<td>17±5.0</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ectopics per minute</td>
<td>0.5±0.13</td>
<td>0.4±0.15</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Threshold stimulation current, μA</td>
<td>146±41</td>
<td>169±37</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Activation time, ms</td>
<td>49±2.0</td>
<td>51±3.2</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6/group. P, comparison between parameters from ω-3 polyunsaturated fatty acids (ω3FA)-pretreated and control rabbits.

---

![Fig. 1. Comparison of the mean action potential of control (gray) vs. ω-3 polyunsaturated fatty acids (ω3FA)-pretreated (black) animals. In the ω3FA-pretreated animals, the plateau phase of the monophasic action potential is flatter and requires more time before the fast, final repolarization begins. APD30, APD60, and APD90, action potential duration at 30%, 60%, and 90%, respectively.](http://ajpheart.physiology.org/...).
Fig. 2. Comparison of the dofetilide-induced prolongation of APD60 in control and ω3FA-pretreated hearts. Every minute the hearts were stimulated for 30 s at a cycle length of 1,000 ms, and their APD60 is plotted as a function of time into the experiment. The first group of data points (0 to 1,700 ms) represents the drug-free period. This is followed by 1 nM (1,700 to 3,300 ms), 3 nM (3,300 to 4,900 ms), 10 nM (4,900 to 6,400 ms), 30 nM (6,400 to 8,100 ms), and finally 100 nM (8,100 to 9,000 ms) dofetilide. In the ω3FA-pretreated group, 1 heart became unexcitable during perfusion with 10 nM dofetilide, and all others during 30 nM, so that, at right, no data points could be measured at 100 nM. The 97.5% confidence interval for APD prolongation in control hearts is shown as a solid horizontal line.

5; P > 0.05). However, as the holding potential became less negative, τre markedly prolonged; by -90 mV, τre increased from 25.2 ± 5.0 ms in control to 49.0 ± 7.3 ms in 1 μM DHA (n = 6; P < 0.01). Thus recovery kinetics of Nav1.5 channels in the presence of ω3FA is an ultrafast process, i.e., not much slower than in control.

Thus block induced by 1 μM DHA was measured at the maximum peak of a 10-ms test pulse to -10 mV from different holding potentials applied after a 10- or a 500-ms prepulse. The degree of block measured at the test pulse after the 10- or 500-ms prepulse from the four different holding potentials (-120, -100, -90, and -80 mV) was similar: 12.0 ± 5.2% vs. 30.3 ± 9.7% at -120 mV (n = 6; P > 0.05), 16.8 ± 5.6% vs. 34.1 ± 8.9% at -100 mV (n = 6; P > 0.05), 18.9 ± 4.8% vs. 35.8 ± 7.2% at -90 mV (n = 5; P > 0.05), and 42.0 ± 7.1% vs. 35.4 ± 5.2% at -80 mV (n = 4; P > 0.05). Thus, upon depolarization, sodium channels quickly become blocked; there is marked block in less than 10 ms, and after only 500 ms most of the channels become blocked. Once the membrane potential becomes sufficiently negative, recovery occurs ultrafast; even at 25°C, τre becomes shorter than 50 ms.

ω3FA pretreatment prevents dofetilide-induced TdP. In control hearts, dofetilide induced TdP in five of the six hearts but in none following ω3FA pretreatment. Since the primary cause for TdP is TRiA-D (21, 38), we characterized the effects of ω3FA pretreatment upon these four parameters.

Triangulation increased with the dofetilide concentration (Fig. 4B), but in both groups starting at 10 nM it exceeded the upper 97.5% confidence limit of 29 ms previously determined in normal rabbit hearts (23). At 30 nM dofetilide, triangulation reached 222 ± 109 ms in control hearts, but it also increased to 91 ± 55 ms (P = 0.29; vs. control hearts) in ω3FA-pretreated hearts.

Reverse use-dependence increased significantly starting from 10 nM and exceeded the upper 97.5% confidence limit of 6 ms in both groups (Fig. 4A) (23). However, in control hearts, at 10 nM dofetilide, reverse use-dependence increased to 32 ± 11 ms and only to 8 ± 6 ms in ω3FA (P = 0.025; vs. control hearts). At 30 nM dofetilide, reverse use-dependence reached 47 ± 24 ms in control hearts but was only -1 ± 25 ms in ω3FA-pretreated hearts.

Instability increased to 943 ± 485 ms in control hearts and to 344 ± 156 ms in ω3FA-pretreated hearts by 10 nM dofetilide (Fig. 4C). Both values exceeded the proarrhythmic upper 97.5% confidence limit of 136 ms previously determined in normal rabbit hearts (23). At 30 nM, instability further increased to reach 3,208 ± 2,013 ms in control hearts and 498 ± 201 ms in ω3FA-pretreated hearts (P = 0.037; vs. control hearts).

Spatial and temporal dispersion of APD60 increased in control hearts and ω3FA (Fig. 4D). At 10 nM it was 60 ± 12 ms in control hearts and 41 ± 18 ms in ω3FA-pretreated hearts, both values exceeding the proarrhythmic upper 97.5% confidence interval of 23 ms, previously determined in normal rabbit hearts (23). At 30 nM, dispersion further increased to
reach 89 ± 24 ms in control hearts and 49 ± 5 ms in ω3FA-pretreated hearts (P = 0.13).

Proarrhythmia. Stalling of repolarization rate (repolarization rate = 0) (38) started at 1 nM in two of six control hearts, but stalling required 10 nM in ω3FA hearts, where it then developed in three of six hearts. EADs began at 3 nM in control hearts and at 10 nM in ω3FA. Finally, TdP and VT started to develop at 3 nM dofetilide in control hearts; moreover, dofetilide induced TdP in five out of six control hearts. In contrast with that of the ω3FA-pretreated hearts, there was a
single heart that exhibited VT at 10 and 30 nM dofetilide; most importantly, not a single TdP was observed in the six ω3FA hearts (Fisher exact test; \( P < 0.01 \)).

**DISCUSSION**

Our results demonstrate that ω3FA pretreatment completely suppresses dofetilide-induced TdP (which occurred in 5 out of 6 control hearts), does not induce TRId, and attenuates dofetilide-induced TRId and that DHA (in acute treatments) blocks sodium channels with ultrafast kinetics.

The pharmacological profile of ω3FA strikingly matches that of amiodarone: block of sodium, potassium, and calcium channels as well as α- and β-adrenoceptors (17, 24, 26, 41, 42). Most importantly, the cardioprotective effects of ω3FA observed in the GISSI Prevenzione study (14) were better than those previously reported with amiodarone (27). Our present observations may account for some of these advantages; whereas amiodarone has been found to induce some triangulation (38), ω3FA do not. Although amiodarone also has fast kinetics of recovery from sodium channel block (32), its onset and offset kinetics are still nearly one order of magnitude slower than those of ω3FA reported in the present study (38).

Since clinical use of amiodarone is hampered by its many toxicities (16), the adverse side effects of ω3FA are limited to weak inflammatory and hemostasis disorders (28). Therefore, it could be speculated that ω3FA could be a highly effective and nontoxic adjunct to other strategies for the prevention of myocardial ischemia-related sudden death. However, contrary to reports of the beneficial effects of ω3FA consumption or supplements on cardiac deaths in some populations (14), other trials have suggested a neutral effect in patients with implanted cardioverter defibrillators (7, 35) and even a deleterious effect in men with angina (8). It is unclear whether these contradictory observations on the effects of ω3FA supplementation can be accounted for by different population characteristics (e.g., angina vs. post-myocardial infarction or heart failure), possible toxic effects of mercury exposure associated with fish intake, background fish consumption in the control population, or the type of arrhythmogenic mechanism (e.g., triggered activity vs. reentry arrhythmias) (12).

The ultrafast onset and recovery kinetics of DHA sodium channel block, never observed before with other agents (≈30 ms), ensures that conduction is not slowed, except for a brief window early in diastole. Since the recovery of the sodium currents is not much slower than normal, the vulnerable period would not be widened much, as is seen with other sodium channel blockers (34); instead, it would only be delayed. At 25°C and at −90 mV, the \( \tau \) for recovery from sodium channel block is about 50 ms. As the membrane potential is made more negative, recovery becomes increasingly faster, until at −120 mV, recovery closely tracks the reactivation of control sodium channels (Fig. 3B). If this time constant were to exhibit a similar temperature dependence as is the case with other sodium channel blockers (25), then at 37°C recovery of block could easily be three times faster. Furthermore, these recovery characteristics would also be shifted by about 20 mV to more positive potentials (25). Such fast kinetics would well explain our observations that, except for a brief early diastolic window, conduction would not be slowed. However, during and before this early diastolic window, sodium channels would not be available. Hence throughout the cardiac action potential and specifically during the rapid repolarization period, no inward sodium current activation would be available. Therefore, upon reaching a normal resting potential, recovery would be complete in a time frame similar to that of reactivation in control. Thus the period of slowed conduction (partial availability of sodium channels) would be little or not prolonged, i.e., just delayed.

This profile of sodium channel block has three important clinical consequences. First, whereas in well-polarized tissue sodium channels would behave normally, except for a small delay of their availability, in ischemic tissue recovery would be very slow or may not occur at all. As a result, tissue depolarized by an ischemic event (responsible for proarrhythmia) would remain unavailable for participation in reentry. This mechanism could contribute to the clinical observation that ω3FA reduce sudden death upon reinfarction (14) but might not protect against ventricular arrhythmia in the absence of ischemia (7). Second, much evidence has accumulated that blockers of \( I_{Kr} \) are less torsadogenic provided they also block inward currents (sodium and/or calcium) at lower concentrations than those needed to block \( I_{Kr} \) (6, 23). Our results on the effects of ω3FA on sodium channels, together with those previously reported on L-type Ca\(^{2+}\) channels (41), may account for the complete suppression of dofetilide-induced TdP in ω3FA-pretreated hearts observed in the present study. Finally, it may be of interest to test in a clinical trial whether the danger of sudden death is reduced by ω3FA feeding in specific clinical diseases such as congenital long-QT syndrome type 2, characterized by human ether-a-go-go-related gene (HERG) channel dysfunction.

Dofetilide-induced TRId was attenuated by pretreatment with dietary supplements of ω3FA. Although these reductions of TRId were not large enough to stay within safe limits (21, 23, 38), nevertheless these simultaneous reductions may also have contributed to the observed reduction of dofetilide-induced TdP. However, the fact that TRId could not be completely suppressed, whereas TdP was entirely prevented, further supports that the block of sodium channels with ultrafast kinetics potentially plays a key role. If so, then ω3FA might reduce the rare occurrences of TdP and render cardiac as well as noncardiac agents with such liability safer to use.

The effects of ω3FA on the APD and, thus, indirectly on the repolarizing currents observed in this chronic rabbit model are at variance with findings in other animal species (39) in different experimental conditions using isolated cells (39) or ω-linolenic acid (4) and after acute administration at different concentrations of ω3FA (31). The rabbit animal model differs from other species in that the repolarization phase of the APD is exclusively dependent upon \( I_{Kr} \), and that could explain in part the differences observed between species. In contrast, block of sodium currents by ω3FA is consistently observed in all species.

**Limitations of the present study.** The present paper has several experimental limitations: 1) the effects of ω3FA on sodium channels were analyzed by studying the effects of DHA (not DHA + EPA) on Nav1.5 channels transiently expressed in HEK293 cells; 2) no voltage-clamp studies on myocytes obtained from ω3FA-fed rabbits were done; 3) the beneficial effects of ω3FA were demonstrated after 30 days of pretreatment, so the time course of development of this benefit
is unknown; and 4) specifically, our study cannot rule out that
the benefits might not be preceded by untoward effects (which
could help account for some of the clinical inconsistencies).
The fast sodium channel block with fast kinetics holds for
chronic exposure, but preliminary experiments suggest that
these effects are more difficult to establish in acute experiments
(unpublished observations). Additional studies will be
needed to address these limitations.

Conclusions

The cardiac effects of ω3FA resemble those of amiodarone;
both block sodium, calcium, and potassium channels, have
antiadrenergic properties, and can prolong the APD, reverse
TRIaD, and suppress TdP. The main difference is that sodium
channel block by ω3FA has a much faster onset and offset
kinetics. As a result, the electrophysiological profile of ω3FA
appears more desirable; the duration of reduced sodium current
(facilitates reentry) is much shorter. These properties, together
with the safer profile of ω3FA versus other antiarrhythmic
agents, may account for the clinical observation that risk
reduction of sudden cardiac death with dietary supplementation
of ω3FA, although not directly compared in a head-to-head
trial, appears larger than with amiodarone in post-myocardial
infarction patients (3, 14, 27). Moreover, if further research
confirms that ω3FA or some components of ω3FA may sup-
press drug-induced TdP, they could be used to salvage certain
valuable medications that would otherwise be rejected for
medical use. Finally, it would be interesting to investigate the
efficacy of the dietary supplementation of ω3FA to reduce the
arrhythmic dangers of certain clinical disease states such as
the congenital long-QT syndrome type 2, characterized by
HERG channel dysfunction.

ACKNOWLEDGMENTS

We thank to E. Beck, B. Hespel, J. Bigneron, G. Pablo and Novartis for
experimental assistance in the project.

GRANTS

This work was funded by Solvay Pharma, Novartis, Grants CICYT
SAF2004-06856 and SAF2007-65868 and Red Temática de Investigación
Cooperativa Grant FIS RD06/0014/0006.

REFERENCES

1. Albert CM, Campos H, Stampfer MJ, Ridker PM, Manson JE, Willett
   WC, Ma J. Blood levels of long-chain n-3 fatty acids and the risk of
2. Albert CM, Hennekens CH, O’Donnell CJ, Ajani UA, Carey VJ, Willett
   WC, Ruskin JN, Manson JE. Fish consumption and the risk of sudden
3. Albert CM, Manson JE, Hennekens CH, Ruskin JN. Fish consumption
   A. Dietary flaxseed protects against ventricular fibrillation induced by
   ischemia-reperfusion in normal and hypercholesterolemic rabbits. J Nutr
5. Billman GE, Kang JX, Leaf A. Prevention of sudden cardiac death by
dietary pure omega-3 polyunsaturated fatty acids in dogs. Circulation 99:
   RH, Ruffolo RR Jr. Combined potassium and calcium channel blocking
   activities as a basis for antiarrhythmic efficacy with low proarrhythmic
   risk: experimental profile of BRL-28972. J Pharmacol Exp Ther 276:
   637–646, 1996.
7. Brouwer IA, Zock PL, Camm AJ, Bocker D, Hauer RN, Wever EF,
   Dullemeijer C, Ronden JE, Katan MB, Lubinski A, Buschler H,
   Schouten EG. Effect of fish oil on ventricular tachyarrhythmia and death
   in patients with implantable cardioverter defibrillators: the Study on
   Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) randomized
8. Burr ML, Ashfield-Watt PA, Dunstan FD, Feihly AM, Breay P,
   Ashton T, Zotos PC, Haboubi NA, Elwood PC. Lack of benefit of
dietary advice to men with angina: results of a controlled trial. Eur J Clin
   PM, Elwood PC, Deadman NM. Effects of changes in fat, fish, and fibre
   intakes on death and myocardial reinfarction: diet and reinfarction trial
    I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha-linolenic
    acid-rich diet in secondary prevention of coronary heart disease. Lancet
12. Den Ruijter HM, Berecki G, Opthoft G, Verkerk AO, Zock PL,
    Coronel R. Pro- and antiarrhythmic properties of a diet rich in fish oil.
13. Geelen A, Brouwer IA, Schouten EG, Maan AC, Katan MB, Zock PL.
    Effects of n-3 fatty acids from fish on premature ventricular complexes
14. GISSI Prevenzione Investigators. Dietary supplementation with n-3
    polyunsaturated fatty acids and vitamin E after myocardial infarction:
    results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della
15. Gonzalez T, Navarro-Polanco R, Arias C, Caballero R, Moreno I,
    Delpo E, Tamargo J, Tamkun MM, Valenzuela C. Assembly with the
    Kv1.5 subunit modulates drug block of hKv15 channels. Mol Pharmacol
    PJ, Trobaugh GB. Toxic and therapeutic effects of amiodarone in the
17. Guizy M, Arias C, David M, Gonzalez T, Valenzuela C. ω-3 and ω-6
    Polyunsaturated fatty acids block HERG channels. Am J Physiol Cell
18. Hennekens KM, Auerd M. Investigation of electrical activity of the
    heart in the high frequency domain. Leuven, Belgium: Katholieke Uni-
    versiteit Leuven, 1997, p. UDC 615.84(043).
19. Hennekens KM. Computer aided development of antiarrhythmic agents
20. Hennekens KM. Thorough QTQTe not so thorough: removes torsado-
genic predictors from the T-wave, incriminates safe drugs, and misses
21. Hennekens KM, Carlsson L, Duker G. Instability and triangulation of
    the action potential predict serious proarrhythmia, but action potential
    duration prolongation is antiarrhythmic. Circulation 103: 2004–2013,
22. Hennekens KM, Hoffmann P. Blinded test in isolated female rabbit
    heart reliably identifies action potential duration prolongation and proar-
rhythmic drugs: importance of triangulation, reverse use-dependence, and
23. Hennekens KM, Lu HR, van Rossem K, De Clerck F. Detection of
    proarrhythmia in the female rabbit heart: blinded validation. J Cardiovasc
    blocking of the major cardiac delayed-rectifier K+ channel (Kv1.5) by
    polyunsaturated fatty acids. Proc Natl Acad Sci USA 91: 1937–1941,
    1994.
25. Johns JA, Anno T, Bennett PB, Snyders DJ, Hennekens LM. Tem-
    perature and voltage dependence of sodium channel blocking and unblock-
    ing by O-demethyl encaïnide in isolated guinea pig myocytes. J Cardio-
    Guenney JY. Peroxidation of docosahexaenoic acid is responsible for its
    effects on TTO and I SS in rat ventricular myocytes. Br J Pharmacol 139:
    Simon P. Randomised trial of effect of amiodarone on mortality in
    patients with left-ventricular dysfunction after recent myocardial infar-

AJP-Heart Circ Physiol • VOL 295 • OCTOBER 2008 • www.ajpheart.org

Downloaded from http://alphaprotein.physiology.org/ by 10.220.33.5 on July 1, 2017


