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COMMENTARY

Compromised vascular endothelial cell SK_{Ca} activity: a fundamental aspect of hypertension?

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Smooth muscle hyperpolarization originating in the endothelium and commonly referred to as the EDHF (endothelium-derived hyperpolarizing factor) response provides a very significant drive to vasodilatation particularly in small resistance arteries. Together with other endothelium-dependent dilator pathways 'EDHF' hyperpolarization is compromised by cardiovascular disease, including hypertension. However, although attenuated vascular hyperpolarization has been described in animal models of hypertension, the underlying mechanisms are not fully understood. In the current issue of the *British Journal of Pharmacology*, Weston *et al.* combine classic pharmacological approaches with electrophysiological and molecular techniques to suggest that attenuated endothelium-dependent hyperpolarization (and as a consequence vasodilatation) reflects major disruption of pathways associated with the activation of endothelial small conductance Ca^{2+}-activated K-channels (SK_{Ca}) in mesenteric arteries from spontaneously hypertensive rats. In addition to reductions in SK_{Ca} and K_{IR} proteins, changes in caveolin-1 isomers were also detected, possibly indicating channel realignment within plasmalemmal structures.
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The acronym 'EDHF' (endothelium-derived hyperpolarizing factor) was coined by *inter alia* one of the senior authors (AHW) of the current paper by Weston *et al.* (2010) (Taylor and Weston, 1988; Chen *et al.*, 1998). The term described endothelium-dependent smooth muscle hyperpolarization and relaxation independent of nitric oxide and was assumed to reflect the action of a diffusible factor (as per EDRF). The intervening years have witnessed quite considerable efforts expended in an attempt to identify this 'EDHF'. At times differing data from groups around the world provided a quite confusing picture, but by and large it seems now this reflected the complexity of a hyperpolarizing pathway that cannot be explained simply on the basis of a single diffusible factor, and in addition that can also involve spread of hyperpolarization through heterocellular, myoendothelial gap junctions.

Despite some variability in the actual EDHFs that operate in specific vessels, the endothelium-dependent hyperpolarizing pathway, or EDH, does display some key unifying characteristics. Initiation of EDH requires an increase in endothelial cell cytoplasmic calcium concentration. Of fundamental importance was the discovery (Edwards *et al.*, 1998) that that this $[Ca^{2+}]_i$ increase then activates two distinct types of K_{Ca}, the small and intermediate subtypes (SK_{Ca} and IK_{Ca}), both shown directly to reside on endothelial not smooth muscle cells (Edwards *et al.*, 1998). These channels then together generate the EDH that leads to smooth muscle hyperpolarization and relaxation, and explains the requirement for a combination of selective channel blockers (apamin and TRAM-34) in order to block EDH-generated vascular relaxation. As a consequence, sensitivity to block with both apamin and TRAM-34, but not apamin and iberiotoxin is regarded as a defining 'fingerprint' for the EDH pathway (see Busse *et al.*, 2002 for a review).

But although both SK_{Ca} and IK_{Ca} operate in parallel to generate EDH and vasorelaxation, they can be activated independently. So in quiescent arteries, EDH is preferentially associated with SK_{Ca} activation, as it is blocked by apamin. But under more depolarizing conditions, EDH is generated by activation of *both* SK_{Ca} and IK_{Ca}. As both channel subtypes are activated by increased cytoplasmic calcium concentration, it was suggested that each subtype might be located in discrete regions of the endothelial cell membrane (Crane *et al.*, 2003). This has indeed proved to be the case. In the rat mesenteric artery, a resistance-size vessel widely utilized in EDH studies and the subject of the current Weston *et al.* paper (Weston *et al.* 2010), SK_{Ca} channels distribute throughout the endothelial cell membrane, but cluster in the proximity of the large

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gap junctions between endothelial cells. In contrast, IK_{Ca} channels are only found in detectable amounts upon endothelial cell projections towards adjacent smooth muscle, where they can form myoendothelial gap junctions (Dora *et al.*, 2008). Furthermore, the SK_{Ca} but not the IK_{Ca} channels are localized in caveolae (Absi *et al.*, 2007), a relationship it is now suggested may be disrupted in the endothelium of spontaneously hypertensive rat (SHR) arteries (Weston *et al.*, 2010). So apart from this very interesting observation, what is the importance of the current work?

There are three key aspects. First, intracellular microelectrode recordings are used to provide a functional readout of the consequence of a *c.* 40% reduction in SK_{Ca} protein (detected by Western blots) in mesenteric arteries from SHRs; and second, related to and significantly enhancing these electrophysiological experiments is the use, for the first time in vascular tissue, of CyPPA, a positive modulator of SK_{Ca} channels. Previous studies have reported that EDH is reduced in SHR mesenteric arteries, but although this was associated with attenuated relaxation no mechanistic explanation was provided (Fujii *et al.*, 1992). What is a very striking observation now is that although SK_{Ca} protein was reduced by 40%, SK_{Ca} hyperpolarization evoked by ACh was effectively abolished (compare figure 1A with B, in the latter apamin does not further reduce the ACh hyperpolarization in SHR vessels). However, (see figure 2) significant hyperpolarization could still be evoked from the SHR arteries by applying the trisubstituted pyrimidine, CyPPA, which appears to be completely selective in its ability to activate SK_{Ca} channels (as hyperpolarization was abolished by apamin). So by directly activating a reduced population of SK_{Ca} channels, it was still possible to evoke a greater hyperpolarization in SHR mesenteric arteries than that generated by ACh acting through these channels in control Wistar-Kyoto rat arteries. So does this mean it is the disruption of the membrane localization of SK_{Ca} relative to other components of the EDH pathway, inferred from the change in caveolin-1 monomer/dimer ratio, that is responsible for the depressed EDH, rather than the reduced channel protein *per se*? This is clearly an important aspect that merits further investigation.

A third observation of note relates to the reduction in K_{IR} protein that was also discovered in the SHR arteries (approximately 50% and similar in magnitude to the reduction in SK_{Ca}), and direct correlation of this reduction to attenuated smooth muscle hyperpolarization in response to exogenous K^+. In the mesenteric artery, EDH invades the adjacent smooth muscle layers by passing through myoendothelial gap junctions *and* as a result of K^+ efflux through endothelial K_{Ca} channels. The latter acts as an intercellular EDHF, activating K_{IR} channels and Na^+/K^+ ATPase to cause smooth muscle hyperpolarization (Edwards *et al.*, 1998; Mather *et al.*, 2005). It has been suggested recently that K^+ efflux although IK_{Ca} is closely coupled solely to Na^+/K^+ ATPase, and clustering of this pump close to these channels on endothelial cell projections supports this contention (Dora *et al.*, 2008; Harno *et al.*, 2008). A close link between SK_{Ca} and K_{IR} would in this context make sense: K^+ passing out through SK_{Ca} serving to relieve the rectifying block of K_{IR} on the smooth muscle and also on the endothelium (exogenous K^+ caused greater hyperpolarization in arteries with an extant endothelium); the latter perhaps serving to amplify endothelial cell hyperpolarization and thus the current available to pass through myoendothelial gap junctions.

So by combining technically challenging electrophysiological experiments with molecular studies and the use of a novel pharmacological modulator of SK_{Ca} channels, the work of Weston *et al.* (2010) provides us with some important new insights into the vascular mechanisms that may underlie hypertension. Inevitably, the novelty of this work means it raises more questions than answers! Of course one is what comes first in SHR arteries, an increase in pressure or disrupted signalling through endothelial cell SK_{Ca} channels? As reducing SK3 protein *in vivo* leads to a significant increase in blood pressure, perhaps the latter is the more likely explanation (Taylor *et al.*, 2003). Also important will be probing whether disrupted signalling correlates with morphological changes in channel distribution, and if there are any alterations in the IK_{Ca}-Na^+/K^+ ATPase signalling axis. Certainly, there is evidence from angiotensin-induced hypertensive rats to suggest IK_{Ca} channel protein, normally not present in caveolae, may also be decreased (Hilgers and Webb, 2007). Time will provide answers to these questions, but SK_{Ca} and its associated signalling pathways may represent promising novel therapeutic targets for blood pressure modulation.

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