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COMMENTARY

Peroxynitrite: *in vivo* cardioprotectant or arrhythmogen?

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The factors that determine susceptibility to lethal ventricular arrhythmias during myocardial ischaemia and reperfusion *in vivo* are complex, but the balance between proarrhythmic and antiarrhythmic endogenous substances is likely to be important. However, in this context, it is not well established what effect endogenously produced peroxynitrite has on arrhythmias that develop during ischaemia/reperfusion *in vivo*. The study by Kiss *et al*., published in this issue of the *BJP*, provides some insights to this problem. We discuss the wider implications of this study as well as issues that still require resolution.
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Ventricular fibrillation, a lethal ventricular arrhythmia, is thought to account for the majority of sudden cardiac death because of ischaemic heart disease (Zipes and Wellens, 1998), but has proved to be an elusive target for drug therapy. This failure is likely to reflect the complex nature of the settings of ischaemia and reperfusion, in which there is a multiplicity of biochemical changes, including depletion of substrates, accumulation of metabolites and biochemicals, and ionic imbalance. In accordance with a biochemical paradigm of arrhythmogenesis, the biochemical changes have the potential to affect the normal electrophysiological properties of the myocardium, and thereby enhance susceptibility to arrhythmias. In support of this paradigm, some biochemicals that accumulate as a result of ischaemia and/or reperfusion have been identified as having proarrhythmic actions (Curtis *et al*., 1993). However, other biochemicals have been proposed to have antiarrhythmic actions (Parratt, 1993). As a consequence, the overall effect on arrhythmia susceptibility is likely to be dependent on the balance between these proarrhythmic and antiarrhythmic biochemicals and other factors.

One biochemical produced in the setting of ischaemia/reperfusion is peroxynitrite, which is formed by the reaction between nitric oxide and superoxide ions. Once formed, peroxynitrite can rapidly decompose by a variety of routes to generate a variety of products including nitrite and nitrate, as well as other oxidant species such as the hydroxyl radical (Szabo *et al*., 2007). The studies that have been performed to look at the effects of peroxynitrite on cardiac function have created a debate on whether peroxynitrite is protective or toxic in the heart (Ferdinandy and Schulz, 2001). This debate has also been played out in the search for the identity of the trigger factors and mediators of myocardial preconditioning, as nitric oxide and superoxide radicals, which are thought to have a function in mediating the cardioprotective effects of preconditioning (Ferdinandy and Schulz, 2003), are also the raw ingredients for the formation of peroxynitrite. However, there is not only a question concerning the role of peroxynitrite in the cardioprotection induced by preconditioning, but also a wider question concerning the role of peroxynitrite in determining the susceptibility to arrhythmias in the setting of ischaemia and reperfusion *in vivo*. That is, is peroxynitrite prarrhythmic or antiarrhythmic?

The study by Kiss *et al*. (2008) provides some resolution to this question. Their study, performed in anesthetized dogs, investigated the effect of delivery of an exogenous peroxynitrite solution through intracoronary infusion on arrhythmias that occurred during a subsequent 25 min period of regional ischaemia and during reperfusion. The authors used a pharmacological preconditioning-type protocol in which there were two such infusions of peroxynitrite before the induction of ischaemia. The study found that dogs pretreated with peroxynitrite in this manner had fewer premature beats and a lower incidence of ventricular fibrillation during ischaemia and during reperfusion compared with controls. These antiarrhythmic effects were accompanied by reductions in epicardial S–T segment elevation and nitrotyrosine formation, an index of peroxynitrite production, measured in reperfused myocardial tissue samples. As these effects were not reversed by coinfusion of

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5-hydroxydecanoate, an inhibitor of the postulated mitochondrial K_{ATP} channel, the effects of peroxynitrite could not be accounted for by opening of the mitochondrial K_{ATP} channel.

These results need careful interpretation. Examination of the effects of peroxynitrite infusion on arrhythmias alone might lead one to conclude that peroxynitrite is antiarrhythmic. However, quite a different conclusion can be made if the reduction in nitrotyrosine formation following pretreatment with peroxynitrite, an observation that is especially intriguing, is taken into account. This is because the nitrotyrosine measurements suggest that pretreatment with exogenous peroxynitrite actually reduces endogenous peroxynitrite production as a result of ischaemia/reperfusion. Is this plausible? It can be pointed out that ischaemic preconditioning appears to accomplish something similar for example, oxidants produced from preconditioning cycles subsequently limit damage, including that because of oxidant production, from a sustained ischaemic insult followed by reperfusion (Tosaki *et al*., 1994). Similarly, pretreatment with other oxidant species before sustained ischaemia mimics preconditioning, lowering oxidant production as a result of ischemia/reperfusion and providing protection (Lebuffe *et al*., 2003). Therefore, it is entirely possible that low-dose peroxynitrite treatment initiates a signalling cascade that limits peroxynitrite generation during ischaemia and reperfusion. The nature of the inferred signalling pathway that mediates these effects of exogenous peroxynitrite was not explored in the study beyond the apparent lack of involvement of the mitochondrial K_{ATP} channel. However, in answer to the question concerning the effect of peroxynitrite on susceptibility to arrhythmias generated by ischaemia and reperfusion *in vivo*, the study by Kiss *et al*. (2008) suggests that although treatment with exogenous peroxynitrite is antiarrhythmic, the production of endogenous peroxynitrite during ischaemia/reperfusion is actually proarrhythmic, and exogenous peroxynitrite treatment produces its effects by limiting endogenous peroxynitrite production.

There are unresolved issues concerning the observed effects of peroxynitrite, such as the identity of the molecular targets of exogenous and endogenous peroxynitrite, in addition to the delineation of any signalling cascades involved. Despite the painstaking efforts of the investigators to prevent the degradation of peroxynitrite before infusion, one may even question whether peroxynitrite itself or one of its decomposition products, such as nitrite, initiated the antiarrhythmic effects as peroxynitrite is notoriously unstable at physiological pH. This is a problem that has plagued many investigations involving peroxynitrite, suggesting that fully decomposed peroxynitrite is the best control for such studies. Nitrite itself appears to be cardioprotective, and the mechanisms responsible for this protective effect have been recently proposed (Hendgen-Cotta *et al*., 2008; Jansson *et al*., 2008). Alternatively, as discussed by Kiss *et al*. (2008), the antiarrhythmic effects of exogenous peroxynitrite may result from nitric oxide generation by *S*-nitrothiols formed from the reaction of peroxynitrite with plasma proteins.

Finally, there is the issue of how the biochemical changes induced by peroxynitrite modify cardiac electrophysiology and thereby the susceptibility to arrhythmias. ECG recordings obtained from a whole animal or heart are likely to provide only limited information about the underlying electrophysiological mechanisms. Therefore, it is not possible to read much into the S–T segment changes found in the study by Kiss *et al*. (2008) other than that these changes may reflect the severity of ischaemia that occurred following occlusion of the left anterior descending coronary artery.

Resolution of these issues is likely to require a significant amount of work and substantially different methodology is used in this study. Nevertheless, the results of the study by Kiss *et al*. (2008) warrant further investigation. It is hoped that future studies will be able to provide further insight regarding the nature of the balance and interplay between proarrhythmic and antiarrhythmic factors in the setting of myocardial ischaemia and reperfusion and how this influences the susceptibility to lethal ventricular arrhythmias.

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