Influence of Dietary Fat on the Pharmacodynamics of Propafenone in Isolated, Perfused Rabbit Hearts

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Background. The fatty acid composition of the phospholipids in sarcolemma may significantly influence cell membrane functions. We evaluated the effects of dietary fat on the pharmacodynamics of the antiarrhythmic drug propafenone in isolated, perfused rabbit hearts.

Methods and Results. Three groups of weanling rabbits (n=9 each group) were fed diets of 10% wt/wt lard, fish oil, or safflower oil for 40 days. Differences in electrophysiological variables were assessed at baseline and during propafenone perfusion. Myocardial concentration–effect relations were determined by plotting electrophysiological effects versus coronary sinus propafenone concentrations. The linoleic acid content of isolated sarcolemma was higher in the safflower group (33.4±11.4%) than in the lard (13.4±2.3%, p<0.01) and fish oil (8.5±1.4%, p<0.01) groups, whereas the ω-3 fatty acid content was higher in the fish oil group (p<0.01). During propafenone perfusion, greater changes in ventricular conduction time were observed in the lard group (22±11 msec) than in the safflower oil group (10±7 msec, p<0.05), whereas changes in ventricular conduction time in the fish oil group (16±7 msec) were intermediate between the lard and safflower oil groups. The slopes of the linear myocardial concentration–effect relations describing changes in QRS duration were steeper in the lard group (0.22±0.07 msec/lug/ml) than in the safflower oil group (0.13±0.04 msec/lug/ml, p<0.01) but not in the fish oil group (0.17±0.08 msec/lug/ml, p=NS). Strength–interval curves were similar at baseline in all three groups. During propafenone perfusion, the threshold current was increased significantly at long coupling intervals (250–380 msec) in the lard group (1.8±1.0 mA) compared with the safflower oil group (0.8±0.6 mA, p<0.05) but not compared with the fish oil group (1.2±0.6 mA, p=NS).

Conclusions. Dietary fat significantly alters the fatty acid composition of the phospholipids in sarcolemma. Propafenone effects on ventricular conduction time and ventricular excitability are significantly influenced by the type of dietary fat. (Circulation 1992;85:1501–1509)

KEY WORDS: ● electrophysiology ● fatty acids ● sarcolemma ● antiarrhythmias

Epidemiological studies have suggested that diets high in unsaturated fats reduce cardiovascular morbidity and mortality from coronary artery disease.1-4 In addition to effects on atherogenesis, dietary fat may influence the genesis of ventricular arrhythmias. McLennan et al5,6 demonstrated a decreased incidence of ventricular arrhythmias during transient or permanent occlusion of coronary arteries in rats fed diets high in unsaturated fats compared with those fed diets high in saturated fat. Furthermore, the incidence of ventricular arrhythmias during reperfusion was reduced in rats fed a fish oil (ω-3) diet but not in rats fed a vegetable oil (ω-6) diet.6 A recent prospective randomized trial showed that patients who were advised to eat fish two or three times a week following a recent myocardial infarction experienced a significant reduction in cardiovascular mortality but not recurrent myocardial infarction.7 It is possible that eating fish prevented fatal ventricular arrhythmias from occurring during ischemia.

It is now recognized that the fatty acid composition of phospholipids in cell membranes can influence cell functions such as membrane permeability to ions,8,9 receptor function,10,11 and membrane-bound enzyme activities.12,13 Some information about the effects of dietary fat on cardiac electrophysiological parameters is available. In isolated myocytes, increasing the linoleic acid content of the sarcolemma has been associated with more rapid repolarization of the action potential,14 whereas depletion of cholesterol content in sarcolemma is associated with more rapid spontaneous depolarization.15 Less shortening of the action potential duration on exposure to hypoxia has been observed in myocytes that have been cultured in media enriched in ω-6 polyunsaturated fatty acids compared with myocytes that have been cultured in media enriched in ω-3 polyunsaturated fatty acids. However, the recovery of the hypoxia-induced shortening of the action potential duration is greater in the myocytes cultured in the ω-3

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fatty acid–rich media after exposure to normoxic conditions. Because significant shortening of the action potential duration may contribute to the development of arrhythmias, these differences may explain the reduction in reperfusion arrhythmias observed in rats fed a fish oil diet compared with those fed a sunflower seed oil diet. The direct exposure of long-chain fatty acids, including arachidonic acid, to nerve axons has been reported to suppress sodium currents in nerve axons. Other investigators have reported that arachidonic acid activates a potassium channel in rat atrial cells and in rat ventricular myocytes. The significance of these latter observations is unclear since fatty acids incorporated into phospholipids of the membrane bilayer may have different effects on membrane proteins than do free fatty acids.

It is presently unknown whether fatty acids modify the electrophysiological effects of antiarrhythmic drugs. We hypothesized that the effects of antiarrhythmic drugs are significantly influenced by the fatty acid composition of phospholipids in the sarcolemma. Accordingly, the purposes of the present study were 1) to evaluate the influence of dietary fat on the fatty acid composition of phospholipids in rabbit sarcolemma, 2) to evaluate the influence of dietary fat on baseline cardiac electrophysiological parameters, and 3) to determine the effects of dietary fat on the pharmacodynamics of the antiarrhythmic drug propafenone.

Methods

Experimental Diets

Weanling New Zealand White rabbits (456±44 g) were divided into three groups and fed diets supplemented with lard, fish oil, or safflower oil for 40 days. Ground rabbit chow (16% Rabbit Pellets, United Grain Growers Ltd.) was mixed with 10% wt/wt lard (ICN Biomedicals, Canada, Ltd.), 10% wt/wt fish oil (Promega, Parke-Davis, USA) or 10% wt/wt safflower oil (ICN Biomedicals, Canada, Ltd.). The antioxidant tertiary butyl hydroquinone (0.04%) was added to each mixture. Diets were prepared fresh each week and stored at 5°C until use. Food intake was measured daily, and feedings were adjusted such that animals consumed approximately the same amount daily. The feed was changed daily, and any uneaten portion was discarded. Animals were weighed weekly.

The fatty acid composition of the lipid in the experimental diets is shown in Table 1. The lard diet consisted primarily of unsaturated and monosaturated fats. The safflower oil diet consisted primarily of linoleic acid. The fish oil diet consisted primarily of polyunsaturated fatty acids with an increased proportion of ω-3 fatty acids.

Experimental Preparation

On the day the rabbits were to be killed, they were pretreated with heparin sulfate (75 units/kg) and then anesthetized with sodium pentobarbital (30–40 mg/kg). Hearts were rapidly removed through a median sternotomy incision and perfused retrogradely via the aorta. The pulmonary veins and venae cavae were ligated, and the pulmonary artery was cannulated for collection of coronary sinus effluent. Hearts were perfused with a modified Krebs-Henseleit buffer consisting of (mM) NaCl 118, KCl 3.3, CaCl₂ 2.0, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24, glucose 10, and Na pyruvate 2.0 and albumin 20 mg/12. The buffer was equilibrated with 95% O₂–5% CO₂, pH of the perfusate was 7.4±0.02, and temperature was 37°C. The perfusate flow rate was maintained constant at 19 ml/min throughout all studies.

Hearts were atri ally paced at a cycle length of 400 msec (pulse width, 2.0 msec; twice diastolic threshold intensity) via a bipolar platinum electrode positioned in the region of the sinus node. A bipolar platinum electrode was also positioned on the lateral wall of the left ventricle for ventricular stimulation protocols. Platinum electrodes were positioned on the epicardial surface of the heart for monitoring of the ECG. Monophasic action potentials (MAP) were recorded from the epicardial and endocardial surfaces of the left ventricle via contact electrode catheters. The endocardial MAP catheter was positioned in the left ventricle via a left atriotomy incision.

Study Protocol

Hearts were perfused for an equilibration period of 20–30 minutes before initiation of perfusion of buffer containing propafenone (0.3 μM), which was continued for 140 minutes. This concentration of propafenone is in the therapeutic range when unbound concentrations are considered. The class I antiarrhythmic drug propafenone was selected for study since it is a lipophilic drug that accumulates extensively in the myocardium and its relatively slow time course of accumulation permits evaluation of myocardial concentration–effect relations. Nine experiments were carried out in each of the lard, fish oil, and safflower oil groups.

Samples of the coronary sinus effluent were collected before commencing propafenone perfusion and then every 5 minutes for 40 minutes and every 10 minutes thereafter. Samples of aortic perfusate were collected at 10 and 20 minutes and then every 20 minutes. Hearts were blotted and weighed after completion of each experiment. Myocardial samples were stored at -70°C.

### Table 1. Fatty Acid Composition of Lipids in Experimental Diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Lard</th>
<th>Fish oil</th>
<th>Safflower oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>ND</td>
<td>8.2</td>
<td>ND</td>
</tr>
<tr>
<td>16:0</td>
<td>27.6</td>
<td>10.7</td>
<td>7.0</td>
</tr>
<tr>
<td>16:1</td>
<td>2.5</td>
<td>12.2</td>
<td>0.3</td>
</tr>
<tr>
<td>18:0</td>
<td>14.9</td>
<td>4.6</td>
<td>2.6</td>
</tr>
<tr>
<td>18:1</td>
<td>43.9</td>
<td>13.2</td>
<td>12.8</td>
</tr>
<tr>
<td>18:2 (ω-6)</td>
<td>11.2</td>
<td>2.0</td>
<td>77.3</td>
</tr>
<tr>
<td>18:3 (ω-3)</td>
<td>ND</td>
<td>1.4</td>
<td>ND</td>
</tr>
<tr>
<td>18:4 (ω-3)</td>
<td>ND</td>
<td>5.6</td>
<td>ND</td>
</tr>
<tr>
<td>20:1</td>
<td>ND</td>
<td>3.8</td>
<td>ND</td>
</tr>
<tr>
<td>20:4 (ω-3)</td>
<td>ND</td>
<td>0.9</td>
<td>ND</td>
</tr>
<tr>
<td>20:5 (ω-3)</td>
<td>ND</td>
<td>26.5</td>
<td>ND</td>
</tr>
<tr>
<td>22:5 (ω-3)</td>
<td>ND</td>
<td>2.3</td>
<td>ND</td>
</tr>
<tr>
<td>22:6 (ω-3)</td>
<td>ND</td>
<td>8.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, nondetectable.
Small quantities of ventricular muscle were homogenized and subsequently analyzed for propafenone concentration. The majority of the myocardium was preserved for subsequent isolation of sarcolemma. Propafenone concentrations in the aortic perfusate samples, coronary sinus effluent samples, and myocardial samples were determined using a high-performance liquid chromatographic procedure previously reported.  

Electrophysiological Measurements  
ECGs were recorded for measurement of QRS duration and QT interval duration. MAP duration was also measured from the intrinsic deflection of the upstroke to the level of 90% repolarization. The left ventricular effective refractory period (VERP) was measured by introducing a single ventricular extrastimulus (S2) after an eight-beat ventricular pacing train (S1) at a pacing cycle length of 400 msec. VERP was defined as the longest S2-S2 interval that failed to depolarize the ventricle. VERP was measured to the nearest 2 msec. Differences in ventricular pacing thresholds were also evaluated over time. The ventricular pacing threshold was defined as the minimal current required to evoke a ventricular depolarization during ventricular pacing at 400 msec, 2.0-msec pulse width. Ventricular pacing thresholds were measured to the nearest 0.1 mA. The above electrophysiological parameters were measured at baseline and every 5–10 minutes during propafenone perfusion, coinciding with collection of coronary sinus samples for measurement of propafenone concentration. In addition, ventricular strength–interval curves were also measured at baseline and at 90 minutes after initiation of propafenone perfusion. After an eight-beat drive train (cycle length, 400 msec), an extra stimulus was introduced late in diastole (380 msec) and decremented by 10–20-msec intervals to the minimum S2, S2 interval that no longer evoked a response at 10 mA. The minimum current output that evoked a ventricular extra stimulus at each coupling interval was measured by decreasing the intensity of the extra stimulus in 0.1-mA decrements until failure to capture was observed. The absolute refractory period (ARP) was defined as the longest coupling interval at which a current of 10 mA failed to capture. The relative refractory period (RRP) was defined as the coupling interval at which an increase in current greater than 0.1 mA was required to shorten the coupling interval by 10 msec. The diastolic threshold was defined as the minimal current required for consistent ventricular capture.  

Isolation of Sarcolemma  
Membrane vesicles enriched in sarcolemma were prepared by the method of Presti et al.  

Lipid/Fatty Acid Analysis  
Lipids were extracted from the sarcolemma membrane fraction by the method of Folch. Thin-layer chromatography of the lipid extract demonstrated the absence of diglycerides and triglycerides. Cholesterol content was determined by enzymatic assay, and the fatty acid composition of the phospholipids was determined by gas liquid chromatography. There was insufficient sample to permit separation and analysis of the fatty acid content of individual phospholipids in the sarcolemma.  

Data Analysis  
The coronary sinus effluent propafenone concentration (Ccs) versus time (t) data were fit to a one-compartment pharmacokinetic model using the nonlinear least-squares fitting program MK MODEL.  

\[ C_{CS} = C_{AO}(1 - e^{-kt}) \]  
The rate constant of accumulation of propafenone was determined from this analysis.  
The electrophysiological effects of propafenone included changes in QRS duration, QT interval, VERP, MAP duration, and ventricular pacing thresholds. Myocardial concentration–effect relations were determined by plotting electrophysiological effects versus the coronary sinus propafenone drug concentrations. The slopes of the coronary sinus concentration–effect relations were determined by linear regression analysis. This analysis is based on the assumption that the coronary sinus propafenone concentration is in dynamic equilibrium with the myocardial propafenone concentrations. In the present study, the measured myocardial propafenone concentrations were compared with the myocardial propafenone concentrations estimated from the changes in the coronary sinus propafenone concentrations. The myocardial propafenone concentration was estimated from the difference between the areas under the aortic perfusate propafenone–time curve and the coronary sinus propafenone–time curve. The areas beneath these curves were determined by the trapezoidal rule. The area between these curves multiplied by the perfusate flow rate is the amount of drug that accumulates in the myocardium. The concentration of propafenone in the myocardium was then determined by dividing the amount by the heart weight.  

Statistical Analysis  
Data are presented as mean±SD. Comparisons between groups were made using two-way ANOVA and the Neuman-Keuls test for multiple comparisons. Changes in electrophysiological variables over time were assessed by ANOVA for repeated measures. A value of \( p<0.05 \) was considered statistically significant.  

Results  
Dietary Fat Effects on Fatty Acid Composition of Sarcolemma  
The weights of the weanling rabbits before initiation of the experimental diets were similar for the lard (0.45±0.04 kg), fish oil (0.46±0.04 kg), and safflower oil
TABLE 2. Fatty Acid Composition of Phospholipids in Sarcolemma Membrane Fraction

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Lard (n=8)</th>
<th>Fish oil (n=9)</th>
<th>Safflower oil (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>1.3±1.3</td>
<td>1.8±0.3</td>
<td>0.9±0.4*</td>
</tr>
<tr>
<td>dHa 16:0</td>
<td>3.0±1.2</td>
<td>3.8±0.4</td>
<td>2.6±0.8</td>
</tr>
<tr>
<td>16:0</td>
<td>20.2±2.6</td>
<td>17.7±1.2</td>
<td>16.0±4.4†</td>
</tr>
<tr>
<td>16:1</td>
<td>1.6±0.8</td>
<td>2.6±0.5†</td>
<td>0.8±0.4*</td>
</tr>
<tr>
<td>dHa 18:0</td>
<td>3.9±1.3</td>
<td>3.0±0.3†</td>
<td>2.7±0.6†</td>
</tr>
<tr>
<td>18:0</td>
<td>22.7±3.7</td>
<td>16.9±2.0</td>
<td>18.8±7.6</td>
</tr>
<tr>
<td>18:1</td>
<td>16.7±2.6</td>
<td>14.3±0.9</td>
<td>11.7±2.7</td>
</tr>
<tr>
<td>18:2 (ω-6)</td>
<td>13.4±2.3</td>
<td>8.5±1.4</td>
<td>33.4±11.9§</td>
</tr>
<tr>
<td>20:3 (ω-6)</td>
<td>2.7±2.1</td>
<td>0.7±0.4†</td>
<td>1.2±1.1</td>
</tr>
<tr>
<td>20:4 (ω-6)</td>
<td>9.9±3.6</td>
<td>6.0±0.6‡</td>
<td>8.2±1.5§</td>
</tr>
<tr>
<td>20:5 (ω-3)</td>
<td>1.7±1.8*</td>
<td>13.2±1.7</td>
<td>1.6±2.0*</td>
</tr>
<tr>
<td>22:5 (ω-3)</td>
<td>1.3±0.2*</td>
<td>3.0±0.4</td>
<td>0.9±0.4*</td>
</tr>
<tr>
<td>22:6 (ω-3)</td>
<td>1.4±0.7*</td>
<td>6.8±0.8</td>
<td>1.1±1.4*</td>
</tr>
<tr>
<td>Cholesterol/PL</td>
<td>0.30±0.12</td>
<td>0.29±0.05</td>
<td>0.32±0.10</td>
</tr>
<tr>
<td>Σ Sat FA</td>
<td>53.4±7.3</td>
<td>43.2±3.0†</td>
<td>41.2±11.5‡</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>17.5±3.2</td>
<td>17.0±1.2</td>
<td>12.1±2.4§</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>28.4±7.0</td>
<td>39.8±2.3</td>
<td>46.6±9.6‡</td>
</tr>
<tr>
<td>DBI</td>
<td>1.12±0.19</td>
<td>1.92±0.11‡</td>
<td>1.36±0.23§</td>
</tr>
</tbody>
</table>

dHa, dimethylacetal derivative; Cholesterol/PL, cholesterol-to-phospholipid ratio; Σ Sat FA, total proportion of saturated fatty acids; Σ MUFA, total proportion of monounsaturated fatty acids; Σ PUFA, total proportion of polyunsaturated fatty acids; DBI, double bond index (sum of percentage of individual unsaturated fatty acids × number of double bonds/100).

Data are mean±SD. Values represent weight percent of total fatty acids in the sarcolemma. Fatty acids are designated by chain length: number of double bonds with the number in parentheses representing the carbon atoms between the terminal bond and methyl group.

* p<0.001 compared with fish oil.
† p<0.05 compared with lard.
‡ p<0.01 compared with lard.
$ p<0.01 compared with fish oil.
|| p<0.05 compared with fish oil.

significantly higher in the safflower oil group than in the lard (p<0.01) and fish oil groups (p<0.01). The cholesterol-to-phospholipid ratios were similar in the lard, fish oil, and safflower oil groups. The double bond index, a measure of the amount of unsaturated fat, was highest in the fish oil group and lowest in the lard group. Significant differences in this index were identified among the three groups (p<0.05).

Myocardial Pharmacokinetics of Propafenone

Figure 1 summarizes the average coronary sinus propafenone concentration–time data for the three diet groups. Assuming that the myocardial and coronary sinus effluent concentrations are in dynamic equilibrium, these coronary sinus perfusate-concentration–time curves parallel the time course of myocardial accumulation of propafenone.2125 Under these experimental conditions, propafenone accumulates slowly in the myocardium and the coronary sinus propafenone concentrations approach steady state (more than five half-lives) only after 100 minutes of perfusion.

The rate constants of accumulation of propafenone in the myocardium for the three diet groups are shown in Table 3. The time to reach equilibrium was greater in the fish oil group than in the lard and safflower oil groups (0.46±0.05 kg, p=NS). Significant differences were not observed in the weights of the rabbits immediately before death (1.12±0.13, 1.25±0.20, and 1.16±0.13 kg for the lard, fish oil, and safflower oil groups, respectively). The mean heart weight of the fish oil group after propafenone perfusion was significantly greater (4.28±0.43 g) than the weight of the lard (3.14±0.54 g, p<0.01) and safflower oil groups (3.33±0.56 g, p<0.01).

The fatty acid composition of the phospholipids isolated from the sarcolemma was significantly altered by the type of dietary fat; these results are presented in Table 2. The proportion of total saturated fat was higher in the sarcolemma isolated from the lard group than from the fish oil (p<0.05) and safflower oil group (p<0.01). In addition, the proportions of individual saturated fats (14:0, 16:0, and 18:0) were higher in the lard group. The proportion of ω-6 polyunsaturated fatty acids (18:2, 20:3, and 20:4) was significantly lower (p<0.05) and the proportion of ω-3 fatty acids (20:5, 22:5, and 22:6) was significantly greater (p<0.001) in the phospholipids isolated from sarcolemma of rabbits fed the fish oil diet than from the lard and safflower oil groups. The concentration of linoleic acid (18:2) was

TABLE 3. Myocardial Accumulation of Propafenone

<table>
<thead>
<tr>
<th></th>
<th>Lard (n=9)</th>
<th>Fish oil (n=9)</th>
<th>Safflower oil (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g)</td>
<td>3.14±0.54</td>
<td>4.28±0.43*</td>
<td>3.39±0.56</td>
</tr>
<tr>
<td>Cao (ng/ml)</td>
<td>87±14</td>
<td>96±10</td>
<td>95±18</td>
</tr>
<tr>
<td>Cfish (μg/g)</td>
<td>23±8</td>
<td>21±4</td>
<td>18±6</td>
</tr>
<tr>
<td>Csafo (μg/g)</td>
<td>19±7</td>
<td>24±11</td>
<td>17±6</td>
</tr>
<tr>
<td>Csafo/Cao</td>
<td>252±96</td>
<td>224±33</td>
<td>192±56</td>
</tr>
<tr>
<td>k (min⁻¹)</td>
<td>0.042±0.015</td>
<td>0.027±0.008†</td>
<td>0.044±0.010</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>19±7</td>
<td>28±9†</td>
<td>17±4</td>
</tr>
</tbody>
</table>

Cao, propafenone concentration in aortic perfusate; Cfish, measured propafenone myocardial concentration; Csafo, propafenone myocardial concentration estimated from aortic perfusate–coronary sinus concentrations differences; Csafo/Cao, myocardial-to-perfusate concentration ratio; k, rate constant of myocardial accumulation; t½, half-life of uptake.

Data are mean±SD.

*p<0.01; †p<0.05.
groups \((p<0.05)\). The myocardial concentration of propafenone measured at the end of the perfusion period was similar in all three groups. The myocardial propafenone concentrations estimated from aortic perfusate–coronary sinus propafenone concentration differences are a close approximation of the measured myocardial propafenone concentrations (Table 3). The myocardial/aortic perfusate concentration ratio was also similar in the three groups.

**Baseline Electrophysiological Effects**

The mean QRS duration measured at baseline was similar in the lard \((29±4\;\text{msec})\), fish oil \((29±5\;\text{msec})\), and safflower oil \((27±4\;\text{msec})\) groups. Differences in baseline electrophysiological measures of ventricular refractoriness and repolarization are shown in Figure 2. The VERP and the endocardial MAP duration tended to be lower in the safflower oil group than in the lard group \((p=0.07)\). Significant differences in the baseline epicardial MAP duration and the QT interval duration were not observed among the three groups.

**Propafenone Electrophysiological Effects**

The time course of changes in ventricular conduction time (QRS duration) and VERP are shown in Figure 3. QRS duration increased and VERP prolonged during propafenone perfusion. No further significant increases in these parameters were observed after 90 minutes of drug perfusion (ANOVA for repeated measures). Propafenone produced greater changes in ventricular conduction time in the lard group than in the safflower oil group throughout the time course of perfusion \((p<0.05)\). Greater changes in ventricular conduction time were also observed in the lard group than in the fish oil group, but these differences were no longer statistically significant at the end of the perfusion period. Although changes in VERP tended to be lower in the lard group compared with the fish oil and safflower oil groups, significant differences were not observed among the three diet groups at the end of the perfusion period.

**Propafenone Effects on Ventricular Excitability**

Changes in ventricular pacing thresholds are shown in Figure 4. Ventricular pacing thresholds increased over time during propafenone perfusion in all three diet groups \((p<0.05)\).
FIGURE 4. Time-dependent changes in ventricular stimulation thresholds during propafenone perfusion in the lard (○), fish oil (□ FO), and safflower oil (▲ SO) groups. Stimulation thresholds were measured at a pacing cycle length of 400 msec and a 2.0-msec pulse width. Lower stimulation thresholds were observed in the SO and FO groups compared with the lard group after initiation of propafenone perfusion (p<0.05). Data are mean±SD, n=9 per group. Data are not shown after t=110 minutes of propafenone perfusion since stimulation thresholds were not measured in all experiments after this time.

groups (p<0.01 by ANOVA for repeated measures). Greater increases in ventricular pacing thresholds were observed over time in the lard group compared with the safflower oil group (p<0.05) and the fish oil group (p<0.05) (Figure 4). However, significant differences were not observed between the fish oil and safflower oil groups.

Strength–interval curves measured at baseline and during perfusion of propafenone are shown for the three diet groups in Figure 5. The mean diastolic thresholds, ARP, and RRP for the three groups at baseline and during propafenone perfusion are shown in Table 5. Significant differences in the curves were not observed at baseline. After perfusion of propafenone, greater increases in the current required to evoke a ventricular depolarization were observed in the lard group than in the safflower oil group. These differences were statistically significant only at long coupling intervals (S1S2, 250–380 msec; p<0.01 by ANOVA). The current required to evoke a premature stimulus in the fish oil group was intermediate between those of the lard and safflower oil groups, but the differences were not statistically significant. Propafenone effects on the ARP and RRP did not differ among the three diet groups.

Myocardial Concentration–Effect Relations

Electrophysiological effects (QRS duration, VERP, and QT interval) were plotted versus the coronary sinus propafenone concentrations. Examples of these concentration–effect relations are shown in Figure 6. Linear concentration–effect relations were observed for QRS duration and the QT interval. The mean values of the slopes and the correlation coefficients (r) of the linear regression analyses are shown in Table 6. The concentration–effect relations describing changes in QRS duration were shifted to the left in the lard group compared with in the safflower oil group, and the slopes were also significantly greater (p<0.01). The slopes of the QRS concentration–effect relationships in the fish oil group were intermediate between the slopes of the lard and safflower oil groups (p=NS). The slopes of the QT interval concentration–effect relations were similar in the three groups. Significant linear correlations were not consistently observed between coronary sinus propafenone concentrations and VERP or MAP duration.

Discussion

The results of the present study indicate that the fatty acid composition of the phospholipids of rabbit sarcolemma and the electrophysiological effects of the antiarrhythmic drug propafenone on rabbit myocardium are significantly influenced by dietary fat. Propafenone significantly increased ventricular conduction time and decreased ventricular excitability in hearts from rabbits treated with a lard diet compared with those treated with a safflower oil diet. The effects of propafenone on these electrophysiological parameters in the group treated with a fish oil diet were intermediate between those of the lard and safflower oil groups.

Table 5. Effects of Propafenone on Ventricular Excitability

<table>
<thead>
<tr>
<th></th>
<th>Lard (n=9)</th>
<th>Fish oil (n=9)</th>
<th>Safflower oil (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic threshold (mA)</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>ARP (msec)</td>
<td>185±14</td>
<td>183±15</td>
<td>178±15</td>
</tr>
<tr>
<td>RRP (msec)</td>
<td>224±20</td>
<td>224±30</td>
<td>201±18</td>
</tr>
<tr>
<td>Propafenone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic threshold (mA)</td>
<td>1.8±1.0</td>
<td>1.2±0.6</td>
<td>0.8±0.6*</td>
</tr>
<tr>
<td>ARP (msec)</td>
<td>214±20</td>
<td>220±18</td>
<td>204±20</td>
</tr>
<tr>
<td>RRP (msec)</td>
<td>285±61</td>
<td>285±44</td>
<td>256±47</td>
</tr>
</tbody>
</table>

ARP, absolute refractory period; RRP, relative refractory period. Data are mean±SD. *p<0.05 compared with lard group.

FIGURE 5. Strength–interval relations. Upper panel: The mean threshold current at each coupling interval for the lard (○), fish oil (□ FO), and safflower oil (▲ SO) groups. Data are mean±SD, n=9 per group. SD bars not shown. Lower panel: The mean threshold current at each coupling interval for the lard (○), fish oil (□ FO), and safflower oil (▲ SO) groups. Data are mean±SD, n=9 per group. *p<0.05 lard compared with SO by ANOVA. SD bars not shown for the FO group or for the lard group at coupling intervals <250 msec.
Modification of Fatty Acid Composition of the Sarcolemma

As expected, significant changes in the fatty acid composition of the phospholipids of sarcolemma were observed among the three diet groups, and these changes reflect the fatty acid content of the dietary lipids.10,34 Higher levels of polysaturated fatty acids and lower levels of saturated fatty acids were present in the phospholipids of sarcolemma isolated from the fish oil and safflower oil groups compared with the lard group. The ratio of ω-6 to ω-3 fatty acids was reversed in the fish oil group. These changes may have significantly influenced the physical properties of the sarcolemma. Although membrane fluidity was not directly measured in the present study, one important determinant of membrane fluidity, the cholesterol-to-phospholipid ratio, was similar among the three groups.35 Another parameter that determines membrane fluidity is the membrane sphingomyelin concentration.35 However, individual phospholipids were not determined in this study. Thus, changes in membrane physical properties resulting from changes in the membrane fatty acid composition might contribute to the differences in the electrophysiological effects of propafenone observed in the present study. However, other factors, including differences in the fatty acid composition of individual phospholipids,35 differences in the concentration of linoleic acid, and possible changes in prostaglandin and leukotriene synthesis,34,35 must also be considered.

Baseline Electrophysiological Parameters

Significant differences in baseline electrophysiological parameters were not observed among the three diet groups. However, the VERP and the endocardial MAP duration tended to be shorter in the safflower oil group than in the lard group. Previous investigators have also observed shorter action potential durations in myocytes that were cultured in media enriched with linoleic acid compared with myocytes that were cultured in media enriched with palmitic acid.14,16 Activation of potassium channels by arachidonic acid might contribute to early repolarization of these myocytes.18,19 Although the content of arachidonic acid measured in the phospholipids isolated from sarcolemma was similar in the lard and safflower oil groups, the content of linoleic acid, the substrate for arachidonic acid synthesis, was significantly higher in the safflower oil group. Thus, differences in local arachidonic acid synthesis might contribute to the slight differences in measures of ventricular repolarization observed in the present study.

Propafenone Effects on Conduction Time and Membrane Excitability

Propafenone prolonged ventricular conduction time and decreased ventricular excitability in the lard-treated group compared with the safflower oil–treated group. These effects cannot be explained by differences in myocardial propafenone concentrations. The possible mechanisms of these effects include dietary fat–induced changes in the passive membrane properties of the sarcolemma,37 changes in sodium channel receptor density, changes in propafenone affinity for the sodium channel,10,11 and/or effects on other ionic channel proteins, such as gap junctions.38 It is possible that changes in the membrane order resulting from differences in the fatty acid composition of the sarcolemma may have altered the interaction of the lipophilic drug propafenone with the sodium channel–membrane interface.39,40 The present study was not designed to investigate these possible mechanisms.

Propafenone Myocardial Concentration–Effect Relations

The myocardial accumulation of propafenone was similar in all three diet groups. Thus, differences in myocardial drug concentrations cannot explain the differences in the electrophysiological effects of propafenone. We have previously demonstrated that the relations between propafenone-induced prolongation of ventricular conduction time and the estimated myocardial propafenone concentrations are linear.21,25,33 This relation is based on the assumption that coronary sinus.

TABLE 6. Slopes of Coronary Sinus Concentration–Effect Relations

<table>
<thead>
<tr>
<th></th>
<th>Lard (n=9)</th>
<th>Fish oil (n=9)</th>
<th>Safflower oil (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QRS slope (msec/ng/ml)</td>
<td>0.22±0.07</td>
<td>0.17±0.08</td>
<td>0.13±0.04*</td>
</tr>
<tr>
<td>r</td>
<td>0.90±0.08</td>
<td>0.93±0.03</td>
<td>0.90±0.05</td>
</tr>
<tr>
<td>QT slope (msec/ng/ml)</td>
<td>0.20±0.09</td>
<td>0.22±0.09</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>r</td>
<td>0.82±0.10</td>
<td>0.86±0.08</td>
<td>0.79±0.13</td>
</tr>
</tbody>
</table>

r, correlation coefficient of linear regression analysis; slope, slope of linear regression analysis.

Data are mean±SD.

*p<0.01.

FIGURE 6. Upper panel: Examples of coronary sinus propafenone concentration–effect relations. QRS duration is plotted versus the coronary sinus propafenone concentration for one experiment in each of the lard (○), fish oil (□ FO), and safflower oil (▼ SO) groups. The slopes of these relations and the correlation coefficients were 0.20, 0.15, and 0.11 msec/ng/ml and 0.92, 0.94, and 0.94 for the lard, FO, and SO groups, respectively. Lower panel: QT interval is plotted versus the coronary sinus propafenone concentration for one experiment in each of the lard (○), FO (□), and SO (▼) groups. The slopes and correlation coefficients were 0.19, 0.20, and 0.24 msec/ng/ml and 0.94, 0.92, and 0.91 for the lard, FO, and SO groups, respectively.
propranolol concentrations are proportional to myocardial propafenone concentrations. In the present study, the myocardial propafenone concentrations estimated from aortic perfusate and coronary sinus effluent propafenone concentration differences were similar to the measured myocardial drug concentrations. The coronary sinus propafenone concentration–effect relations describing changes in ventricular conduction time were shifted in the lard group compared with the safflower oil group, and the slopes of these relations describing changes in ventricular conduction time were significantly greater in the lard group (p < 0.01). This difference confirms the presence of a pharmacodynamic interaction secondary to the dietary interventions that is independent of the small differences in myocardial drug concentrations.

Significant differences in the concentration–effect relations describing changes in the QT interval were not observed among the three groups. The measurement of the QT interval is dependent on both action potential duration and ventricular conduction time. Greater changes in the endocardial MAP duration were observed in the unsaturated fat diet groups compared with the lard group. Thus, the failure to observe significant differences in propafenone effects on the QT interval, despite the increased effects on ventricular conduction time observed in the lard-treated group compared with the safflower oil–treated group, may be explained by differences in the effects of propafenone on ventricular repolarization in the lard group compared with the fish oil and safflower oil groups.

Clinical Implications

Therapeutic plasma propafenone concentrations span several orders of magnitude.24 Factors that contribute to the wide range of therapeutic concentrations include variability in the biotransformation of propafenone to the active metabolite 5-hydroxyproprafenone24 and variability in plasma protein binding.41 In the present study, we have shown that dietary fat significantly modulates the electrophysiological effects of propafenone; the magnitude of the effects on ventricular conduction time and ventricular excitability differed by as much as 100% between the lard and safflower oil groups. Thus, dietary fat is another factor that contributes to the considerable variability of propafenone’s electrophysiological effects. If the observations of the present study can be confirmed in humans, changes in dietary fat could potentially modulate the antiarrhythmic effects of propafenone. An increase in the consumption of saturated fats might change a therapeutic concentration of propafenone to a toxic concentration, whereas an increase in an unsaturated fat diet might change a therapeutic concentration to a subtherapeutic concentration. Certainly, the current interest in dietary intervention to reduce the risk of coronary artery disease highlights a population that might be subjected to changes in dietary fat consumption. Studies in humans will be required to evaluate the clinical significance of these experimental observations.

Conclusions

Dietary fat significantly alters the fatty acid composition of phospholipids in rabbit sarcolemma. Baseline difference in ventricular conduction time and ventricular excitability were not observed among the three diet groups. Propafenone decreased ventricular excitability and increased ventricular conduction time in hearts removed from rabbits fed a lard diet compared with those from rabbits fed a safflower oil diet. The mechanisms of these effects require further study.

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References


Influence of dietary fat on the pharmacodynamics of propafenone in isolated, perfused rabbit hearts.
A M Gillis, R Keashly, P A Watson, H J Mathison and H G Parsons

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