BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 507–509

0007-1188/09
www.brjpharmacol.org

COMMENTARY

Aminoethyl nitrate – the novel super nitrate?

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Long-term use of most organic nitrates is limited by development of tolerance, induction of oxidative stress and endothelial dysfunction. In this issue of the *BJP*, Schuhmacher *et al.* characterized a novel class of organic nitrates with amino moieties (aminoalkyl nitrates). Aminoethyl nitrate was identified as a novel organic mononitrate with high potency but devoid of induction of mitochondrial oxidative stress. Cross-tolerance to nitroglycerin or the endothelium-dependent agonist acetylcholine after *in vivo* treatment was not observed. Like all nitrates, aminoethyl nitrate induced vasorelaxation by activation of soluble guanylate cyclase. Thus, in contrast to the prevailing view, high potency in an organic nitrate is not necessarily accompanied by induction of oxidative stress or endothelial dysfunction. This work from Daiber's group is an important step forward in the understanding of nitrate bioactivation, tolerance phenomena and towards the development of better organic nitrates for clinical use.
British Journal of Pharmacology (2009) **158,** 507–509; doi:10.1111/j.1476-5381.2009.00414.x

This is a Commentary on the Research Paper in this issue by Schuhmacher *et al.* (pp. 510–520). To view this article visit http://www3.interscience.wiley.com/journal/121548564/issueyear?year=2009

Keywords: endothelium; nitric oxide; organic nitrates; mitochondrial aldehyde dehydrogenase; oxidative stress; vascular function

Abbreviations: ALDH, aldehyde dehydrogenase; cGMP, cyclic guanosine monophosphate; GTN, glycerol trinitrate (nitroglycerin); PETN, pentaerithrityl tetranitrate; PTIO, 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; sGC, soluble guanylate cyclase

Organic nitrates are still an important part of the therapeutic armamentarium in symptomatic patients with coronary artery disease. Mechanistically, by liberation of nitric oxide (NO) or a related molecule, nitrates stimulate soluble guanylate cyclase (sGC) in smooth muscle cells, leading to enhanced formation of cyclic guanosine monophosphate (cGMP) and relaxation, thus replacing the diminished endogenous vasodilator capacity. Although there is no data to show prognostic improvement by use of organic nitrates in coronary artery disease, the symptomatic effectiveness during an acute episode of angina pectoris is well proven and seen by physicians on a daily basis. More problematic is the long-term use of organic nitrates, which is limited by side effects, such as headache, as well as by development of tolerance associated with impaired efficiency to prevent episodes of angina. Furthermore, the induction of oxidative stress and endothelial dysfunction demonstrated during long-term treatment with organic nitrates with the suspicion of potential aggravation of atherosclerosis has led to some reluctance to use these compounds (Munzel *et al.*, 2007). While considerable efforts have been made to find novel compounds stimulating the NO/sGC/cGMP axis, such as enhancers of endogenous endothelial NO synthase, activators of sGC or inhibitors of phosphodiesterases (Schafer *et al.*, 2006; Fraccarollo *et al.*, 2008), so far, none of these drugs have been proven useful to replace nitrates in treatment of patients with symptomatic coronary artery disease.

However, research efforts during the last years revealed that there are considerable differences among organic nitrates with regard to development of tolerance, as well as induction of endothelial dysfunction. Münzel's group, as well as that of Parker, have published seminal papers demonstrating in mice and man that the induction of vascular tolerance and oxidative stress during long-term exposure to nitrates such as nitroglycerin and isosorbide-5-mononitrate is not shared by other nitrates, such as pentaerithrityl tetranitrate (PETN) (Münzel *et al.*, 1996; Thomas *et al.*, 2007; Thum *et al.*, 2007). In addition to the considerable importance for the clinical application of organic nitrates, these research efforts also generated novel concepts in terms of nitrate bioactivation and the development of tolerance (Sydow *et al.*, 2004).

In this issue of the *British Journal of Pharmacology*, Schuhmacher *et al.* (2009) describe the characterization of a novel class of organic nitrates with amino moieties (aminoalkyl nitrates) (see Figure 1). Their results challenge traditional concepts regarding nitrate bioactivation and tolerance development. Aminoethyl nitrate was characterized as a novel

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Received 13 March 2009; revised 2 April 2009; accepted 29 April 2009

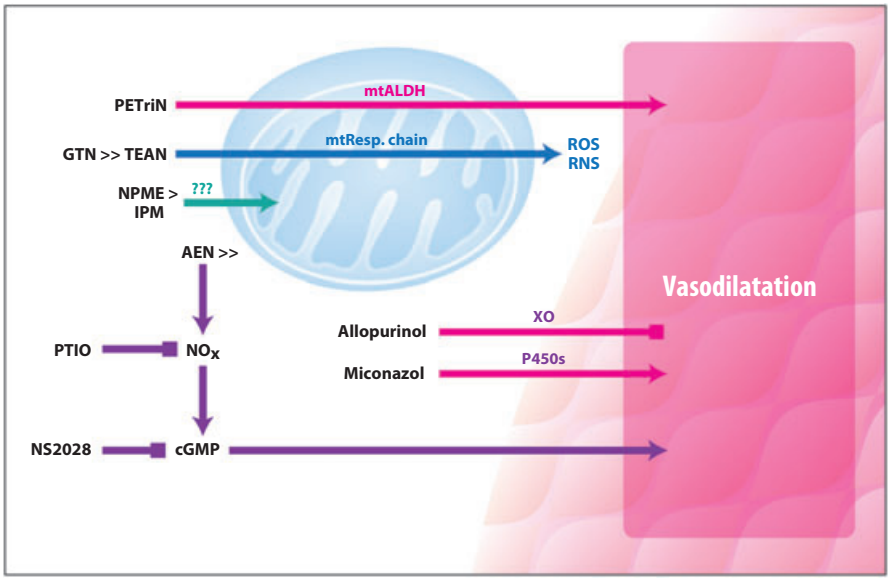

Figure 1 Bioactivation, mitochondrial effects and mechanisms of vasodilatation induced by aminoethyl nitrate (AEN) and other organic nitrates in the study of Schuhmacher *et al.* (2009). Both the highly potent nitroglycerin (GTN) and the less potent triethanolamine trinitrate (TEAN) are bioactivated by mitochondrial aldehyde dehydrogenase (mtALDH) and produce considerable amounts of mitochondrial reactive oxygen and nitrogen species (ROS/RNS, most probably peroxynitrite). In contrast, the trinitrate metabolite of pentaerithrityl tetranitrate, PETriN, also bioactivated by the mtALDH, did not induce mitochondrial oxidative stress. None of the mononitrates was bioactivated by mtALDH. Nevertheless, AEN was almost as potent as GTN and much more potent than methyl-3-nitrooxypropanoate (NPME), which showed significantly higher vasodilator potency than isopropyl nitrate (IPM). None of the mononitrates increased ROS/RNS levels in isolated mitochondria. Whether AEN, NPME and IPM undergo mitochondrial metabolism remains to be established. AEN-induced vasodilatation was attenuated by the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) and the inhibitor of soluble guanylate cyclase, NS2028. The lack of effect of miconazol on AEN-induced relaxation makes it unlikely that AEN was metabolized by cytochrome P450 enzymes. Attenuation of AEN-induced relaxation by allopurinol indicated that xanthine oxidase (XO) may be involved in bioactivation of AEN.

organic mononitrate with high potency but devoid of induction of mitochondrial oxidative stress and cross-tolerance to nitroglycerin or the endothelium-dependent agonist acetylcholine after *in vivo* treatment. Although the intermediate role of NO for nitroglycerin-induced vasorelaxation has been questioned (Kleschyov *et al.*, 2003), aminoethyl nitrate, like most nitrates, elicited vasorelaxation by the liberation of NO (proven by use of the NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide) and sGC stimulation (proven by use of the selective sGC inhibitor NS2028, Figure 1).

As the nitrates were delivered by osmotic minipumps, important questions of enteral uptake and the bioavailability after oral application remain to be settled. Furthermore, the bioactivation pathway is not unequivocally defined, but does not appear to involve aldehyde dehydrogenase or cytochrome P450 enzymes. Aminoethyl nitrate did not cause tolerance to any of the other vasodilators tested, but did induce *in vivo* tolerance to itself, implying that aminoethyl nitrate has its own bioactivation system. However, the metabolism of aminoethyl nitrate was apparently not inactivated by other nitrates or oxidative stress, as nitroglycerin therapy *in vivo* did not induce cross-tolerance to aminoethyl nitrate. This work from Daiber's group is an important advance in our fuller understanding of nitrate bioactivation, of tolerance phenomena, and, from the view of drug discovery, an important step towards the development of better organic nitrates for clinical application.

Conflict of interest

J.B. has received research grant support and honoraria from Actavis, related to PETN.

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