440P A PROGRAM FOR FITTING DATA FOR A SINGLE CURVE TO THE OPERATIONAL MODEL (BLACK EQUATION)

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441P EDHF EXISTS

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Endothelial cells synthesize and release vasoactive mediators in response to various neurohumoral substances (e.g. acetylcholine, adenosine triphosphate, bradykinin, thrombin, ...) and physical stimuli (e.g. 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 the cyclooxygense pathway have been identified as endothelium-derived vasodilators. However, not all endothelium-dependent relaxations can be fully explained by the release of either NO or/and prostacyclin. Another unidentified substance(s) which hyperpolarizes the underlying vascular smooth muscle cells, termed endothelium-derived hyperpolarizing factor (EDHF), may contribute to endothelium dependent relaxations.

In blood vessels from various species, endothelium-dependent relaxations are partially or totally resistant to inhibitors of NO synthase and cyclooxygenase. In these blood vessels endothelium-dependent hyperpolarizations, which are resistant to NO scavengers and to inhibitors of NO synthase and cyclo-oxygenase, are also observed without an increase in intracellular level of cyclic nucleotides (cGMP and cAMP) in the smooth muscle. In canine, porcine and human blood vessels, the hyperpolarization cannot be mimicked by nitrovasodilators or exogeneous NO. However in other species (rat, guinea-pig, rabbit), endothelium-dependent hyperpolarizations resistant to NO synthase and cyclooxygenase and hyperpolarizations to endothelium-derived or exogeneous NO can be observed in the same vascular smooth muscle cells. In most blood vessels where NO causes hyperpolarization, the response is blocked by glibenclamide suggesting the involvement of ATP-dependent potassium channels. However, hyperpolarizations caused by EDHF are insensitive to glibenclamide but, depending on the tissue, are inhibited by relatively small concentration of TEA, or by apamin or the combination of charybdotoxin plus apamin, clearly indicating that NO and EDHF interacts with two different targets.

The existence of EDHF as a diffusable substance has been demonstrated under superfusion bioassay conditions whereby the source of EDHF was either native vascular segments or cultured endothelial cells, using either conventional intracellular microelectrode or patch-clamp techniques. Under the same bioassay conditions, EDHF released from cultured endothelial cells reduces the intracellular calcium concentration in vascular smooth muscle cells, supporting the involvement of this factor in the endothelium-dependent relaxation of the vascular smooth muscle cells. The technical difficulties in demonstrating the diffusable nature of EDHF could be explained either by a very short half-life of the substance, its preferential abluminal release, the simultaneous release of a hypothetical endothelium-derived depolarizing factor or a combination of these possibilities.

Theoretically, endothelium-dependent hyperpolarization may also involve electrical coupling through myo-endothelial junctions. Indeed, substances which produce endothelium-dependent hyperpolarization of vascular smooth muscle cells also hyperpolarize, with the same time course, endothelial cells. However, dye studies do not demonstrate coupling between endothelial and smooth muscle cells. Furthermore, although electrical coupling from smooth muscle to endothelial cells exists, electrical propagation in reverse direction does not seem to occur. Finally, halothane or heptanol, agents which uncouple cells linked by gap junctions, do not inhibit endothelium-dependent hyperpolarizations.

Altogether these results suggest the existence of a third pathway, besides the L-arginine-NO synthase and the cyclooxygenase pathways, in the endothelium production and release of vasoactive factors. The identification of EDHF and/or the discovery of specific inhibitors of its synthesis and its action may allow a better understanding of its physiological and pathophysiological role(s).