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COMMENTARY

Peroxynitrite: just an oxidative/nitrosative stressor or a physiological regulator as well?

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It is widely accepted now that enhanced peroxynitrite ($ONOO^-$) formation contributes to oxidative and nitrosative stress in a variety of cardiovascular and other pathologies (see for reviews: Ferdinandy & Schulz (2001, 2003), Denicola & Radi (2005)). Therefore, targeting $ONOO^-$ directly by $ONOO^-$ decomposition catalysts and $ONOO^-$ scavengers or indirectly by inhibitors of downstream targets of peroxynitrite such as poli(ADP-ribose)-polimerase or matrix metalloproteinases are exciting new strategies for cytoprotection (Salvemini *et al*., 1998; Ferdinandy *et al*., 2000; Virag *et al*., 2003; Giricz *et al*., 2006). In contrast, increasing evidence suggests that physiological levels of $ONOO^-$ may act as a regulator of several physiological functions (Ferdinandy & Schulz, 2001; 2003; Herold & Fago, 2005; Ji *et al*., 2006). However, still very little is known about the physiological roles of endogenous peroxynitrite formation, possibly due to the number of technical limitations of detecting low, physiological levels of $ONOO^-$ in biological systems (Tarpey & Fridovich, 2001; Daiber *et al*., 2003).

$ONOO^-$ is a powerful oxidant species, which can be formed *in vivo* by the nonenzymatic reaction of nitric oxide (NO) and superoxide anion at an extremely rapid rate limited only by diffusion (Figure 1). At physiological pH, $ONOO^-$ is protonated to form peroxynitrous acid which rapidly decomposes forming highly reactive oxidant species especially in the presence of CO_2 (see for review Szabó, 1996). Unfortunately, due to its very short half life at physiological pH, endogenous formation of $ONOO^-$ cannot be directly detected in biological systems (Tarpey & Fridovich, 2001; Alvarez & Radi, 2003; Daiber *et al*., 2003). Although nitration of tyrosine residues is being recognized as a marker for $ONOO^-$ formation, the specificity and sensitivity of nitrotyrosine formation, especially in case of physiological rate of $ONOO^-$ production, is not sufficient (van der Vliet *et al*., 1995; Ferdinandy & Schulz, 2001; 2003; Tarpey & Fridovich, 2001). Nitrotyrosine can be formed by $ONOO^-$-independent pathways as well, for example, *via* the actions of peroxidases in the presence of nitrite (Eiserich *et al*., 1998). Moreover, the exogenous administration of $ONOO^-$ in experimental settings (e.g. *via* the blood) does not accurately reflect the effects of endogenous generation of $ONOO^-$ within the cells. Exogenous $ONOO^-$ rapidly reacts with plasma proteins and thiols to form the NO donor *S*-nitrosothiols (see for review Ferdinandy & Schulz (2001)). Thus, $ONOO^-$ is likely to be detoxified before it has a chance to reach tissues downstream of the injection site, let alone the intracellular compartment (Ishida *et al*., 1999). As NO itself is a cardioprotective and antioxidant molecule (Wink *et al*., 1993; Rubbo *et al*., 1996; Ferdinandy & Schulz, 2003) tissue protection may be seen when exogenous $ONOO^-$ is administered intravenously (Lefer *et al*., 1997; Nossuli *et al*., 1997; 1998). Exogenously applied $ONOO^-$, however, may show toxic effects when it does not have the opportunity to combine with sulphydryl groups or other antioxidant defenses before reaching its cellular targets. This is dependent upon the concentration of $ONOO^-$ and the antioxidant capacity of the cell or tissue of interest. Indeed, $ONOO^-$ has been shown to be detrimental to cellular functions when it was applied for example, in crystalloid buffer systems, in which the concentrations of extracellular antioxidants and both free and protein-bound thiols are limited (Schulz *et al*., 1997; Digerness *et al*., 1999; Ferdinandy *et al*., 2000).

In this issue of *British Journal of Pharmacology*, Graves *et al*. (2006) show that L-β,β-dimethylcysteine (L-penicillamine), a potential $ONOO^-$ scavenger, inhibits the dose-dependent vasodilator responses to moderate doses of peroxynitrite administered repeatedly *in vivo*. This group has also shown recently that the vasodilator response elicited by exogenous $ONOO^-$ involves activation of ATP-sensitive potassium channels (K_{ATP}) (Graves *et al*., 2005b). As the glibenclamide-sensitive vasodilator response was still seen after repeated injections of increasing doses of $ONOO^-$, when depletion of antioxidants is suspected, $ONOO^-$ may open K_{ATP} independently from generation of *S*-nitrosothiols (Graves *et al*., 1998). However, when 10 repeated injections of a high dose of $ONOO^-$ ($10\,\mu mol\,kg^{-1}$) were administered, a loss of K_{ATP} function has been observed (Graves *et al*., 2005a). Vasodilation and opening of K_{ATP} is not the only potential

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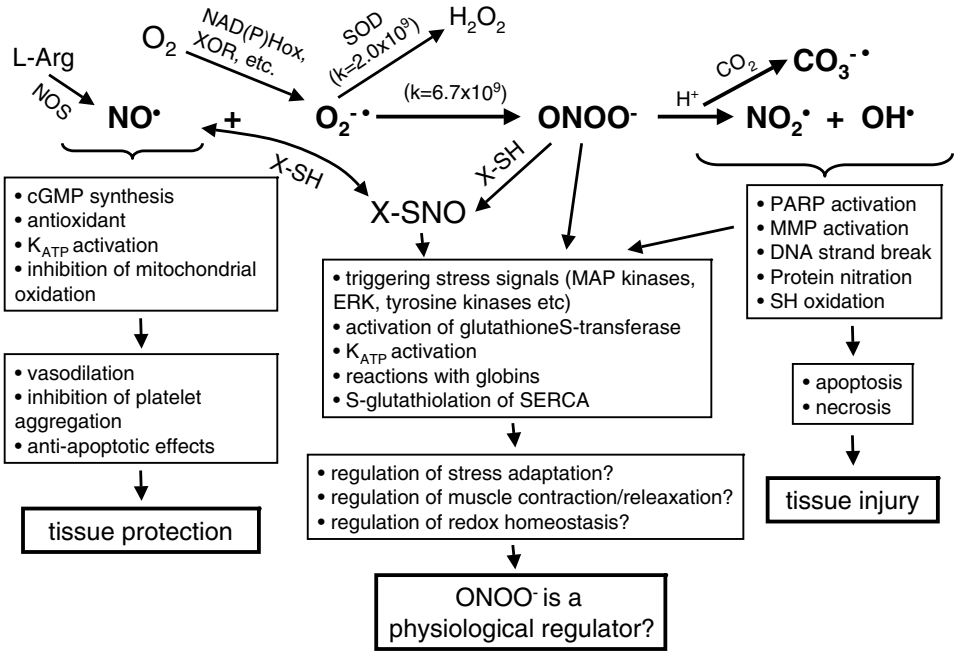

Figure 1 Cellular mechanisms of the actions of NO, superoxide ($O_2^{-\bullet}$), and $ONOO^-$. NO is an important cardioprotective molecule *via* its vasodilator, antioxidant, antiplatelet, and antineutrophil actions and it is essential for normal cellular function. However, excess NO could be detrimental if it combines with $O_2^{-\bullet}$ to form $ONOO^-$ which rapidly decomposes to highly reactive oxidant species leading to tissue injury. There is a critical balance between cellular concentrations of NO, $O_2^{-\bullet}$, and superoxide dismutase (SOD) which physiologically favor NO production but in pathological conditions such as, for example, ischemia and reperfusion result in $ONOO^-$ formation. $ONOO^-$ might be converted to NO donors if it combines with SH-group containing molecules (X-SH) to form *S*-nitroso compounds (X-SNO) including *S*-nitrosoglutathione. *S*-nitrosylation and *S*-glutathiolation are proposed mechanisms by which $ONOO^-$ regulates protein functions. Increasing evidence suggests that physiological levels of $ONOO^-$ act as regulator of several physiological functions. MMP, matrix metalloproteinase; NOS, NO synthase; PARP, poly-ADP ribose polymerase; XOR, xanthine oxidoreductase; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; K_{ATP}, ATP sensitive potassium channel.

regulator function of $ONOO^-$, where $ONOO^-$ is not definitely detrimental to the tissues.

Increasing evidence suggests that $ONOO^-$ may act as a regulator of various physiologic cellular functions. Endogenous $ONOO^-$ has been shown to trigger ischemic stress adaptation of the rat myocardium (Altug *et al*., 2000; Csonka *et al*., 2001; see for review: Ferdinandy & Schulz, 2003), and to activate stress response pathways such as the tyrosine kinase-dependent MAP-kinase and ERK pathways (see for review Klotz *et al*. (2002)). Cerioni *et al*. (2006) has recently demonstrated that nontoxic concentrations of peroxynitrite induced mitochondrial translocation of PKC-alpha and activated cell survival pathways in U937 cells. It has been recently shown that activation of microsomal glutathione-*S*-transferase-1 by peroxynitrite is mediated by nitration of tyrosine residue 92, and represents one of the few examples in which a gain in function has been associated with nitration of a specific tyrosine residue by $ONOO^-$ (Ji *et al*., 2006). Reactions of $ONOO^-$ with globins are suspected to play crucial roles in regulating normal physiological responses (see for review Herold & Fago, 2005). Moreover, $ONOO^-$ appears to be essential to the reversible *S*-glutathiolation of sarcoplasmic reticulum Ca^{2+}-ATPase, thereby regulating muscle relaxation (Viner *et al*., 1999; Adachi *et al*., 2004). $ONOO^-$ and NO donors can stimulate myocardial contractility independently of guanylyl cyclase activation, suggesting a role for *S*-nitrosylation reactions in the positive inotropic effects of NO/peroxynitrite in intact hearts (Paolocci *et al*., 2000). *S*-nitrosylation and *S*-glutathiolation are proposed mechanisms by which $ONOO^-$ regulates protein functions, although it should be noted that the role of NO and $ONOO^-$ in these reactions is still not clear and little is known about the oxidative actions $ONOO^-$ which seems to be more important than the nitrosative effect of $ONOO^-$ (Ji *et al*., 1999; Viner *et al*., 1999; Okamoto *et al*., 2001; Steffen *et al*., 2001). Nevertheless, it is plausible to speculate that $ONOO^-$ *via* its oxidative and nitrosative actions plays an important role in several physiological regulatory mechanisms that is becoming increasingly clear.

In summary, although it is widely accepted that enhanced $ONOO^-$ formation is cytotoxic, increasing evidence suggests that physiologic levels of $ONOO^-$ contribute to regulation of normal cellular functions. However, due to the numerous limitations of $ONOO^-$ detection using the currently available techniques, the conclusions should be drawn cautiously from studies based on $ONOO^-$ measurements. The development of more sensitive techniques to detect $ONOO^-$ and/or the discovery of specific and sensitive markers for endogenous $ONOO^-$ formation at a physiological rate will definitely enhance the exploration of the physiological roles of $ONOO^-$.

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