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COMMENTARY

Evidence against C-type natriuretic peptide as an arterial 'EDHF'

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C-type natriuretic peptide (CNP) is found in and released from vascular endothelial cells. Recently, a novel role has been suggested for this peptide, that of an endothelium-derived hyperpolarizing factor or EDHF. Implicit in this proposal is a widespread role for CNP as a key mediator of vascular dilatation. In this issue of the British Journal of Pharmacology, Leuranguer *et al.* compare the profile of membrane potential changes evoked with this putative EDHF or with endogenous EDHF (activated with ACh) in small carotid arteries. Marked differences between the two profiles lead them to discount a possible role for CNP as an EDHF.
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The term endothelium-derived hyperpolarizing factor (EDHF) was first introduced in 1988 to distinguish hyperpolarization-associated vascular relaxations from the endothelium-derived relaxing factor 'EDRF' response due to nitric oxide (NO) (Chen *et al*., 1988). The EDHF pathway is now known to represent a fundamental control mechanism within the mammalian vasculature, with a particular influence on the diameter of the smaller resistance arteries. As such, EDHF intimately influences both blood pressure and flow, and most importantly this influence is modified by cardiovascular disease (see Feletou and Vanhoutte, 2006, for a review on EDHF). The EDHF response *per se* describes the NO- and prostacyclin-independent vascular relaxations that follow an increase in endothelial cell Ca^{2+} levels, the subsequent activation of endothelial cell Ca^{2+}-activated K-channels (SK_{Ca} and IK_{Ca}) and spread of the resultant hyperpolarization from the endothelium to the adjacent vascular smooth muscle. Following the recently resurrected proposal that C-type natriuretic peptide (CNP) may act as a diffusible EDHF (Chauhan *et al*., 2003; Hobbs *et al*., 2004), the work in guinea-pig carotid artery by Leuranguer and colleagues in this issue provides convincing evidence against the possibility that CNP may represent an EDHF.

The original suggestion that CNP might be an EDHF was based on observations that CNP can be released from endothelial cells and on microelectrode experiments in porcine coronary arteries in which CNP stimulated smooth muscle cell hyperpolarization (Wei *et al*., 1994 and references therein). However, this potentially interesting idea was soon discounted, as exogenous CNP, assumed to be acting on natriuretic receptor-B (NPR-B), failed to mimic either bradykinin-evoked EDHF-mediated hyperpolarization or relaxation in these arteries (Barton *et al*., 1998). It is now known that the EDHF response in this artery can be explained by the presence of myoendothelial gap junctions between the endothelial and smooth muscle cells, and an action of arachidonic acid metabolites (epoxyeicosatrienoic acids) on both the endothelium and the smooth muscle (Weston *et al*., 2005). However, in addition to acting on NPR-B, which activates particulate guanylyl cyclase, CNP also binds to NPR-C, a receptor distributed widely throughout the vasculature and allocated a 'clearance' receptor role. CNP was recently proposed to act as an EDHF, by activating vascular smooth muscle NPR-C (Chauhan *et al*., 2003; Hobbs *et al*., 2004). Although this suggestion was based largely on experiments with the rat small mesenteric artery, the CNP pathway is now proposed to represent a major and widespread dilator mechanism within the mammalian cardiovascular system (Chauhan *et al*., 2003; Villar *et al*., 2007).

The importance of the observations reported by Leuranguer *et al.* (2007) is twofold. First, the guinea-pig small carotid artery, like the rat mesenteric artery, is a vessel in which the EDHF pathway has been extensively investigated and characterized. So it is in many ways regarded as a 'reference' vessel for EDHF studies. Second, the extensive use of intracellular microelectrode recordings to measure smooth muscle hyperpolarizations (the axiomatic feature of the EDHF pathway) has allowed the authors to reveal major differences between ACh (EDHF)-evoked hyperpolarizations and those of CNP. Key observations in the carotid artery are that, in marked contrast to ACh-evoked EDHF responses, CNP causes only relatively very weak hyperpolarizations,

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which (a) are due to activation of glibenclamide-sensitive K_{ATP} channels via NPR-B, and (b) display rapid tachyphylaxis. Neither of these is a characteristic of 'EDHF'.

In spite of the widespread vascular distribution of NPR-C, the ability of CNP to evoke vascular relaxation associated with at least some hyperpolarization reflects NPR-B activation in porcine coronary artery, human subcutaneous resistance arteries and guinea-pig small carotid arteries (Barton *et al.*, 1998; Garcha and Hughes, 2006; Leuranguer *et al.*, 2008). The concept that CNP is an EDHF (Chauhan *et al.*, 2003; Hobbs *et al.*, 2004) then rests almost exclusively on evidence derived from the rat small mesenteric artery, which in common with the guinea-pig small carotid artery is well characterized in terms of EDHF. So how might CNP fit within the mechanisms already defined in this resistance artery?

Following release in the mesenteric bed, it is suggested that CNP activates GIRK channels on the muscle via NPR-C, causing hyperpolarization and relaxation (Chauhan *et al.*, 2003). While this is certainly an interesting possibility, some very fundamental questions remain to be answered. The evidence for and against this proposed role for CNP has been comprehensively discussed recently and the reader is directed to a review by Sandow and Tare (2007) for a detailed picture. Some key questions are the following: (1) Does CNP truly mimic agonist-evoked EDHF-mediated hyperpolarization? The membrane potential data available to date are very limited, and while they do show CNP can cause an increase in membrane potential, they do not really answer this question. ACh (by activating/releasing EDHF) evokes a true hyperpolarization as it increases resting membrane potential, or if prior smooth muscle depolarization and contraction has been stimulated, ACh repolarizes (reverses depolarization) and then hyperpolarizes the cells. In both cases, the potential ends up close to E_K at around $-70/80$ mV. Data with CNP and supramaximal concentrations of ACh (10 μM) show only repolarization, which may in part reflect the properties of the agonist, U46619, employed to stimulate depolarizing/constriction, which progressively removes the endothelial SK_{Ca} then IK_{Ca} activity underlying EDHF (Plane and Garland, 1996; Crane and Garland, 2004). (2) How does activation of endothelial cell SK_{Ca} (and IK_{Ca}?) cause the release of CNP from the endothelium? (3) Does inhibition of CNP synthesis/release prevent agonist-evoked EDHF responses? (4) Are functional GIRK channels really present on the smooth muscle cells of arteries and able to mediate the action of CNP? (5) How does CNP selectively activate NPR-C in the mesenteric artery, when reverse transcription-PCR analysis indicates NPR-A and -B are also present, and in other vessels with a similar receptor profile how does it appear to act only through NPR-B, causing relatively weak hyperpolarization and relaxation due to BK_{Ca} activation? Finally, and perhaps most fundamental, how does the CNP story fit with the known presence and central role of heterocellular (myoendothelial) gap junctions in the EDHF pathway?

Answering these and related questions may help to define a role for CNP in the vasculature, in addition to recognized effects on smooth muscle proliferation and aldosterone production. However, on reviewing the available literature, Sandow and Tare (2007) concluded that the evidence in favour of CNP as an EDHF was not yet convincing, an opinion now elegantly reinforced by Leuranguer *et al*. (2008).

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