Cardiac Ca\textsuperscript{2+} Regulation and the Tuna Fish Sandwich

Stephen O’Neill
Unit of Cardiac Physiology, Department of Medicine, Manchester University, Manchester M13 9PT, UK

Following myocardial ischemia and reperfusion, there is a risk of fatal arrhythmias that result from damage to cellular Ca\textsuperscript{2+} homeostasis mechanisms. n-3 Polyunsaturated fatty acids seem to protect against these arrhythmias by mechanisms involving the sarcoplasmic reticulum and the sarcolemma.

Despite a diet rich in fats, Eskimos don’t die of heart disease (7). This rather blunt statement was the original reason for the interest in n-3 polysaturated fatty acids (PUFAs) and cardiac muscle (1). n-3 PUFAs are present in many foodstuffs but are particularly high in fish oils, which obviously form an important part of the Eskimo diet. The reasoning goes, therefore, that people eating diets rich in these fatty acids suffer less from heart disease. The focus of this review is to explain some of the effects that n-3 PUFAs have on cardiac muscle and to relate these to the occurrence of arrhythmias triggered by spontaneous release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum (SR). By the time you finish reading, you should have a clear understanding of why the taste of cod liver oil might actually be worth it. If they could only genetically modify cream cakes....

Antiarrhythmic properties of n-3 PUFAs

The case for an antiarrhythmic effect of n-3 PUFAs comes from clinical trials and from animal studies. Clinical trials have shown that the risk of sudden death in patients who have survived myocardial infarction is greatly reduced by inclusion of n-3 PUFAs in the diet (18). The risk may be reduced to 50% by including as little as one meal of oily fish per week (18). Thus it seems that fatal ventricular fibrillation is less likely to occur if the diet contains sufficient n-3 PUFAs. Animal studies confirm this. Coronary ligation studies in a variety of species have shown that the incidence of ventricular fibrillation is lower in animals fed a diet rich in n-3 PUFAs before ligation (e.g., Ref. 11). In isolated cells, the story is similar. In neonatal rat cardiac myocytes, eicosapentaenoic acid (EPA) prevents the arrhythmogenic action of many interventions, including high external Ca\textsuperscript{2+} and ouabain (5).

Electrophysiological effects of n-3 PUFAs

n-3 PUFAs have a number of effects on the electrophysiological properties of ventricular myocytes that might be relevant to their antiarrhythmic effects. Work in neonatal rat ventricular myocytes (6) has shown that the resting membrane potential hyperpolarizes, the action potential shortens, and membrane excitability is reduced; the combination of these effects increases the refractory period. Each of these changes has obvious antiarrhythmic effects. Several studies have shown effects of n-3 PUFAs that might explain these changes in electrophysiology. Inhibition of the Na\textsuperscript{+} current has been reported (8, 9) in ventricular myocytes. This will lead to a higher threshold for the action potential and will increase the refractory period of the membrane following an action potential. The shortening of the action potential may also owe something to the reduced Na\textsuperscript{+} current, but inhibition of L-type Ca\textsuperscript{2+} current (9, 13, 20) will also be heavily involved. The overall effect of n-3 PUFAs on the plateau phase of the action potential may depend on the actual concentration used, because the transient outward current appears to be more sensitive than Na\textsuperscript{+} and L-type Ca\textsuperscript{2+} currents. Indeed, in rat ventricular myocytes where transient outward current is well developed, lower concentrations of n-3 PUFAs actually increase the action potential duration (9). Although the specific current involved has not been identified, it has been shown that the equilibrium potential of the resting steady-state current is shifted more negative by n-3 PUFAs, as would be required for a resting hyperpolarization (9). Of themselves, these effects on surface membrane ionic currents will produce an antiarrhythmic effect. They will make reentrant arrhythmias more unlikely, and delayed afterdepolarizations (DADs) will also be less likely to reach the threshold for an action potential. However, this second category of potential arrhythmia depends on the function of the SR; if n-3 PUFAs affect the SR, this too might influence the likelihood of arrhythmias.

n-3 PUFAs and SR Ca\textsuperscript{2+} release

All studies of the effect of n-3 PUFAs on electrically stimulated Ca\textsuperscript{2+} release or contraction of cardiac myocytes have found that they induce a negative inotropic effect (9, 13, 20), e.g., Fig. 1. However, when the effectiveness of the trigger for Ca\textsuperscript{2+} release from the SR is measured, i.e., the amount of Ca\textsuperscript{2+} release or contraction produced by a given L-type Ca\textsuperscript{2+} current, it appears that Ca\textsuperscript{2+} release is normal (13, 20). This is illustrated in Fig. 1. The L-type Ca\textsuperscript{2+} currents b and d (Fig. 1B) are both smaller than control, b because of the inhibition of the current by EPA, d because of a smaller depolarization. Importantly, currents b and d are the same size and stimulate similar amounts of contraction.

The reduction in contraction produced by EPA, therefore, is entirely due to the reduction in the L-type Ca\textsuperscript{2+} current trigger...
for Ca\(^{2+}\) release. However, this is only a partial description of the effects of n-3 PUFAs on the behavior of the SR. Although the amount of Ca\(^{2+}\) released from the SR triggered by a given L-type Ca\(^{2+}\) current may be normal, this does not mean that the sensitivity of Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR) is normal. In the presence of n-3 PUFAs, there is a substantial increase in the amount of Ca\(^{2+}\) stored by the SR (13), as is shown in Fig. 2. The integral of the caffeine-induced Na\(^{+}/Ca^{2+}\) exchange current can be used as a measure of Ca\(^{2+}\) stored in the SR; EPA clearly increases this current and its integral. This means that, although Ca\(^{2+}\) release may be normal in absolute terms, in fractional terms less Ca\(^{2+}\) is released, i.e., the sensitivity of CICR is normal. In the presence of n-3 PUFAs, there is a substantial increase in the amount of Ca\(^{2+}\) stored by the SR (13), as is shown in Fig. 2. The integral of the caffeine-induced Na\(^{+}/Ca^{2+}\) exchange current can be used as a measure of Ca\(^{2+}\) stored in the SR; EPA clearly increases this current and its integral. This means that, although Ca\(^{2+}\) release may be normal in absolute terms, in fractional terms less Ca\(^{2+}\) is released, i.e., the sensitivity of CICR is reduced.

To understand how SR Ca\(^{2+}\) content is increased, we must refer to the fluxes of Ca\(^{2+}\) taking place across the surface membrane of the cell rather than just across the SR membrane. Over any given period of time, the cell must remain in Ca\(^{2+}\) balance such that, on average, the amount of Ca\(^{2+}\) entering the cell is the same as the amount leaving. If this were not true, the cell would either gain or lose Ca\(^{2+}\). In the case of a rapid inhibition of CICR, as follows application of tetracaine, this balance is upset because Ca\(^{2+}\) release is inhibited. Efflux of Ca\(^{2+}\) from the cell is greatest during systole, when intracellular Ca\(^{2+}\) is high. Inhibition of Ca\(^{2+}\) release, therefore, reduces efflux of Ca\(^{2+}\), and if Ca\(^{2+}\) influx remains unchanged the cell gains Ca\(^{2+}\). It appears that in such a case this Ca\(^{2+}\) gained by the cell is accommodated in the SR and is available for release on the next stimulus. With each cycle, therefore, the SR fills more and systolic release is increased. Therefore, Ca\(^{2+}\) release is initially depressed but recovers until efflux and influx are once more in balance. With low concentrations of tetracaine that have little effect on L-type Ca\(^{2+}\) current (i.e., Ca\(^{2+}\) influx), balance is reached once again at the same systolic release of Ca\(^{2+}\) as was achieved in control. Although Ca\(^{2+}\) release is normalized, clearly this is a smaller fraction of the SR Ca\(^{2+}\) content.

The picture for n-3 PUFAs is more complicated than for tetracaine because they are effective inhibitors of both L-type Ca\(^{2+}\) current and CICR. It is still the case, however, that influx and efflux of Ca\(^{2+}\) must balance. The inhibition of L-type current means that Ca\(^{2+}\) influx is reduced and so the requirement for efflux is less. As a result, sufficient Ca\(^{2+}\) efflux to balance influx can be achieved at a lower systolic Ca\(^{2+}\); hence the negative inotropic effect. Nevertheless, for a given Ca\(^{2+}\) current trigger, the SR releases a smaller fraction of its content in the presence of n-3 PUFAs.

**Inhibition of CICR and the antiarrhythmic effects of n-3 PUFAs**

Systolic Ca\(^{2+}\) release, therefore, may be a poor indicator of reduced sensitivity of CICR, because it is possible that no steady-state effect is present. Inhibition of CICR in a Ca\(^{2+}\)-overloaded cell exhibiting spontaneous waves does, however, produce a steady-state reduction in the frequency of spontaneous release events. This reduced frequency is seen even though SR Ca\(^{2+}\) content is increased (15). To explain the apparent paradox of increased SR Ca\(^{2+}\) content and reduced wave frequency, we must refer to the conditions required for propagation of a wave of CICR. Release at one point in the cell has to be an effective trigger at neighboring Ca\(^{2+}\) release sites for propagation to proceed. If the sensitivity to Ca\(^{2+}\) of release sites is depressed, a larger Ca\(^{2+}\) trigger is required. When CICR is inhibited, wave propagation will be delayed until SR Ca\(^{2+}\) content has increased to the point where sufficient trigger Ca\(^{2+}\) is provided. It follows that this delay of release will lead to increased SR Ca\(^{2+}\) content because efflux of Ca\(^{2+}\) from the SR during a wave limits the amount of Ca\(^{2+}\) the SR can hold (3) (once a certain threshold amount of SR Ca\(^{2+}\) is reached, spontaneous release of Ca\(^{2+}\) takes place, limiting the SR content to that threshold amount). By raising the threshold for spontaneous Ca\(^{2+}\) release, inhibition of CICR allows the SR Ca\(^{2+}\) content to rise. Put another way, normally the limit to SR Ca\(^{2+}\) content appears not to be set by the ability of SR Ca\(^{2+}\)-ATPase to accumulate Ca\(^{2+}\) but by the ability of the ryanodine receptor (RyR) to remain closed.

An initial delay in release on inhibition of CICR, therefore, allows SR Ca\(^{2+}\) content to rise. In turn, this leads to a lower steady-state frequency of waves because the waves are larger. With a greater SR Ca\(^{2+}\) content, when a wave finally does occur, more Ca\(^{2+}\) release takes place, activating more Ca\(^{2+}\) influx than normal (15). After each wave, therefore, the SR has been depleted more, in absolute terms, than by a typical wave in control conditions. The SR Ca\(^{2+}\) content is a major determinant of whether propagation can take place; there is evidence (19) that if too little release takes place propagation fails. Therefore, propagation of another wave is unlikely until the SR regains Ca\(^{2+}\) lost in the previous wave. More time will be required when CICR is inhibited to replace the greater loss of Ca\(^{2+}\), and so wave frequency is reduced. Thus the effects of inhibition of CICR are explained by the need for a higher SR Ca\(^{2+}\) content to overcome inhibition and allow propagation and the effects of this higher SR Ca\(^{2+}\) content on the efflux of Ca\(^{2+}\) generated by waves.

Again, the situation with n-3 PUFAs is more complicated, in two ways. First, EPA is likely to enter the cell slowly. It is unlikely, therefore, that the initial delay of release that follows application of tetracaine (which can be applied rapidly) will be...
seen with n-3 PUFAs. Nevertheless, the lower frequency of spontaneous waves at a higher SR content is seen. Further complication arises because inhibition of release is not the only mechanism at play with n-3 PUFAs. EPA also causes the resting intracellular Ca\(^{2+}\) to fall (13). Because this represents the pool of Ca\(^{2+}\) from which the SR must refill, the frequency of waves is further reduced. More evidence of this fall of resting Ca\(^{2+}\) comes from measurements of Ca\(^{2+}\) efflux activated by waves. Inhibition of CICR on its own does not alter the efflux of Ca\(^{2+}\) per unit time activated by waves (15). This makes sense if influx of Ca\(^{2+}\) has been unaffected; then the requirement for efflux remains unchanged. Therefore, when CICR is inhibited waves are less frequent, but their greater size compensates and efflux is maintained. In EPA, however, wave-activated Ca\(^{2+}\) efflux is reduced per unit time. Individual waves activate more efflux than control, as we would expect from an inhibition of CICR, but as influx is reduced, the efflux required to balance influx is also reduced (14).

In the absence of such a fall of resting Ca\(^{2+}\), EPA can still reduce the frequency of spontaneous waves. The Ca\(^{2+}\) available for uptake into the SR in a chemically permeabilized cell is set by the composition of the mock cytoplasmic solution applied. Figure 3 shows that the frequency of spontaneous waves of contraction in a permeabilized cardiac myocyte is reduced after exposure to 10 \(\mu\)M EPA, even under these conditions in which availability of Ca\(^{2+}\) to the SR is unchanged. Recently, an attempt to quantify the relative importances of the two means for reducing spontaneous wave frequency has concluded that up to 75% of the reduction in frequency is due to inhibition of CICR, with the remaining 25% due to lower availability of Ca\(^{2+}\) for refilling the SR (14).

How are these effects of n-3 PUFAs at the level of the SR able to contribute to their antiarrhythmic action? Following an action potential, the membrane potential may transiently depolarize in Ca\(^{2+}\)-overloaded muscle; this is known as a DAD. Such a depolarization may be large enough to reach the threshold for an action potential and therefore is arrhythmogenic. DADs appear to be due to spontaneous release of Ca\(^{2+}\) from the overloaded SR (10). The wave of Ca\(^{2+}\) release activates the Ca\(^{2+}\) efflux pathways in the surface membrane of the cell (generating the loss of Ca\(^{2+}\), referred to earlier, that the SR must replace before another wave is possible). This Ca\(^{2+}\) efflux depolarizes the cell because the bulk of it is generated by the electrogenic Na\(^{+}/Ca\(^{2+}\) exchanger. If n-3 PUFAs can reduce the frequency of spontaneous waves of Ca\(^{2+}\) release by the SR, then the risk from this particular arrhythmogenic mechanism is reduced. This is particularly relevant to ischemic cardiac muscle. Damage to cardiac myocytes resulting from ischemia and/or reperfusion reduces their ability to regulate intracellular Ca\(^{2+}\); inevitably, this leads to Ca\(^{2+}\) overload. The effects of n-3 PUFAs on the electrophysiology and Ca\(^{2+}\) regulation mechanisms of the cell will combine to reduce the risk of the Ca\(^{2+}\) overload leading to arrhythmias.

Where do n-3 PUFAs come from?

Now that we have seen how n-3 PUFAs might benefit the heart following ischemia, we should ask some questions about how they reach the cells that have been damaged. There are two possible routes: via the bloodstream or released by the cells themselves. Given the high-affinity binding of fatty acids to albumin, even if the total fatty acid present in plasma is such that the free concentration will be low. This seems to rule out carriage and delivery by the bloodstream; however, we should remember that the n-3 PUFAs will be in competition with other fatty acids for binding sites on albumin. The total amount of fatty acids in the blood may reach millimolar levels; if 5% of this were EPA and the affinity of albumin for EPA is similar to that for other fatty acids, then the free concentration of EPA could reasonably reach 10–20 \(\mu\)M. Thus the concentration of EPA shown to be effective against arrhythmias can be reached in the bloodstream. The other possibility, however, seems rather more elegant. During myocardial ischemia, phospholipases are activated, releasing large amounts of fatty acid from the sarcolemma (4). Although it appears that phospholipase A\(_2\) has some selectivity for arachidonic acid release from phospholipids, it is capable of releasing other fatty acids (4). Considering being the case, it makes sense that cells in which the sarcolemmal phospholipids have been enriched with n-3 PUFAs derived from the diet will release a greater proportion of n-3 PUFAs during ischemia. Of course, these n-3 PUFAs released by phospholipases at exactly the point where their antiarrhythmic properties will be of most benefit to the organ, i.e., in the cells most prone to DADs. It is well documented that activation of phospholipase A\(_2\) under ischemic conditions leads to release of arachidonic acid and lysophospholipids, both of which have been found to be proarrhythmic (5). The liberation of n-3 PUFAs will help reduce this drive toward arrhythmia.
Possible mechanisms of action

n-3 Polyunsaturates get their name from the position of the first of their several double bonds between the 3rd and 4th carbons on the acyl chain. Many of the experiments reviewed here have been carried out using EPA, which consists of a 20-carbon chain containing 5 double bonds (20:5n-3). The other commonly used example is docosahexaenoic acid (22:6n-3). Given the large number of double bonds, it is likely that their presence either in phospholipids or as free acids will change the physical properties of the membrane. Clearly, if the physical properties of the membrane change, this will alter the environment in which membrane proteins have to operate and may affect their function. Such arguments have been put forward to explain the effects of n-3 PUFAs on surface membrane ion channels (8) as well as phospholipase A2 activity and receptor coupling to intracellular messenger systems such as inositol 1,4,5-triphosphate (2). It should be pointed out that membrane fluidity increases when the free n-3 PUFA inserts, whereas incorporation of the fatty acids into membrane phospholipids appears to decrease fluidity, perhaps through secondary effects on membrane cholesterol levels (2). It is to be expected therefore that the long-term effects of exposure to n-3 PUFAs and those of acute exposure could be quite different, at least as far as the effects that are mediated via changes in membrane fluidity are concerned. As yet, the mechanism for inhibition of the Ca2+ release mechanism of cardiac SR is not known. Insertion of the fatty acid into the SR membrane where it might alter the working environment of the RyR is a possibility, but it is also possible that second-messenger systems are involved.

It is well known that PUFAs, n-3 and n-6, can act as second messengers that may activate or inhibit various kinases (12) and alter cAMP levels (16). They also are substrates for ion channels (8) as well as phospholipase A2 activity and receptor coupling to intracellular messenger systems such as inositol 1,4,5-triphosphate (2). It should be pointed out that membrane fluidity increases when the free n-3 PUFA inserts, whereas incorporation of the fatty acids into membrane phospholipids appears to decrease fluidity, perhaps through secondary effects on membrane cholesterol levels (2). It is to be expected therefore that the long-term effects of exposure to n-3 PUFAs and those of acute exposure could be quite different, at least as far as the effects that are mediated via changes in membrane fluidity are concerned. As yet, the mechanism for inhibition of the Ca2+ release mechanism of cardiac SR is not known. Insertion of the fatty acid into the SR membrane where it might alter the working environment of the RyR is a possibility, but it is also possible that second-messenger systems are involved.

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Due to constraints on the number of references set by editorial policy, this review has not been able to refer to many works that have made important contributions to the area of interest. I would like to apologize to the authors whose work I have had to omit as a result.

References