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Polyunsaturated fatty acid-derived I_{Ks} Channel Activators Shorten the QT interval ex-vivo and in-vivo

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Abstract

Aim—We aimed to assess the ability of natural and modified polyunsaturated fatty acids to shorten QT interval in *ex-vivo* and *in-vivo* guinea pig hearts.

Methods—The effect of one natural (docosahexaenoic acid) and three modified (Lin-GLY, DHA-GLY, N-AT) polyunsaturated fatty acids on ventricular action potential duration (APD) and QT interval was studied in a E4031 drug-induced long QT2 model of *ex-vivo* guinea pig hearts. The effect of DHA-GLY on QT interval was also studied in *in-vivo* guinea pig hearts upon intravenous administration. The effect of modified polyunsaturated fatty acids on I_{Ks} was studied using *Xenopus laevis* oocytes expressing human KCNQ1 and KCNE1.

Results—All tested polyunsaturated fatty acids shortened ADP and QT interval in *ex-vivo* guinea pig hearts, however with different ability in restoring baseline APD/QT interval with specific modified polyunsaturated fatty acids being most efficacious. Despite comparable ability in activating the human KCNQ1/KCNE1 channel, Lin-GLY was not as effective in shortening APD/QT interval as DHA-GLY in *ex-vivo* hearts. By constructing a guinea pig-like KCNE1, we found Lin-GLY to induce less activating effect compared with DHA-GLY on human KCNQ1 co-expressed with guinea pig-like KCNE1. DHA-GLY was studied in more detail and was found to shorten QT interval in *in-vivo* guinea pig hearts.

Conclusion—Our results show that specific polyunsaturated fatty acids shorten QT interval in guinea pig hearts. The tendency of modified polyunsaturated fatty acids with pronounced I_{Ks} channel activating effect to better restore QT interval suggests that modifying polyunsaturated fatty acids to target the I_{Ks} channel is a means to improve the QT-shortening effect.

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Conflict of Interest

A patent application based on these results has been submitted by the University of Miami with S.I.L. and H.P.L. identified as inventors.

Keywords

Guinea pig heart; IKs channel; KCNQ1; Kv7.1; Long QT Syndrome; PUFA

Introduction

One of the major cardiac potassium currents, I_{Ks}, is generated by the slowly activating I_{Ks} potassium channel[1] that is composed of 4 alpha (K_V7.1, KCNQ1) subunits and 1 to 4 beta (KCNE1) subunits[2–4]. Over 300 different mutations in the genes encoding KCNQ1 and KCNE1 subunits are linked to a prolonged QT interval in the electrocardiogram (ECG), leading to a condition known as congenital Long QT Syndrome (LQTS)[1]. In the general population, one in 2000 has congenital LQTS[5], where mutations in the alpha-subunit (KCNQ1) of the I_{Ks} channel complex is the most common form of LQTS (called LQT1)[1]. Such mutations decrease the current through the I_{Ks} channel, which impairs repolarization of the cardiac action potential and results in prolonged action potential duration. The prolonged ventricular action potential duration is seen in the surface ECG as a prolonged QT interval. LQTS is a risk factor for ventricular fibrillation and sudden cardiac death[6–8]. 4000 Americans die yearly from sudden cardiac death caused by congenital LQTS[9], most of which occur in children and young adults. Additionally, QT-prolonging medications (such as specific anti-arrhythmics and antibiotics) may cause drug-induced LQTS, of which block of the delayed rectifier current I_{Kr} (i.e. drug-induced LQT2) is the most common cause[10,11]. At present, LQTS is treated using β blockers and implantable cardioverter-defibrillators[12,13]. Although these approaches reduce cardiac arrhythmias by reducing the occurrence of pro-arrhythmic sympathetic triggers (in the case of β blockers) or injecting currents to stop arrhythmias (in the case of defibrillators), neither of these approaches treat the underlying defect. In addition, β blockers do not work in all patients, are contraindicated in some patients, and have side effects, such as fatigue, diarrhea, vomiting, heart failure, and depression[14–16], which may limit their utility. The cost of defibrillator implantation is high and implantation might cause damage to heart and lung tissue, and post-hospitalization infections, device related complications and most importantly in-appropriate shocks[12,13,17]. Another treatment strategy would be to restore physiological QT interval by directly targeting the underlying cause of the disease, which most commonly are defective I_{Ks} channels. However, there is no clinically approved activator of the I_{Ks} channel, highlighting the need to develop I_{Ks} channel activators whose QT-shortening potential are evaluated in different models. In this study we assess the ability of a set of I_{Ks} channel activators to shorten QT interval in guinea pig hearts.

Polyunsaturated fatty acids (PUFAs) have been put forward as anti-arrhythmic compounds[18]. The American Heart Association recommends intake of fish rich in PUFAs, particularly in individuals with cardiovascular problems[19]. However, results from both basic research and clinical trials have been mixed with some studies showing clear positive effects of PUFAs on cardiovascular parameters, while other trials showed no significant effect and some basic research showed even pro-arrhythmic effects[19–26]. These divergent results could be due to several factors, including the heterogeneity of the patients (or animal models), small patient populations in some studies, and potential differences in the efficacy of PUFAs

for different types of arrhythmias (triggered versus re-entry)[23,26]. In addition, most of these studies did not measure the blood levels of PUFAs to determine their bioavailability[19]. A large prospective cohort study that measured blood levels of PUFAs found that there was a strong inverse correlation of mortality from cardiac arrhythmias and blood levels of PUFAs: the highest quintile had 45% less chance of dying from cardiac arrhythmias compared to the lowest quintile[27].

It has been suggested that beneficial effects of PUFAs, such as docosahexaenoic acid (DHA, the most abundant PUFA in fish oils), are due to inhibition of voltage-gated Na^+ and Ca^{2+} channels[20,28–31]. We and others have shown that natural PUFAs, such as DHA, have small or no effect on I_{Ks} channels[32–34]. In contrast, we recently showed that modified PUFAs (such as N-arachidonoyl taurine, N-AT, and DHA glycine, DHA-GLY) have large activating effects on I_{Ks} channels[34] and restore the function of I_{Ks} channels with LQT1 mutations[35]. This suggests that modified PUFAs would be more effective at shortening the QT interval compared to natural PUFAs by adding an effect of PUFAs on I_{Ks} channels. In this study we assess the ability of natural and modified PUFAs to shorten the QT interval in *ex-vivo* and *in-vivo* guinea pig hearts. Guinea pigs are often used for cardiac studies because their cardiac action potential resembles the human, but still has the benefit of a relative small size as compared to rabbits and larger mammals[36–41].

Our results show that specifically modified and natural PUFAs shorten the QT interval in *ex-vivo* guinea pig hearts. The most efficacious PUFA analogue, DHA-GLY, fully restored baseline QT interval in *ex-vivo* guinea hearts with drug-induced LQTS and significantly shortened QT interval in *in-vivo* guinea pig hearts. Our findings suggest that modified PUFAs, which directly increase the I_{Ks} currents, have a tendency towards increased QT-shortening efficacy compared with PUFAs that have no or very little effect on the I_{Ks} currents. We propose that these modified PUFAs could be developed into novel LQTS drugs that could potentially prevent prolonged QT intervals in LQTS patients and, thereby, reduce the risk of developing ventricular fibrillation and sudden cardiac deaths. Such developed modified PUFAs may offer a complement to the current treatments of LQTS, such as β blockers and implantable cardioverter-defibrillators.

Results

PUFA and PUFA analogues shorten QT interval in ex-vivo guinea pig hearts with drug-induced LQT2

We used the I_{Kr} blocker E4031 to induce LQTS in *ex-vivo* guinea pig hearts (Fig. 1a, see Methods for details). This model of drug-induced LQT2 gives the advantage of prolonging the QT interval by affecting another channel than the I_{Ks} channel. On average, 20 minutes of application of 0.03 μM of E4031 resulted in a prolongation of QT interval and action potential duration (APD_{90}) of 18 ± 1 ms and 16 ± 1 ms (n = 23), respectively. The QT and APD prolongation induced by continuous application of E4031 was stable for a minimum of 30 minutes (Supplementary Fig. 1).

We next assessed the ability of one PUFA (DHA) and three different PUFA analogues (DHA-GLY, Lin-GLY and N-AT) to restore baseline QT interval and APD in *ex-vivo*

hearts with established drug-induced LQT2 (E4031 was continuously applied). Molecular structures of PUFA and PUFA analogues are provided in Figure 1b. We have previously described that DHA-GLY gradually shortens the QT interval and APD in *ex-vivo* guinea pig hearts with prolonged QT interval over an application period of 30 minutes[34]. In line with these previously published data, we here find that application of 10 μM of DHA-GLY for 30 minutes shortened the QT interval and APD_{90} compared to E4031 values and restored baseline QT interval (Fig. 1c, Table I). Application of 10 μM of DHA shortened QT interval and APD_{90} compared to E4031 values (Fig. 1d, Table I). However, the QT interval was not fully restored to baseline values with a trend towards statistically significant difference from baseline ($P = 0.06$) (Fig. 1d, Table I). The APD_{90} was restored to baseline values by 10 μM of DHA, but there was a trend towards larger effects of 10 μM of DHA-glycine on the APD_{90} as compared to DHA ($P = 0.10$) (Fig. 1d). The two PUFA analogues Lin-GLY and N-AT showed variable ability to shorten QT interval: Application of 10 μM of Lin-GLY for 30 minutes shortened the QT interval and APD_{90} compared to E4031 values but did not fully restore baseline QT interval or APD (Fig. 1e, Table I). Application of 30 μM of N-AT for 30 minutes significantly shortened the QT interval and APD_{90} compared to E4031 values and fully restored baseline QT interval and APD (Fig. 1f, Table I). The reason for the higher concentration of N-AT used in these experiments is that N-AT has an apparent lower affinity to the I_{Ks} channel compared with DHA-GLY and Lin-Gly[42].

Because DHA-GLY was most efficacious in restoring the prolonged QT-interval to baseline values, we investigated DHA-GLY effects on other parameters of cardiac function. Application of 10 μM of DHA-GLY did not affect QRS-duration (Table II) or retrograde coronary flow (Table II). However, 10 μM of DHA-GLY significantly increased the PR interval (Table II).

Altogether, data from *ex-vivo* guinea pig hearts show that all tested PUFA/PUFA analogues significantly shortened QT interval and APD (by about 4–16 ms and 9–23 ms, respectively) in *ex-vivo* guinea pig hearts with prolonged QT interval (Table I). However, in contrast to DHA-GLY and N-AT, Lin-GLY and DHA failed to fully restore baseline QT interval, while DHA, but not Lin-GLY, did normalize APD (Table I).

DHA-GLY shortens QT interval in in-vivo guinea pig hearts

To investigate whether the effects of DHA-GLY observed in *ex-vivo* hearts translate into *in-viv*o experiments, we utilised a novel closed chest cardiac electrophysiology model[43], in which QT interval was monitored with custom-made ECG needle electrodes and cardiac parameter determined during intra-cardiac pacing at 300 BPM (Figure 2a, see Methods for details). QT interval, PR interval and QRS duration remained stable during 30 minutes of vehicle infusion (Fig. 2b–d, Table III). In contrast, infusion of 10 mg/ml of DHA-GLY (corresponding to 40 mg/kg/hr) gradually shortened QT interval over the 30 minutes DHA-GLY was infused (Fig. 2b). After 30 minutes of infusion of 10 mg/ml of DHA-GLY, the changes in QT interval was significantly larger in the DHA-GLY group as compared to the time-matched controls (−17.3 ± 2.8 ms vs 1.2 ± 1.6 ms) (Fig. 2b, Table III). No significant effect of DHA-GLY was observed on the QRS duration whereas the PR interval was prolonged by DHA-GLY (Fig. 2c–d, Table III).

Lin-GLY is less effective than DHA-GLY in activating a guinea-pig like I_{Ks} channel

Lin-GLY was as effective as DHA-GLY in activating the human I_{Ks} (hKCNQ1/hKCNE1) channel expressed in *Xenopus* oocytes (Fig. 3a): 20 μM of Lin-GLY or DHA-GLY shifted the midpoint for channel activation, V_{50}, by about −34 mV (Fig. 3a, $P > 0.05$ for 20 μM using Student's t-test). Because DHA-GLY was clearly effective in restoring the QT interval in guinea pig hearts, we were surprised that Lin-GLY shortened but did not restore the QT interval in guinea pig hearts. One hypothesis for the similar effect on human I_{Ks} channel but different effect in guinea pig heart is that DHA-GLY is not shortening the QT interval in guinea pig hearts by activating guinea pig I_{Ks} channels, but through some other mechanism that is specific for DHA-GLY, but not Lin-GLY. Another hypothesis is that DHA-GLY activates the guinea pig I_{Ks} channels, whereas Lin-GLY does not activate the guinea pig I_{Ks} channels. To test the second hypothesis, we looked for differences in the sequences of human and guinea pig KCNQ1 and KCNE1 subunits. The sequences of human and guinea pig KCNQ1 subunits are very similar in the region on which PUFAs have been suggested acting in I_{Ks} channels (external ends of S3, S4, and S6 of KCNQ1[34,44] (Supplementary Fig. 2). There are, however, three amino acids that are very different between human and guinea pig KCNE1 at the external end of the transmembrane region of KCNE1 (Fig. 3b), which are also located close to the putative PUFA binding site. We therefore mutated these three amino acids in hKCNE1 to the corresponding guinea pig amino acids (RSS to LRD), to create a guinea pig-like KCNE1 (referred to as gplKCNE1). Surprisingly, 20 μM of Lin-GLY had less effect compared with 20 μM of DHA-GLY on hKCNQ1 co-expressed with gplKCNE1 in *Xenopus* oocytes (Fig. 3c). Whereas DHA-GLY shifted V_{50} by about −24 mV Lin-GLY shifted V_{50} by only −17 mV (Fig. 3c, $P < 0.05$ for 20 μM using Student's t-test). No significant difference in the effect was observed at 7 μM ($P > 0.05$ using Student's t-test). However, because of challenges in exact translation of relevant concentrations between model systems, the different effects of the slightly higher concentrations of DHA-GLY and Lin-GLY on I_{Ks} current when using the guinea pig-like KCNE1 subunit may contribute to the different effects of DHA-GLY and Lin-GLY on shortening the QT interval in guinea pig hearts.

Discussion

In this study, we assessed the ability of natural and modified PUFAs to shorten the QT interval in guinea pig hearts. DHA, DHA-GLY, Lin-GLY and N-AT all significantly shortened the QT interval in a drug-induced model of LQTS in *ex-vivo* guinea pig hearts. However, they restored baseline QT interval to different extent. In addition, for the first time we demonstrate that modified PUFAs shortened the QT interval *in-vivo* in guinea pig. Altogether, this study suggests that modifying PUFAs to target the I_{Ks} channel is a means to improve the QT-shortening effect of PUFA.

What may be the mechanistic basis underlying the variable ability of tested compounds to shorten the QT interval? We have previously suggested that natural and modified PUFAs activate the I_{Ks} channel by a lipoelectric mechanism, in which the PUFA inserts itself in the otherwise membrane-filled cleft between two voltage-sensing domains of KCNQ1 subunits (Supplementary Fig. 2)[34,44]. From this position, the negatively-charged head

group of the PUFA would electrostatically interact with the positively charged residues in the KCNQ1 voltage sensor S4 and a positively charged lysine in S6 to activate the I_{Ks} channel by shifting the voltage-dependence of channel opening and increasing the maximal conductance, respectively[44]. We have previously shown that a low pKa of the PUFA head group is important to keep the head group negatively charged when bound to KCNQ1/KCNE1 channels[33,34]. For example, DHA has no significant effect on human KCNQ1/KCNE1 channels expressed in oocytes at pH 7.4, most likely because the carboxyl head group of DHA is protonated and uncharged[33,34]. In contrast, DHA-GLY has a much lower pKa and N-AT even a lower pKa[34], such that DHA-GLY and N-AT would be partly and fully deprotonated, respectively, at physiological pH. A protonated DHA, with a limited ability to activate KCNQ1/KCNE1 channels in guinea pig hearts, could explain why DHA did not shorten the QT interval as much as DHA-GLY and N-AT in *ex vivo* guinea pig hearts. Even if DHA did restore the ADP_{90} to baseline values, there was a trend that DHA did not shorten the APD_{90} as much as DHA-GLY or N-AT, suggesting that these modified PUFAs would be more effective than regular PUFAs to treat Long QT Syndrome.

Surprisingly, although Lin-GLY and DHA-GLY had similar activating effects on human KCNQ1/KCNE1 (hKCNQ1/hKCNE1) channels expressed in *Xenopus* oocytes, we found that Lin-GLY shortened the QT interval and APD to a lesser extent than DHA-GLY. Based on our two-electrode voltage-clamp recordings, this is likely because Lin-GLY has less activating effect than DHA-GLY on “guinea pig-like” KCNQ1/KCNE1 (hKCNQ1/gplKCNE1) channels, and hence shortens the QT interval to a smaller extent. The three residues on the guinea pig KCNE1 subunit that when introduced in the human KCNE1 subunit make guinea pig like KCNQ1/KCNE1 channels less sensitive to Lin-GLY are located at the external end of the KCNE1 transmembrane segment (Fig. 3C). Why these three residues determine the DHA-GLY and Lin-GLY selectivity for KCNQ1/KCNE1 channels is not clear. However, in a recent model of KCNQ1/KCNE1 channels, these three KCNE1 residues would be located very close to our proposed PUFA binding site[44] (Supplementary Fig. 2) and these residues could therefore interact with the PUFA. Further experiments are necessary to determine the molecular mechanism for the PUFA specificity of KCNQ1/KCNE1 channels. As importantly, our findings of species difference in the efficacy of PUFAs stresses the importance of thorough assessment of novel drug candidates in multiple species and in human cell systems prior to clinical testing, in order to approve the possibility for translation of preclinical findings to clinical efficacy.

Several observations suggest that natural and modified PUFAs target multiple ion channels. Firstly, although DHA in our hands does not activate KCNQ1/KCNE1 expressed in *Xenopus* oocytes[33,34], we here observed that DHA shortened QT interval and APD in *ex-vivo* hearts. The QT shortening DHA effect could be caused by previously described inhibiting effects of DHA on cardiac voltage-gated Na^+ and Ca^{2+} channels[20,28–31]. In line with this, DHA has been shown to shorten action potential duration in isolated guinea pig cardiomyocytes by inhibiting Na^+ and Ca^{2+} currents, whereas the effect on delayed potassium current was limited[45]. We did observe a significant prolongation of PR-interval after DHA application (from 60 ± 2 to 65 ± 3 ms, P = 0.006 with one-way ANOVA followed by Tukeý́s multiple comparison test), which could be caused by Ca^{2+} channel inhibition by DHA. However, as DHA has been shown to increase I_{Ks} in a fraction of guinea pig cardiomyocytes[32],

it is possible that DHA effects on guinea pig I_{Ks} also has a minor contribution to the QT shortening effect. We did also observe a significant prolongation of PR-interval after DHA-GLY application, which could be caused by Ca^{2+} channel inhibition by DHA-GLY. We have in a recent study shown that modified PUFAs inhibit Nav1.5 and Cav1.2 expressed in *Xenopus* oocytes, in addition to activating KCNQ1/KCNE1 channels[42]. Altogether, this suggests that natural and modified PUFAs shorten QT interval and APD in guinea pig hearts by targeting several ion channels. Notably though, the diverse effects of the different PUFAs on guinea pig hearts observed in this study mirror the effects of different PUFAs on KCNQ1/KCNE1 channels. This suggests that modified PUFAs shorten the QT interval, at least in part, by acting on I_{Ks} channels in guinea pig hearts and that the efficacy of PUFAs may be increased by engineering an activating effect on the I_{Ks} channel.

Regular PUFAs, such as DHA and EPA, have shown mixed results in clinical trials in preventing cardiac arrhythmia and sudden cardiac death. DHA did significantly reduce the prolonged QT interval and APD in our drug-induced model of LQTS in *ex-vivo* guinea pig hearts. There is a trend that two of the three modified PUFA analogues shorten the QT interval and APD more than DHA in our study, suggesting that these modified PUFAs would be more effective in treating LQTS than regular PUFAs. We tested DHA-GLY *in-vivo* and found it to significantly shorten the QT interval. Further studies are needed to test whether these modified PUFAs can prevent cardiac arrhythmia and sudden cardiac death *in vivo*.

Materials and Methods

The animal experiments followed the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes and were approved by The Ministry of Environment and Food of Denmark under License No. 2017-15-0201-01296 or The Linköping University Animal Ethics Committee (Ethical Permit 1659). Surgery of *Xenopus laevis* to isolate oocytes were approved by The Linköping University Animal Ethics Committee (Ethical Permit 1941). The study conforms with good publishing practice in physiology[46].

Drugs

DHA (Docosahexaenoic acid), DHA-GLY (Docosahexaenoyl Glycine), Lin-GLY (Linoleoyl Glycine), N-AT (N-Arachidonoyl Taurine) were purchased from Cayman Chemical Company (Michigan, USA). E-4031 was purchased from Tocris (Bristol, UK). 10% albumin in 0.85% sodium chloride and 0.05% sodium azide was purchased from Sigma-Aldrich, St. Louis MO, USA. DHA-GLY was delivered as a stock solution of 130 mM in EtOH. DHA, Lin-GLY and N-AT were prepared as stock solutions of 30 mM (N-AT) or 100 mM (DHA and Lin-GLY) in EtOH and stored at −20°C. Test solutions were prepared at the day of experiments.

Isolated ex-vivo heart experiments

Female Dunkin Hartley guinea pigs (300–450 g. Charles River, France) were used for the *ex-vivo*, Langendorff, experiments. The guinea pigs were anesthetised by an intraperitoneal injection of 200 mg/ml pentobarbital and 20 mg/ml lidocaine hydrochloride (Glostrup

Apotek, Denmark), 1.5 ml/kg. A tracheostomy was performed, and the guinea pigs were ventilated using a rodent ventilator, 60 strokes/minute and 5 ml volume, (Model 7025 Ugo Basile, Italy). The ribcage was cut open and hearts excised and cannulated *in-situ* by a small incision near the aortic arch and connected to the Langendorff apparatus (Hugo Sachs, Harvard Apparatus, Germany). The hearts were mounted on a Langendorff-perfusion apparatus and retrogradly perfused at a constant pressure of 60 mmHg with a 37°C modified Krebs'–Henseleit solution (in mmol L-1: NaCl 120, $NaHCO_3$ 25, KCl 4, MgCl 0.6, NaH_2PO_4 0.6, $CaCl_2$ 2.5, Glucose 11) saturated with 95% O_2 and 5% CO_2. The hearts were submerged into a temperature-controlled organ bath containing 37°C carbonated Krebs-Henseleit buffer. Perfusion pressure, coronary flow, ECG and MAP signals were continuously sampled at 2k/s and digitised by a Powerlab 16/30 (ADinstruments) and monitored using LabChart 7 software (ADinstruments). Volume conducted ECGs and monophasic action potentials (MAPs) were recorded throughout the experiments and the four MAP electrodes were placed on the right and left ventricle. The heart rate was controlled by electrical stimulation of the right atrial appendage using a bipolar pacing electrode. Atrial epicardial pacing was performed at 240 BCL/250 BPM with square pulses of 2 ms.

We previously showed that DHA-GLY successfully restored a prolonged QT-interval induced by hERG blocker E4031[34]. Testing of PUFA and modified PUFA in this study followed the same procedure. In brief, after a stabilisation period of 30 minutes, baseline recordings were measured for 2 minutes during pacing at 250 BPM. This was followed by 20 minutes of perfusion of 0.03 μM E4031. At this concentration E4031 is believed to affect the I_{Kr} current exclusively[47,48]. After 20 minutes of perfusion with E4031, the hearts were paced for 2 minutes at 250 BPM. If the QT had prolonged by less than 10 ms the experiment was excluded from the study. This was followed by 30 minutes of perfusion with 10 μM of DHA, DHA-GLY, Lin-GLY or 30 μM of N-AT together with 0.03 μM of E4031 at intrinsic heart rate. Every 10^{th} minute the hearts were paced for 2 minutes at 250 BPM. Left ventricular MAP recordings were used for the analysis of APD_{90} unless the signal was lost during the experiments. In this case the right ventricular APD signal was used.

In-vivo closed-chest experiments

Female Dunkin Hartley guinea pigs weighing 300–450 g were sedated with 5% isoflurane/oxygen in a sedation box and transferred to the procedure table. It should be noted that isoflurane is reported to mediate QT-prolongation through inhibition of the I_{Ks} and I_{Kr} current in guinea pigs[49,50]. The isoflurane level was reduced to a maintenance level of 2.5–3% and delivered through a mask. The body temperature was continuously monitored using a MicroTherma 2 rectal thermometer (ThermoWork Inc, UT, USA) and the guinea pigs were kept at 38–39°C using an (watts) infrared lamp. Custom-made ECG needle electrodes were attached to each limb and connected to an ADinstruments Octal Bio Amp and the signals were processed by an ADinstruments Powerlab 16/30 data acquisition system (Dundedin, New Zealand). An electrophysiological catheter for intracardiac pacing (EPR-802 Millar Inc. USA) was placed in the right atrium via a small surgical incision in the jugular vein. The pacing catheter was connected to a Digitimer DS3 isolated current stimulator (Settings; current amplitude × 100 μA, duration 100 μs × 4) and the hearts were paced with 2 ×

diastolic threshold. An intravenous catheter was also placed in the jugular vein (I.D. 0.5 mm O.D. 0.8 mm Natsume Seisakusho Japan) for drug administration and connected to a syringe pump (New Era Syringe Pump Systems NE300, Farmingdale, NY, USA).

The guinea pigs were randomised to receive either DHA-GLY 10 mg/ml (corresponds to 40 mg/kg/hr, n = 5) dissolved in vehicle or corresponding amount of vehicle (n = 4) (10% albumin in 0.85% sodium chloride and 0.05% sodium azide). The 15-minute baseline recording contained 1-minute trains with 200 BCL/300 BPM atrial stimulation every 5th minute. At time 0, the infusion pump was started and either vehicle (10% albumin in 0.85% sodium chloride and 0.05% sodium azide) or DHA-GLY was constantly infused over a 30-minute period. During infusion, 1-minute trains with 200 BCL/300 BPM atrial stimulation was performed every 5th minute.

Xenopus oocyte experiments

Xenopus oocytes were surgically isolated at Linköping University. KCNQ1 and KCNE1 channel cRNA were transcribed using the mMessage mMachine T7 kit (Ambion, Fischer Scientific, Sweden). Site-directed mutagenesis was performed using the Quickchange II XL Mutagenesis Kit (QuikChange II XL with 10 XL Gold cells, Agilent, CA, USA) for mutations in KCNE1 to construct the guinea pig like KCNE1 construct. 50 ng of cRNA was injected at a 3:1, weight:weight (KCNQ1:KCNE1) ratio into defolliculated *Xenopus laevis* oocytes for I_{Ks} channel expression. Injected oocytes were incubated at 16°C for two to five days before performing two-electrode voltage clamp experiments using a Dagan CA-1B Amplifier (Dagan, MN, USA). Currents were filtered at 500 Hz and sampled at 5 kHz. The holding voltage was set to −80 mV. Activation curves were generated in steps between −80 to +60 mV in increments of 20 mV (5 s duration). The tail voltage was set to −30 mV. The control solution contained (all in mmol L-1) 88, NaCl, 1 KCl, 15 HEPES, 0.4 $CaCl_2$, and 0.8 $MgCl_2$. pH was set to 7.4 using NaOH. All compounds were bought from Sigma-Aldrich (Stockholm, Sweden). Control or PUFA solution was applied using a Minipuls 3 peristaltic pump (Gilson, WI, USA) until the effect on current amplitude reached steady state (typically 3–8 minutes depending on concentration). The chamber was cleaned in-between each oocyte using ethanol-supplemented control solution.

Electrophysiological analysis was performed in GraphPad Prism 8 (GraphPad Software Inc., CA, USA). To quantify the voltage dependence for channel opening, tail currents were measured shortly after stepping to the tail voltage and plotted against the preceding activation voltage. A Boltzmann function was fitted to the data to generate the conductance *versus* voltage ($G(V)$) curve:

$$G(V) = G_{min} + (G_{max} - G_{min}) / \left(1 + \exp\left(\frac{(V_{50} - V)}{s}\right)\right),$$

where $G_{\min}$ is the minimal conductance, $G_{\max}$ the maximal conductance, V_{50} the midpoint (i.e., the voltage at which the conductance is half the maximal conductance determined from the fit) and s the slope of the curve. The difference in V_{50} induced by DHA-GLY

or Lin-GLY in each oocyte (i.e., ΔV_{50}) was calculated to quantify the shift in the voltage dependence for channel opening.

Statistics

Average values are expressed as mean ± sem. Statistics were calculated using repeated measures one-way ANOVA followed by Tukeý́s multiple comparison test, unpaired Student's-t test followed by Holm-Sidak's multiple comparisons method, or Student's t-test. Used statistical tests for each calculation is indicated in the text or figure or table legend. $P < 0.05$ was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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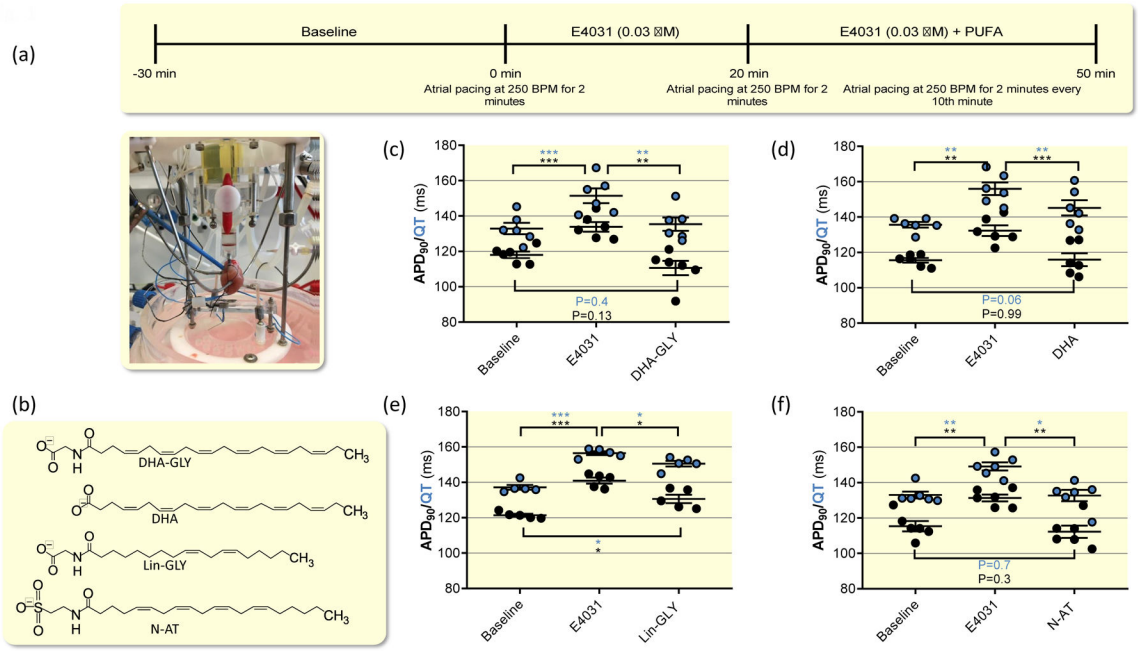

Figure 1. PUFAs shorten and partially or fully restore prolonged QT interval in isolated *ex-vivo* guinea pig hearts.

(a) Overview of study design for *ex-vivo* guinea pig heart experiments along with photo of experimental configuration. (b) Molecular structure of indicated PUFAs. (c) Effects of 0.03 μM E4031 and 10 μM DHA-GLY together with 0.03 μM E4031 on QT interval and action potential duration. Data shown as mean ± sem. Statistical analysis used was repeated measures one-way ANOVA, followed by Tukeýs multiple comparison test. n = 6. (d-f) Same as in C but for 10 μM DHA (d, n = 6), 10 μM Lin-GLY (e, n = 5), and 30 μM N-AT (f, n = 6).

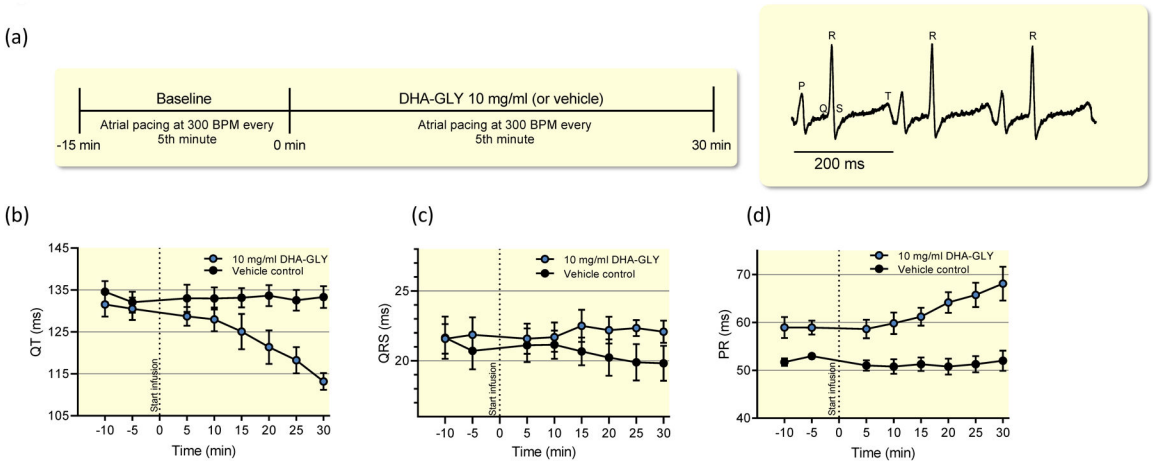

Figure 2. DHA-GLY shortens QT interval in *in-vivo* guinea pig hearts.

(a) Study design for *in-vivo* experiments in guinea pigs with representative ECG recording. (b) Effects of 10 mg/ml (40 mg/kg/hr) DHA-GLY and vehicle control on QT interval during intra-cardiac pacing. (c-d) same as (b) but for QRS-duration and PR-interval. Data shown as mean ± sem, n = 5. Data is summarized in Table III.

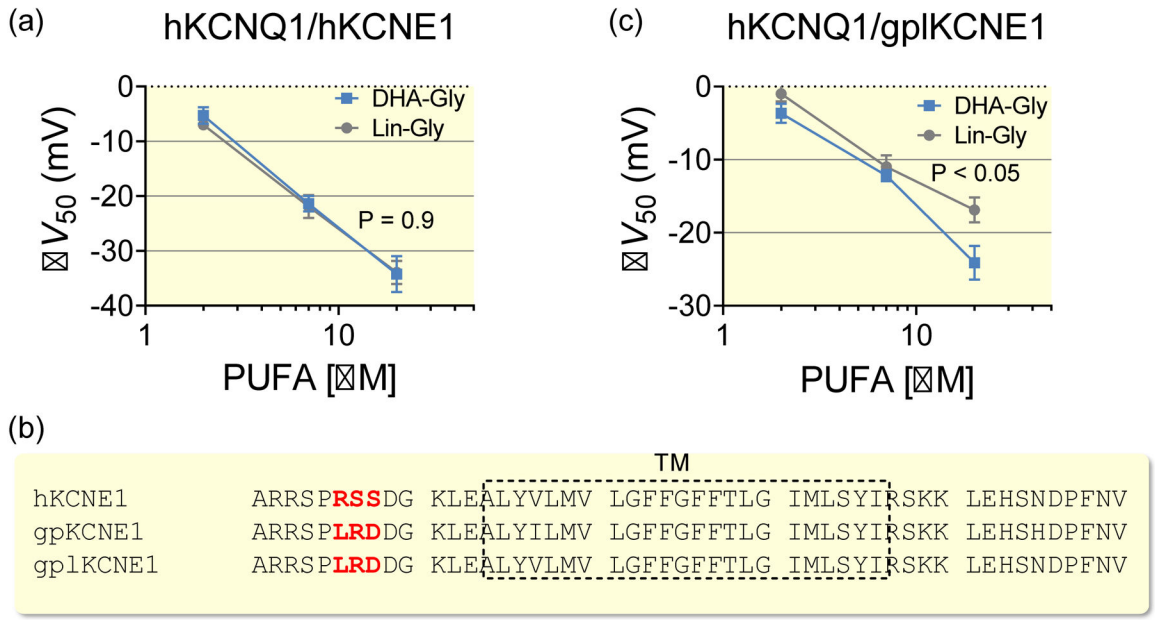

Figure 3. Lin-GLY effects depend on guinea pig KCNE1 residues

(a) Concentration-response relationship for the ability of DHA-GLY and Lin-GLY to shift the voltage dependence of activation (i.e. ΔV_{50}) of hKCNQ1/hKCNE1 channels expressed in *Xenopus* oocytes. Data shown as mean ± sem, n = 8. Statistics denote Student's t-test for the effect at 20 μM. (b) Sequence alignment of human KCNE1 (hKCNE1), guinea pig KCNE1 (gpKCNE1), and the constructed guinea pig like KCNE1 (gplKCNE1) subunits. Deviating residues in the extracellular terminal are marked in red. The transmembrane (TM) region is indicated by the dashed box. (c) Concentration-response relationship of the ability of DHA-GLY and Lin-GLY to shift V_{50} of hKCNQ1/gplKCNE1 channels expressed in *Xenopus* oocytes. Data shown as mean ± sem, n = 8–11. Statistics denote Student's t-test for the effect at 20 μM.

Table I.

Summary of PUFA effect on QT-intervals and APD_{90} in *ex-vivo* guinea pig hearts.

Compound		Baseline (ms)	E4031 0.03 μM (ms)	ΔQT/APD_{90}, compared with baseline (ms)	PUFA (ms)	ΔQT/APD_{90}, compared with E4031 (ms)	ΔQT/APD_{90}, compared with baseline (ms)	n
DHA-GLY (10 μM)	QT	132.9 ± 3.2	151.5 ± 4.1	+18.6 ± 1.9 (P < 0.001)	135.4 ± 3.7	−16.2 ± 2.4 (P < 0.01)	+2.4 ± 1.7 (P = 0.4)	6
	APD_{90}	118.1 ± 1.9	133.8 ± 2.7	+15.8 ± 1.7 (P < 0.001)	110.6 ± 4.1	−23.2 ± 3.6 (P < 0.01)	−7.4 ± 3.2 (P = 0.13)	6
DHA (10 μM)	QT	135.7 ± 1.5	156.0 ± 1.5	+20.3 ± 2.7 (P < 0.01)	145.2 ± 4.3	−10.8 ± 1.4 (P < 0.01)	+9.6 ± 3.1 (P = 0.06)	6
	APD_{90}	115.5 ± 1.4	132.3 ± 3.0	+16.8 ± 2.3 (P < 0.01)	115.9 ± 3.7	−16.4 ± 1.2 (P < 0.001)	+0.3 ± 3.2 (P = 0.99)	6
Lin-GLY (10 μM)	QT	134.6 ± 1.7	152.7 ± 1.0	+18.1 ± 1.8 (P < 0.01)	148.4 ± 1.3	−4.3 ± 1.3 (P ≤ 0.05)	+13.8 ± 1.1 (P ≤ 0.05)	5
	APD_{90}	121.4 ± 0.7	141.0 ± 1.7	+19.6 ± 1.7 (P < 0.001)	132.4 ± 2.7	−8.6 ± 1.5 (P ≤ 0.05)	+11.0 ± 3.0 (P ≤ 0.05)	5
N-AT (30 μM)	QT	133.0 ± 1.9	149.2 ± 2.3	+16.2 ± 3.1 (P < 0.01)	132.7 ± 3.2	−16.4 ± 2.6 (P < 0.01)	−0.3 ± 2.7 (P = 0.7)	6
	APD_{90}	115.4 ± 2.9	131.3 ± 2.0	+15.9 ± 2.5 (P < 0.01)	112.3 ± 3.4	−19.0 ± 3.0 (P < 0.01)	−3.1 ± 1.8 (P = 0.29)	6

QT/APD_{90} determined during pacing at 250 BPM as described in Methods. Data shown as mean ± sem. Statistics denote repeated measures one-way ANOVA followed by Tukeýs multiple comparison test.

Table II.

Summary of DHA-GLY effect on cardiovascular parameters in *ex-vivo* guinea pig hearts.

Parameter	Baseline	E4031 0.03 μM	Δ_{E4031}, compared with baseline	DHA-GLY 10 μM	$\Delta_{DHA-GLY}$, compared with E4031	$\Delta_{DHA-GLY}$, compared with baseline	n
PR interval	58.8 ± 3.6†	61.2 ± 4.2†	+2.4 ± 0.9† (P = 0.09)	69.4 ± 4.2†	+8.2 ± 2.2† (P ≤ 0.05)	+10.6 ± 2.7† (P ≤ 0.05)	6
QRS duration	18.8 ± 0.6†	18.8 ± 0.6†	+0.1 ± 0.2† (P = 0.9)	19.1 ± 0.6†	+ 0.3 ± 0.3† (P = 0.7)	+ 0.4 ± 0.3† (P = 0.5)	6
Coronary flow	16.2 ± 2.0‡	14.3 ± 2.0‡	−1.8 ± 0.3‡ (P < 0.01)	12.2 ± 1.8‡	−2.0 ± 2.3‡ (P = 0.7)	−3.9 ± 2.2‡ (P = 0.3)	6

Parameters determined during pacing at 250 BPM as described in Methods. Data shown as mean ± sem. Statistics denote repeated measures one-way ANOVA followed by Tukeýs multiple comparison test.

† denotes ms.

‡ denotes ml/min.

Table III.

Changes in PR interval, QRS duration and QT interval in *in-vivo* guinea pig experiments.

Treatment	ΔPR (ms)		ΔQRS (ms)		ΔQT (ms)		n
TMC (vehicle)	−1.0 ± 2.3		−0.9 ± 1.1		+1.2 ± 1.6		5
DHA-GLY (10 mg/ml)	+9.2 ± 3.0	(P = 0.03†)	+0.2 ± 0.8	(P = 0.561†)	−17.3 ± 2.8	(P = 0.001†)	5

Parameters determined during pacing at 300 BPM as described in Methods. TMC denotes time-matched controls receiving vehicle infusion. Δ-values refers to the absolute changes (test-baseline) in PR interval, QRS duration and QT interval between test values and baseline values. Test values (for vehicle or DHA-GLY) were determined after 30 minutes of infusion (time = +30 min). Baseline values were determined 5 minutes prior to DHA-GLY/vehicle infusion (time = −5 min). Data shown as mean ± sem.

†P-values refer to the comparison of Δ-values between TMC and DHA-GLY groups using unpaired Student's-t test..