

Is nitric oxide the only answer?

Michel Félétou, PhD*[†]; Paul M. Vanhoutte, MD, PhD[†]

*IdRS - Suresnes; [†]IRIS - Courbevoie - FRANCE

Endothelium-dependent relaxations cannot be explained only by the release of nitric oxide and/or prostacyclin. Another still unidentified substance—endothelium-derived hyperpolarizing factor (EDHF)—which hyperpolarizes the underlying vascular smooth muscle cells, may also contribute to endothelium-dependent relaxations. In human, endothelium-dependent hyperpolarizations are observed in blood vessels that exhibit endothelium-dependent relaxations partially or totally resistant to inhibitors of nitric oxide synthase and cyclooxygenase. The contribution of the EDHF response is more important in smaller than in larger arteries. The suggestion that EDHF could be a metabolite of arachidonic acid formed through cytochrome P450 is still controversial. The identification of EDHF and/or the discovery of specific inhibitors of its synthesis action will permit a better understanding of its physiological and pathophysiological role(s).

Keywords: EDHF; endothelium; hyperpolarization; hyperpolarizing factor; membrane potential; potassium channel; prostacyclin; smooth muscle; vasodilatation

Address for correspondence:

Michel Félétou, Département de Diabétologie, Institut de Recherches Servier, 11 rue des Moulineaux, 92150 Suresnes, France (e-mail: feletou@servier.fr)

Nitric oxide (NO) produced from L-arginine by endothelial nitric oxide synthase (NOS) and prostacyclin produced from arachidonic acid by cyclooxygenase have been identified as endothelium-derived vasodilators¹ (see also article by Boulanger and Vanhoutte on page 3). However, in various blood vessels from different species, endothelium-dependent relaxations are partially or totally resistant to inhibitors of

cyclooxygenase and NOS (Figure 1). In these blood vessels, muscarinic agonists, bradykinin, or substance P elicit endothelium-dependent hyperpolarizations of vascular smooth muscle cells that are also partially or totally resistant to inhibitors of cyclooxygenase and NOS, suggesting the existence of an additional mechanism for endothelial control of vasomotor tone (Figure 2). These endothelium-dependent responses are observed without an

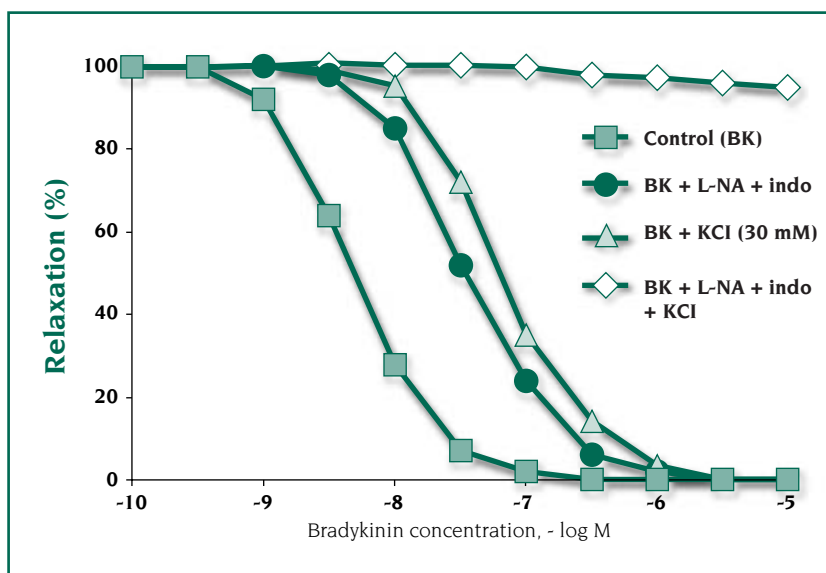


Figure 1. Concentration-relaxation curves to cumulative addition of bradykinin (BK) in porcine coronary artery. Bradykinin induces an endothelium-dependent relaxation that is minimally affected by the presence of L-nitro-arginine (L-NA: 10^{-4} M) and indomethacin (indo: 5×10^{-6} M), inhibitors of nitric oxide synthase (NOS) and cyclooxygenase, respectively. The component of the relaxation that subsists in the presence of these inhibitors is attributed to the release of a factor inducing hyperpolarization of the vascular smooth muscle cells (endothelium-derived hyperpolarizing factor, EDHF). The hyperpolarization is blocked by raising the potassium concentration (KCl: 30 mM) of the extracellular medium. This also produces an inhibition of the endothelium-dependent relaxation to bradykinin. The combination of inhibitors of NOS and cyclooxygenase and the elevated potassium concentration abolishes the relaxation to bradykinin. These experiments suggest that the activation of three pathways (NOS, cyclooxygenase, and the release of unidentified EDHF) contributes to endothelium-dependent relaxations in the porcine coronary artery. Similar observations have been reported for various animal and human isolated arteries.

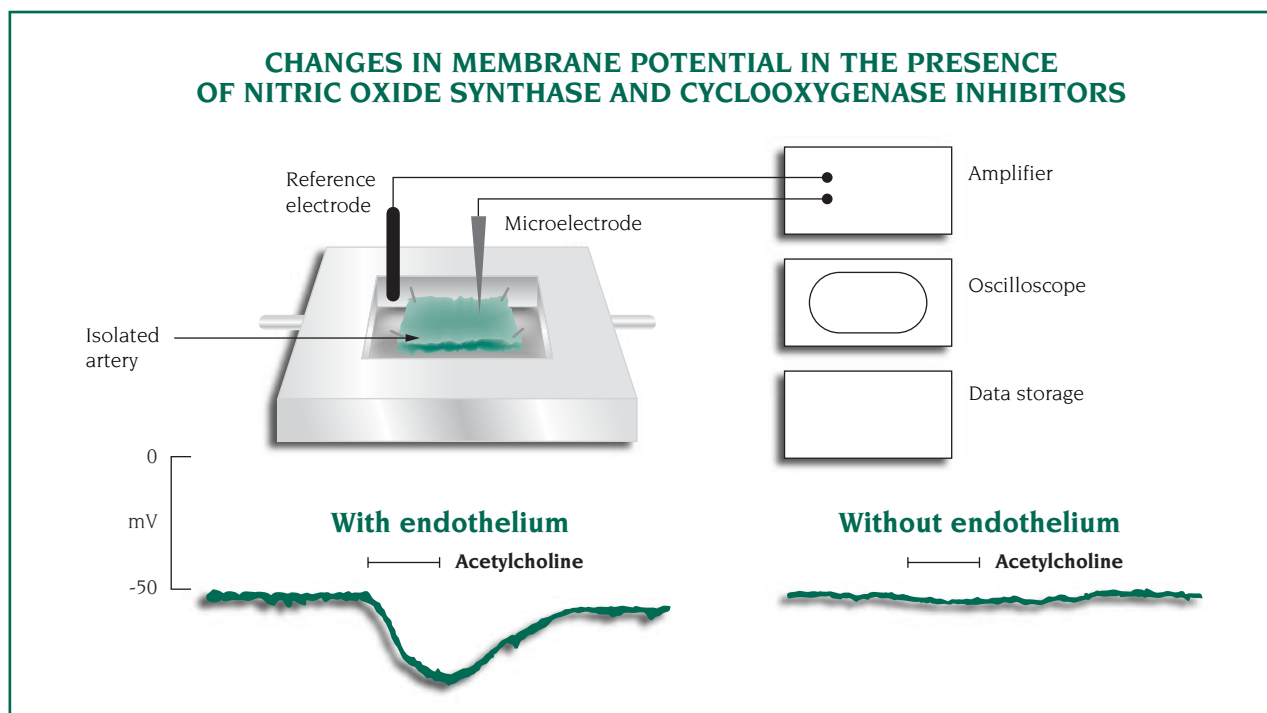


Figure 2. Recording of endothelium-dependent hyperpolarization. The membrane potential of vascular smooth muscle cells is measured with a microelectrode inserted into a single cell impaled at random from the media of a strip of an isolated artery. Drugs such as acetylcholine produce hyperpolarization of the vascular smooth muscle cells only in the presence of the endothelial cells. This hyperpolarization is not affected by the presence of inhibitors of nitric oxide synthase and cyclooxygenase. This again suggests that EDHF is responsible for the endothelium-dependent hyperpolarization.

increase in intracellular levels of cyclic nucleotides (cyclic GMP and cyclic AMP) in the smooth muscle cells, indicating that neither NO nor prostacyclin are responsible. Substances that produce endothelium-dependent hyperpolarization of vascular smooth muscle cells also hyperpolarize endothelial cells, with the same time course. Thus, cell-to-cell conduction could explain endothelium-dependent hyperpolarization. Indeed, direct electrical coupling between endothelial and vascular smooth muscle cells may be relevant at the microcirculatory level where myoendothelial junctions are numerous. However, this is unlikely to play an important role in the larger blood vessels in which endothelium-dependent hyperpolarizations have been reported so far (Figure 3). Thus, endothelium-dependent hyperpolarizations resistant to inhibitors of NOS and

cyclooxygenase have been attributed to the release of an as yet unidentified diffusible substance, termed endothelium-derived hyperpolarizing factor (EDHF), and may contribute to endothelium-dependent relaxations.^{2,3}

DOES EDHF EXIST IN HUMAN BLOOD VESSELS?

Endothelium-dependent hyperpolarizations associated with endothelium-dependent relaxations resistant to inhibitors of NOS and cyclooxygenase have been recorded in human coronary, pial, and gastropiloric arteries.⁴⁻⁶ Endothelium-dependent relaxations resistant to inhibitors of NOS and cyclooxygenase, which are generally attributed to EDHF release, have also been observed in subcutaneous, omental, renal, and radial arteries. By contrast, EDHF-dependent

responses are minimal in the internal thoracic artery and in the basilar artery. As in animal arteries, the contribution of the EDHF response is significantly greater in smaller than in larger human arteries.^{6,7} Therefore, EDHF may play a significant role in the local regulation of peripheral vascular resistance and thus of the distribution of blood flow.

It is difficult to evaluate, in the intact human, the involvement of EDHF in the vasodilator responses to various stimuli, as specific inhibitors of its synthesis or action are not available. An EDHF mechanism is often suggested to explain vasodilatations in the intact organism that are resistant to inhibitors of NOS. However, other interpretations are possible. First of all, most of the human studies do not involve the administration of an inhibitor



of cyclooxygenase. Although in normal subjects inhibitors of cyclooxygenase do not appear to affect vasodilatation in response to mediators such as acetylcholine, the continuous release of vasodilator prostanoids contributes to the regulation of resting forearm blood flow in humans.⁸ Furthermore, complete blockade of NOS is difficult to obtain, and/or the non-endothelial effect of the vasodilators, such as a direct effect on the smooth muscle cells or an inhibitory effect on the sympathetic nerve endings, cannot be excluded easily. Therefore, the exact role of EDHF in the control

of human blood vessel tone is still unknown.⁷

HOW ARE HYPERPOLARIZATION AND RELAXATION RELATED?

The hyperpolarization of the cell membrane of vascular smooth muscle cells and the resulting reduction in Ca²⁺ entry explain the endothelium-dependent relaxations caused by EDHF. Indeed, hyperpolarization of smooth muscle cells reduces the open-state probability of voltage-dependent calcium channels, thereby decreasing calcium

influx and lowering intracellular calcium levels. In addition, the hyperpolarization may reduce the increase in intracellular phosphatidylinositol turnover caused by agonist-induced receptor activation and therefore decrease the release of calcium from intracellular stores.

MECHANISM UNDERLYING ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION

The amplitude of the endothelium-dependent hyperpolarization is inversely related to the extracellular

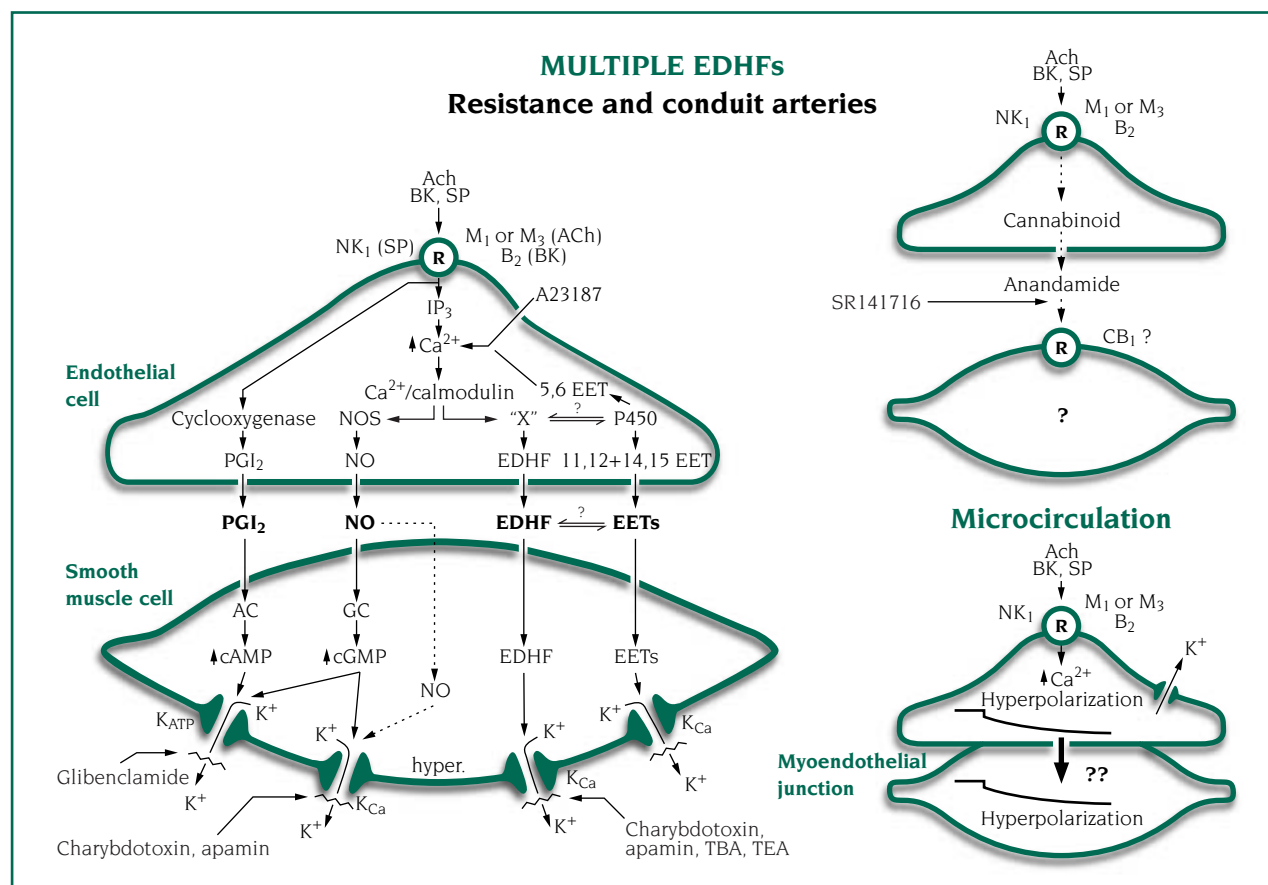


Figure 3. Possible mechanisms leading to endothelium-dependent hyperpolarizations. A23187, calcium ionophore; AC, adenylate cyclase; Ach, acetylcholine; ATP, adenosine triphosphate; B₂, B₂ bradykinin receptor subtype; BK, bradykinin; cAMP, cyclic adenosine monophosphate; CB₁, cannabinoid receptor; cGMP, cyclic guanosine monophosphate; EDHF, endothelium-derived hyperpolarizing factor; 5,6 EET, 5,6-epoxyeicosatrienoic acid; 11,12 EET, 11,12-epoxyeicosatrienoic acid; 14,15 EET, 14,15-epoxyeicosatrienoic acid; GC, guanylate cyclase; IP₃, inositol triphosphate; K_{ATP}, K_{Ca}, potassium channels; M₁, M₃, muscarinic receptor subtype M₁ or M₃; NK₁, NK₁ neurokinin receptor subtype; NO, nitric oxide; NOS, nitric oxide synthase; PGI₂, prostacyclin; R, receptor; SR141716, an antagonist of CB₁ receptors; SP, substance P; TBA, tetrabutylammonium; TEA, tetraethylammonium. TBA and TEA are nonspecific inhibitors of potassium channels when used at high concentrations (>5 mM). However, at lower concentrations (1-3 mM) these drugs act selectively on calcium-activated potassium channels (K_{Ca}).

concentration of K⁺ ions, and it disappears at K⁺ concentrations higher than 25 mM. Endothelium-dependent hyperpolarizations are associated with an increase in rubidium flux confirming the importance of potassium movement in the response. Nonselective potassium channel inhibitors, such as tetraethylammonium or tetrabutylammonium, prevent the hyperpolarization in the cell membrane of vascular smooth muscle cells. These observations concur, suggesting that endothelium-dependent hyperpolarization involves the opening of a potassium channel. In animal as well as human blood vessels, endothelium-dependent hyperpolarizations are insensitive to glibenclamide, indicating that they are independent of the activation of ATP-sensitive potassium channels. In most blood vessels, they are minimally or not at all affected by toxins that block either large conductance calcium-activated potassium channels (charybdotoxin, iberiotoxin) or small conductance calcium-activated potassium channels (apamin, scillatoxin) when given individually. However, hyperpolarizations can be prevented by the combination of charybdotoxin plus apamin (*Figure 3*). Although the potassium conductance involved in the endothelium-dependent hyperpolarization in human blood vessels has not been identified with precision, the limited data available are consistent with observations already made in other species.^{2,7}

IS EDHF A CYTOCHROME P450 DERIVATIVE?

In blood vessels from species such as the rat, guinea pig, and rabbit, endothelium-derived or exogenously added NO as well as prostacyclin (and its stable analog iloprost) hyperpolarize the vascular smooth

muscle cells. Responses attributed to EDHF are observed in the presence of inhibitors of NOS and cyclooxygenase. Nevertheless, a residual release of NO and/or prostacyclin, due to an uncomplete inhibition of NOS and/or cyclooxygenase, is conceivable, and this could explain endothelium-dependent hyperpolarizations. However, hyperpolarization in response to NO, depending on the species, involves either ATP-sensitive potassium channels (sensitive to glibenclamide), or large conductance calcium-activated potassium channels (sensitive to iberiotoxin, charybdotoxin, or low concentrations of tetraethylammonium or tetrabutylammonium), while that evoked by prostacyclin involves ATP-sensitive potassium channels exclusively (*Figure 3*). Therefore, the mechanism underlying these hyperpolarizations clearly differs from that attributed to EDHF.^{2,7}

Molecules such as anandamide, carbon monoxide, hydroxyl radicals, and hydrogen peroxide are all putative EDHFs, as they are produced by the endothelial cells and induce hyperpolarization of the smooth muscle cells. However, the evidence confirming the role of these molecules as EDHF is either weak or inexistent.^{3,7}

EDHF may be a short-lived metabolite of arachidonic acid, possibly produced through the cytochrome P450 monooxygenase pathway.⁹ Inhibitors of this pathway inhibit endothelium-dependent relaxations resistant to inhibitors of NOS and cyclooxygenase in the perfused heart and kidney of the rat and in the isolated porcine and bovine coronary arteries. Some metabolites of arachidonic acid, formed through cytochrome P450, activate potassium channels in vascular smooth muscle cells.

Muscarinic agonists induce not only endothelium-dependent relaxation and hyperpolarization, but also the release of epoxyeicosatrienoic acids from the endothelial cells.

These responses are inhibited by reasonably selective inhibitors of P450 monooxygenase. The cytochrome P450 metabolites produced by the endothelial cells increase the open-state probability of calcium-activated potassium channels sensitive to tetraethylammonium or charybdotoxin, and induce hyperpolarization of coronary arterial smooth muscle cells. Taken together, these observations support the hypothesis that epoxyeicosatrienoic acids could be EDHFs at least in blood vessels such as the bovine coronary artery (*Figure 3*).¹⁰

However, in human coronary and omental arteries, the involvement of metabolites of arachidonic acid formed through cytochrome P450 is unlikely, as several inhibitors of the enzyme have no effect on the responses to EDHF. This is in agreement with what is observed in blood vessels of rats, guinea pigs, dogs, and pigs, in which chemically unrelated inhibitors of cytochrome P450 do not inhibit the EDHF responses, or do so in a nonspecific way.¹¹ Indeed, at high concentrations, inhibitors of cytochrome P450 are unspecific and can inhibit hyperpolarizations induced by potassium-channel openers such as levcromakalim.

In human renal arteries, the inhibition of the relaxation attributed to EDHF by two anesthetic agents, etomidate and thiopental, may indicate the involvement of cytochrome P450. Cultured endothelial cells from the human umbilical vein synthesized a transferable β -naphthoflavone-inducible hyperpolarizing substance.¹² However, activation of cytochrome P450 in human



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 (Figure 3). The fundamental endothelial function of products of cytochrome P450 may confuse the issue when interpreting results of studies investigating the effects of inhibitors of cytochrome P450 on EDHF-mediated responses.^{2,7}

Although its existence has been established, the identity of EDHF is still elusive. The possibility of multiple EDHF(s) depending on the species or the size of the blood vessel has to be considered (Figure 3).

DO CARDIOVASCULAR DISEASES ALTER EDHF-MEDIATED RESPONSES?

In aging animals and in various animal models of diseases including hypertension, diabetes, and endotoxemia, as well as in humans (although the number of observations is limited), endothelium-dependent hyperpolarizations are diminished. The absence of endothelium-dependent hyperpolarization may contribute to the abnormal vascular responses observed under these pathologic conditions.^{4,6} Conversely, enhancement of EDHF-mediated responses contributes to the antihypertensive and cardioprotective action of drugs such as angiotensin-converting enzyme inhibitors,⁴ estrogen, diets rich in ω_3 -unsaturated fatty acids, and exercise training.

CONCLUSION

Besides the release of NO and prostacyclin, endothelial cells cause hyperpolarization of the underlying

vascular smooth muscle cells and this contributes to the endothelium-dependent relaxations. Studies in animal blood vessels show that this phenomenon is due to a diffusible factor, termed endothelium-derived hyperpolarizing factor (EDHF), which activates potassium channels on the smooth muscle cells. The identity of EDHF and the exact nature of the potassium channel involved remain to be determined. Only through the development of selective inhibitors of the synthesis or action of EDHF, will its role be able to be evaluated fully in humans.

REFERENCES

- 1. Furchgott RF, Vanhoutte PM.** *Endothelium-derived relaxing and contracting factors.* *FASEB J.* 1989;3:2007-2018.
- 2. Félétou M, Vanhoutte PM.** *Endothelium-derived hyperpolarizing factor.* *Clin Exp Pharmacol Physiol.* 1996;23:1082-1090.
- 3. Mombouli JV, Vanhoutte PM.** *Endothelium-derived hyperpolarizing factor(s): updating the unknown.* *Trends Pharmacol Sci.* 1997;18:252-256.
- 4. Nakashima M, Mombouli JV, Taylor AA, Vanhoutte PM.** *Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries.* *J Clin Invest.* 1993;92:2867-2871.
- 5. Petersson J, Zygmunt PM, Brandt L, Högestätt ED.** *Substance P-induced relaxation and hyperpolarization in human cerebral arteries.* *Br J Pharmacol.* 1995;115:889-894.
- 6. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A.** *Importance of endothelium-derived hyperpolarizing factor in human arteries.* *J Clin Invest.* 1997;100:2793-2799.
- 7. Félétou M, Vanhoutte PM.** *Endothelium-derived hyperpolarizing factor.* In: Vallance PJ, Webb DJ, eds. *Vascular Endothelium in Human Physiology and Pathophysiology.* Amsterdam, The Netherlands: Harwood Academic Publishers; 1998. In press.
- 8. Duffy SJ, Tran BT, New G, et al.** *Continuous release of vasodilator prostanoids contributes to regulation of resting forearm blood flow in humans.* *Am J Physiol.* 1998;274(4, pt 2):H1174-H1183.
- 9. Komori K, Vanhoutte PM.** *Endothelium-derived hyperpolarizing factor.* *Blood Vessels.* 1990;27:238-245.
- 10. Campbell WB, Gebremedhin D, Pratt PF, Harder DR.** *Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors.* *Circ Res.* 1996;78:415-423.
- 11. Chataigneau T, Félétou M, Duhault J, Vanhoutte PM.** *Epoxyeicosatrienoic acids, potassium channel blockers and endothelium-dependent hyperpolarisation in the guinea-pig carotid artery.* *Br J Pharmacol.* 1998;123:574-580.
- 12. Popp R, Bauersachs J, Sauer E, Hecker M, Fleming I, Busse R.** *A transferable, β -naphthoflavone-inducible, hyperpolarizing factor is synthesized by native and cultured porcine coronary endothelial cells.* *J Physiol (London).* 1996;497:699-709.