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COMMENTARY

Reduced vascular reactivity after chronic nitroglycerine administration: EDHF mechanism is also downregulated

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Abbreviations: AVECs, aortic valve endothelial cells; EC, endothelial cell; EDHF, endothelium-derived hyperpolarizing factor; IK_{Ca}, Ca^{2+}-activated K^+ channels of intermediate conductance; NO, nitric oxide; NTG, nitroglycerin; PGI_2, prostacyclin; SK_{Ca}, Ca^{2+}-activated K^+ channels of small conductance

Organic nitrates such as nitroglycerin (NTG) have been used as potent vasodilators in medicine for more than a century to treat cardiovascular diseases such as angina pectoris, acute myocardial infarction and congestive heart failure. Unfortunately, the usefulness of these nitrates is limited by a decrease in efficacy during long-term continuous administration, a phenomenon known as nitrate tolerance. Although numerous candidate enzymes for NTG metabolism, as well as a multiplicity of mechanisms, have been proposed to be involved in tolerance development, the biochemical mechanisms of action of nitrate are still incompletely resolved.

Nitrates have been traditionally thought to bypass the endothelium to exert their dilatory action on the vascular smooth muscle through the liberation of nitric oxide (NO) (Chung & Fung, 1990). However, recent evidence shows that among their actions, nitrates may interact with the vascular endothelium, and chronic nitrate administration may have a deleterious effect on endothelial function (Munzel, 2001). In particular, long-term NTG administration leads to smaller relaxations to NO and prostacyclin (PGI_2), two endothelium-dependent vasodilators crucial to blood vessel tone modulation. The exact pathways by which nitrates may exert these actions have not been elucidated. In the present issue of *Br J Pharmacol*, Kusama *et al*. (2005) provide a possible additional explanation for the tolerance and/or endothelial dysfunction that develops during NTG treatment showing that, besides altering NO and PGI_2 pathways, long-term administration of NTG may also affect a third endothelium-dependent dilatory mechanism occurring in blood vessels, the endothelium-derived hyperpolarizing factor (EDHF) phenomenon.

Despite the passage of two decades since Suzuki, Weston and co-workers (Chen *et al*., 1988) first described the phenomenon of EDHF, a single chemical that can universally explain the EDHF hyperpolarizing and relaxing actions in all blood vessels has not been identified (Coleman *et al*., 2004). A substantial weight of evidence suggests that products of cytochrome P450, the EETs, have a role in EDHF in some vessels. EETs have a range of actions, including as second messengers in endothelial cells (EC), modulators of endothelial ion channels or enzymes, or as diffusible factors released from EC to effect hyperpolarization and relaxation in vascular smooth muscle cells. Recent evidence indicates that they may act in parallel with the other EDHF mechanisms described below, depending on the conditions (Weston *et al*., 2005). Importantly, agents that evoke vasodilation *via* smooth muscle hyperpolarization first induce an obligatory hyperpolarization in the endothelium. It is now widely accepted that this endothelial hyperpolarization plays a major role in the EDHF phenomenon and is consequent to opening of Ca^{2+}-activated K^+ channels of intermediate (IK_{Ca}) and small (SK_{Ca}) conductance. These channels, now also implicated in the hyperpolarization of aortic valve endothelial cells (AVECs) as reported by Kusama *et al*. (2005), are responsible for a K^+ efflux from EC, which could account for EDHF in some arteries, according to an elegant hypothesis by Edwards, Weston and co-workers (Edwards *et al*., 1998). The endothelial hyperpolarization led to another explanation for the EDHF phenomenon, which represents the electrotonic spread of this hyperpolarization from the endothelium to the smooth muscle *via* gap junctions between these two cell types. It is now well established that such myoendothelial electrical coupling occurs in a range of vessels and, particularly in arterioles, this coupling can be sufficiently strong such that the two layers function essentially as a single electrical syncytium. Although the syncytial nature of native EC/smooth muscle poses problems in the identification of the source of changes in membrane potential in intact vessels (von der Weid & Bény, 1993; Marchenko & Sage, 1994; Allen *et al*., 2002), the problem was largely circumvented in the study by Sandow *et al*. (2002) through the judicious choice of preparation. The

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presence of myoendothelial electrical coupling correlated with the identification of myoendothelial gap junctions at the electron microscopy level in an artery with EDHF (rat mesenteric), while an absence of electrical coupling was associated with no detectable EDHF in rat femoral artery. The *in situ* preparation of intact AVECs from the rabbit heart used by Kusama *et al*. (2005) elegantly takes advantage of the fact that these ECs are not in contact with smooth muscle cells. These steps are necessary since, although cultured or acutely isolated EC could provide the means of avoiding the issue of syncytial coupling, concerns as to whether these systems reflect the physiological situation are repeatedly raised, as receptor systems and ion channels may be lost in cell culture or enzymatic dissociation processes.

This paper by Kusama *et al*. (2005) addresses issues related to NO-induced modulation of EDHF. It has been suggested that EDHF may function more as a backup relaxatory mechanism during conditions in which the NO bioavailability is diminished (Kemp *et al*., 1995). This idea has developed to include basal inhibition of EDHF by NO under physiological conditions. Diminished NO production, such as by NO synthesis inhibitors or in some diseases, would remove this brake on EDHF such that the resulting enhanced EDHF could at least partly compensate for the loss of NO relaxation (Bauersachs *et al*., 1996). Kusama *et al*. (2005) have found that inhibition of either NO or prostaglandin synthesis had no immediate effect on endothelial hyperpolarization evoked by ACh. On the basis that EDHF involves myoendothelial current flow, this provides evidence that basal NO may not inhibit EDHF. On the other hand, chronic NO administration *via* an NTG patch, did result in downregulation of AVEC hyperpolarization. There was no change in the endothelial $[Ca^{2+}]_i$, suggesting that the effect occurred at the level of the AVEC K^+ channels. This result strongly implies that EDHF, in the smooth muscle, would also be downregulated by this treatment. This has important clinical implications since subjects on NTG treatment require optimal vasodilator capability, yet the NTG treatment is likely to downregulate the important endothelium-dependent vasodilator, EDHF. Increased superoxide production is thought to play a major role in nitrate-induced EC dysfunction (Munzel *et al*., 1995; Gori & Parker, 2002). Thus, also of clinical importance is the finding by Kusama *et al*. (2005) that chronic *in vivo*, but not acute *in vitro* administration of the antioxidant, ascorbate, prevented the deleterious effect of NTG on EC hyperpolarizing capacity. Thus antioxidants would be expected to maintain EDHF-mediated vasodilation that appears to be increasingly important in the smallest arteries and arterioles. Whether NTG treatment affects the channels' properties, directly or *via* regulatory proteins, or decreases the number of functional channels expressed has not been addressed and awaits further studies.

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