Anti-arrhythmic actions of polyunsaturated fatty acids in cardiac muscle exerted via the sarcoplasmic reticulum

S. O’Neill
Unit of Cardiac Physiology, Oxford Road, Manchester M13 9PT, U.K.

Abstract
Cardiac arrhythmias can be triggered from ischaemic cardiac muscle due to the damage inflicted on individual myocytes. During an ischaemic episode free fatty acids accumulate in the ischaemic tissue. The importance of these fatty acids lies in the apparent ability of some classes of fatty acid to protect against cardiac arrhythmias. As cardiac sudden death is a likely cause of death in patients who have suffered an initial ischaemic insult, protection against such arrhythmias may be of crucial importance. The following review discusses how this protection may be produced, dealing specifically with changes in electrophysiological properties of cells and intracellular calcium regulation.

Introduction
It has been known for many years now that certain groups of people seem to be much less prone to cardiovascular disease than others, e.g. the Inuit peoples of Greenland have much lower incidence of coronary heart disease than their Danish counterparts despite having a diet equally rich in animal fats [1,2]. It seems, crucially, that the types of fats eaten by the Inuit are rich in the n−3 class of PUFAs (polyunsaturated fatty acids). An example of this class is shown in Figure 1.

Epidemiological evidence of protection provided by these PUFAs is backed up by studies carried out in animals and clinical studies. Clinical studies have shown that patients who have suffered one coronary event have their risk of sudden cardiac death reduced by about 50% if they have added to their diet one meal of oily fish per week [3]. Animal studies of the effects of altering diet show that addition of n−3 PUFAs to the diet leads to an increased amount of eicosapentaenoic and docosahexaenoic acids in the cell membranes throughout the body [4]. It is thought that this acts as a ‘reservoir’ of PUFAs for release during ischaemia. It has also been shown that direct application of a mixture of these fatty acids to the blood decreases the incidence of arrhythmic events and ventricular fibrillation in animals undergoing experimental occlusion of the coronary arteries to produce ischaemia and its sequelae [5,6]. Thus there is abundant evidence that n−3 PUFAs, either in the diet or directly applied to ischaemic tissues, can protect against cardiac arrhythmias.

Where do PUFAs come from during ischaemia?
It is known that fatty acids are released from cardiac muscle during ischaemia due to activation of phospholipase A2 in the ischaemic tissue [7,8]. Of course, the phospholipase A2 can only release n−3 PUFAs if they are present in the tissues. It has been shown in many animal studies that the cell membrane levels of n−3 fatty acids incorporated into phospholipids increases when the diet has been supplemented [4]. This increase leads to a change in the ratio of n−3:n−6 PUFAs present in the membranes [4]. The n−6 class of PUFAs includes arachidonic acid and as a result of the change in proportions of the two classes of PUFAs present there ought to be a change in the mixture of fatty acids released during ischaemia. This is indeed the case, but the change is even greater than expected from the changed representation in the membrane phospholipids. It seems that there is a preferential release of the n−3 fatty acids when their representation is increased in the membrane. This preferential release of n−3 PUFAs will increase their effectiveness in protecting against arrhythmias. We should now consider what cellular mechanisms might be involved in this protection.

Cellular mechanisms of anti-arrhythmic actions: electrophysiology
Several studies in isolated cardiac myocytes have shown that n−3 PUFAs alter the electrical properties of the surface membrane of the cell. Thus it has been reported that there is inhibition of sodium current [9–11], L-type calcium current [10,12,13] and transient outward current [10]. The net effect of these changes seems to be a reduction of the electrical excitability of the surface membrane, i.e. the threshold for an action potential is raised. Therefore, any potentially arrhythmogenic stimulus would have to be larger to be
effective. This has been directly demonstrated in neonatal cardiac cells by the requirement for a larger voltage pulse in field-stimulated cells to produce an action potential [14]. In whole-animal experiments the voltage required to induce ventricular fibrillation is also increased [6].

n–3 PUFAs reduce the frequency of spontaneous action potentials in neonatal cardiac cells [14] and also can reduce the effect on spontaneous activity in the same cell type of interventions that lead to calcium overload, e.g. inhibition of the Na⁺/K⁺-ATPase with ouabain [15]. This last result suggests there might be some effect on intracellular calcium regulation that mediates part of the protection of n–3 PUFAs.

**Cellular mechanisms of anti-arrhythmic actions: Ca²⁺ regulation**

One mechanism by which damaged cardiac cells can produce arrhythmias is through generation of waves of CICR (calcium-induced calcium release). These waves of Ca²⁺ release from the SR (sarcoplasmic reticulum) occur when the cells are overloaded with calcium. In such cases, localized release of calcium from the SR is able to propagate along the cell as release of calcium at one site triggers release from its neighbours. Such behaviour will have consequences for contraction of the myocyte but much more dangerous are the electrical consequences. The wave of raised intracellular calcium activates calcium efflux from the cell, largely on Na⁺/Ca²⁺ exchange. The stoichiometry of this exchange means that one positive charge enters the cell for each calcium ion that leaves, i.e. there is an inward, depolarizing current as the wave propagates. Such a wave of CICR can lead to spontaneous action potentials [16]. Although, as set out above, the presence of n–3 PUFAs will reduce the likelihood of these waves taking the cell membrane over the threshold for an action potential, if wave frequency were also reduced this too would be anti-arrhythmic. There is evidence of just such an effect from permeabilized and intact cardiac myocytes.

In permeabilized cells, calcium overload can be produced by raising the concentration of calcium in the solution mimicking the cell cytoplasm. When this is done waves of CICR are produced that are very similar to those seen in cardiac myocytes with intact cell membranes. Addition of EPA (eicosapentaenoic acid; 10 µM) caused the frequency of waves to be reduced [17]. As the concentration of calcium available to the SR is not changed in these experiments, a reduced frequency of waves must indicate either inhibition of the calcium-release mechanism or reduced uptake of calcium into the SR. The data presented in this study did not allow further conclusions to be drawn on these other mechanisms but work in my own laboratory has allowed us to look further into these possibilities.

In intact cardiac myocytes application of EPA (10 µM) reduces the frequency of spontaneous waves of CICR (Figure 2) [12,18]. This is as we would expect from the work of Rodrigo and colleagues [17]; however, what was not previously known was that at the same time as wave frequency is falling, so SR calcium content is rising [12,18]. This seems paradoxical: if

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**Figure 1 | Structure of one of the n–3 PUFA class**

The class of fatty acids is named after the position of the first double bond on the chain, i.e. position 3.

**n-3 PUFA**

[Diagram showing Eicosapentaenoic Acid]

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**Figure 2 | Spontaneous waves of CICR are reduced in frequency by 10 µM EPA**

Note also that the resting Ca²⁺ level also falls. EPA is removed by application of 1 mg/ml BSA.
spontaneous waves of CICR are due to calcium overload of the SR then it seems reasonable that increasing the SR content should exacerbate the overload. However, this conjunction of lower frequency of spontaneous events with higher content is what happens when the calcium release mechanism of the SR is inhibited [19–21].

This study also confirmed earlier observations [22] that EPA caused a fall of the resting level of intracellular calcium in isolated neonatal cardiac myocytes [12] (see also Figure 2). This too would certainly contribute to a lower frequency of spontaneous waves. By reducing the availability of calcium for refilling the SR after each wave, the time for refilling would be increased, i.e. frequency would be lower. In further work we have attempted to measure the contribution of each of these effects; inhibition of CICR and lower availability of calcium to the overall inhibition of spontaneous waves. We related the frequency of waves to the overall influx of calcium into the cell via the average calcium concentration over a period of time. This can be justified if the cell is in equilibrium for calcium movements, i.e. influx = efflux, and the calcium efflux depends on the level of calcium in the cytoplasm. In this case the average level of intracellular calcium over a period of time will be determined by the level of influx. Using this approach we were able to determine that up to 75% of the effect of EPA on wave frequency was exerted via inhibition of the calcium release mechanism of the SR, the remainder being due to lower calcium influx [18].

In another study that looked at the effect of EPA on intracellular calcium regulation it was reported that there was no effect on systolic release of calcium in cardiac myocytes [23]. This seems at odds with the results presented above but is, in fact, as we would expect for inhibition of the calcium release mechanism of the SR. In the steady state, the efflux of calcium from the cell must equal the influx. If systolic release of calcium from the SR activates most of the calcium efflux from the cell, then inhibition of the calcium-release mechanism will reduce efflux from the cell. However, as a consequence, SR calcium content will rise, making more calcium available for release. This situation will continue until the rise in SR content is sufficient to allow enough release to take place to balance influx of calcium [24]. Thus it has been demonstrated that factors that alter the sensitivity of the ryanodine receptor to calcium have only transient effects on calcium release from the SR and changes in SR calcium content take place that compensate for the initial inhibition of calcium release [19–21].

What effects might n–6 PUFAs have?

Although it is clear that n–3 PUFAs have several effects that can be considered anti-arrhythmic, some consideration should be given to the effects that the n–6 PUFAs might have. These are the fatty acids released during ischaemia when n–3 PUFAs are not available and might, therefore, be considered the ‘default’ condition. Evidence does exist that these PUFAs too can have anti-arrhythmic actions. It has been shown that arachidonic acid causes a reduction of the open probability of the isolated ryanodine receptor incorporated into lipid bilayers [25]. In addition a decreased binding of [3H]ryanodine to cardiac heavy SR vesicles in the presence of arachidonic acid also indicates reduced ryanodine receptor open probability (although this same study failed to find an effect on the ryanodine receptor incorporated into lipid bilayers [26]). In intact cells arachidonic acid has been shown to reduce the frequency of delayed afterdepolarizations in guinea pig myocytes calcium overloaded by isoprenaline application [27]. It was not possible, however, to say whether calcium influx or SR properties were being altered to produce this effect. Measurements of intracellular calcium do show that arachidonic acid alters calcium regulation in cardiac cells, apparently emptying the SR store of calcium [28].

Given these effects on the SR calcium release channel we ought to expect arachidonic acid to be anti-arrhythmic but it is clear that n–3 PUFAs are thought of in this context and not n–6 fatty acids. This may arise from the ‘default’ status of arachidonic acid release referred to earlier. The anti-arrhythmic properties of n–3 PUFAs are measured with release of n–6 PUFAs as the reference, i.e. n–6 PUFAs may also be anti-arrhythmic but less so than the n–3 class.

References


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