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The electrical and mechanical response of adult guinea pig and rat ventricular myocytes to ω3 polyunsaturated fatty acids

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Abstract

Single adult guinea-pig and rat ventricular cardiac myocytes were used to study the effects of two members of the ω3 class of polyunsaturated fatty acids, docosahexaenoic acid and eicosapentaenoic acid, on the electrical and mechanical activity of cardiac muscle. Docosahexaenoic acid and eicosapentaenoic acid reduced the electrical excitability of both guinea-pig and rat cells in a dose-dependent manner. Both agents produced a dose-dependent negative inotropic response in guinea-pig cells but in the rat cells there was first a dose-dependent positive inotropic effect at low concentrations (< 10 μM) followed by a negative inotropic effect at higher concentrations (> 10 μM). Possible mechanisms by which these agents affect contraction were studied using conventional electrophysiological techniques. The polyunsaturated fatty acids reduced the action potential duration and the plateau potential of the guinea-pig cells in a simple, dose-dependent manner. In contrast, the effect on the rat action potential mirrored the inotropic effect. At low concentrations (< 10 μM) there was a concentration-dependent increase in action potential duration followed by a concentration-dependent decrease at higher concentrations (> 10 μM). Both polyunsaturated fatty acids decreased the fast Na^+ current and the L-type Ca^{2+} current in a concentration-dependent but not use-dependent manner in cells from both species. In the rat cells these agents inhibited the transient outward current resulting in an increase in the duration of the rat action potential. The effects of polyunsaturated fatty acids on the Ca^{2+}, Na^+ and K^+ currents underlie these changes in the action potentials in guinea-pig and rat heart cells. The effects on the L-type Ca^{2+} current and action potential duration can also explain both the simple negative inotropic effects of the agents on the guinea-pig cells and the more complex effects on the rat cells. These effects of polyunsaturated fatty acids on membrane currents may account for their anti-arrhythmic properties.

Keywords: Eicosapentaenoic acid; Docosahexaenoic acid; Action potential; Ca^{2+} current; Transient outward current

1. Introduction

Polyunsaturated fatty acids have beneficial anti-arrhythmic properties in ischaemic cardiac preparations. For example, the ω3 class of polyunsaturated fatty acids, docosahexaenoic acid and eicosapentaenoic acid, when infused acutely, prevented the development of exercise-induced ventricular fibrillation in an intact dog model of myocardial infarction (Billman et al., 1994). In addition, reperfusion-induced ventricular fibrillation was minimised in isolated hearts from adult rats fed a diet rich in docosahexaenoic acid and eicosapentaenoic acid (McLennan and Dallimore, 1995; McLennan et al., 1996) and these compounds, when added to the incubation medium, either prevented or terminated chemically-induced arrhythmic activity in isolated, rhythmically-beating layers of neonatal rat cardiac myocytes (Kang and Leaf, 1996c).

The arrhythmias generated in single cells originate from abnormal electrical potentials which may develop as a result of a direct change in ion channel activity of the cell membrane or from changes in cell membrane permeability leading to Ca^{2+} overload. Compounds that alter the behaviour of membrane Na^+, Ca^{2+} and K^+ channels can reduce the incidence of arrhythmias and many of the important and established anti-arrhythmic drugs target specific membrane channels (Ducceschi et al., 1996). Another potential target for anti-arrhythmic agents is the sarcoplasmic reticulum at the sites of Ca^{2+} release and Ca^{2+} uptake. This is particularly so for those arrhythmias associated with a cellular Ca^{2+}-overload (Du Toit and Opie, 1994; Kang and Leaf, 1996c). Here, the increase in cytosolic Ca^{2+} can lead to spontaneous release of Ca^{2+} from the sarcoplasmic reticulum, triggering not only an automatic

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mechanical event but also automatic electrical activity in the form of a delayed after-depolarisation (Woodley et al., 1991; Lakatta and Guarnieri, 1993). Thus, the ability of ω3 polyunsaturated fatty acids to terminate established arrhythmias or to prevent their development could reflect an action at either the cell membrane or the sarcoplasmic reticulum.

Neonatal rat cardiac myocytes do not show a transient outward current (I_{to}). However, this current is expressed throughout the adult heart where it repolarizes the cells and thus shortens the duration of the action potential. In dog and human hearts, the transient outward current is localised to the epicardium of the ventricular wall, with little or no expression in the endocardium and; it is absent altogether in the adult guinea pig heart. In both rat and dog, this transient outward current has been implicated in the development of ventricular arrhythmias in the ischaemic heart (Pike et al., 1993; Di Diego and Antzelevitch, 1994; Chang et al., 1996). It is stimulated by a high cytosolic Ca^{2+}, which leads to the development of electrical inhomogeneity in the ventricle, and arrhythmias result as a consequence of 'phase 2 re-entry'. Inhibition of the transient outward current with 4-aminopyridine, reduces the incidence of arrhythmias in the dog heart (Krishnan and Antzelevitch, 1993).

At the single cell level, most of the reported results that relate to the mechanism of action of the polyunsaturated fatty acids have been obtained from isolated neonatal rat cardiac cells. In the present study we have used electrophysiological techniques in isolated adult heart cells from both the rat and guinea pig to examine the effects of ω3s on the membrane Na^+, Ca^{2+} and K^+ currents. We have also investigated the contractile effects of these agents in both species. These species were chosen as the adult rat ventricular cells manifest a transient outward current, which results in a characteristically short action potential. In contrast, guinea-pig ventricular cells lack this transient outward current and have a pronounced inwardly rectifying K^+ current (I_{K1}), which helps to maintain the plateau of the action potential, resulting in a prolonged action potential. Using whole cell current- and voltage-clamp techniques, we have found that the effects of the ω3 compounds eicosapentaenoic acid and docosahexaenoic acid are both concentration- and species-dependent. In rat cells there is a concentration-dependent increase in action potential duration (< 10 μM eicosapentaenoic acid), an

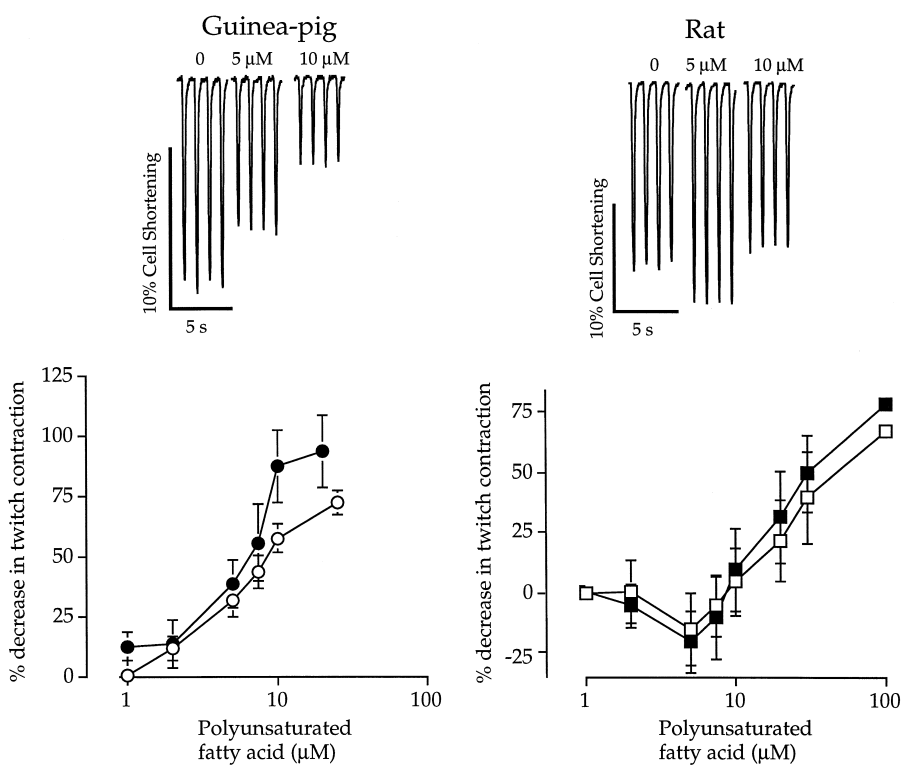

Fig. 1. The top part of the figure shows the effect of increasing concentrations of eicosapentaenoic acid on the magnitude of cell shortening of a single guinea-pig (left) or rat (right) ventricular myocyte. The cells are contracting in response to a field stimulation at 1 Hz and contraction is expressed as percentage of the change in diastolic cell length. The figure shows a control record made in normal Tyrode (0), and records after 5 min exposure to 5 and then 10 μM eicosapentaenoic acid, at which time a steady-state was achieved. The lower part of the figure shows a plot of % inhibition of cell shortening against the log of the [polyunsaturated fatty acid] for guinea-pig cells (left) and rat cells (right). The effects of docosahexaenoic acid are shown as open symbols and eicosapentaenoic acid as filled symbols. The plots show a simple, concentration-dependent reduction in twitch size in the guinea-pig cells but a more complex relationship in the rat. In the rat cells, a positive inotropic response between 1–7.5 μM is followed by a reduction in twitch size at concentrations above 10 μM. Data is the mean of at least 6–8 cells ± S.E.M.

effect that is reversed at higher concentration (> 10 μM). In guinea pig cells, however, there is only a reduction in action potential duration. At low concentrations these agents have a positive inotropic effect on the rat heart cells which is reversed at higher concentrations, whereas they have a simple negative inotropic effect at all concentrations in guinea-pig cells. These electrical and mechanical effects can be explained largely by effects of ω3s on the Na^+, Ca^{2+} and K^+ currents which underlie the action potential.

2. Materials and methods

2.1. Cell isolation

Cardiac ventricular myocytes were isolated from the hearts of adult male Wistar rats (300–350 g) and male guinea pigs (450–650 g) using the technique of Mitra and Morad (1985) modified by the inclusion of 1% bovine serum albumin (fraction V, Sigma) in the solution containing the collagenase and protease enzymes. Guinea pigs were killed by cervical dislocation and rats were anaesthetised with an i.p. injection of pentobarbitone before removing the heart. Ethical permission was obtained from the University of Otago committee on ethics in the care and use of laboratory animals. After digestion with the enzyme solution, the heart was perfused with normal Tyrode solution for 5 min. The digested heart was then removed from the cannula, cut in half and placed in 10 ml of normal Tyrode at 35°C. Gentle agitation liberated single cells that were precipitated and washed twice in normal Tyrode solution. Cells were stored at 10°C and used within 12 h. The yield of quiescent, rod-shaped cells isolated in this way was around 60–80% for the guinea-pig and 70–90% for the rat hearts.

2.2. Measurement of cell contraction

Cells were placed in 0.4 ml of normal Tyrode in the well of a tissue bath and continuously perfused with this solution at 32 ± 2°C. The temperature of the solution in the bath was regulated with a feedback temperature controller (FCP SCTC-02/30 NPI). Cells were observed through an inverting microscope (Nikon, diaphot) incorporating a CCD camera (Pulnix, TM-460). To record contrac-

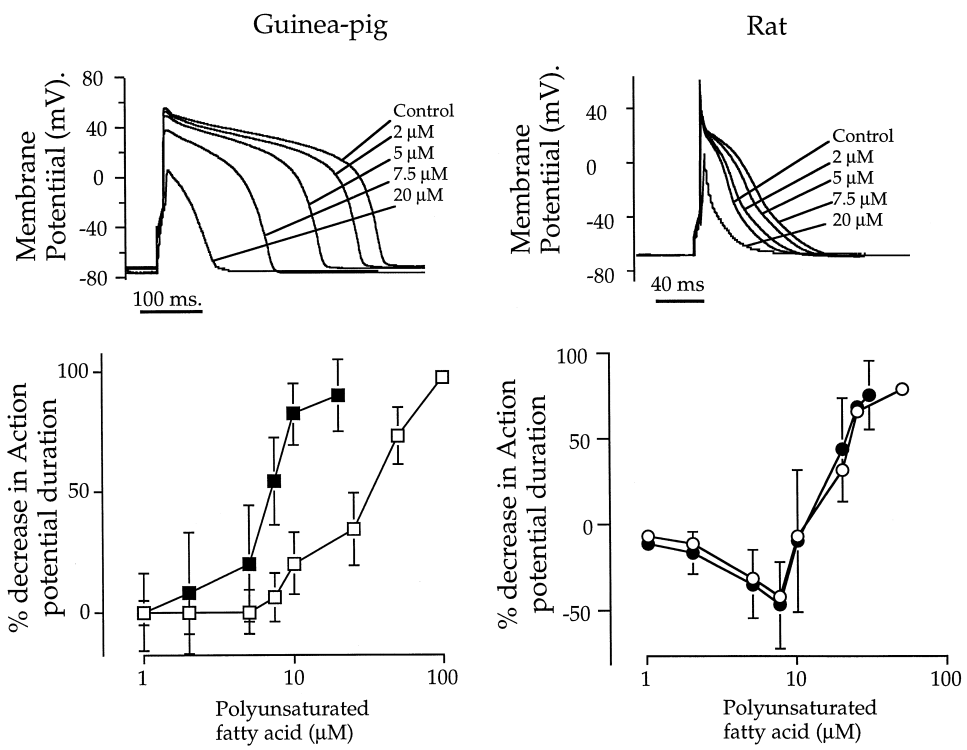

Fig. 2. The top panels of this figure show the effects of increasing concentrations of eicosapentaenoic acid on the action potential recorded from a single ventricular myocyte. Records of action potentials from an adult guinea-pig (left) and rat (right) ventricular myocyte, stimulated at 1 Hz show the control action potential recorded in normal Tyrode and the effects of the addition of 2, 5, 7.5 and 20 μM eicosapentaenoic acid to the perfusate. The steady-state response is shown after 5 min exposure to the each concentration of eicosapentaenoic acid. The lower panels show a plot of the action potential duration against the log of [polyunsaturated fatty acid] for guinea-pig cells (left) and rat cells (right). The action potential duration is determined as the time to repolarize to 80% of maximum diastolic potential and is expressed as the percentage of the duration in normal Tyrode. The effects of docosahexaenoic acid are shown as open symbols and eicosapentaenoic acid as filled symbols. The plot shows a clear reduction in the action potential duration of guinea-pig cells but a prolongation of the rat action potential duration at between 1–7.5 μM and a concentration-dependent reduction in rat action potential duration at concentrations above 10 μM. The data are plotted as the mean ± S.E.M. Number of observations is 12–16 for the guinea pig and 11–14 for the rat.

tions from single cells, platinum electrodes were placed at either end of the tissue bath. Cells were exposed to electrical field stimulation by passing an electrical current between these electrodes. Contractions of an individual cell were assessed from change in cell length, using a video edge detector to continuously monitor the cell's edges (Steadman et al., 1988). Cell contractions were induced at a constant stimulus frequency and the amplitude of contraction was allowed to come to a steady level before experiments were started. Contraction strength is determined from cell shortening, expressed as the percentage change in the diastolic length.

2.3. Measurement of action potentials and membrane currents

To record action potentials, the cells were stimulated under current-clamp through a patch-style micro-electrode, and membrane currents were recorded with a whole-cell voltage-clamp (EPC-7 amplifier, List). The patch electrode was filled with a solution containing (mM) 150 KCl, 3 Na_2ATP, 5 $MgCl_2$, 10 Hepes, buffered to a pH of 7.2 with KOH. In some experiments, CsCl was substituted for KCl to block outward K^+-currents. In the experiments where the effects of the polyunsaturated fatty acids on the Ca^{2+} current were investigated, the Ca^{2+} chelator 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) at a concentration of 10 mM was added to the electrode. BAPTA was introduced into the cell in this way to buffer intracellular Ca^{2+} and thereby limit any possible additional effects of the ω3 compounds on the intracellular Ca^{2+} activity that might otherwise affect the size and shape of the L-type Ca^{2+} current or transient outward current (White and Terrar, 1992). In the case of the action potential data, inclusion of BAPTA prolongs the action potential and it was therefore ommitted. In all other experi-

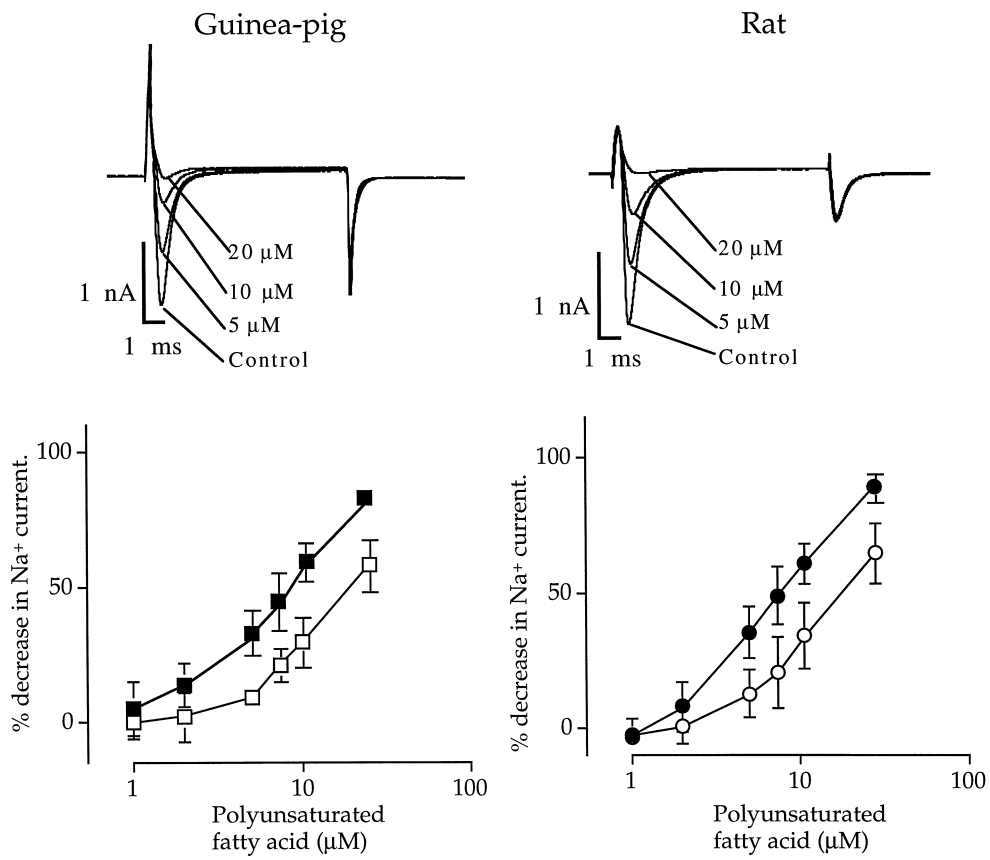

Fig. 3. The top panels of the figure illustrate the effects of eicosapentaenoic acid on the fast Na^+ current recorded from a single guinea-pig ventricular cell (left) and rat cell (right), and show a dose-dependent reduction in the peak amplitude of the Na^+ current with increasing concentrations of eicosapentaenoic acid. The Na^+ current was recorded from single ventricular cells voltage-clamped through a low resistance patch-style micro-electrode (<2 MΩ) and filled with an electrode solution containing 150 mM CsCl to block outward K^+ currents. The extracellular Na^+ concentration was lowered to 10% of normal (Na^+ replaced with *n*-methyl-glucamine) and experiments performed at 25°C. The Na^+ current was activated by a depolarising voltage-clamp pulse to -50 mV from a holding potential of -80 mV. The lower panels show plots of the inhibition of the Na^+ current against the log of [polyunsaturated fatty acid], for guinea-pig cells (left) and rat cells (right). The inhibition is expressed as a percentage of the Na^+ current recorded in normal Tyrode. The effects of docosahexaenoic acid are shown as open symbols and eicosapentaenoic acid as filled symbols. There was a simple dose-dependent inhibition of the Na^+ current. The data are plotted as the mean ± S.E.M. (number of observations is 8–10 for guinea-pig data and 6–8 for rat).

ments (Na^+, the transient-outward current and steady-state currents) BAPTA was also absent as there was no evidence that ω3 polyunsaturated fatty acids could be affecting these currents indirectly through their action on intracellular Ca^{2+} (see Section 3 for each current).

2.4. Experimental solutions

Normal Tyrode contained (mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 0.33 NaH_2PO_4, 5 sodium pyruvate, 10 glucose and 10 Hepes buffered to pH 7.3 with NaOH. In the experiments where the extracellular Na^+ was removed to either reduce the size of, or inhibit completely, the Na^+ current, the extracellular Na^+ was replaced with *n*-methyl-glucamine. The ω3 compounds, eicosapentaenoic acid and docosahexaenoic acid, were made up as a stock solution of 50 mM in ethanol and stored at −20°C.

2.5. Data recording and analysis

Records were digitized and analysed either with a CED interface and voltage-clamp software (Cambridge Electronic Design) using a PC-computer, or as chart or scope records using the MacLab system (ADInstruments) and a MacIntosh computer. The duration of the action potential was defined as the time to repolarize to 80% of the maximum diastolic potential. Electrophysiological and contraction parameters were normalized to the comparable response seen in normal Tyrode. The concentration of ω3s which produced half the maximal percentage inhibition were calculated using a least squares fit of the data to a Hill equation assuming a coefficient of 1 and are shown as the mean ± S.E.M. The figures show the means ± S.E.M. In some cases the size of the plot symbols exceeded the S.E.M. Statistical significance was checked using a 2-tailed *t*-test and the Instat software package.

3. Results

3.1. Effects of the polyunsaturated fatty acids on the twitch contraction of single ventricular myocytes

The strength of contraction of cells was estimated from measurements of cell shortening in response to an electrical field stimulation. Contraction is shown as a change in the length of the cell expressed as a percentage of the maximum diastolic length. The cells were stimulated at 1

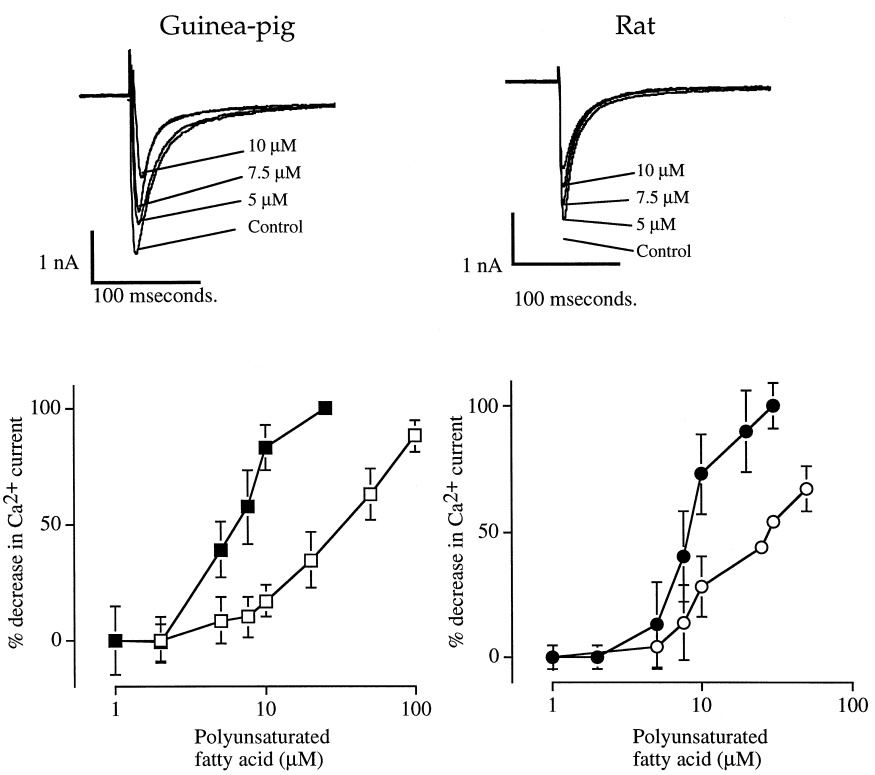

Fig. 4. The top panel of the figure illustrates the effects of eicosapentaenoic acid on the L-type Ca^{2+} current recorded from a single guinea-pig ventricular cell (left) and rat cell (right), and show a dose-dependent reduction in the peak Ca^{2+} current by eicosapentaenoic acid. The cells were voltage-clamped through a patch-style, micro-electrode filled with an electrode solution containing 150 mM CsCl to block outward K^+ currents and BAPTA (10 mM) to buffer cell Ca^{2+}. The Ca^{2+} current was activated by a depolarising voltage-clamp pulse to 0 mV from a holding potential of −45 mV. The lower panels show plots of the inhibition of the Ca^{2+} current against the log of [polyunsaturated fatty acid] for guinea-pig cells (left) and rat cells (right). Inhibition is expressed as a percentage on control recorded in normal Tyrode. The results for docosahexaenoic acid are shown as open symbols and those for eicosapentaenoic acid as filled symbols. The data are plotted as the mean ± S.E.M. (number of observations is 6–10 for guinea-pig data and 5–8 for rat).

Hz with a voltage 1.5 to 2 times threshold. At high concentrations of eicosapentaenoic acid (> 10 μM) it was often necessary to increase the stimulus voltage due to the reduction in excitability. This results from the inhibition of the Na^+ current (Xiao et al., 1995; Section 3.3). In guinea pig cells, docosahexaenoic acid and eicosapentaenoic acid produced a simple, concentration-dependent reduction in the degree of cell shortening. In rat cells the effects were more complex. At low concentrations (< 7.5 μM) there was a positive inotropic effect; at concentrations above 10 μM, there was a concentration-dependent negative inotropic response (Fig. 1).

3.2. Effects of eicosapentaenoic acid and docosahexaenoic acid on the action potential

The configuration of the action potential was determined in both guinea-pig and rat ventricular myocytes. The action potential duration was taken as the time to repolarise to 80% of the maximum diastolic potential. Guinea-pig ventricular myocytes were quiescent in normal Tyrode at 32°C and had a resting membrane potential of -77.2 ± 0.4 mV ($n = 37$). The action potential recorded from these cells reached $+50.6 \pm 0.5$ mV ($n = 16$) and plateaued at around +35 to +40 mV. The action potential duration, measured as the time to repolarize to 80% of the maximum diastolic potential, was variable at between 210–270 ms. Both docosahexaenoic acid and eicosapentaenoic acid reduced the plateau potential and the duration of the action potential in a concentration-dependent manner, with eicosapentaenoic acid being appreciably more effective than docosahexaenoic acid (Fig. 2).

The resting potential recorded from rat cells was -72.4 ± 0.6 mV ($n = 22$). The action potentials reached $+43.8 \pm 3.4$ mV and plateaued at +18 to +20 mV, with an action potential duration of 30–50 ms. The effects of docosahexaenoic acid and eicosapentaenoic acid on the rat cell action potential were more complex than those observed in guinea-pig cells. At low concentrations (1 to 7.5 μM), eicosapentaenoic acid produced a dose-dependent increase in the duration of the action potential, characterised by a lengthening of the early plateau potential (Fig. 2). With higher concentrations of eicosapentaenoic acid (above 10 μM), there was a dose-dependent reduction in the plateau potential and the duration of the action potential.

3.3. Effects on the Na^+-current and L-type Ca^{2+}-current

The electrophysiological mechanisms by which the polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid produce these effects on the action potential were investigated in both guinea pig and rat cells. To study the effect on the Na^+ current, whole-cell, voltage-clamp experiments were performed with a low resistance patch electrode (1–3 MΩ) filled with an electrode solution containing CsCl to block outward K^+ currents. To decrease the size of the Na^+ current and therefore facilitate accurate clamping of the membrane potential during the passage of the fast Na^+ current, the Na^+ concentration of the perfusing Tyrode was reduced to 10% of normal and the experiments were performed at 25°C. The fast Na^+

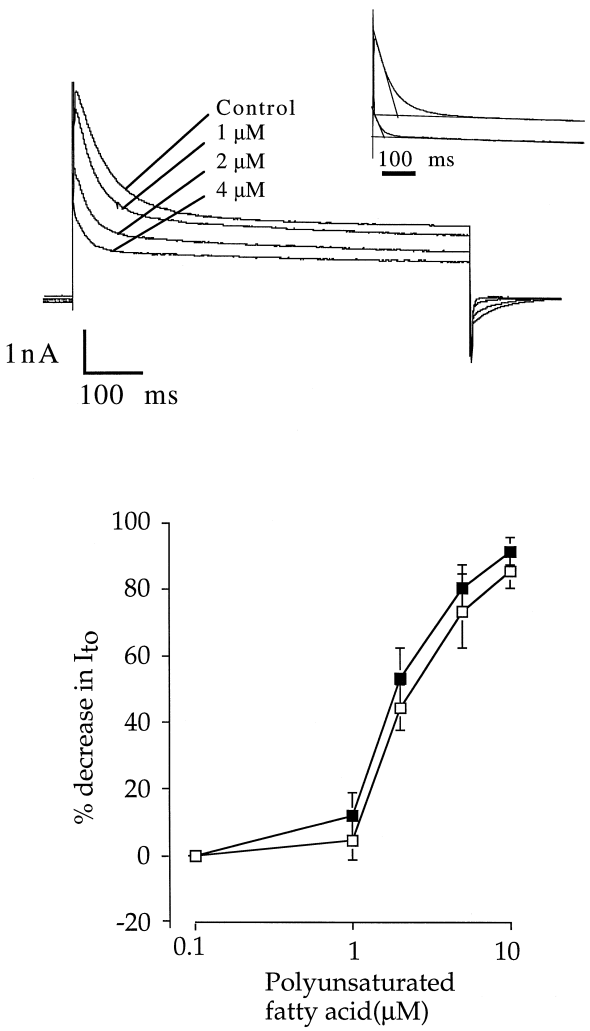

Fig. 5. The top panel of the figure shows the effect of increasing concentrations of eicosapentaenoic acid on the transient outward current recorded from a rat ventricular myocyte. The cells were perfused with Na^+-free Tyrode solution containing 10 μM nifedipine to inhibit the fast Na^+ current and L-type Ca^{2+} current. The transient outward current was activated by a voltage-clamp pulse from a holding potential of −80 mV to +20 mV for 200 ms. The size of the transient outward current was derived by subtracting the maintained outward current, which was extrapolated back to the start of the voltage pulse, from the peak outward current (see inset). The lower panel shows a plot of the inhibition of the transient outward current (I_{to}) against the log of the concentration of polyunsaturated fatty acid, for docosahexaenoic acid (open symbols) and eicosapentaenoic acid (filled symbols). Data are plotted as the mean ± S.E.M (number of observations is 5–8).

Table 1
The IC_{50} values for the inhibition of twitch contraction, the fast Na^+- and L-type Ca^{2+} currents by docosahexaenoic acid and eicosapentaenoic acid, recorded from guinea-pig and rat ventricular cells and the transient outward current from rat cells

		Docosahexaenoic acid	Eicosapentaenoic acid	Significance
Twitch size	Guinea pig	8.5 ± 1.1 μM (6)	6.7 ± 2.2 μM (7)	NS
	Rat	63 ± 8.3 μM (6)	51 ± 5 μM (6)	NS
Na^+ current	Guinea pig	15.7 ± 0.9 μM (7)	8.9 ± 0.5 μM (11)	$P < 0.005$
	Rat	12.8 ± 0.8 μM (6)	7.9 ± 0.6 μM (6)	$P < 0.005$
Ca^{2+} current	Guinea pig	34.7 ± 2.6 μM (6)	8.6 ± 1.5 μM (7)	$P < 0.001$
	Rat	27.9 ± 2.5 μM (5)	9.4 ± 0.8 μM (6)	$P < 0.003$
I_{to} current	Rat	2.6 ± 0.7 μM (5)	1.9 ± 0.3 μM (6)	NS

P value indicates level of significance using a 2-tailed *t*-test.
Contraction was measured as the percentage cell shortening. The IC_{50} values were obtained by fitting the data shown in Fig. 1 and Figs. 3–5 with a Hill equation using a least squares fit. The difference between docosahexaenoic acid and eicosapentaenoic acid were tested for significance using a 2-tailed *t*-test.

current was activated by depolarising the cell to −50 mV from a holding potential of −80 mV. Both docosahexaenoic acid and eicosapentaenoic acid produced a concentration-dependent reduction in the maximal Na^+ current in cells from both species (Fig. 3).

The effects of the ω3 polyunsaturated fatty acids on the L-type Ca^{2+} current were also investigated. The patch electrode had CsCl in the solution to block outward K^+ currents and the Ca^{2+} chelator BAPTA (10 mM) to buffer the Ca^{2+} in the cells during excitation. Both docosahexaenoic acid and eicosapentaenoic acid produced a concentration-dependent reduction in the magnitude of the L-type Ca^{2+} current in cells from both species (Fig. 4).

3.4. The transient outward current in rat ventricular myocytes

As expected, we identified a large transient outward current in the adult rat cells, which is absent from neonatal cells. The cells were perfused with a Tyrode solution containing 10 μM nifedipine to block L-type Ca^{2+} currents and all of the Na^+ was replaced with *n*-methyl-glucamine, to inhibit the fast Na^+ currents. The current was activated by depolarising the cell to the plateau potential of +20 mV, from a holding potential of −70 mV. This current could be blocked by internal Cs^+ and 1 mM external 4-aminopyridine. Both docosahexaenoic acid and eicosapentaenoic acid produced a concentration-dependent reduction in the magnitude of this transient outward current (Fig. 5). Note that this current is much more sensitive to polyunsaturated fatty acids than either the Ca^{2+} or Na^+ currents (Table 1).

3.5. Steady-state currents (delayed rectifier I_K and inward rectifier I_{K1})

The steady-state currents were investigated in both guinea-pig and rat cells using a ramp voltage-clamp protocol. The cells were voltage-clamped with a patch-style micro-electrode filled with a conventional solution. To activate the voltage-dependent transient currents, the cells

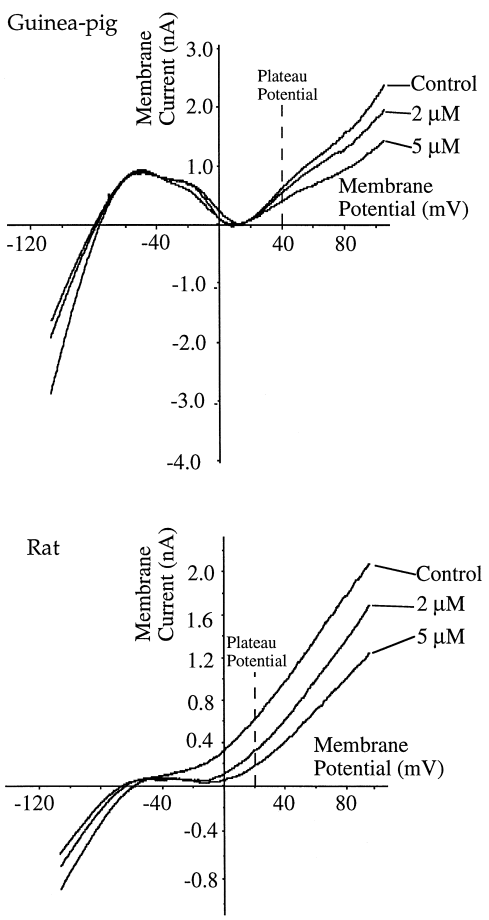

Fig. 6. Steady-state current–voltage relationships recorded from voltage clamped ventricular myocytes in response to a voltage ramp pulse, showing the effects of increasing concentrations of eicosapentaenoic acid. The cell was depolarized to +110 mV for 500 ms and then the potential was ramped to −110 mV at a speed of −0.25 V/s. The current recorded during the voltage ramp is plotted against the ramp voltage. Relationships were determined in normal Tyrode and after 5 min perfusion with Tyrode containing 2 and 5 μM eicosapentaenoic acid. Top panel is the steady state current voltage relationship recorded from a guinea-pig cell and the lower panel is that from a single rat ventricular myocyte. The plateau potential for the species is indicated by the dashed line.

were depolarized to +60 mV for 500 ms from a holding potential set to the cell's resting potential. These voltage-dependent transient currents inactivate over the next 500 ms, and any Ca^{2+} current activated at this potential was small, so that the additional effects of the ω3s on intracellular Ca^{2+} could be ignored. In addition, the delayed rectifier (I_K) was fully activated and any background currents would also be expected to be active. The membrane potential was then ramped back down to −110 mV at a ramp speed of −0.25 V/s. The resulting current which flows across the membrane during the voltage ramp reflects the activity of the delayed rectifier (I_K) and the inward rectifier (I_{K1}). This current was plotted against the ramp potential to generate a current–voltage relationship (Fig. 6). The steady-state outward current measured at the plateau of the rat action potential of +20 mV was inhibited 30–40% by 2 μM and 50–60% by 5 μM eicosapentaenoic acid (Fig. 6). For the guinea-pig cells, the inhibition of the steady-state current measured at the plateau of the action potential of +40 mV, was much less marked, with an inhibition of 10% by 2 μM and 30–40% by 5 μM eicosapentaenoic acid. Thus, these agents have a smaller effect on the outward currents which contribute to the repolarization of the action potential in the guinea-pig cells than they have in the rat cells.

4. Discussion

We have examined the effects of the ω3 polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid on the electrical and mechanical activity of single ventricular myocytes isolated from the adult rat and guinea pig. Both docosahexaenoic acid and eicosapentaenoic acid inhibited the fast Na^+ current and the slow L-type Ca^{2+} current in a concentration-dependent manner, with eicosapentaenoic acid being more effective than docosahexaenoic acid (Table 1). These results can explain (i) the loss of excitability seen in neonatal cells (Kang et al., 1995), (ii) the reduction in the action potential duration and plateau potential and (iii) the negative inotropic effect of the drug. These results confirm some of the findings reported previously for neonatal rat cells (Hallaq et al., 1992; Kang et al., 1995), but also show in the adult rat a pronounced positive inotropic effect, which is concentration-dependent. In addition, we have found that lower concentrations (< 7.5 μM) of both eicosapentaenoic acid and docosahexaenoic acid prolong the cardiac action potential in the adult rat. The positive inotropic effect is due to the prolongation of the action potential which is linked to the inhibition of the transient outward current. The transient outward current is prominent in adult rat cells and its inhibition by polyunsaturated fatty acids may explain the anti-arrhythmic properties of these agents in hearts which have a transient outward current (Adamantidis, 1995).

4.1. The effects on the action potential

In neonatal rat cardiac myocytes the ω3 polyunsaturated fatty acids have been found to bind to Na^+ channels, thereby inhibiting the fast Na^+ current (Kang and Leaf, 1996b). Our results show an essentially similar action of the drug in both adult guinea-pig and rat cells. This inhibition of the Na^+ channels will decrease the electrical excitability of these cells.

It has been suggested by Kang and Leaf (1996b) that the ability of the drug to inhibit Na^+ channels results from a reversible binding of the polyunsaturated fatty acids directly to the ion channel protein at the lipid–protein interface of the channel. They also speculate that the ω3s may interact with similar domains in other voltage-sensitive channels. This idea is supported by the observations that in rat neonatal and adult cells, ω3s interact with the Ca^{2+} current (Xiao et al., 1997). Paradoxically, however, they are without effect on contraction strength even at 10 μM (Kang and Leaf, 1996c). The reduction of the L-type Ca^{2+} current in adult guinea-pig and rat cells by ω3s reported in this study, can explain their effects on both the plateau potential and the duration of the guinea-pig action potential at all concentrations and of the rat at the higher concentrations.

4.2. The transient outward current

The prolongation of the adult rat cardiac action potential which occurs at low concentrations of both eicosapentaenoic acid and docosahexaenoic acid contrasts with the effect in neonatal rat cells. This difference reflects the inhibitory effect of these ω3s upon the transient outward current (I_{to}) which is not present in the neonatal rat cardiac cells. Thus, in the neonatal heart cells, the ω3 polyunsaturated fatty acids reduce only the action potential duration (Kang et al., 1995). The appearance of the channels responsible for I_{to} during development is paralleled by a gradual reduction in the duration of the action potential (Kilborn and Fedida, 1990).

In dogs and humans, there are regional differences in the distribution of the I_{to} channel in the ventricle, with a larger expression in the epicardium in comparison to the endocardium (Wettwer et al., 1994). In the dog, this results in a characteristic notch in the early part of the action potential, a pronounced dome shape to the plateau, and a shorter action potential (Litovsky and Antzelevitch, 1988). We have shown here that, in the rat, docosahexaenoic acid and eicosapentaenoic acid inhibit the transient outward current at the same concentration as required to produce the prolongation of the action potential duration (Table 1). Inhibition of the transient outward current will reduce the current which normally repolarizes the adult rat membrane potential and, therefore, will prolong the action potential. The effects of ω3s on the Ca^{2+} current, which would reduce the action potential duration, occur at much higher

concentrations. The overall effect of low concentrations of polyunsaturated fatty acids (< 10 μM) in increasing the duration of the rat action potential can thus be explained by its dominant effect on the transient outward current (Table 1).

4.3. The inotropic effects

The reduction in contraction in adult guinea-pig and rat ventricular cells is in contrast to the results reported in neonatal rat cells (Kang and Leaf, 1996c). The negative inotropic effect seen at all concentrations in guinea-pig cells and in rat cells at higher concentrations could be explained simply by the inhibition of the Ca^{2+} current, resulting in a smaller influx of Ca^{2+} and, therefore, less Ca^{2+} released from the sarcoplasmic reticulum, the combined effect being a reduction in the cytosolic Ca^{2+} transient.

The positive inotropic response of the adult rat ventricular cell to low concentrations of eicosapentaenoic acid, is the opposite of that predicted from its effect on Ca^{2+} channels. However, at the lower concentrations of both eicosapentaenoic acid and docosahexaenoic acid (< 7.5 μM), the dominant effect of the agents in the rat is inhibition of the transient outward current, thereby prolonging the cardiac action potential. This maintained plateau results in the cell remaining in a depolarised state for longer. This could result in a prolonged influx of Ca^{2+} through L-type Ca^{2+} channels. In addition, the prolonged depolarised potential would favour the maintenance of a high $[Ca^{2+}]_i$ via modulation of the Na^+/Ca^{2+} exchanger (Noma et al., 1991) and could result in a prolonged Ca^{2+} transient (Bers et al., 1990). A rise in the systolic Ca^{2+} can result in an increase in the Ca^{2+} content of the sarcoplasmic reticulum (Frampton et al., 1991). These effects of a maintained plateau potential will work to increase contractile strength over a period of time. The maximal effects of eicosapentaenoic acid on inhibition of the transient outward current and prolongation of the action potential occur at concentrations of 2–5 μM, with an increase in the action potential duration of between 20–30%. At this low concentration, the Ca^{2+} current is not yet affected significantly (Table 1 and Fig. 4). The prolongation of the rat cardiac action potential at these low concentrations is, therefore, the dominant effect and results in a positive inotropism.

4.4. Possible anti-arrhythmic action of eicosapentaenoic acid and docosahexaenoic acid

An anti-arrhythmic action of the ω3 polyunsaturated fatty acids docosahexaenoic acid and eicosapentaenoic acid has been suggested from both epidemiological and controlled dietary studies in humans (Riemersma and Sargent, 1989), acute studies in conscious dogs (Billman et al., 1994) and results from experiments on single neonatal cells (Kang and Leaf, 1996c). These anti-arrhythmic properties have been ascribed to the ability of the agents to inhibit voltage-sensitive Na^+ and Ca^{2+} channels, leading to a reduction in excitability and an increase in the refractoriness of the cells, together with a decreased tendency to Ca^{2+} overload during ischaemia and metabolic injury (Leaf, 1995; Kang and Leaf, 1996a). These actions of anti-arrhythmic agents are well documented. The Vaughan–Williams classification of anti-arrhythmic agents states that the type I drugs inhibit the Na^+ channels leading to a reduction in cell excitability and that the type IV drugs inhibit Ca^{2+} channels and thus alleviate atrioventricular node conduction and Ca^{2+} overload damage.

However, simply inhibiting the Na^+ channels in hearts which have a regional presence of the transient outward current, can in itself be pro-arrhythmic. Inhibition of the Na^+-current under conditions where the transient outward current has been stimulated (Ca^{2+} overload) can result in heterogeneity within the ventricles, leading to the establishment of re-entry circuits.

The ability of ω3s to inhibit the transient outward current suggests another anti-arrhythmic action of these agents. The class III anti-arrhythmic agents, some of which inhibit K^+ channels, work by prolonging the cardiac action potential duration and thereby increasing the effective refractory period. This class III effect would complement the inhibitory actions on the Na^+ channels. By increasing the refractory period, the incidence of re-entry circuits will diminish (Ducceschi et al., 1996). This effect of the ω3 polyunsaturated fatty acids may explain their action in preventing the arrhythmias which developed in exercising dogs with myocardial infarction (Billman et al., 1994). The regional distribution of I_{to} between the epi- and endocardium of canine ventricles and the subsequent activation of I_{to} by intracellular calcium during ischaemia, has been suggested as a possible mechanism for the generation of ischaemia-induced arrhythmias (Di Diego and Antzelevitch, 1994).

In conclusion, the different inotropic responses of guinea-pig and rat ventricular muscle to the ω3 compounds docosahexaenoic acid and eicosapentaenoic acid can be explained by effects of these agents on the action potential. This in turn is a reflection of the presence of the transient outward current in the rat ventricle and the many ion channels which appear to be affected by the polyunsaturated fatty acids in both species. These compounds may have potential anti-arrhythmic actions through their effects upon Na^+, Ca^{2+}, and K^+ channels. It should be noted, however, that polyunsaturated fatty acids are not carried in the blood as free acids but are bound to plasma albumins. The concentration at which these polyunsaturated fatty acids had their action on ion channels in this and other studies is higher than the free acid levels measured in the plasma. However, when they are present in the diet at high concentrations or can be synthesised from dietary precursors, they become inserted into, and concen-

trated in cell membranes. They may then be available to affect channel activity.

Finally, it is interesting to note that the actions of docosahexaenoic acid and eicosapentaenoic acid on the Na^+ current, Ca^{2+} current and I_{to}, which we report here, are paralleled by a plant alkaloid, liridenine, which has also recently been found to have anti-arrhythmic properties in isolated rat hearts (Chang et al., 1996).

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