locally regulated vascular tone is generally determined by a variety of autonomic nerves, circulating vasoactive compounds, tissue metabolites, and endothelium-derived autacoids. The best characterized autacoids are the potent vasodilators nitric oxide (NO) and prostacyclin (PGI₂) and the vasoconstrictor peptide endothelin-1. Several studies have, however, convincingly demonstrated the existence of an NO/PGI₂-independent component of endothelium-dependent relaxation in various arterial beds, most notably in mesenteric, carotid, cerebral, coronary, and renal arteries. Because the NO/PGI₂-independent vasodilation originally described was coincident with vascular smooth muscle hyperpolarization and was abolished by depolarizing concentrations of potassium, it was proposed to be mediated by an endothelium-derived hyperpolarizing factor or “EDHF.”¹

When the term EDHF was initially coined, researchers expected to be able to identify a specific chemical entity synthesized in, and released from, the endothelium which hyperpolarizes vascular smooth muscle cells and elicits relaxation. However, there does not seem to be a specific EDHF, as at least 3 principal mechanisms have been linked to the EDHF phenomenon: (1) an increase in endothelial [Ca²⁺], after cell stimulation triggers the synthesis of a cytochrome P450 metabolite which is essential for the subsequent EDHF-mediated responses; (2) K⁺, released from endothelial cells via Ca²⁺-dependent K⁺ (K⁺Ca) channels induces smooth muscle hyperpolarization by activating inwardly rectifying K⁺ channels or the Na⁺-K⁺-ATPase on vascular smooth muscle cells; and (3) endothelial cell hyperpolarization is transmitted to the vascular smooth muscle via gap junctions. The strengths and weaknesses of the arguments for each of these specific types of EDHF have been reviewed recently.²,³

The resting membrane potential of smooth muscle cells in normal pressurized arteries and arterioles is mainly determined by the open probability of K⁺ and, to a lesser extent, Cl⁻ channels and ranges in vivo between −55 and −35 mV, reflecting the fact that arteries are usually partially contracted, a state from which they can constrict or dilate. The mechanism by which hyperpolarization elicits relaxation is controversial, but the most direct and obvious explanation is that the opening of K⁺ channels hyperpolarizes the smooth muscle cell membrane, reducing the open probability of voltage-dependent Ca²⁺ channels and activating Ca²⁺ sequestration and extrusion mechanisms, so that [Ca²⁺], is lowered and relaxation can take place. However although the literature relating to EDHF seems confusing, there is widespread agreement that the initial step in all of the EDHF-mediated responses studied to date is activation of small (SKCa) and intermediate conductance K⁺Ca (IKCa) channels in the endothelium.² This explains why NO/PGI₂-independent hyperpolarization and relaxation are exquisitely sensitive to the combination of charybdotoxin and apamin,⁴⁻⁷ or the more specific inhibitors TRAM-348 and UCL1684,⁹ and insensitive to iberiotoxin, an inhibitor of large conductance (BKCa) K⁺Ca channels. However, the lack of selectivity of the available tools has made it difficult to determine whether the activation of the endothelial IKCa or SKCa or the release of a hyperpolarizing factor such as the cytochrome P450 epoxygenase-derived epoxyeicosatrienoic acids underlies the NO/PGI₂-independent responses.¹⁰,¹¹ For example, clotrimazole, an inhibitor of cytochrome P450 epoxygenases, also blocks IKCa whereas charybdotoxin blocks IKCa as well as BKCa.¹² Thus it seems that a genetic approach is the only way to elucidate the relative importance of the two channels in EDHF- as well as NO- and PGI₂-dependent responses.

In this issue of Circulation Research, Si et al¹³ report that the targeted deletion of KCa3.1 (to give the IKCa its proper name¹⁴) attenuates the acetylcholine (ACh)-induced hyperpolarization of endothelial and vascular smooth muscle cells. These effects were associated with an attenuated EDHF-dependent relaxation of the isolated carotid artery as well as resistance-sized vessels in the cremaster microcirculation indicating that a significant portion of the NO and PGI₂-independent relaxation in the arteries studied was related to the opening of KCa3.1 (Figure).

With the type of genetic approach used there is a risk that compensation occurs and that the relative importance of remaining KCa channels, in this case the SKCa (or KCa2.3), increases after specific deletion of the KCa3.1. Although resting membrane potential was not different in aortic and carotid artery endothelial cells from wild-type and KCa3.1⁻/⁻ mice, the response to the application of ACh was significantly smaller in cells from animals lacking KCa3.1 and completely reversed by the UCL1684, a specific KCa2.3 blocker. In wild-type mice, UCL1684 had a minimal effect on the...
ACh-induced hyperpolarization whereas the KCa3.1 blocker TRAM-34 induced complete repolarization. These data indicate that while there does not seem to be a significant role of the endothelial KCa3.1 in the regulating of the resting membrane potential, it plays a key role in the hyperpolarization elicited by agonist stimulation. That the effects described are related to, and have consequences on, EDHF-mediated responses was demonstrated using a number of approaches. Firstly, there was a marked attenuation (by 88%) of the maximal ACh-induced hyperpolarization of carotid artery smooth muscle cells from KCa3.1+/− compared with wild-type mice. Secondly, ACh-induced relaxation in the presence of a NO synthase and cyclooxygenase inhibitor was significantly attenuated in carotid artery smooth muscle cells from KCa3.1−/− mice compared with wild-type mice. It should be noted that vasodilator responses were also attenuated in arteries from KCa3.1−/− mice even in the absence of a NO synthase and cyclooxygenase inhibitor. As the effects of NO synthase inhibition were similar in KCa3.1+/− and wild-type animals Si et al. conclude that NO is unable to compensate for the attenuated EDHF response. This may indeed be the case as while NO has been reported to affect IKCa channels is reported to be NO-independent (at least in rat cerebral arteries) and SKCa are thought to contribute to hyperpolarization only when the endothelial NO synthase is active. Finally, the vasodilatation measured in resistance-sized arteries in the cremaster microcirculation was also significantly attenuated by the loss of KCa3.1. In none of the experiments performed was the lack of KCa3.1 associated with any impairment of contractile responses elicited with either an agonist (phenylephrine) or by an increase in transmural pressure (myogenic tone); similarly, endothelium-independent relaxation to sodium nitroprusside was similar in arteries from KCa3.1−/− and wild-type animals.

A number of studies relying on a combination of genetic and pharmacological approaches have indicated a potential role for an EDHF in the regulation of blood pressure. In agreement, the loss of KCa3.1 led to a significant increase in arterial blood pressure (assessed using tail cuff plethysmography and telemetry), and to mild left ventricular hypertrophy. Although the article by Si et al. indicates the potential importance of KCa3.1 in EDHF-mediated responses, some points regarding the potential interaction between KCa3.1 and KCa2.3 remain to be cleared up, especially as conditional knockout of the latter channel increases myogenic and agonist (phenylephrine)-induced tone as well as systemic blood pressure. Unfortunately, there is currently no information regarding EDHF-dependent responses in KCa2.3−/− mice. Determining the relevance of an EDHF in the acute regulation of vascular tone as well as in the development of certain pathologies such as hypertension or heart failure is hampered by the fact that there is a certain amount of redundancy in the palette of endothelium-derived vasodilator autacoids that are generated by most vascular beds. The availability of KCa3.1−/− animals is likely to prove invaluable in the elucidation of the pathophysiological relevance of the EDHF pathway in different organs.

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None.

References


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Realizing Its Potential: The Intermediate Conductance Ca2+-Activated K+ Channel (KCa3.1) and the Regulation of Blood Pressure
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