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Review article

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THE THIRD PATHWAY: ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION

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In response to various neurohumoral substances endothelial cells release nitric oxide (NO), prostacyclin and produce hyperpolarization of the underlying vascular smooth muscle cells, possibly by releasing another factor termed endothelium-derived hyperpolarizing factor (EDHF). EDHF-mediated responses are sensitive to the combination of two toxins, charybdotoxin plus apamin, but do not involve ATP-sensitive or large conductance calcium-activated potassium channels. As hyperpolarization of the endothelial cells is required in order to observe endothelium-dependent hyperpolarization, and electrical coupling through myo-endothelial gap junctions may explain the phenomenon. An alternative explanation is that the hyperpolarization of the endothelial cells causes an efflux of potassium that in turn activates the inwardly rectifying potassium conductance and the Na^+/K^+ pump of the smooth muscle cells. Endothelial cells produce metabolites of the cytochrome P450-monooxygenase that activate BK_{Ca}, and induce hyperpolarization of coronary arterial smooth muscle cells. The elucidation of the mechanism underlying endothelium-dependent hyperpolarization and the discovery of specific inhibitors of the phenomenon are prerequisite for the understanding of the physiological role of this alternative endothelial pathway involved in the control of vascular tone in health and disease.

Key words: *cytochrome P450 monooxygenase, endothelium, gap junction, hyperpolarization, potassium channels, smooth muscle.*

INTRODUCTION

Endothelial cells synthesize and release vasoactive mediators in response to various neurohumoral substances (e.g. acetylcholine, adenosine triphosphate, bradykinin, substance P, thrombin) and physical stimuli (e.g. the shear stress exerted by the flowing blood). Nitric oxide (NO) produced by the L-arginine-NO synthase pathway and prostacyclin produced from arachidonic

acid by cyclooxygenase have been identified as endothelium-derived vasodilators. However, some endothelium-dependent relaxations cannot be explained by the release of either NO or/and prostacyclin. In various blood vessels endothelium-dependent relaxations are accompanied by endothelium-dependent hyperpolarization of the vascular smooth muscle cells. With the discovery of specific inhibitors of the NO production, it became obvious that endothelium-dependent relaxations and hyperpolarizations can be partially or totally resistant to inhibitors of cyclooxygenases and NO synthases suggesting the existence of an additional endothelial mechanism (1—5). Under these conditions, the hyperpolarization of the smooth muscle membrane and the following decrease in the intracellular Ca^{2+} concentration explains the endothelium-dependent relaxations (6—8). Indeed, hyperpolarization of smooth muscle cells induces relaxation by reducing the open probability of voltage-dependent calcium channels and the turnover of intracellular phosphatidylinositol (9—10). Endothelium-dependent hyperpolarizations and/or relaxations resistant to inhibitors of nitric oxide synthase and cyclooxygenase are also present in various human blood vessels including coronary arteries (11) *(Fig. 1)*.

MECHANISM OF ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION

The mechanism of endothelium-dependent hyperpolarization involves the opening of a potassium conductance. Indeed, the amplitude of the hyperpolarization is inversely related to the extracellular concentration of K^+ ions, and it disappears in K^+ concentrations higher than 25 mM (12—15). Non selective inhibitors of calcium-dependent potassium channels, such as tetraethylammonium or tetrabutylammonium prevent the hyperpolarization (13, 16, 17). Endothelium-dependent hyperpolarizations are associated with an increase in rubidium efflux (18, 19) and a decrease in membrane resistance which suggest that the hyperpolarization is due to the opening and not to the closing of a conductance (e.g. chloride or non-specific cationic conductances) (12, 20, 21).

In all the species studied so far, including human (22—28), endothelium-dependent hyperpolarizations are insensitive to glibenclamide (29) (an inhibitor of ATP-sensitive potassium channels) They are blocked by apamin (30) (a specific inhibitor of small conductance calcium-activated potassium channel) or by the combination of apamin plus charybdotoxin (15, 29, 31—38) (a non specific inhibitor of large and intermediate conductance calcium-activated potassium channels as well as some voltage-dependent potassium channels) but not by the combination of apamin plus iberiotoxin (32, 36—39) (a specific inhibitor of large conductance calcium-activated potassium channels: BK_{Ca}) indicating that BK_{Ca} are not involved in EDHF-mediated responses. The site

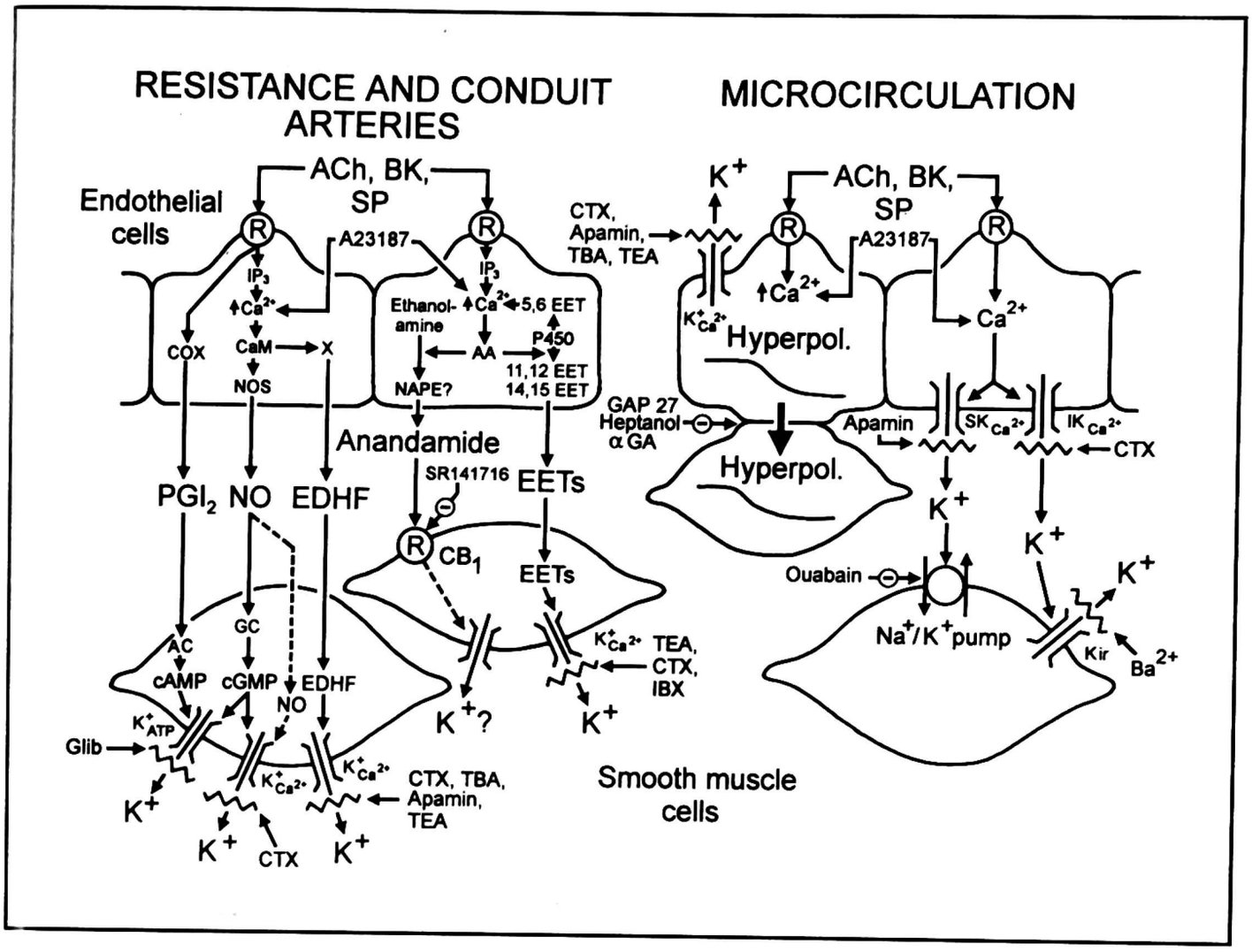

Fig. 1. Endothelium-dependent hyperpolarizations (modified from reference 5)

Acetylcholine (ACh), bradykinin (BK) and substance P (SP), through the activation of their respective receptor subtypes (M_3 = muscarinic, B_2 = bradykinin and NK_1 = neurokinin receptors), and agents that increase intracellular calcium, such as the calcium ionophore A23187, provoke endothelium-dependent hyperpolarization.

R: receptor; NOS: nitric oxide synthase; COX: cyclooxygenase; X: putative EDHF synthase; P450: cytochrome P450 monooxygenase; CaM: calmodulin; NO: nitric oxide; PGI_2: prostacyclin; EDHF: endothelium-derived hyperpolarizing factor; 5,6 EET: 5,6-epoxy-eicosatrienoic acid; 11,12 EET: 11,12-epoxy-eicosatrienoic acid; 14,15 EET: 14,15-epoxy-eicosatrienoic acid; NAPE: N-acylphosphatidylethanolamine; GC: guanylate cyclase, cGMP: cyclic guanosine monophosphate; cAMP: cyclic adenosine monophosphate; ATP: adenosine trisphosphate; IP_3: inositol trisphosphate; Hyperpol.: hyperpolarization.

SR 141716 is an antagonist of the cannabinoid CB1 receptor subtype (CB1). Glibenclamide (Glib) is a selective inhibitor of ATP sensitive potassium channels (K^+_{ATP}). Tetraethyl ammonium (TEA) and tetrabutyl ammonium (TBA) are non specific inhibitors of potassium channels when used at high concentrations (>5 mM) while at lower concentrations (1—3 mM) these drugs are selective for calcium-activated potassium channels ($K^+_{Ca^{2+}}$). Iberiotoxin (IBX) is a specific inhibitor of large conductance $K^+_{Ca^{2+}}$. Charybdotoxin (CTX) is a non selective inhibitor of large conductance $K^+_{Ca^{2+}}$, intermediate conductance $K^+_{Ca^{2+}}$ ($IK^+_{Ca^{2+}}$) and some voltage-dependent potassium channels. Apamin is a specific inhibitor of small conductance $K^+_{Ca^{2+}}$ ($SK^+_{Ca^{2+}}$). Barium (Ba^{2+}) in the micromolar range, is a specific inhibitor of inward rectifyer potassium channel (K_{ir}). Gap27, an eleven amino acid peptide possessing conserved sequence homology to a portion of the second extracellular loop of connexin, 18β-glycyrrhetinic acid (αGA) and heptanol are gap junction uncouplers.

of action of the two toxins (apamin and charybdotoxin) is more likely to be the endothelial cells (inhibition of endothelial hyperpolarization) than the smooth muscle cells [inhibition of the action of endothelium-derived hyperpolarizing factor (EDHF)]. Indeed, calcium-activated potassium channels are expressed in endothelial cells (40). The combination of the two toxins blocks EDHF-mediated responses if selectively applied to the endothelium (41), and inhibits the hyperpolarization of the endothelial cells produced by acetylcholine (42, 43). Finally, the existence of a potassium conductance specifically sensitive to the combination of charybdotoxin plus apamin could not be detected in isolated vascular smooth muscle cells (39, 44).

In some vascular tissue, prostacyclin and NO can also be considered as endothelium-derived hyperpolarizing factors since the two endothelial mediators hyperpolarize the vascular smooth muscle cells. However, the mechanisms of the hyperpolarizations produced either by prostacyclin or NO differ from the mechanism of the endothelium-dependent hyperpolarizations attributed to EDHF. Prostacyclin and/or its stable analogues open ATP-sensitive potassium channels blocked by sulfonylureas such as glibenclamide (29, 30, 45—48) and in some instance BK_{Ca} (49—52), or 4-aminopyridine-sensitive delayed rectifier potassium channel (53). Similarly, NO and/or NO donors can open ATP-sensitive potassium channels (29, 31, 47, 54—58) and BK_{Ca} (58—69). In some tissue NO can activates both BK_{Ca} and delayed rectifier voltage-dependent potassium channels (53). In most of the tissues, the activation of BK_{Ca} by NO is dependent upon cyclic-GMP-dependent protein kinase. However, NO can also produce a direct, cyclic-GMP-independent activation of BK_{Ca} (62, 67, 70, 71).

NATURE OF EDHF

Endothelium-dependent hyperpolarization could involve electrical coupling through myo-endothelial junctions (72). Indeed, substances which produce endothelium-dependent hyperpolarization of vascular smooth muscle cells, also hyperpolarize endothelial cells, with the same time course (73). Gap junctions couple smooth muscle and endothelial cells, and conduction of depolarization and hyperpolarization from smooth muscle cells to endothelial cells has been demonstrated (74, 75) as well as conduction of hyperpolarization from endothelial to smooth muscle cells (76, 77). Specific blockers of gap junctions, 18β-glycyrrhetinic acid and Gap27, a peptide which possesses a conserved sequence homology with a portion of connexin, inhibit EDHF-like responses in rabbit and guinea-pig arteries (72, 76—78). However, the respective role of myo-endothelial and of myo-myo gap junction coupling has to be established to better understand the potential contribution of gap junction in EDHF responses.

An alternative explanation is that the hyperpolarization of the endothelial cells causes an efflux of potassium from the intracellular space that could lead to the accumulation of potassium ions in the intercellular space between endothelial and smooth muscle cells. A moderate increase in potassium concentration can provoke the hyperpolarization of vascular smooth muscle cells by activating the inwardly rectifying potassium conductance (79) and the Na^+/K^+ pump (80). Therefore, potassium ions could be EDHF. This hypothesis has been successfully demonstrated in the hepatic and mesenteric arteries of the rat (42) but does not seem to be verified in other blood vessels from other species (15).

The existence of a diffusable substance has been demonstrated under bioassay conditions in which the source of EDHF was either native vascular segments or cultured endothelial cells (81—83). EDHF could be a short-lived metabolite of arachidonic acid produced through the cytochrome P450 monooxygenase pathway (84). Experiments performed mainly in bovine and porcine coronary arteries show that EDHF-responses are inhibited by inhibitors of cytochrome P450 monooxygenases and are associated with the release from endothelial cells of epoxyeicosatrienoic acid, substances that produce hyperpolarization of vascular smooth muscle (85, 86). However, inhibitors of cytochrome P450, studied at high concentration, are notoriously unspecific. In other blood vessels of the pig as well as in various arteries from humans, chemically unrelated inhibitors of cytochrome P450 do not produce an inhibition of EDHF-mediated responses (14, 24, 87, 88). Finally, activation of cytochrome P450 in endothelial cells may be a more general requirement for increasing the intracellular calcium concentration and thus the release of endothelium derived factors such as NO and EDHF (89) or producing endothelial hyperpolarization by allowing the opening of calcium-activated potassium channels.

Theoretically, adenosine, anandamide, the endogenous ligand for the cannabinoid CB_1 receptor as well as short-lived molecules such as carbon monoxide, hydroxyl radicals and hydrogen peroxide could all be putative endothelial-derived hyperpolarizing factors as they are produced by the endothelial cells and induce hyperpolarization of the smooth muscle cells, but the role of these molecules as EDHF has not been demonstrated convincingly (5, 11, 90, 91).

CONCLUSION

The elucidation of the mechanism underlying endothelium-dependent hyperpolarizations and the discovery of specific inhibitors of the phenomenon are prerequisite for the understanding of the physiological role of this alternative endothelial pathway involved in the control of vascular tone in health and disease.

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