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ORIGINAL ARTICLE

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Antiarrhythmic and electrophysiological effects of long-chain ω-3 polyunsaturated fatty acids

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Abstract Recent studies indicate that a diet enriched in ω-3 polyunsaturated fatty acids may prevent sudden cardiac death. The goal of the present study was to elucidate how ω-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and α-linolenic acid (ALA; 1–20 μM) may affect the cardiac activation and repolarization pattern. For this reason, DHA, EPA or ALA was infused in spontaneously beating isolated rabbit heart (Langendorff technique) and subjected to 256 electrodes epicardial mapping. All compounds exhibited a negative inotropic and chronotropic effect. EPA and ALA, but not DHA, prolonged QTc. The dispersion was enhanced at higher concentrations (>5 μM) by DHA and less (or not affected) by the others. The total activation time, reflecting ventricular conduction, was prolonged predominantly by DHA and to a lower extent by the other drugs. Atrioventricular conduction time was slowed only by DHA and EPA. To analyze of the pattern of activation, we determined the timepoint of activation as $t(dU/dt_{min})$ for all 256 electrodes. The beat-to-beat similarity of these patterns was moderately reduced by all drugs. Regarding antiarrhythmic activity we found that the threshold for elicitation of a ventricular extrasystole was concentration-dependently enhanced by DHA and EPA, but not by ALA. DHA dose-dependently reduced longitudinal propagation velocity V_L and to a lower extent transverse velocity V_T. Anisotropy was not significantly changed. EPA and ALA did not exhibit a systematic effect on V_L or V_T. These results clearly demonstrate that DHA, EPA, and ALA exhibit direct electrophysiological effects with different profiles.

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Abbreviations ALA: α-Linolenic acid · ARI: Activation–recovery interval · BCL: Basic cycle length · BTP: Breakthrough-point similarity · CF: Coronary flow · DHA: Docosahexaenoic acid · EPA: Eicosapentaenoic acid · LVP: Left ventricular pressure · TAT: Total activation time · VEC: Similarity of vector fields · V_L: Longitudinal propagation velocity · V_T: Transverse propagation velocity

Introduction

Cardiovascular mortality is among the most common causes of death in Western industrialized countries. A serious life-threatening complication in many cardiovascular diseases is the occurrence of ventricular arrhythmia, especially in the course of myocardial ischemia and infarction as well as in the course of other structural heart diseases such as heart failure. Besides this, sudden cardiac death, which may represent arrhythmogenic death in a considerable number of cases, contributes to cardiovascular mortality.

In consequence, efforts have been undertaken to find therapeutic strategies against the occurrence of arrhythmia. Classical antiarrhythmic drugs, which have been often used in the past, are substances which block cardiac ionic channels, thereby altering the cardiac action potential, resulting in alterations of the spread of activation or of the pattern of repolarization, finally suppressing cardiac arrhythmia. The problem, however, with these drugs is their proarrhythmic potential as became evident in the CAST trial (Echt 1991) and in animal studies (e.g., Dhein et al. 1993). While these drugs are effective in suppressing manifest ongoing arrhythmia, they are problematic in the use for prevention or prophylaxis of arrhythmia due to their proarrhythmic potential. Thus, there is an urgent need to find alternative strategies to prevent cardiac arrhythmia. In that context recent studies (Albert et al. 1998, 2002; Billman et al. 1999; GISSI Prevenzione Investigators 1999; Marchioli and GISSI-

Prevenzione Investigators 2002) point to an antiarrhythmic potential of polyunsaturated fatty acids (PUFAs) preventing sudden cardiac death.

Fatty acids can be classified in saturated, unsaturated and polyunsaturated fatty acids. For classification the number of carbon atoms is given followed by the number of unsaturated bonds (e.g., C18:1=oleic acid, a C18 fatty acid with one unsaturated bond). The position of the unsaturated bond is given after a Δ as an exponent (C18:3 $\Delta^{(9,12,15)}$= cis-,cis-,cis-9, 12, 15-octadecatrienic acid = α-linolenic acid). In a fatty acid the carbon atom which is at maximum distance to the carboxyl moiety (with the α-carbon atom) is designated the ω-carbon atom. It is also possible to give the position of the unsaturated bond relative to the ω-carbon atom. Consequently, arachidonic acid is ω-6 polyunsaturated acid (C20:4 ω-6) and α-linolenic acid is a ω-3 polyunsaturated acid (C18:3 ω-3). The most relevant polyunsaturated fatty acids are cis-5,8,11,14,17-eicosapentaenoic acid (C20:5 ω-3), cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6 ω-3) and cis-9,12,15-α-linolenic acid (C18:3 ω-3).

Natural sources of polyunsaturated fatty acids comprise vegetable oils such as olive oil or linseed-oil, fish (such as mackerel, salmon or herring) and shellfish. α-linolenic acid, which is found in low amounts in vegetable oils, can only be synthesized by plants from oleic and linolenic acid and therefore it is an essential fatty acid for mammals. In the mammalian metabolism other unsaturated fatty acids such as eicosatetraenoic acid are metabolized from α-linolenic acid. However, eicosapentaenoic acid and docosahexaenoic acid are mainly obtained from diets containing fatty fish and fish oils.

ω-3 Polyunsaturated fatty acids are normally bound to albumin which contains binding sites for free fatty acids. Eight to ten fatty acid molecules can bind to one molecule of albumin so that 10 g albumin can carry 1.5 to 2.6 mmol docosahexaenoic acid (=0.86 g pure docosahexaenoic acid; Cistola et al. 1987; Hamilton 1992). This is necessary to take into account if these substances are used in in vivo experiments.

Until recently, the classical view of the effects of these fatty acids was that they incorporate into lipid bilayers and exert their antithrombotic, antiatherosclerotic and antiarrhythmic effects via affection of membrane characteristics and eicosanoid production. Thus, α-linolenic acid may reduce platelet aggregability (McDonald et al. 1989; Indu and Ghafoorunissa 1992; Chan et al. 1993) by influencing the effects of arachidonic acid (Renaud et al. 1986; Budowski 1989) or by conversion to eicosapentaenoic acid (Valsta et al. 1996; Sanders and Younger 1981; Weaver et al. 1990; Seppänen-Laakso et al. 1992). However, due to recent clinical and nonclinical studies new aspects on antiarrhythmic effects became evident and this former view has to be—at least in part—revised or completed. In the literature there are reports on effects of ω-3 polyunsaturated acids on sodium (Xiao et al. 1995, 2001; Pound et al. 2001), calcium (Xiao et al. 1997) and potassium channels (Honoré et al. 1994; Poling et al. 1995; Macleod et al. 1998). However, it is unclear, how these drugs affect the cardiac process of electrical activation propagation and repolarization. Comparative data on cardiac activation and repolarization process or mapping data on the effects on transverse or longitudinal velocity for DHA, EPA and ALA, as the most commonly used ω-3 polyunsaturated acids, are still missing. Thus, the goal of the present study was to find out whether the ω-3 polyunsaturated acids DHA, EPA, and ALA and a combination of the ethylesters of DHA and EPA exert direct electrophysiological effects, and—in that case—which type of electrophysiological effects can be observed. Moreover, the effects of these drugs on activation and repolarization wavefronts as well as on anisotropy should be investigated.

Materials and methods

Heart preparation and epicardial mapping All experiments were performed in accordance with the ethical rules of the Council for International Organization of Medical Science and the German laws for animal welfare. The method of heart preparation and epicardial potential mapping has been described in more detail previously (Dhein et al. 1993) and will be explained only briefly in the following paragraph.

Male white New Zealand rabbits (conventional, normally fed ad libitum, 1,500–1,800 g, Charles River, Kisslegg, Germany) were treated with 1,000 IU/kg heparin i.v. 5 min before they were stunned by a sharp blow on the neck and killed rapidly by subsequent exsanguination as approved by the local committee for animal care. The heart was excised, prepared and perfused according to the Langendorff technique at constant pressure (70 cm H_2O) with Tyrode's solution of the following composition: Na^+ 161.02, K^+ 5.36, Ca^{++} 1.8, Mg^{++} 1.05, Cl^- 147.86, HCO_3^- 23.8, PO_4^{2-} 0.42 and glucose 11.1 mM, equilibrated with 95% O_2 and 5% CO_2 (pH=7.4). The surface temperature of the heart was 37°C. The hearts were connected to a 256 channel mapping system HAL4 (Ing.Buero Peter Rutten, Hamburg, Germany; temporal resolution 20 kHz per channel; amplitude resolution 0.04 mV, interchannel coupling <−60 dB; bandwidth of the system 0.5 Hz–100 kHz; data were not filtered) as described previously (Dhein et al. 1988). Two hundred fifty-six AgCl electrodes (diameter 70 μm) were cast in four polyester plates (in 8*8 orthogonal matrices with 1 mm interelectrodes distance), which were attached to the heart surface in an elastic manner, so that they could follow the heart movements easily without dislocation. The hearts were beating at their spontaneous rate. The four plates were located at the right wall (64 channels), the front wall (64 channels), the left wall (64 channels), and the back wall (64 channels), so that both ventricles were mapped with a total of 256 electrodes.

We administered DHA, EPA and ALA in cumulative concentrations of 1, 2, 5, 10 and 20 μM, each concentration being applied for a period of 15 min via intracoronary infusion. Each experimental series was carried out with $n \geq 6$ experiments. Epicardial potential mapping was performed in each experimental phase during periods of constant cycle length of at least 4 min, in order to make it possible to com-

pare the activation patterns (of single heart beats) or their alterations.

In addition, the functional parameters maximum systolic left ventricular pressure (LVP), basic cycle length (BCL) and coronary flow (CF) were assessed continuously as described (Dhein et al. 1993). The delay between the beginning of the atrial potential and the first normal ventricular activation was assessed as a measure for the atrioventricular conduction time.

For evaluation of the mapping data the activation time points at each electrode were determined as t(dU/dt_{min}) (Dhein et al. 1993; Durrer and Van der Tweel 1954). Next, the repolarization time points were determined as t(dU/dt_{max}) during the T wave as described (Dhein et al. 1993; Millar et al. 1985). After automatic determination activation and repolarization timepoints were verified (or corrected if necessary) manually by the experimentator. From these data for each electrode an activation–recovery interval (ARI, corresponding to the epicardial potential duration) was calculated. The corresponding distribution of ARI was analyzed for each area of the heart (i.e., front, left, right or back wall) calculating the standard deviation of ARI at 64 electrodes and expressed as ARI dispersion.

Moreover, to assess the heart-rate independent changes of ARI, we measured the basic cycle length (BCL) and calculated QTc as $QTc = (ARI/1{,}000)/\sqrt{(BCL/1{,}000)}$. This was calculated for each electrode, and the mean was calculated either for all of 256 electrodes or as local QTc separately for each region from the 64 respective electrodes.

From the activation time points an activation sequence was determined. We determined those electrodes which were activated before any of the neighboring ones and defined them as "breakthrough points" which can be considered as the origins of epicardial activation (Arisi et al. 1983). These breakthrough points were determined for heart beats under control conditions and for heart beats under treatment. Heart beats in the various phases of the experiment were compared to those under control conditions by calculating the percentage of breakthrough points with identical location as compared to their location under control conditions (identical = deviating not more than 1 mm from their location under control conditions, this parameter was named BTP). That means, that two identical heart beats should reveal a breakthrough-point similarity of 100%. It is, however known from previous studies, that identical heart beats do occur only rarely and that arrhythmogenic stimuli can reduce breakthrough-point similarity (Dhein et al. 1988, 1989, 1990, 1993). In the above studies we defined a lower limit of 50% breakthrough-point similarity beneath which it was only a matter of time until arrhythmia would occur.

In a similar way the spread of epicardial excitation was analyzed. In order to allow a quantitative and comparative description of the activation process for each electrode an activation vector was calculated from the activation times and the locations of the surrounding electrodes which were activated after the central electrode (i.e., a maximum number of 8), as described by Müller et al.(1991). These vectors give direction and apparent velocity of local activation. The percentage of similar vectors (VEC) between heart beats in the various experimental phases compared with those under control conditions was determined (vectors deviating not more than 5° from their original direction were considered to be similar). The critical value beneath which arrhythmia often occurs (see above) for VEC similarity is 10% as determined in previous studies (Dhein et al. 1988, 1989, 1990, 1993).

Taken together, the parameters BTP and VEC characterize the geometry of the epicardial activation process, and represent the beat similarity of the cardiac impulse as compared to heart beats under control conditions. Thus, decreasing values for BTP or VEC indicate progressive deviation from the initial (control) activation pattern. Moreover, the total activation time (TAT, ms) was assessed as the delay between activation of the first and activation of the last electrode.

Programmed stimulation In additional experiments we have delivered rectangular pulses of 2 ms duration of increasing amplitude at a frequency of 5 Hz to the back wall of the ventricles in order to elicit a ventricular extrasystole. We have determined the threshold at which a ventricular extrasystole could be induced in absence and presence of DHA, EPA, and ALA (1–20 μM).

Determination of anisotropy In order to assess the degree of anisotropy under the influence of the fatty acids, additional mapping experiments were carried out in which the ventricle was stimulated via a bipolar electrode (rectangular pulses of 2 ms duration and double threshold) and the activation pattern isochrones around this stimulation point were analyzed. From the isochrones and the fiber orientation of the ventricle the longitudinal and transversal conduction velocity was calculated as described by Wit and Dillon (1993) and by Haverkamp et al. (1993). Briefly, longitudinal conduction velocity was evaluated from electrodes (distant from the stimulus site) on the long axis showing the shortest conduction time interval. Transverse conduction velocity was evaluated from electrodes on a line perpendicular to the long axis (90±5°) across the more closely spaced isochrones. Velocities were calculated by dividing the distance traveled by the wave front by the corresponding conduction time interval between the selected electrodes. We avoided such areas for electrode selection which exhibited sudden changes in the density of isochrones. V_L and V_T were determined at least at two sites in each map and the mean was taken for further analysis (for further details see Haverkamp et al. 1993).

Chemicals Docosahexaenoic acid, eicosapentaenoic acid, and α-linolenic acid were obtained from Sigma (Taufkirchen, Germany). A 20 mM stock solution was prepared by dissolving the fatty acids in dimethylsulfoxide (DMSO). This was dissolved in Tyrode solution to the final fatty acid concentration. The maximum final concentration of DMSO did not exceed 0.1%. This concentration has been tested (Dhein et al. 1999) and does not exhibit any effects on the heart regarding the parameters tested in this study. All

chemicals were purchased from Sigma (Taufkirchen, Germany), except heparin which was from Serva (Heidelberg, Germany). All chemicals used were of analytical grade.

Statistics Each experimental series was carried out with $n \geq 6$ experiments. All data are given as means ± SEM of n experiments. Concentration–response curves were fitted using the GraphPadPrism software, version 2.01 for Windows (GraphPad Software, San Diego, CA, USA) for iterative nonlinear regressions and concentration–response curves.

For statistical analysis ANOVA was performed. If ANOVA indicated statistically significant differences nonpaired and

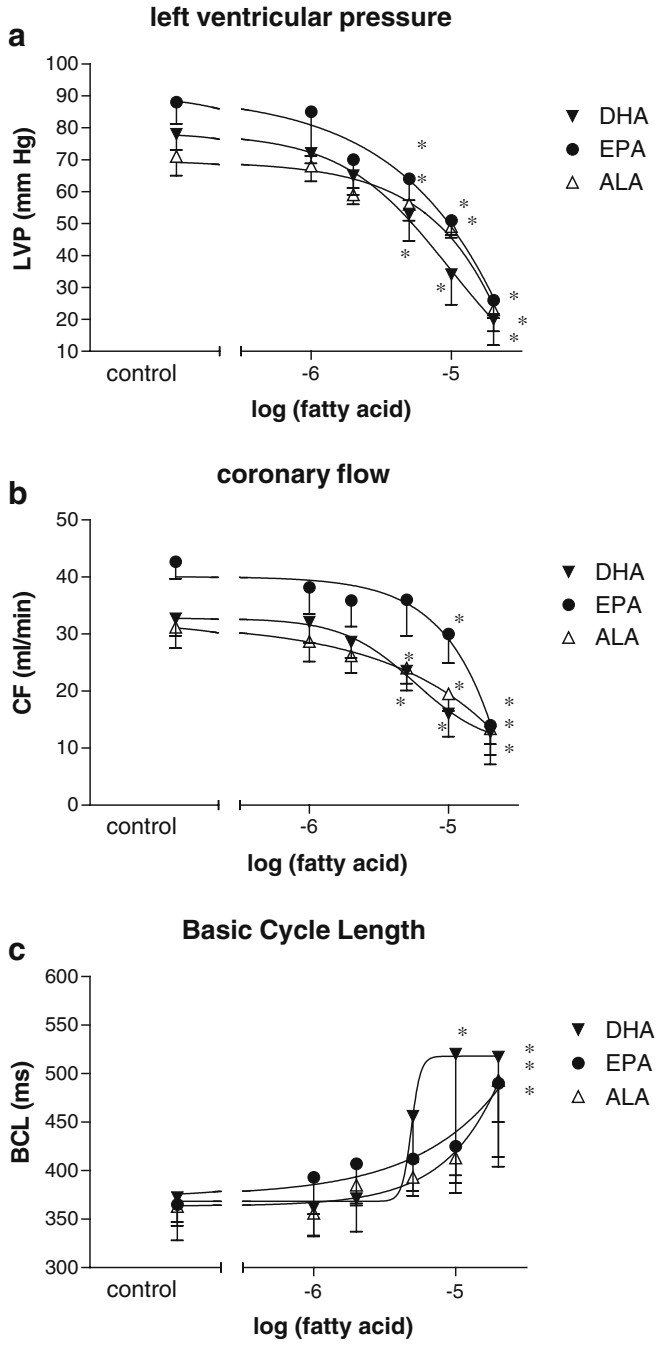


Fig. 1 Effects of polyunsaturated fatty acids on functional parameters of the isolated rabbit heart. **a** Effect of docosahexaenoic acid (*DHA*, n=7), eicosapentaenoic acid (*EPA*, n=7), and α-linolenic acid (*ALA*, n=6) on left ventricular pressure (*LVP*) given as means ± SEM of n experiments. **b** Effects of DHA, EPA, and ALA on coronary flow (*CF*). **c** The response of the basic cycle length (*BCL*) to DHA, EPA, and ALA in the same hearts. Significant changes vs. control conditions are indicated by an *asterisk* (p<0.05).

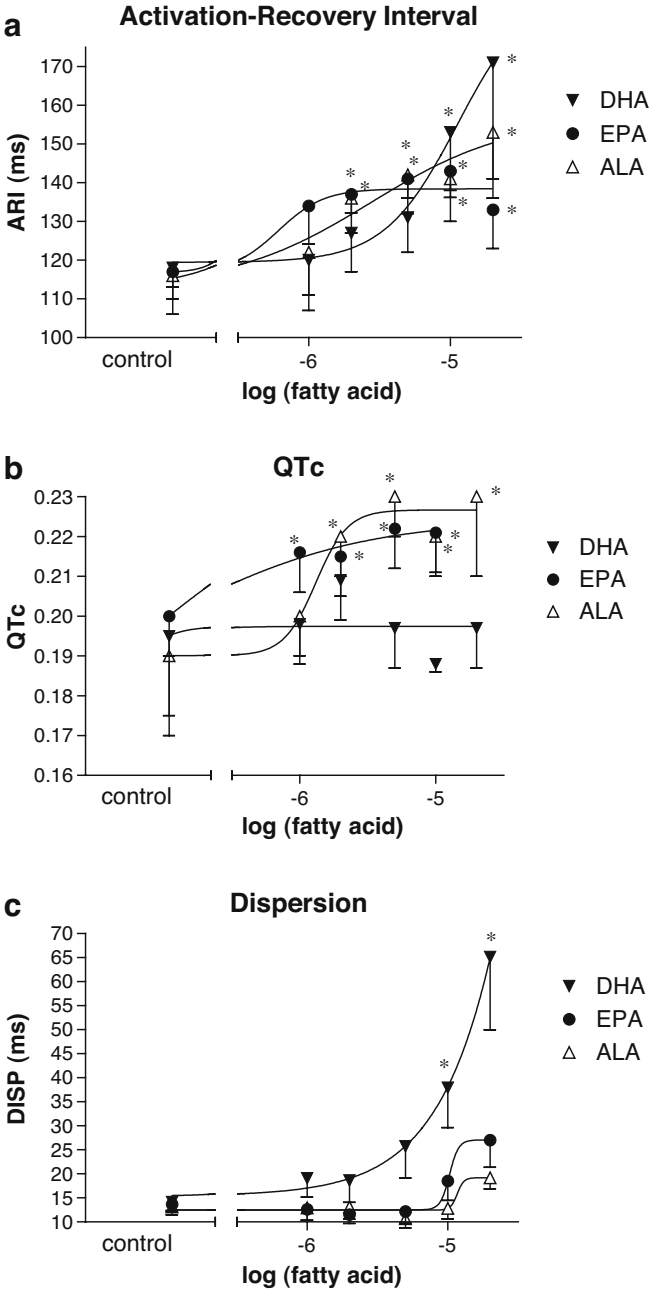


Fig. 2 Effects of polyunsaturated fatty acids on the epicardial activation–recovery interval (*ARI*) and its dispersion. Epicardial potential duration was assessed as the ARI at 256 epicardial ventricular electrodes. **a** Effect of DHA (n=7), EPA (n=7), and ALA (n=6) on epicardial ARI given as means ± SEM of n experiments. **b** The corresponding QTc values to those in panel **a** calculated as $\mathrm{QTc} = (\mathrm{ARI}/1{,}000)/\sqrt{(\mathrm{BCL}/1{,}000)}$. **c** The data for the dispersion of ARI at the 256 electrodes in the three experimental series. Dispersion was calculated as SD of ARI at 256 electrodes. Significant changes vs. control conditions are indicated by an *asterisk* (p<0.05).

Table 1 Local changes in QTc at the right, front, left and back walls of the ventricles of the isolated perfused rabbit heart without treatment (control) or under the influence of 10 μM docosahexaenoic acid (*DHA*, *n*=7), eicosapentaenoic acid (*EPA*, *n*=7) or α-linolenic acid (*ALA*, *n*=6) given as means ± SEM of *n* experiments (given as original data or as percentage of control)

	Right	Front	Left	Back
Control	0.178±0.021	0.180±0.016	0.157±0.016	0.186±0.016
DHA 10 μM	0.226±0.011*	0.187±0.009	0.179±0.024	0.182±0.006
Percentage	147±24*	108±12	118±18	103±12
Control	0.217±0.008	0.206±0.013	0.203±0.011	0.198±0.015
EPA 10 μM	0.233±0.015	0.223±0.017	0.217±0.018	0.223±0.011*
Percentage	103±7	108±4	105±8	118±13*
Control	0.223±0.013	0.202±0.009	0.172±0.012	0.197±0.007
ALA 10 μM	0.0229±0.010	0.213±0.015	0.214±0.011*	0.213±0.007*
Percentage	105±7	107±10	127±11*	109±5*

*Significant changes vs. control conditions ($p<0.05$)
Note that DHA, EPA, and ALA prolonged QTc in specific areas

paired two-tailed Student's *t*-test corrected for multiple comparisons (Bonferroni correction) was used at a level of significance of $p<0.05$. Statistical calculations were performed with the SYSTAT software, version 5.02 for Windows (SYSTAT, Evanston, IL, USA).

Results

Epicardial mapping

Regarding the functional parameters DHA, EPA, and ALA exhibited a clear negative inotropic effect (Fig. 1a). LVP was diminished from values around 70–90 mmHg down to values in the range of 45–20 mmHg at 20 μM. The coronary flow was concomitantly decreased (Fig. 1b). In high concentrations the relative coronary flow rCF (calculated as CF/LVP, ml/min mmHg) was enhanced with DHA, EPA and ALA (by 42±35%, 27±23%, 28±21% at 20 μM).

All drugs slowed the heart rate as indicated by a prolongation of BCL (Fig. 1c), which was maximal with DHA. Concomitantly, we found a prolongation of the atrioventricular conduction time (see below and Fig. 3b). Regarding this parameter we found a similar order of magnitude of effects.

All agents prolonged the ARI (Fig. 2a). However, this effect has to be related to the heart rate, since ARI is frequency-dependent. Therefore, QTc was calculated as mean QTc of all 256 electrodes. The ARI-prolonging effect was mainly due to the bradycardic action of the drugs. However, there was a slight frequency independent QTc prolonging effect by EPA and ALA, while DHA alone did not significantly affect QTc (Fig. 2b). In a more detailed analysis, we evaluated the QTc prolonging effects in the four regions of the ventricles calculating QTc for each region from the 64 electrodes for each area. The QTc-prolonging effects were mostly expressed at the right ventricular wall for DHA and at the left wall for ALA, while EPA prolonged mostly at the back wall (Table 1).

The ARI dispersion, indicating local inhomogeneities of ARI, was enhanced at higher concentrations (>5 μM) by DHA and less (or not affected) by the others (Fig. 2c).

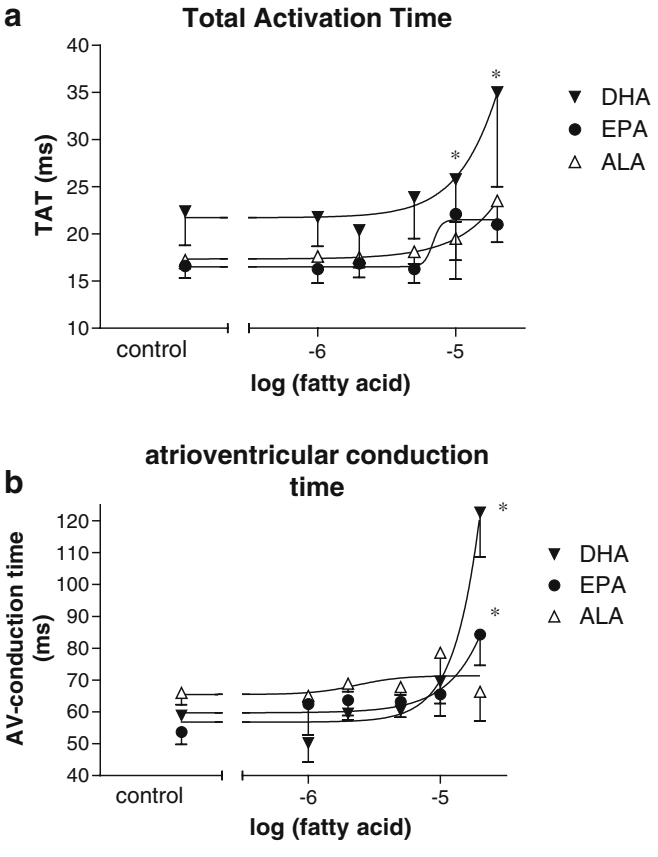


Fig. 3 Effects of the polyunsaturated fatty acids on the atrioventricular and ventricular conduction in the isolated rabbit heart. **a** Effects of DHA (*n*=7), EPA (*n*=7), and ALA (*n*=6) on total activation time (*TAT*) defined as the time delay between activation of the first and last ventricular electrode, thus giving the duration of the ventricular activation process. **b** The changes in atrioventricular conduction time (ms; means ± SEM of *n* experiments) in response to DHA (*n*=7), EPA (*n*=7), and ALA (*n*=6). Significant changes vs. control conditions are indicated by an *asterisk* ($p<0.05$).

Regarding the activation process, the TAT, reflecting ventricular conduction, was prolonged predominantly by DHA and to a lower extent by the other drugs (Fig. 3a). Taken together, these changes indicate a slowing of ventricular conduction mainly by DHA. Atrioventricular conduction was also prolonged by DHA and—to a minor extent—by EPA, but not by ALA (Fig. 3b).

Regarding the pattern of activation, the location of ventricular breakthrough points of activation was only slightly affected by all drugs, leading to a slightly lower breakthrough-point similarity (Fig. 4a). Thus, the starting points of ventricular activation were not affected by the drugs. If the activation breaks through a wavefront is initiated which spreads over the ventricle. These wavefronts were mathematically described as vectors and we investigated possible alterations of these vectors. The percentage of vectors with unchanged direction (= vectorfield similarity) was reduced by all drugs. However, this reduction was only moderate.

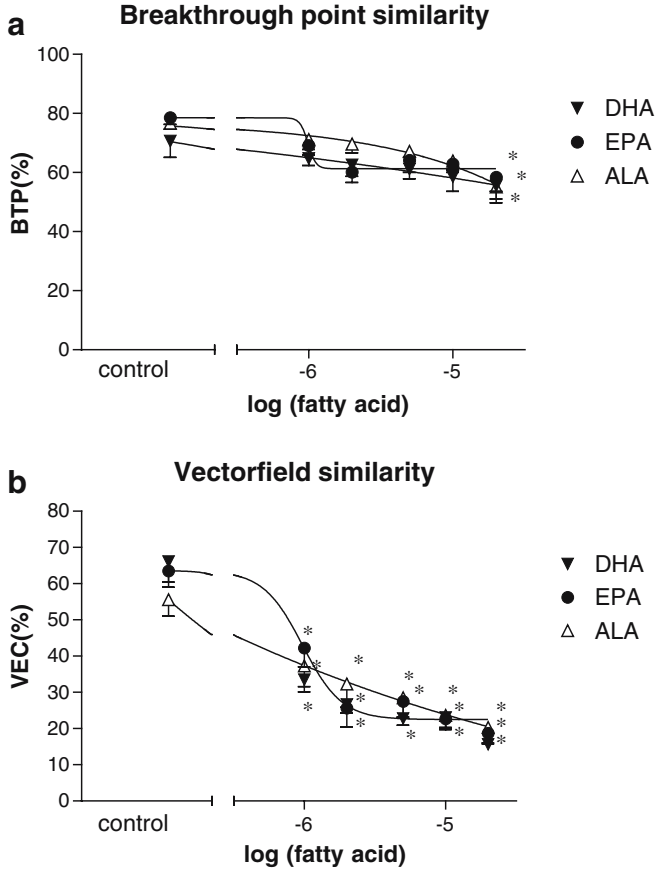


Fig. 4 Effects of polyunsaturated fatty acids on the beat-to-beat similarity of epicardial activation patterns in the isolated rabbit heart. **a** Effects of DHA (n=7), EPA (n=7), and ALA (n=6) on breakthrough point similarity (*BTP*) given as the percentage of breakthrough points with unchanged localization (means ± SEM of n experiments). **b** The effects of DHA, EPA, and ALA on the vectorfield similarity giving the percentage of vectors with unchanged direction (deviation less than 5°; means ± SEM of n experiments). The control value gives the maximum similarity of two succeeding heart beats under control conditions. For further details see text (Materials and methods). Significant changes vs. control conditions are indicated by an *asterisk* (p<0.05).

Table 2 Stimulation thresholds (mA) necessary to elicit a ventricular extrasystole at the back wall

Drug	Control	1 μM	2 μM	5 μM	10 μM	20 μM
DHA	1±0.3	1±0.3	1.5±0.6	1.7±0.5*	2.8±0.6*	–
EPA	1.1±0.2	1.2±0.3	1.5±0.4	1.9±0.4*	2.2±0.2*	2.2±0.2*
ALA	1.1±0.3	1.1±0.2	1±0.3	1.2±0.3	1±0.2	1.1±0.2

*Significant changes vs. control conditions (p<0.05)
Pulse duration was 2 ms, stimulation rhythm was 5 Hz. At 20 μM DHA it was not possible to elicit a response to the extrastimulus

The most prominent reduction in vectorfield similarity to values below 20% (at 20 μM) was observed with DHA (Fig. 4b). Time control experiments (no treatment, vehicle control) revealed that the vectorfield similarity decreases with time. Under control conditions the maximum similarity of two succeeding beat is 45–65% and decreases with time so that after 1 h 25±3% of the vectors still have the same direction (tolerance 5°) as initially as was published earlier (Müller et al. 1991; Dhein et al. 1993). That means, that the drop in VEC under the influence of the fatty acids is only slightly below the time control.

Programmed stimulation

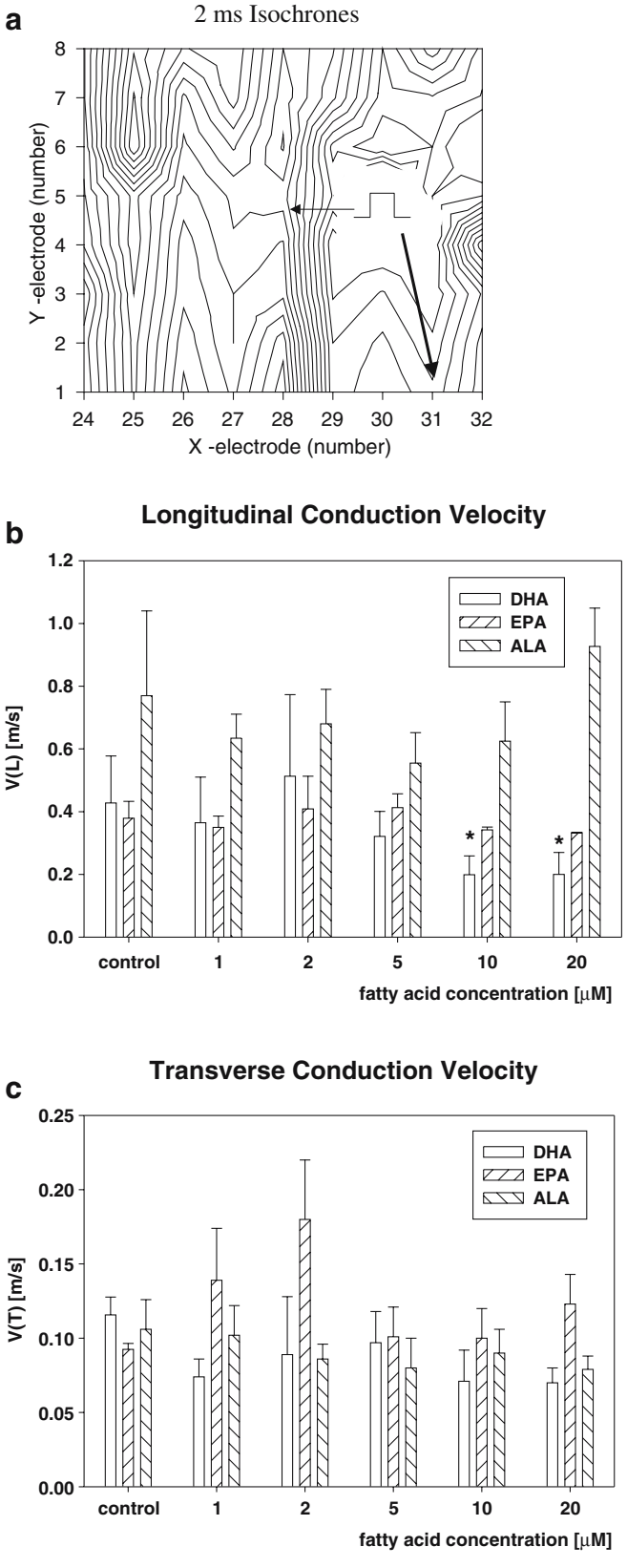
In order to assess the antiarrhythmic activity of the drugs we performed additional experiments using programmed stimulation. We found that the threshold for elicitation of a ventricular extrasystole was concentration-dependently enhanced by DHA, and EPA and the combination, but not by ALA, indicating antiarrhythmic activity for DHA and EPA (Table 2).

Determination of anisotropy

Rectangular pulses of double threshold were delivered to the back wall of the heart and the propagation of the elicited action potential was analyzed transverse and longitudinal to the fiber axis (Fig. 5a). The figure shows the isochrones on the cardiac surface around the stimulation site with maximum $V(L)$ and $V(T)$ indicated by arrows. The interelectrode distance was 1 mm. The numbers refer to the isochrone, the inter-isochrone delay shown is 2 ms. Longitudinal conduction velocity ($V(L)$; Fig. 5b) was concentration-dependently diminished by DHA, however reaching the level of significance only at 10 and 20 μM. DHA had only a minor decreasing effect on transverse conduction velocity ($V(T)$; Fig. 5c). Thus, anisotropy (V_L/V_T) was only slightly but not significantly changed by DHA. ALA and EPA had no concentration-dependent effect on $V(L)$, $V(T)$ or anisotropy.

Discussion

The above results demonstrate that ω-3 polyunsaturated fatty acids exert direct electrophysiological effects. How-



◀ **Fig. 5** Effects of polyunsaturated fatty acids on the longitudinal and transverse propagation of activation. **a** Isochrones under control conditions at the back wall, where an activation was elicited as indicated by a rectangular pulse. Isochrones of activation are given for every 2 ms (starting at the stimulation site). The interelectrode distance was 1 mm in *X* and *Y* direction. Back wall X electrodes are numbered from 24 to 32 (prior X numbers refer to the other regions of the hearts). Note the elliptical propagation of the activation wavefront (*arrows*). **b**, **c** Effects of DHA, EPA, and ALA on longitudinal propagation velocity (V_L) (**b**) and transverse propagation velocity (V_T) (**c**) as means ± SEM of n=6 experiments/series. Significant changes vs. control conditions are indicated by an *asterisk* (p<0.05).

ever, as became evident in this study the profile of electrophysiological effects is different for the various compounds. Regarding the clinical data indicating a possible antiarrhythmic activity (Albert et al. 2002) we found that the threshold for initiation of ventricular extrasystoles was enhanced by DHA and EPA. Although this cannot be transferred uncritically to the clinical situation in which arrhythmia is initiated by other mechanisms, these results show a possible suppression of ventricular arrhythmia. However, this effect could not be verified for ALA. This seems to be in good correspondence with clinical data, since a significant reduction in sudden death in the Physicians Health Study was found in patients without coronary heart disease depending on eicosapentaenoic acid and docosahexaenoic acid, while α-linolenic acid did not exert significant effects (Albert et al. 2002). A suppression of electrically stimulated ventricular extrasystoles by DHA and EPA may—with all caution—indicate a possible inhibition of focal activity.

Regarding the functional parameters we observed a negative inotropic and chronotropic effect with a concomitant reduction in coronary flow. The negative inotropic and chronotropic effect may indicate a calcium-antagonistic effect. This is further supported by the finding of an enhanced relative coronary flow (CF/LVP) which indicates vasodilation at high concentrations of the fatty acids. If these compounds really exert calcium-antagonistic effects one should expect that they slow atrioventricular conduction. Accordingly, we found a prolongation of the atrioventricular conduction time. Taken together, negative inotropic and chronotropic effects, enhanced relative coronary flow and slowed atrioventricular conduction indicate a possible calcium-antagonistic effect. According to our data this would be mostly pronounced with DHA. Regarding the effects of polyunsaturated fatty acids on $I_{Ca.L}$ Macleod et al. (1998) compared the effects of EPA and DHA. EPA resulted in a 50% $I_{Ca.L}$ inhibition at concentrations of about 8.6±1.5 μM (guinea pig ventricular cardiomyocytes). In contrast, in the same cells IC_{50} for DHA was considerably higher with 34.7±2.6 μM. Similar results were found in rat cardiomyocytes (IC_{50} 9.4±0.8 [EPA]; 27.9±2.5 μM [DHA]). The inhibition of $I_{Ca.L}$ was accompanied by a negative inotropic effect in adult guinea pig and rat cardiomyocytes (Macleod et al. 1998), while this was not seen in neonatal rat cardiomyocytes (Kang and Leaf 1996; Kang et al. 1995). In neonatal rat cardiomyocytes EPA resulted in an inhibition of L-type Ca^{++} current with an IC_{50} of 0.8 μM in a voltage- and time-dependent manner. Other polyunsaturated fatty acids such as docosahexaenoic acid, linolenic acid and arachidonic acid produced a similar inhibitory effect on $I_{Ca.L}$ (Xiao et al. 1997). In the rabbit heart, according to our data, the strongest negative inotropic effect and prolonging

effect on atrioventricular conduction was found with DHA with an EC_{50} of 9.5 or 12.5 μM, respectively.

According to our data EPA and ALA prolong QTc indicating frequency-independent prolongation of the action potential with EC_{50} of 0.59 μM (EPA) and 1.47 μM (ALA), while DHA did not significantly affect QTc (as mean at all 256 electrodes, but see below). The most common explanation for this class III-like effect is an inhibition of potassium channels. Thus, it has been shown that the transient outward current $I_{t.o.}$, the delayed rectifier I_K, and the inward rectifier I_{K1} can be inhibited by polyunsaturated fatty acids. Thus, inhibition of I_K (or Kv1.5) with a decrease in peak current and acceleration of the apparent inactivation was seen with DHA and arachidonic acid (30 μM; Honoré et al. 1994). Linolenic acid was reported in that study not to affect I_K. Fifty percent I_K inhibition was achieved using 30 μM DHA. However, in rat pineal gland cells I_K inhibition with DHA exhibited an IC_{50} value of 2.5±0.3 μM (Poling et al. 1995). Using a ramp protocol in their voltage clamp experiments Macleod et al. (1998) found 30–40% inhibition of I_K with 2 μM and 50–60% inhibition with 5 μM EPA. Regarding the transient outward current $I_{t.o.}$ EPA and DHA seemed to be equally effective leading to 50% inhibition in concentration of about 2 μM. In this concentration I_K and I_{K1} were also blocked but apparently to a smaller extent (Macleod et al. 1998). The inhibition of $I_{t.o.}$ would prolong the action potential which might enhance the plateau duration enabling Na^+/Ca^{++} exchange thereby leading to slight positive inotropic effects as observed with concentrations below 10 μM in rat cardiomyocytes (Macleod et al. 1998). The inhibition of $I_{t.o.}$. by polyunsaturated fatty acids was also seen by others (Bogdanov et al. 1995). According to our data, in the rabbit, DHA seems to have only minor effects on QTc (all 256 electrodes, see Fig. 2b) while EPA and ALA significantly prolong QTc. Thus, probably species differences have also to be taken into account as well.

Another aspect of ARI prolongation is the homogeneity of the effect. We found that DHA increased dispersion of ARI indicating that the action potential duration becomes inhomogeneous with a predominant prolongation at the right wall (see Table 1), which might be caused by regional differences in the expression of potassium channels, in sensitivity to the fatty acids or by an inhibition of intercellular gap junctional communication as formerly also seen with palmitoleic acid (Dhein et al. 1999), a substance known to inhibit gap junction conductivity. From this point of view, DHA might possess a possible proarrhythmic risk. However, if DHA would predominantly inhibit gap junctions, one would expect a clear effect on transverse conduction velocity (as previously seen with the gap junction inhibitor palmitoleic acid; Dhein et al. 1999), which was not seen. The QTc prolonging effects of ALA and EPA, however, also showed some inhomogeneity between the four regions of the ventricles which might mean a possible proarrhythmogenic risk, although we have not observed any proarrhythmia within these experiments.

In addition, we found class I-like effects as evident from the prolongation of the total activation time indicating slowing of ventricular conduction. Since the propagation velocity along the fibre axis is directly dependent on the availability of sodium channels (Buchanan et al. 1985), it could be expected that DHA reduces the longitudinal conduction velocity as shown in this study. These effects might indicate a sodium channel inhibiting effect, which seems to be most prominent with DHA and to a lower degree with EPA. Accordingly, it has been shown that both DHA and EPA can inhibit sodium channels. EPA produced a concentration-dependent suppression of I_{Na} in cultured neonatal rat cardiomyocytes and shifted the steady state inactivation to more negative potentials. The IC_{50} value of I_{Na} suppression was 4.8 μM (Xiao et al. 1995) (with 64±5% inhibition at 10 μM and 51±8% inhibition at 5 μM) or 0.51 μM in HEK293t cells (Leaf 2001). In another study the IC_{50} values were calculated with 8.9±0.5 μM in guinea pig ventricular cardiomyocytes and 7.9±0.6 μM in rat ventricular cardiomyocytes (Macleod et al. 1998). Interestingly, the inhibitory action on I_{Na} was not use-dependent in that study, i.e., similar degrees of inhibition were achieved in cells stimulated at frequencies ranging from 0.03 to 1.0 Hz (Xiao et al. 1995). However, to completely exclude use-dependence higher frequencies (such as 3 or 5 Hz) should also be investigated (but these data are not available at present). A similar inhibition of I_{Na} was found with DHA (although an IC_{50} value was not calculated in the study of Xiao et al.) using concentrations of 5 and 10 μM (Xiao et al. 1995). Macleod et al. (1998) found IC_{50} for DHA on I_{Na} of ventricular cardiomyocytes of 15.7±0.9 (guinea pig) and 12.8±0.8 μM (rat), i.e., DHA exhibited slightly lower potency in inhibiting I_{Na} than eicosapentaenoic acid. Ten micromolars ALA also exhibited similar I_{Na} inhibition as compared to EPA (Xiao et al. 1995). In contrast, in the rabbit in our study ALA exerted only minor effects on parameters usually affected by sodium channel antagonists.

An interesting aspect regarding the molecular mechanism comes from a recent study (Xiao et al. 2001): in HEK293t cells expressing the wild type of the hH1α Na^+ channels voltage- and concentration-dependent inhibition of I_{Na} by eicosapentaenoic acid was observed. However, the mutant N406K of the channel was significantly less sensitive to eicosapentaenoic acid. These results demonstrated elegantly that substitution of a single amino acid (N406K) in the domain-1-segment-6 region significantly affected the effect of eicosapentaenoic acid indicating a direct effect of the fatty acid on the channel. Domain-1-segment-6 is also the region containing the binding site for batrachotoxin, while local anesthetics such as lidocain seem to act at the domain-4-segment-6 region. Thus, the site of interaction with the channel where polyunsaturated fatty acids bind seems to be different from the site of action for local anesthetics. This might explain differences in the kinetics and type of block between the two groups of substances. Thus, the study of Xiao et al. (2001) is in further support of a direct binding of polyunsaturated fatty acids to the sodium channel. In addition, in concentrations affecting the I_{Na} (i.e., 1–10 μM) the effect of polyunsaturated fatty acids on the packing of phospholipids in the membrane is considerable low (Pound et al. 2001), so that a direct bind-

ing of polyunsaturated fatty acids to the channel was supposed as the dominant mechanism.

If the activation process is slowed, the pattern of activation might also be affected. Thus, we analyzed the breakthrough-point pattern and its beat-to-beat similarity as well as the vectorfield similarity. The fatty acids had only minor influence on BTP. Since the location of the breakthrough points is defined by the endings of Purkinje fibers, probably the dynamics of the activation of the Purkinje fibers is not affected by the fatty acids. In contrast, we found some effect on the vectorfield similarity which means that the ventricular activation propagation pattern changes. However, as compared to palmitoleic acid or to typical class I antiarrhythmics-like flecainide (which reduce VEC down to 10± 1%; Dhein et al. 1993), the effects of DHA, EPA and ALA on VEC are moderate and only slightly below the time control (which is 25±3%). Strong alterations of the vectorfields indicate proarrhythmic activity (Dhein et al. 1993). However, a direct comparison with flecainide is not possible since in that case use-dependent block has also to be taken into account and would be out of the scope of the present study. Our present data might indicate a certain proarrhythmic risk for DHA, while the others did show minor effects. In principle the class III-like effects of ALA and EPA might bear the risk of torsade-de-pointes arrhythmia, but we have never observed such an arrhythmia with these drugs. Probably, in comparison to classical class III antiarrhythmics such as amiodarone, sotalol or dofetilide, the prolongation of QTc is too small to evoke this type of arrhythmia.

A further point worth discussing is the question of concentrations. In the concentration range in which we could observe direct antiarrhythmic and electrophysiological effects a marked negative inotropic effect was also seen. Under in vivo conditions this effect might be compensated by activation of the sympathoadrenergic system, which is not possible to investigate in a denervated isolated Langendorff heart. Moreover, under therapeutic conditions, the polyunsaturated fatty acids are administered chronically and it has been assumed that the fatty acids accumulate in the membranes and in the adipose tissue (Albert et al. 2002). These authors described the antiarrhythmic effects to occur at levels of 4–7% polyunsaturated ω-3 fatty acids of total fatty acids. Thus, it is difficult to extrapolate from the concentration–response curves as investigated in our present acute study to the chronic in vivo situation.

Taken together, DHA, EPA and ALA exert direct electrophysiological effects and an antiarrhythmic effect could be shown for DHA and EPA. While all fatty acids exhibited effects similar to calcium antagonists, DHA might affect sodium channels acting in a class I-like manner according to this data and the literature. EPA and ALA predominantly affected QTc in a class III-like manner and therefore may be supposed to act on potassium channels besides an action on I_{Na} by EPA. However, this interpretation needs some caution since in other species the prevalence for a certain type of channel might be different as the literature shows.

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