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THEMED SECTION: ENDOTHELIUM IN PHARMACOLOGY

COMMENTARY

Nitroxyl anion – the universal signalling partner of endogenously produced nitric oxide?

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Although it is generally assumed that the primary product of the three isoforms of NO synthase is the nitric oxide radical ($NO^{\bullet}$), growing evidence suggests that the one-electron reduced form of nitrogen monoxide, nitroxyl anion (NO^{-}), may be a natural co-product. Thus, evidence from conduit and resistance arteries and nitrergically innervated tissues indicates that NO^{-} exerts widespread signalling functions alongside $NO^{\bullet}$ in the cardiovascular and autonomic nervous systems, and perhaps beyond. In this issue of the *BJP* Andrews *et al.* add to this debate by providing strong evidence that $NO^{\bullet}$ and HNO both contribute to the EDRF-mediated component of in mouse (MMA) and rat (RMA) mesenteric resistance arteries.
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This article is a commentary on Andrews *et al.*, pp. 540–550 of this issue and is part of a themed section on Endothelium in Pharmacology. For a list of all articles in this section see the end of this paper, or visit: http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: endothelium-derived relaxing factor (EDRF); endothelium-derived hyperpolarizing factor (EDHF); nitroxyl anion (NO^{-}); nitric oxide ($NO^{\bullet}$)

Abbreviations: MMA, mouse mesenteric artery; NOS, nitric oxide synthase; RMA, rat mesenteric artery; sGC, soluble guanylate cyclase

It is now well established that vascular tone is regulated by three distinct vasodilator signals generated by the vascular endothelium, namely, prostacyclin, endothelium-derived relaxing factor (EDRF) and endothelium-derived hyperpolarizing factor (EDHF). Although there is general acceptance that the nitric oxide radical ($NO^{\bullet}$) formed from L-arginine by endothelial nitric oxide synthase (eNOS) mediates the dilatation attributed to EDRF, a growing number of reports suggest that a component of this dilatation may instead by mediated by the one-electron reduced form of nitrogen monoxide, nitroxyl anion (NO^{-}), which exists as HNO in aqueous solution. In this issue of the *British Journal of Pharmacology*, Andrews *et al.* (2009) add to this debate by providing strong evidence that $NO^{\bullet}$ and HNO both contribute to the EDRF-mediated component of dilatation in mouse (MMA) and rat (RMA) mesenteric resistance arteries.

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In yet another twist, the authors demonstrate that HNO-induced smooth muscle hyperpolarization contributes to the dilator actions of acetylcholine in both the MMA and RMA. The term EDHF was initially introduced to distinguish from EDRF, a distinct, newly emerging pathway whereby vascular smooth muscle relaxation was associated with its hyperpolarization (Chen *et al.*, 1988). This term has endured because there is still debate about whether EDHF is a chemical entity, the electrotonic spread of endothelial hyperpolarization to smooth muscle through myoendothelial gap junctions, or a combination of the two. Indeed, its utility withstood the subsequent realization that $NO^{\bullet}$ itself can promote smooth muscle hyperpolarization at certain sites (Tare *et al.*, 1990) through opening of calcium-activated potassium channels. The term is therefore likely to survive the finding by Andrews *et al.* (2009) that endothelium-derived HNO, released together with $NO^{\bullet}$, can promote smooth muscle relaxation in association with hyperpolarization. Thus, even although both $NO^{\bullet}$ and HNO may promote smooth muscle hyperpolarization, they are distinct from the EDHF that is so readily characterized by its susceptibility to blockade by the inhibitors of small

conductance- and intermediate-conductance calcium-activated potassium channels, apamin and charybdotoxin (or TRAM-34) respectively.

A number of major obstacles have thwarted attempts to differentiate with certainty biological actions resulting from endogenous production of HNO and those due to NO•. Foremost among these is the lack of a method for measuring the presence of HNO in biological experiments either *in vitro* or *in vivo*. Further confusion arises through the ability of HNO to be oxidized to NO• by certain tissues, as was found by Andrews *et al.* (2009) in the MMA. Allied to this is the controversy surrounding the actions of HNO on soluble guanylate cyclase (sGC). Specifically, although there is general agreement that both HNO and NO• promote dilatation in association with an elevation of cyclic guanosine monophosphate levels and that these actions are blocked by the inhibitor of sGC, ODQ, only the latter oxide of nitrogen is reported to activate sGC (Dierks and Burstyn, 1996). The elevation of cyclic guanosine monophosphate levels stimulated by HNO could therefore be easily explained if it were readily oxidized to NO•. There are, however, valid reasons for questioning the generality of this explanation. For example, assays of sGC activity are routinely conducted in the presence of millimolar concentrations of dithiothreitol, and thiols are now known to scavenge HNO. Thus, although they would not have known it at the time, the experimental conditions employed by Dierks and Burstyn (1996) would almost certainly have masked any ability of HNO to stimulate sGC. Thus, the possibility that HNO itself does indeed activate sGC must remain open, particularly as there was no evidence that HNO was oxidized to NO• when it induced ODQ-sensitive dilatation in the RMA (Andrews *et al.*, 2009).

In spite of the difficulties described above, enormous strides have been made in differentiating the distinct actions of endogenous HNO from those of NO• by the use of well-characterized pharmacological tools. Specifically, we can now have reasonable confidence that hydroxocobalamin binds and inactivates NO• but not HNO (Li *et al.*, 1999; Wanstall *et al.*, 2001); L-cysteine and other thiols bind and inactivate HNO but not NO• (Pino and Feelisch, 1994; Ellis *et al.*, 2000; Wanstall *et al.*, 2001); and 4-aminopyridine selectively blocks the smooth muscle hyperpolarization elicited by HNO through activation of voltage-sensitive potassium channels (Irvine *et al.*, 2003), whereas when NO• produces hyperpolarization, it does so by activation of calcium-activated potassium channels. It was through the skilful application of these tools, together with the use of the NO• donor, DEA/NO, and the HNO donor, Angeli's salt, that Andrews *et al.* (2009) were able to provide such compelling evidence that HNO, acting in concert with NO•, underpins the EDRF-mediated component of dilatation in the MMA and RMA.

The cellular source of the HNO contributing to EDRF-mediated dilatation was also considered by Andrews *et al.* (2009). Their findings in the MMA and RMA that L-NAME abolishes and reduces, respectively, the HNO component of EDRF-mediated dilatation, identifies eNOS as the source. In support of this conclusion, the authors cite a number of reports in the literature describing the formation of HNO by NOS, either as a natural product or when the enzyme is uncoupled. Less convincing, however, is the authors' contention that in the RMA 'HNO appears to be, at least in part, non-NOS derived'. While it is possible that their concept of cellular stores of *S*-nitrosothiols acting as a source of HNO for the EDRF response might be correct, an alternative explanation should be considered. Specifically, others have cautioned that the use of a NOS inhibitor will reduce, but not abolish NO• production in vascular tissue (Cohen *et al.*, 1997). Consequently, it could be argued that the residual L-cysteine-sensitive, HNO component of dilatation seen in the RMA following treatment with L-NAME arose from incomplete blockade of NOS. Indeed, this alternative explanation seems more plausible because in the presence of L-NAME, a residual hydroxocobalamin-sensitive, NO• component of dilatation was also seen in the RMA (Andrews *et al.*, 2009).

The concept that HNO mediates a component of EDRF-induced dilatation is not new. Others, using similar pharmacological approaches have produced evidence that HNO operates together with NO• to mediate EDRF-induced dilatation in conduit arteries, namely, the rat and mouse aorta (Ellis *et al.*, 2000; Wanstall *et al.*, 2001). The new findings of Andrews *et al.* (2009) that HNO operates together with NO• in resistance vessels (MMA and RMA), does, however, point to a more widespread role for HNO in regulating tone throughout the vasculature. In fact, as HNO appears to operate together with NO• in mediating nitrergic neurotransmission (Li *et al.*, 1999), it is possible that all forms of NOS operate by simultaneously producing both these forms of nitrogen monoxide.

In conclusion, there is a growing realization that HNO and NO• have distinct biological actions and pharmacological properties. Moreover, both of these nitrogen monoxides appear to be produced endogenously by NOS. These findings open up exciting new opportunities for exploring their distinct contributions in health and disease, and for the separate development of novel therapeutic agents targeted at each. The reader is referred to recent authoritative reviews that explore more fully the biological actions of HNO (Fukuto *et al.*, 2008; Irvine *et al.*, 2008).

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