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COMMENTARY

Mitochondria, the calcium uniporter, and reperfusion-induced ventricular fibrillation

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The role of the mitochondria, and in particular the calcium (Ca) uniporter, in mediating reperfusion-induced arrhythmias is a novel investigative area. This commentary assesses the importance of a new article on this topic, published in this issue of the journal. Ventricular arrhythmogenesis remains an important area of research in the search of novel targets. The article by García-Rivas *et al* in this issue represents a possible novel focus for investigation.
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Reperfusion following a short period of myocardial ischaemia is known to be a double-edged sword. On the one hand, the necessity of reperfusion for the salvage of ischaemic cardiac tissue was established in the 1980s. Although it was initially thought that infarct size limitation could be achieved by pharmacological means alone (see e.g., DeBoer *et al*., 1980), it became apparent that at best drugs could merely delay the occurrence of tissue necrosis and not prevent it (Reimer and Jennings (1984)). Reperfusion thus became established as the only possible means of salvaging ischaemic tissue (Campbell *et al*., 1986). On the other hand, it is known that the occurrence of reperfusion itself can cause cardiac dysfunction and necrosis, including the long standing observation of the appearance of ventricular fibrillation (VF) in whole hearts (Tennant and Wiggers, 1935), a lethal cardiac arrhythmia. As a result, much research has been devoted to understanding the phenomena associated with reperfusion and to limiting the extent of reperfusion injury.

VF has itself been a long-term research focus owing to its lethal consequences and the current lack of any clinical drugs to prevent its occurrence. In the laboratory, VF is studied from two main perspectives that require models in which it can be reliably evoked. It is studied as a primary end point in itself in the evaluation of the efficacy of putative antiarrhythmic drugs, and it is also evoked as a means to investigate its molecular and cellular mechanisms. In this regard, it is well established that VF can be triggered in several different conditions, including myocardial ischaemia, infarction and reperfusion (Manning and Hearse, 1984; Curtis *et al*., 1987), but it can also be triggered as a result of genetic abnormalities (Makielski, 2006) or drug treatment (Fenichel *et al*., 2004). The ease of triggering of VF by reperfusion – which requires only short periods of ischaemia, and can be evoked simply by restoring flow to a globally ischaemic heart – compared to the relative difficulty of obtaining a reliable model of ischaemia-induced VF led to its use as a common means of studying VF in the laboratory. However, it has been argued that the mechanisms of VF occurring in these different conditions are likely to be different, and that occurrences of VF other than following reperfusion may be more relevant in terms of the relative contributions to sudden cardiac death (Clements-Jewery *et al*., 2005). Despite this, reperfusion VF is a real clinical entity that can be triggered following bypass surgery, angioplasty or thrombolysis (Lake *et al*., 1984). Its occurrence outside of the hospital has also been deduced in individuals with coronary artery disease who had suffered heart attacks but did not subsequently develop an actual infarction (Goldstein *et al*., 1981). Therefore, it can be argued that reperfusion VF deserves its research focus.

The mechanisms of reperfusion VF remain a matter of controversy. In the late 1980s it was established at the 'syncitial' level that reperfusion VF, but not ischaemic VF, could be induced in a globally ischaemic heart (Curtis and Hearse, 1989), implying that reperfusion VF does not require a sharp interface between ischaemic and nonischaemic regions for its initiation, in contrast to ischaemic VF. However, at the molecular and cellular levels the precise mechanisms that may be involved in reperfusion VF have proven harder to establish conclusively. Several major mechanisms have been proposed. A potential electrophysiological mechanism (Cascio *et al*., 2001) involves development of cellular uncoupling/decreased gap junctional conductance during ischaemia, whereas a proposed 'bio-

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chemical' mechanism involves the production of reactive oxygen species (Manning and Hearse, 1984), on the basis that VF can be prevented by free radical scavengers (Woodward and Zakaria, 1985) and by reperfusion with anoxic solution in Langendorff hearts (Carbonin *et al*., 1981). Another 'biochemical' mechanism involves cellular Ca overload instigated by reverse mode Na/Ca exchange-mediated Ca influx, on the basis that Ca overload and reperfusion VF can be prevented by relatively selective Na/Ca exchange inhibitors (Elias *et al*., 2001).

A more recently proposed mechanism, which may not be exclusive of the other mechanisms above, involves mitochondria. Mitochondria are thought to play a central role in cell death in ischaemia and reperfusion (Jennings *et al*., 1990), with the opening of the permeability transition pore (PTP), a large ion channel found on the inner mitochondrial membrane, proposed to mediate cell death during reperfusion (Halestrap *et al*., 1997). Furthermore, it has been suggested that the mitochondrial inner membrane potential may play a key role in determining susceptibility to reperfusion arrhythmias (Akar *et al*., 2005). This potential, which is essential for oxidative phosphorylation, can be dissipated by the opening of either the PTP or the mitochondrial inner membrane anion channel (IMAC), thus halting oxidative phosphorylation and activating sarcolemmal K_{ATP} channels (Aon *et al*., 2003). This has the consequence of shortening the action potential, thereby increasing the opportunity for re-entry (and VF) to occur. In theory then, pharmacological blockade of inner mitochondrial ion channels might reduce susceptibility to reperfusion VF. Indeed, one recent study in guinea-pig isolated hearts showed that an inhibitor of the IMAC, namely an antagonist of the mitochondrial benzodiazepine receptor, prevented oscillations of the inner mitochondrial potential and concurrent shortening of the action potential during reperfusion, with the result that the occurrence of reperfusion VF was greatly diminished (Akar *et al*., 2005).

The article by García-Rivas *et al*. (2006) adds to this body of work that suggests a critical role for mitochondria in mediating reperfusion VF. The hypothesis tested was that cellular Ca overload during reperfusion leads to elevation of mitochondrial Ca via the mitochondrial Ca uniporter, eventually causing opening of the PTP, with consequent dissipation of the inner mitochondrial potential and reperfusion VF. Rather than use inhibitors of the PTP or IMAC, an analogue of Ruthenium Red, Ru_{360}, which has been shown to be a relatively selective inhibitor of the mitochondrial Ca uniporter *in vitro*, was used in an *in vivo* rat model of ischaemia–reperfusion (García-Rivas *et al*., 2006). The striking result in the study was a dose-dependent inhibition of reperfusion VF incidence following 5 min of ischaemia. There was almost complete inhibition at the highest dose used, and a dramatic increase in the duration of sinus rhythm, implying a reduction in the occurrence of other arrhythmias. Mitochondria isolated from cardiac tissue taken from rats treated with Ru_{360} that were subjected to ischaemia–reperfusion demonstrated slowed uptake of ^{45}Ca *in vitro* versus that of vehicle controls, suggesting indeed that inhibition of the mitochondrial Ca uniporter had been achieved. These mitochondria also appeared to be able to tolerate greater uptake of Ca as control mitochondria from hearts also subjected to ischaemia–reperfusion appeared to undergo PTP opening (which could be inhibited by cyclosporin A) following initial uptake of Ca. Therefore, inhibition of mitochondrial Ca overload may prevent reperfusion VF.

However, some questions remain. As the inner mitochondrial potential was not recorded, it is not known exactly if prevention of dissipation of this potential was the precise mechanism by which reperfusion VF was prevented, or even if sarcolemmal K_{ATP} channels have any role to play in reperfusion VF in this rat model. In addition, although Ru_{360} appears to have no inhibitory effects on the development of contractile force, L-type Ca channels, the sarcolemmal Na/Ca exchanger or intracellular Ca handling by the sarcoplasmic reticulum at appropriate concentrations (de Jesus Garcia-Rivas *et al*., 2005), it remains unclear whether Ru_{360} possesses any nonselective effects on cardiac Na or K channels that might account for the antiarrhythmic effects observed. Nonetheless, the challenge will be to understand and define the exact contributions to initiation of reperfusion VF made by cellular uncoupling versus mitochondrial/cellular Ca overload and reactive oxygen species production. One might add that the role of mitochondria in causing VF under other conditions such as ischaemia and infarction is not at all well understood. That aside, there is now a growing body of evidence that there are a number of drug targets within mitochondria, which may aid in the search for drugs that reduce sudden cardiac death.

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