ANTIARRHYTHMIC EFFECTS OF EICOSAPENTAENOIC ACID DURING MYOCARDIAL INFARCTION

Enhanced Cardiac Microsomal (Ca$^{2+}$-Mg$^{2+}$)-ATPase Activity

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The effects of dietary supplementation with eicosapentaenoic acid (EPA) on ventricular arrhythmias during myocardial infarction were examined in a canine model. EPA was incorporated into cellular membranes after ingestion of EPA-ester (100 mg/kg body weight/day) for 8 weeks. The ratio of EPA to arachidonic acid (AA) in platelet cell membranes and myocardial microsomes was significantly increased (7% to 37% in platelet cell membranes; p<0.01, 3% to 12% in non-infarcted cardiac microsomes; p<0.01, and from 2% to 8% in infarcted cardiac microsomes; p<0.01). Dietary supplementation with EPA significantly reduced the incidence and severity of arrhythmias during coronary artery occlusion. Immediately after coronary artery occlusion, all of the animals in the control group that were given a toxic dose of digitalis developed ventricular tachycardia (VT) or ventricular fibrillation (VF), whereas none of the animals in the EPA-supplement group developed VT or VF within 15 min after administration of digitalis. Regardless of the presence of an infarcted area, the specific activity of the Ca$^{2+}$-pump enzyme ((Ca$^{2+}$-Mg$^{2+}$)-ATPase) within the myocardial microsomal fraction of the EPA-supplemented group was significantly higher than in that of the control group (Vmax: 140.5±19.1 vs 94.8±28.9 nmol/mg/min in non-infarcted cardiac microsomes, p<0.01, 130.9±18.4 vs 90.2±26.4 nmol/mg/min in infarcted cardiac microsomes, p<0.01, EPA vs control group, respectively). The specific activities of the Na$^{+}$-pump enzyme ((Na$^{+}$-K$^{+}$)-ATPase) and NADPH-dependent cytochrome C reductase in infarcted and non-infarcted cardiac microsomes did not differ between these groups. These results indicate that EPA supplementation increases the (Ca$^{2+}$-Mg$^{2+}$)-ATPase activity within myocardial membranes that is involved in Ca$^{2+}$ metabolism in myocardial cells by increasing the ratio of EPA to AA within cellular membranes. These cellular alterations are likely to reduce the severity of ventricular arrhythmias by inhibiting the rapid accumulation of intracellular Ca$^{2+}$ following ischemia. (Jpn Circ J 1994; 58: 903–912)

METABOLIC changes during acute myocardial infarction have been previously examined in a canine model following ligation of the circumflex branch of the left coronary artery! We demonstrated that intra-myocardial Ca$^{2+}$ levels increase in ischemic myocardium during the early phase of infarction! Similarly, levels of lipoxygenase-metabolites of arachidonic acid (AA), such as the 12-,5-, and 15-, hydroxy-

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eicosapentaenoic acids (HETEs), also increase within ischemic myocardium. These increased lipoygenase-metabolites elicit an inflammatory reaction and exacerbate the injury to ischemic myocardium.

Eicosapentaenoic acid (EPA), an n-3 polyunsaturated fatty acid (PUFA), has been used clinically for the prevention and treatment of atherosclerotic heart disease with encouraging results. EPA competes with AA for incorporation into plasma phospholipid membranes. Following appropriate stimulation, phospholipids which have incorporated EPA produce various lipoygenase and cyclooxygenase products that have less biological activities than those from AA.

The effects of EPA on ischemic myocardium during myocardial infarction have been previously described by our group. We found that EPA-supplementation attenuates cardiac injury during myocardial infarction and lowers the incidence of ventricular fibrillation (Vf). Other investigators have also reported that animals treated with n-3 PUFA before experimental coronary occlusion have significantly smaller infarcts, and fewer arrhythmic and sudden death events when compared to control animals. However, the mechanism of this protective effect is still unknown. Based on previous experiments, this mechanism may involve the regulation of Ca$^{2+}$ flux through myocardial membrane, since intracellular Ca$^{2+}$ concentrations appear to have various and profound effects on myocytes. The reduced susceptibility of cardiac tissue to arrhythmias and necrosis following ischemia in EPA-supplemented animals may be explained by changes in Ca$^{2+}$ transport by cardiac microsomes. In particular, (Ca$^{2+}$-Mg$^{2+}$)-ATPase is the main Ca$^{2+}$-extrusion pump in cardiac microsomes. Therefore, it is of considerable importance to assess the ability of dietary supplementation with EPA to alter the specific activity of (Ca$^{2+}$-Mg$^{2+}$)-ATPase. Diet-induced changes in the activity of this cation pump have been shown to modify the distribution of ionic calcium in the cell, thereby influencing the extent of cardiac necrosis and arrhythmia following ischemia or reperfusion. In this study, we examined the role of intracellular Ca$^{2+}$ mobilization, particularly on cellular membrane ion-transport proteins, to elucidate the mechanism(s) responsible for the antiarrhythmic effects of EPA-supplementation during myocardial infarction.

**MATERIALS AND METHODS**

**Diets**

Thirty adult mongrel dogs (8–12 kg) were used in this study. The control group consisted of 15 dogs who were fed a standard diet prepared by the Oriental Yeast Co. The EPA group consisted of 15 dogs who were fed the same standard diet supplemented with EPA ester (100 mg/kg body weight/day, Mochida Pharmaceutical Co. Tokyo, Japan) for 8 weeks. Digitalis-induced arrhythmias were evaluated immediately after coronary artery ligation in 5 dogs from each group.

**Induction of Experimental Myocardial Infarction and Quantification of Arrhythmias**

After anesthesia was induced using thiopental (20 mg/kg, iv), the dogs were intubated and artificially ventilated with room air via a Harvard respirator. Holter monitoring (Marquette Inc.) was then initiated. Left thoracotomy was performed within the fifth intercostal space. The pericardium was then opened and the heart was exposed. To induce myocardial infarction, the left circumflex coronary artery was isolated from adipose tissue and ligated 0.5 cm distal to its origin. The thoracic cage was closed following the appearance of electrocardiographic ST-segment elevation. We assessed the protective effect of dietary supplementation of EPA on the early phase of ischemia-induced arrhythmia. After ligation, the dogs were continuously observed using Holter-ECG monitoring (Lead II) for 3 h. Thereafter, the hearts were excised and 6 preparations from each group were analyzed biochemically. We continuously monitored the ECG for 3 h because our previous study demonstrated that most of the ventricular tachyarrhythmia induced by coronary ligation appeared within 3 h and also that intramyocardial Ca$^{2+}$ levels were significantly increased 3 h following coronary ligation. The entire protocol followed the local ethical standards for the treatment of animals in our hospital.

The incidence of arrhythmias which oc-
curred within the first 3 h after coronary ligation was assessed by counting the number of ventricular extra beats (VEBs), including those occurring as tachycardia (VT, seven or more consecutive ventricular extra-systoles at a rate faster than the normal sinus rhythm), and the incidence and total duration of all episodes of VT and Vf. In addition, the severity of arrhythmias was assessed quantitatively by an "arrhythmia score" on a scale of 0 to 8 during occlusion, as described by Curtis et al. A score of 0 was given for 0–49 VEBs only; 1, for 50–499 VEBs only; 2, for >500 VEBs, or one spontaneously reversible episode of VT or Vf; 3, for more than one spontaneously reversible episode of VT/Vf, or one or more episodes of nonsynchronously reversible VT/Vf lasting less than 60 sec; 4, for VT/Vf episodes lasting 60–120 sec; 5, for VT/Vf episodes lasting more than 120 sec; 6, for irreversible Vf causing death within 15–240 min after coronary ligation; 7, for fatal Vf within 4–15 min; 8, for fatal Vf within 4 min.

Fatty Acid Composition of Platelet Cell Membrane and Microsomes from Non-Infarcted and Infarcted Myocardium

Since EPA is believed to be incorporated into cardiomyocyte membranes as well as those of platelets, we used the latter to estimate the incorporation of EPA into cell membranes. Platelets were used because purified membrane fractions were easily obtained. Platelets were isolated from peripheral blood immediately before coronary artery ligation. Cell membrane fractions were obtained using the method employed by Minkes et al. Isolated heart preparations were separated into infarcted areas (the 5 mm-thick endocardial layer from the top area of the posterior papillary muscle which reflects the presence of myocardial necrosis induced by coronary occlusion) and non-infarcted areas (the root area of the anterior papillary muscle which reflects the absence of myocardial necrosis). Microsomal vesicles were obtained from each area by sucrose gradient centrifugation as described by Jones and Besch. The lipid component of the membranes was extracted with Folch's solution and the fatty acid composition was analyzed by gas chromatography, as previously described.

Measurement of (Ca²⁺-Mg²⁺)-ATPase, (Na⁺-K⁺)-ATPase, and NADPH Cytochrome C Reductase Activities in Myocardial Microsomes

Activities of microsomal (Ca²⁺-Mg²⁺)-ATPase, (Na⁺-K⁺)-ATPase and NADPH cytochrome C reductase from infarcted and non-infarcted myocardium were measured to examine changes in cellular membrane ion-transport proteins. (Ca²⁺-Mg²⁺)-ATPase activity was determined by a modification of the method of Itoh et al. The reaction mixture contained 30–40 mg of myocardial microsomes, 50 mM Tris-HCl (pH 7.2), 20 mM NaN₃ (an inhibitor of mitochondrial ATPase), 0.1 mM ouabain (an inhibitor of (Na⁺-K⁺)-ATPase), 1 mM ATP and the desired submicromolar free Ca²⁺ concentrations, adjusted by addition of Ca-EGTA buffer. The association constants for Ca²⁺-EGTA and Ca²⁺-ATP at a pH of 7.2 are 6.8×10⁷ and 8.5×10³ respectively. The reaction was initiated by adding ATP and terminated by adding ice-cold trichloroacetic acid (TCA) after incubation at 37°C for 30 min. The mixture was then centrifuged. The inorganic phosphate (Pi) concentration within the supernatant was determined by the method of Youngberg and Youngberg. The (Ca²⁺-Mg²⁺)-ATPase activity was calculated by subtracting the values obtained with EGTA alone from those with Ca²⁺ and EGTA. The (Na⁺-K⁺)-ATPase activity was measured by a similar method except that the reaction medium consisted of 3–4 μg of myocardial microsomes, 50 mM Tris-HCl (pH 7.2), 110 mM NaCl, 15 mM KCl, 5 mM NaN₃, 0.5 mM EGTA, 4 mM MgCl₂ and 1 mM ATP. The activity of NADPH cytochrome C reductase, a marker enzyme of sarcoplasmic reticulum (SR), was also determined by a previously described method.

Evaluation of Digitalis-Induced Arrhythmias

To examine the effects of EPA supplementation on digitalis glycoside toxicity, a fatal dose of digoxin (0.025 mg/kg/min) was administrated intravenously over a 60 sec period to 5 dogs in each group immediately after coronary artery ligation. The administration of digitalis after coronary occlusion is assumed to enhance the elevation of the intracellular cytoplasm free Ca²⁺, which is believed to be the principle cause of the
inotropic effect of digitalis glycosides on the heart. The time of onset of VT or Vf was measured in 5 dogs with EPA-supplementation and compared with that in 5 control animals.

Data Analysis
Data are presented as means ± SEM. Student’s t test or the Wilcoxon test was used to analyze continuous variables. The frequency of events was compared by the chi-square test. P values of <0.05 were considered to be statistically significant.

RESULTS
Fatty Acid Composition of Total Phospholipids from Cell Membrane Fractions After EPA Supplementation
We examined the fatty acid composition of platelet membranes and microsomes from non-infarcted and infarcted myocardium to ensure that dietary supplementation with EPA significantly replaces AA. Fig. 1 shows the phospholipid fatty acid composition of the total phospholipids from the platelet membrane and myocardial microsomes of non-infarcted and infarcted myocardium. In the EPA-supplemented group, levels of linoleic acid and AA, which are n-6 PUFA, tended to account for a lower percentage of the total phospholipids within both platelet membranes and myocardial microsomes. In contrast, levels of EPA and docosahexaenoic acid (DHA), which are n-3 PUFA, in these membranous vesicles were significantly increased. Consequently, the EPA/AA and n-3/n-6 PUFA ratios were markedly higher in the EPA-supplemented group than in the control group. The EPA/AA ratio in the control group vs that in the EPA group was 7±1% vs 37±5% in platelet cell membranes, 3±1% vs 12±5% in non-infarcted myocardial microsomes, and 2±1% vs 8±2% in infarcted myocardial microsomes, (p<0.01). Additionally, in the EPA group,
there was no significant difference in the EPA/AA and n-3/n-6 PUFA ratios in the myocardial microsomes between non-infarcted and infarcted areas.

These findings suggest that dietary EPA competes efficiently with AA for incorporation into myocardial microsomes.

**Arrhythmias Following Coronary Ligation**

Ventricular arrhythmias that developed within 3 h after coronary ligation were analyzed using a Holter monitor. As shown in Fig. 2A, the total number of VEBs was significantly higher in the control group than in the EPA-treated group (393±115 vs 221±98, p<0.05). However, there was no difference in the incidence of VF between the two groups (control group: 2/10, EPA group: 2/10, NS, Fig. 2B). We then analyzed the severity of ventricular arrhythmias using an “arrhythmia score”, which is particularly valuable in statistical evaluation when a low incidence of VT and/or VF precludes comparisons of the duration of episodes. The arrhythmia score obtained within 3 h after coronary ligation was significantly reduced by EPA-supplementation during occlusion (3.6±1.2 vs 2.2±0.8, control vs EPA, p<0.05, Fig. 2C). These findings indicate that the arrhythmic vulnerability in the early stage after coronary occlusion was attenuated under EPA-supplementation.

All of the animals that were given digitalis in the control group to induce arrhythmias developed VT or VF after about 10–15 min. However, in all of the animals in the EPA-supplemented group, VT or VF did not develop until at least 25 min after the administration of digitalis (Fig. 3). These results indicate that the toxic effect of digitalis was suppressed in the EPA-supplemented group, suggesting that the incorporation of EPA into myocardial microsomes prevents the toxic accumulation of cytosolic Ca^{2+} which may subsequently increase after the administration of digitalis.

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\[(\text{Ca}^{2+}-\text{Mg}^{2+})-\text{ATPase and (Na}^{+}-\text{K}^{+})-\text{ATPase Activities in Myocardial Microsomal Vesicles}\]

Ca^{2+} and Na^{+} pump function in myocardial microsomal vesicles was estimated by measuring the ATPase activities of ion-transport proteins in cell membranes. In microsomal vesicles from both non-infarcted and infarcted myocardium, the activity of
TABLE I NADPH CYTOCHROME C REDUCTASE, (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase AND (Na\(^+-\)K\(^+-\))-ATPase ACTIVITIES IN NON-INFARCTED AND INFARCTED MYOCARDIAL MICROSONES

<table>
<thead>
<tr>
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<th>non-infarcted area (n=6)</th>
<th>infarcted area (n=6)</th>
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<tr>
<td><strong>NADPH cytochrome C reductase</strong></td>
<td></td>
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<tr>
<td>EPA (+)</td>
<td>341 ± 68</td>
<td>322 ± 54</td>
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<tr>
<td>EPA (-)</td>
<td>360 ± 109</td>
<td>348 ± 78</td>
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<tr>
<td><strong>(Ca(^{2+})-Mg(^{2+}))-ATPase</strong></td>
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<tr>
<td>EPA (+)</td>
<td>140.5 ± 19.1*</td>
<td>130.9 ± 18.4*</td>
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<td></td>
<td>(4.0 ± 1.0) x 10(^{-8})</td>
<td>(2.5 ± 0.9) x 10(^{-8})</td>
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<tr>
<td>EPA (-)</td>
<td>94.8 ± 28.9*</td>
<td>90.2 ± 26.4*</td>
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<td>(3.9 ± 1.0) x 10(^{-8})</td>
<td>(2.5 ± 0.7) x 10(^{-8})</td>
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<td><strong>(Na(^+-)K(^+-))-ATPase</strong></td>
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</tr>
<tr>
<td>EPA (+)</td>
<td>1997 ± 742</td>
<td>1213 ± 218</td>
</tr>
<tr>
<td></td>
<td>(5.4 ± 1.3) x 10(^{-7})</td>
<td>(5.6 ± 1.3) x 10(^{-7})</td>
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<tr>
<td>EPA (-)</td>
<td>2033 ± 342</td>
<td>1270 ± 310</td>
</tr>
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<td>(5.6 ± 1.3) x 10(^{-7})</td>
<td>(5.0 ± 1.2) x 10(^{-7})</td>
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EPA (+); EPA-treated group, EPA (-); control group.
Specific activities are expressed as n mol/mg/min at 37°C.
*p < 0.01, EPA vs control group

Fig. 4A shows the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activities in microsomal vesicles from non-infarcted myocardium of both EPA-supplemented and control subjects. The enzyme activity increased in proportion to the Ca\(^{2+}\) concentrations in both the control and EPA-supplemented dogs. The Km value, calculated from the double reciprocal plot, was approximately 4.0 x 10\(^{-8}\) (M) for both groups. In contrast, the apparent Vmax per mg protein in the EPA-supplemented group was significantly higher than that in the control group (149.3 vs 90.7 nmol/mg/min, respectively).

As shown in Table I, similar mean apparent Vmax values were also obtained in non-infarcted myocardium from another 6 dogs (140.5 ± 19.1 vs 94.8 ± 28.9 nmol/mg/min, EPA vs control group, p < 0.01). However, in non-infarcted myocardium, there was no significant difference in the Km values of the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activity in microsomes between the two groups ((4.0 ± 1.0) x 10\(^{-8}\) vs (3.9 ± 1.0) x 10\(^{-8}\) (M); EPA vs control, respectively, NS, Table I.). As shown in Table I, the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activity in microsomal vesicles from infarcted myocardium in each group showed results similar to those in non-infarcted myocardium: i.e., apparent Vmax in the EPA group was significantly higher than that in the control group, while there was no difference in Km values (130.9 ± 18.4 vs 90.2 ± 26.4 nmol/mg/min).

Fig. 5. The effect of trifluoroperazine (TFP) (a calmodulin antagonist) on (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activity in cardiac microsomal fractions from a non-infarcted area. The enzyme activity was estimated at a free Ca\(^{2+}\) concentration of 0.1 μM using Ca-EGTA buffer. Activities were calculated as percentages of the control activity. The reaction mixture is the same as that in Fig. 4A, except for the addition of various concentrations of TFP.

NADPH-dependent cytochrome C reductase, a marker enzyme of sarcoplasmic reticulum (SR), was the same in the EPA and control groups (Table I, upper). Therefore, these vesicular samples represent myocardial microsomal vesicles. The results show that the (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activities in microsomal vesicles from non-infarcted myocardium of both EPA-supplemented and control subjects were significantly higher than those in the control group (149.3 vs 90.7 nmol/mg/min, respectively).

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In the EPA-treated group, there was no difference in apparent Vmax between the non-infarcted and infarcted areas (140.5 ± 19.1 vs 130.9 ± 18.4 nmol/mg/min, respectively, NS), while the values in the control group were significantly lower than those in the EPA group. In addition, Km values in non-infarcted myocardium were significantly higher than those in infarcted tissue regardless of EPA-treatment.

(Ca²⁺-Mg²⁺)-ATPase activity was inhibited in a dose-dependent manner by trifluoroperazine (TFP), a calmodulin inhibitor, indicating that calmodulin is responsible for Ca²⁺-dependent enzymatic activity (Fig. 5).

These findings also suggest that the EPA-dependent increase in (Ca²⁺-Mg²⁺)-ATPase activity within myocardial microsomes from either non-infarcted or infarcted myocardium results from increased levels of the enzyme, rather than from changes in its properties.

(Na⁺-K⁺)-ATPase activity within microsomal vesicles from non-infarcted and infarcted myocardium of both the EPA and control groups was inhibited in a dose-dependent fashion by ouabain. The ouabain concentration which led to 50% inhibition (IC₅₀) was similar in the two groups, i.e., approximately 5 × 10⁻⁷ M (Table I, lower). These results suggest that (Na⁺-K⁺)-ATPase activity remains unchanged after the incorporation of EPA into myocardial microsomes.

DISCUSSION

The epidemiological studies performed by Dyerberg, Bang, et al. have created considerable interest in the role of fish oil or EPA in the prevention and treatment of atherosclerotic heart disease, although other studies have failed to confirm their findings. In addition, a large, prospective, randomized clinical trial of 2033 patients with myocardial infarction demonstrated that the mortality rate due to ischemic heart disease was reduced by 29% over a 2-year period in patients who were advised to consume fish two or three times a week, relative to patients who were not given such advice. These results suggested that the ingestion of fish oil stabilizes cardiac rhythm, and prevents fatal ischemic-mediated ventricular arrhythmias. Benediktsdottir and Guobjarnason have shown that rats which consumed cod liver oil were less prone to ventricular fibrillation after isoprenaline, as compared to rats that were fed maize oil. This result supports findings made by Murnaghan who demonstrated that ingestion of saturated fatty acids increased ventricular arrhythmias in rabbits, while ingestion of unsaturated fatty acids had the opposite effect. Culp et al. examined the incidence of extrasystole in dogs after occluding a branch of the left coronary artery. They noted that within 19 h after coronary occlusion, ventricular extrasystoles comprised about 30% of the total heart beats in dogs that had been treated with fish oils, while in control animals this value was 80%. McLennan et al. also reported that supplementation with fish oil in rats completely prevented the development of Vf after reperfusion following coronary ligation. In an experimental canine model of myocardial infarction induced by ligation of the circumflex-branch of the left coronary artery, we previously reported that dietary supplementation with EPA, a n-3 PUFA found in fish oil, attenuates not only ischemic myocardial injury but also the induction of ventricular fibrillation. However, the exact mechanism by which EPA supplementation exerted this effect is unknown.

The purpose of this study was to determine how EPA-ingestion reduces the severity of ischemia-induced ventricular arrhythmias and to examine the role of myocardial cellular membranes in this salutary effect. In previous studies, EPA-supplementation changed the EPA/AA ratio in phospholipids of cellular membranes, which plays a significant role in the function of cell membranes. The results of the present study indicate that in the dog, supplemental dietary EPA efficiently competes with AA for incorporation into cell membranes, and that it can also reduce the susceptibility to ventricular arrhythmias during the early phase of coronary artery occlusion. Although previous experiments in our laboratory revealed that the incidence of Vf was significantly reduced in the EPA-treated group (5/15 vs 0/10, control group vs EPA
group, p<0.05), the present series revealed that the incidence of Vf was similar during the early phase of coronary occlusion (2/10 vs 2/10, control group vs EPA group, NS). One explanation for this discrepancy may involve the lower ratio of EPA/AA in the cellular membrane in the EPA group of the present study. Since EPA was given to the experimental animals as part of their daily diet, the amount of EPA in incorporated into the cellular membranes via absorption from the gastrointestinal tract may have differed between animals. In fact, under EPA-feeding at the same dose, individual differences in EPA/AA in platelet membranes were found in a previous study. The extent of individual histological damage in infarcted myocardium correlated with the EPA/AA ratio in platelet membranes of individual dogs in the EPA group. In the present study, the EPA/AA ratio in platelet membranes of in the EPA group was 0.37 ± 0.05, which is less than that (0.54) in our previous study. On the other hand, the EPA/AA ratio in the control group in the present study was 0.07 ± 0.01, which is similar to that (0.09) in the previous study. In the present study, the EPA/AA ratio within platelet cell membranes in the EPA group vs the control group was 0.37 ± 0.05 vs 0.07 ± 0.01. As shown in Fig. 1, EPA-feeding resulted in an increase in EPA/AA within both platelet membranes and microsomal vesicles from myocardium, indicating that individual differences in EPA/AA observed in platelet membranes in the EPA group may have also been observed in cardiac microsomes, which are related to the occurrence of Vf through alterations in the ability of cardiac cell membrane to pass ions selectively. Therefore, in the present study, the lower EPA/AA ratio in cellular membranes was associated with a reduced efficacy of EPA-treatment in affecting the incidence of Vf, as compared with that in the previous study. Considering both our present and previous data, the incidence of Vf tended to be lower in the EPA-treated group than in the controls (2/20 vs 7/25, p=0.10).

It has been shown that ventricular arrhythmias which occur during the early phase of infarction are due to the formation of re-entry circuits resulting from electrical changes due to ischemic dysfunction in myocardial membrane. Therefore, it is important to examine metabolic and ionic conductance changes in the cell membranes of cardiomyocytes, including cells related to specialized conducting systems. In particular, it is of considerable importance to examine alterations in Ca^{2+} transport, as well as that of Na^{+} and K^{+}, through myocardial cell membranes, since this can greatly influence intracellular Ca^{2+} loading. Thus, we assessed Ca^{2+} and Na^{+} myocardial membrane pump functions by measuring the activities of (Ca^{2+}-Mg^{2+})-ATPase and (Na^{+}-K^{+})-ATPase in myocardial microsomes. The latter can also serve as a marker enzyme of plasma membrane. The activity of NADPH-dependent cytochrome C reductase, a marker of sarcoplasmic reticulum (SR) activity, was significant, indicating that the microsomal vesicles studied may contain plasma membrane as well as SR. We noted an increase in the enzymatic activity of (Ca^{2+}-Mg^{2+})-ATPase in the EPA-supplemented group. These results suggest that EPA is incorporated into myocardial microsomes and leads to stabilization of the myocardial membrane by modulating ion-transport proteins (such as (Ca^{2+}-Mg^{2+})-ATPase). This membrane modification may decrease intracellular Ca^{2+} overload during the early phase of infarction, compared to that in the control group, and thereby reduce the severity of ischemia-mediated ventricular arrhythmias.

To evaluate how acute ischemia affects the fatty acid composition of myocardial microsomes and subsequently affects both the Ca^{2+} and Na^{+} pump functions in myocardial membrane, we examined heart preparations that had been separated into infarcted and non-infarcted areas in both the control and EPA groups. There were no measurable differences between the infarcted and non-infarcted areas with respect to fatty acid composition, except that the EPA/AA and n-3/n-6 PUFA ratios were significantly higher in the EPA group. Both areas in the EPA group contained significantly increased levels of (Ca^{2+}-Mg^{2+})-ATPase activities, compared with those in the control group. These results indicate that, even in infarcted cardiac microsomes, EPA supplementation-enhanced (Ca^{2+}-Mg^{2+})-ATPase activity was unaffected within at least the first 3 h after

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coronary ligation.

Swanson et al\(^{35}\) reported decreased (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activity in the SR of mice that had incorporated large amounts of n-3 PUFAs. However, Ca\(^{2+}\)-EGTA buffer was not used in their study. Thus, the free Ca\(^{2+}\) concentrations in the reaction medium were not clearly determined, and the measured (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activity may have reflected low affinity Ca\(^{2+}\) enzyme pumps rather than physiologically important high affinity Ca\(^{2+}\) pumps.

Hallaq et al\(^{36}\) reported that nascent rat myocytes incubated in EPA-enriched culture medium were less susceptible to the formation of lethal contracture after subsequent exposure to toxic doses of ouabain. Furthermore, myocardial intracellular Ca\(^{2+}\) concentrations were significantly lower in media containing EPA than in that containing AA, although no measurable difference in the inhibition of (Na\(^{+}\)-K\(^{+}\))-ATPase activity was noted between EPA- and AA-enriched myocytes. They suggested that the incorporation of EPA into the cellular membranes of isolated cardiac myocytes protects them from the fatal effects of ouabain, in part by preventing the accumulation of toxic levels of cytosolic calcium. Our data are consistent with these findings: i.e., there were no measurable differences in the inhibition of Na\(^{+}\) pump function in either infarcted or non-infarcted areas between the EPA and control groups, indicating that a toxic dose of digitalis did not inhibit (Na\(^{+}\)-K\(^{+}\))-ATPase pump activity in the presence of dietary EPA. These results further suggest that the increase in (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase due to EPA-supplementation also plays an important role in preventing digitals-enhanced intracellular Ca\(^{2+}\) overload after coronary occlusion. Furthermore, the inhibition of intracellular Ca\(^{2+}\) overload during the early phase of infarction by increased (Ca\(^{2+}\)-Mg\(^{2+}\))-ATPase activities in cardiac microsomes may also have favorable effects on myocardial infarction by decreasing the incidence and severity of ventricular extrasystoles as well as by attenuating the progression of ischemic injury. Various Ca\(^{2+}\) transport systems, including L- and T-type Ca\(^{2+}\) channels and Na\(^{+}\)-Ca\(^{2+}\) exchangers, should be studied in relation to the anti-arrhythmic effects of dietary supplementa-

tion with EPA.

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