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Endothelium-dependent Vasoactive Modulation in the Ophthalmic Circulation

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Abstract—The vascular endothelium is strategically located between the circulating blood and the vascular smooth muscle cells. Different agonists or stimuli transported by the circulating blood can trigger the endothelium to release potent relaxing (nitric oxide, prostacyclin, endothelium-derived hyperpolarizing factor) or contracting factors (endothelin, cycloxygenase products). These endothelium-derived vasoactive factors can modulate blood flow locally. Heterogeneity exists from one vascular bed to the other, or even between vessels, in the agonists able to stimulate the release of endothelium-derived vasoactive factors. In the ophthalmic circulation, nitric oxide and endothelin are strong vasoactive modulators. In many vascular diseases that are of importance in ophthalmology (hypercholesterolemia, arteriosclerosis, hypertension, diabetes, vasospastic syndrome, ischemia and reperfusion, etc) the function of the endothelium can be impaired. There exist different drugs that can modulate the vasoactive function of the vascular endothelium. In other words, it appears that the vascular endothelium plays an important role in both the physiology and pathophysiology of the regulation of blood flow. The modulation of this regulatory system by different drugs might open new therapeutical approaches to treat vascular disorders in ophthalmology.

1. INTRODUCTION

The endothelium consists of a monolayer of cells lining the inner wall of the vasculature. Endothelial cells are startegically located between the circulating blood and the vascular smooth muscle cells. It long had been known that the endothelium regulates permeability (Shepherd and Vanhoutte, 1979), but more recently it has been recognized that it also exerts metabolic functions by activating and inactivating hormones (Dzau, 1986; Lüscher and Vanhoutte, 1990; Ng and Vane, 1967). Furthermore, endothelial cells strongly affect coagulation, platelet function, and fibrinolysis (Chesterman, 1988; Moncada *et al.*, 1977; Radomski *et al.*, 1987a,b). In addition, vasoactive substances that either inhibit (i.e. endothelium-derived relaxing factors: EDRF) or activate (i.e. endothelium-derived contracting factors: EDCF) the underlying smooth muscle cells can also be released by endothelial cells (Furchgott and Zawadzki, 1980; Lüscher and Vanhoutte, 1990; Miller and Vanhoutte, 1983; Moncada and Vane, 1978; Vanhoutte *et al.*, 1986; Yanagisawa *et al.*, 1988a,b). The present review briefly updates (Haefliger *et al.*, 1994a) and summarizes some of the current knowledge about endothelium-derived vasoactive substances with a special emphasis to the ophthalmic circulation (Fig. 1).

2. ENDOTHELIUM-DERIVED RELAXING FACTORS

2.1. L-arginine/nitric oxide pathway

Nitric oxide (NO), which has a very short half-life, is a powerful endothelium-derived vasodilator as well as an inhibitor of platelet function (Busse *et al.*, 1987; Furchgott, 1988; Ignarro *et al.*, 1986; Moncada *et al.*, 1991; Palmer *et al.*, 1987; Radomski *et al.*, 1987a,b). Nitric oxide is formed from the amino acid, L-arginine, into the amino acid, L-citrulline, by the enzyme nitric oxide synthase (NOS) (Moncada *et al.*, 1991) (Fig. 2). There are two major NOS isoforms, one of them is Ca^{2+}- and calmodulin-dependent, while the other one is Ca^{2+}- and calmodulin-independent (Bredt and Snyder, 1990; Palmer *et al.*, 1988a,b, 1987; Palmer and Moncada, 1989; Moncada, 1992; Moncada *et al.*, 1991; Nathan, 1992). The Ca^{2+}-calmodulin-dependent isoform is constitutive and has a widespread tissue distribution in endothelial cells (NOS III or eNOS) as well as in some non-adrenergic-non-cholinergic nerves (NOS I or nNOS). The Ca^{2+}-calmodulin-independent isoform (NOS II or iNOS), which is usually not expressed in normal conditions, is inducible after stimulation by different factors (i.e. endotoxin, tumor necrosing factor, interleukin-1, etc.) (Moncada *et al.*, 1991).

In endothelial cells, NO can be released in response to platelet-derived products (adenosine diphosphate, serotonin, thrombin, etc.), hormones, and autacoids (acetylcholine, bradykinin, histamine, noradrenaline, substance P, and vasopressin, etc.) (Cohen *et al.*, 1983a,b; De Mey and Vanhoutte, 1985; Houston *et al.*, 1985, Cocks and Angus, 1983; Cocks *et al.*, 1985; Katusic *et al.*, 1984; Van de Voorde and Leusen, 1983; Zawadzki *et al.*, 1981). Mechanical forces, such as shear stress, can also stimulate the release of NO, which mediates flow-dependent vasodilation in vivo (Pohl *et al.*, 1986; Rubanyi *et al.*, 1986).

In vascular smooth muscle cells and in pericytes, NO binds to the iron of the hemic structure of soluble guanylate cyclase, and stimulates the

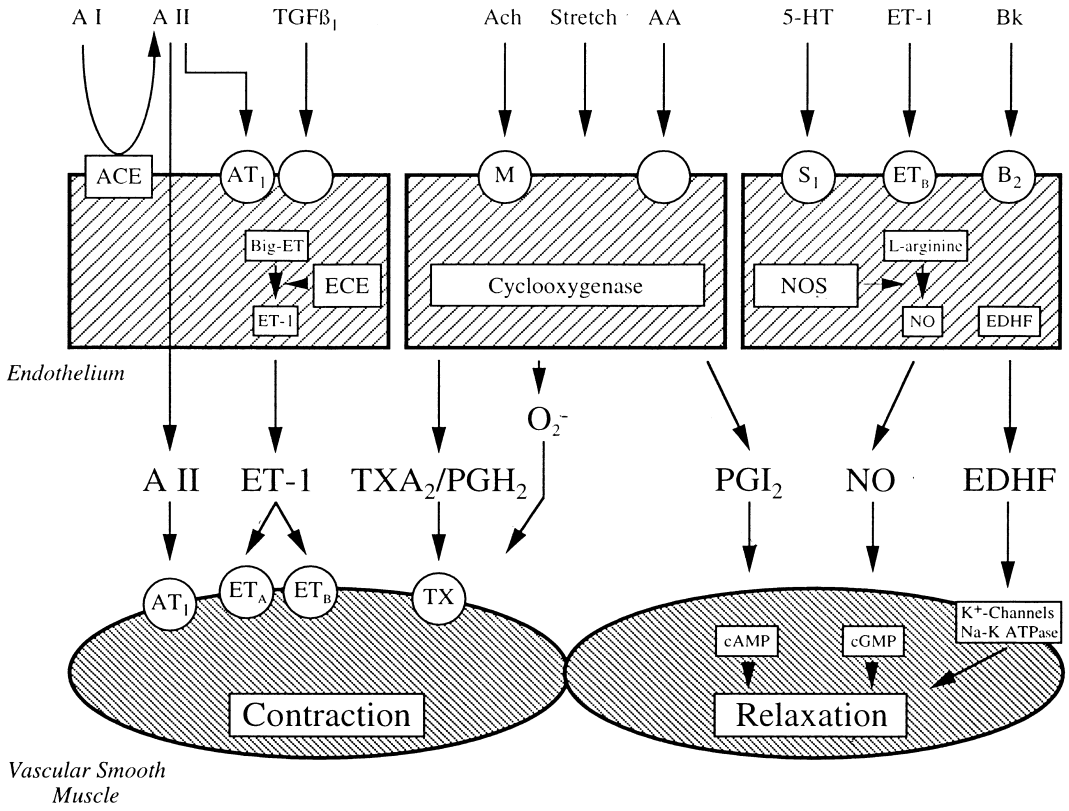

Fig. 1. Schematic diagram of endothelium-derived vasoactive substances released by the endothelium after activation of specific receptors (open circles) or by physical forces. After activation, the endothelium can either produce endothelium-dependent relaxing factors (EDRF), such as nitric oxide (NO), prostacyclin (PGI_2), or the endothelium-derived hyperpolarizing factor (EDHF). The endothelium can also release endothelium-derived contracting factors (EDCF) such as endothelin-1, angiotensin II (A II), thromboxane A_2 (TXA_2), prostaglandin H_2 (PGH_2) or the superoxide anion radical (O_2^-). Bradykinin (Bk), serotonin = 5-hydroxytryptamine (5-HT), arachidonic acid (AA), acetylcholine (Ach), transforming growth factor β ($TGF\beta_1$), angiotensin I (A I), big-endothelin (big-ET), angiotensin converting enzyme (ACE), endothelin converting enzyme (ECE), nitric oxide synthase (NOS), 3′5′-cyclic adenyl cyclase (cAMP), 3′5′-cyclic guanylate cyclase (cGMP).

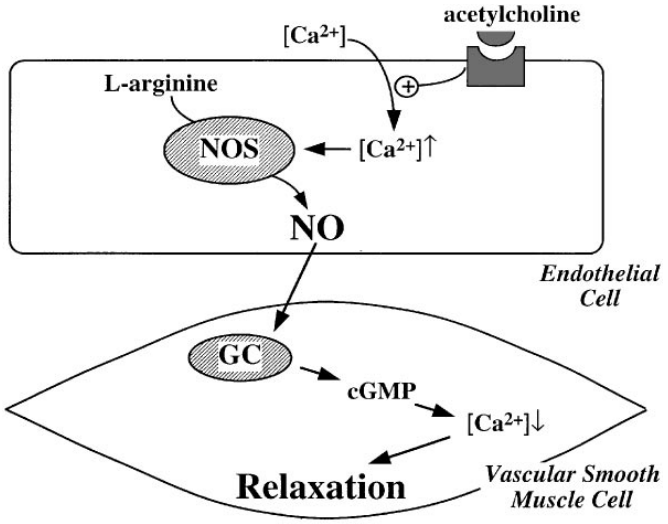

Fig. 2. Schematic representation of the nitric oxide synthase/guanylate cyclase pathway in a blood vessel wall. In endothelial cells nitric oxide (NO) is synthesised from L-arginine via the activation of a calcium (Ca^{2+})-dependent nitric oxide synthase (NOS). NO production can be inhibited by false L-arginine analogs, such as L-N^G-monomethyl arginine (L-NAME). In vascular smooth muscle cells, NO activates a soluble guanylate cyclase (sGC), which increases 3′5′-cyclic guanylate cyclase (cGMP) leading eventually to a relaxation. Receptor-operated agonists (R), such as acetylcholine (Ach) can stimulate the production of NO.

formation of cyclic guanosine 3′, 5′-monophosphate (cGMP) (Busse *et al.*, 1987; Radomski *et al.*, 1987a,b; Rapoport *et al.*, 1983; Haefliger *et al.*, 1994a,b). In these cells, the increase in intracellular cGMP concentration leads to a relaxation via a decrease in intracellular Ca^{2+} (most likely by increasing Ca^{2+} efflux and reuptake into intracellular stores) and dephosphorylation of myosin light chains (Lüscher and Vanhoutte, 1990) (Fig. 2).

The production of NO can be inhibited by synthetic analogs of L-arginine (L-N^G-monomethyl-arginine: L-NMMA; nitro-L-arginine methyl ester: L-NNA) (Palmer *et al.*, 1988a,b), whereas methyl- and dimethylarginines circulate in plasma as endogenous inhibitors of NO (Vallance *et al.*, 1992). Furthermore, hemoglobin and oxygen-derived free radicals inactivate NO (Gryglewski *et al.*, 1986; Moncada *et al.*, 1986; Rubanyi *et al.*, 1985; Rubanyi and Vanhoutte, 1986).

2.2. Prostacyclin

In addition to NO, endothelial cells produce prostacyclin (PGI_2, ecoprostenol). Prostacyclin is a major metabolite from arachidonic acid via the activation of the enzyme cyclooxygenase (Chesterman, 1988). Prostacyclin is a potent inhibitor of platelet aggregation (Moncada *et al.*, 1976; Moncada and Vane, 1978) as well as a vasodilatator (Lüscher and Vanhoutte, 1990; Moncada *et al.*, 1977). It activates adenylcyclase and increases the intracellular production of cyclic adenosine 3′,5′-monophosphate (cAMP) (Fig. 1). Hence, at sites where platelets and/or the coagulation cascade are activated, the endothelium releases vasodilators and platelet inhibitors, such as NO, prostacyclin, and tissue plasminogen activator, which provide local protection against vasospasm, ischemia, and thrombus formation (Mombouli and Vanhoutte, 1999).

2.3. Endothelium-derived hyperpolarizing factor

A putative hyperpolarizing factor (EDHF) (Feletou and Vanhoutte, 1988; Beny and Brunet, 1988) which increases the membrane potential of vascular smooth muscle cells is also formed by the endothelium (Fig. 1). EDHF could be the resultant of an electrotonic conduction of the endothelial cell hyperpolarization to the neighbouring smooth muscle cells (Chaytor *et al.*, 1998; Dora *et al.*, 1999; Yamamoto *et al.*, 1999). In endothelial cells exposed to shear stress a potassium-ion current across the cell membrane can be activated (Olesen *et al.*, 1988). This flow-activated hyperpolarization may be eletrotonically transfered to the smooth muscle cells. Hence, flow-induced release of endothelium-derived relaxing factors modulate vascular tone of conduit and resistance arteries (Griffith *et al.*, 1987, 1988a,b). In some vessels, EDHF could also be potassium ions released by the endothelial cells (Edwards *et al.*, 1998). Depending upon the tissue, potassium ion would hyperpolarize the smooth muscle cells either by gating inward rectified potassium channels or by activating the eletrogenic sodium-potassium ATPase (Na–K ATPase), of both (Knot *et al.*, 1996; Edwards *et al.*, 1998; Prior *et al.*, 1998). The third hypothesis concerning the identity of the EDHF is that it is a product of the cytochrome P450 2C, an epoxyeicosatrienoic acid (Fisslthaler *et al.*, 1999).

The physiological role of EDHF is uncertain, but it may contribute to endothelium-dependent relaxation particularly with certain agonists, such as bradykinin (Mombouli *et al.*, 1996). The release of EDRF from the endothelium can be mediated by both pertussis toxin-sensitive (α_2-adrenergic activation, serotonin, thrombin, aggregating platelets) and insensitive (adenosine diphosphate, bradykinin) G-proteins. In blood vessels from animals with regenerated endothelium, and/or atherosclerosis, there is a selective loss of the pertussis-toxin sensitive mechanism of EDRF-release which favors the occurrence of vasospasm, thrombosis and cellular growth (Mombouli and Vanhoutte, 1999; Feletou and Vanhoutte, 1999).

3. ENDOTHELIUM-DERIVED CONTRACTING FACTORS

3.1. Endothelin

Endothelial cells can produce the 21-amino-acid peptide, endothelin (Dzau, 1986; Gillespie *et al.*, 1986; Hickey *et al.*, 1985; Lüscher and Vanhoutte, 1990; Miller and Vanhoutte, 1983; Vanhoutte

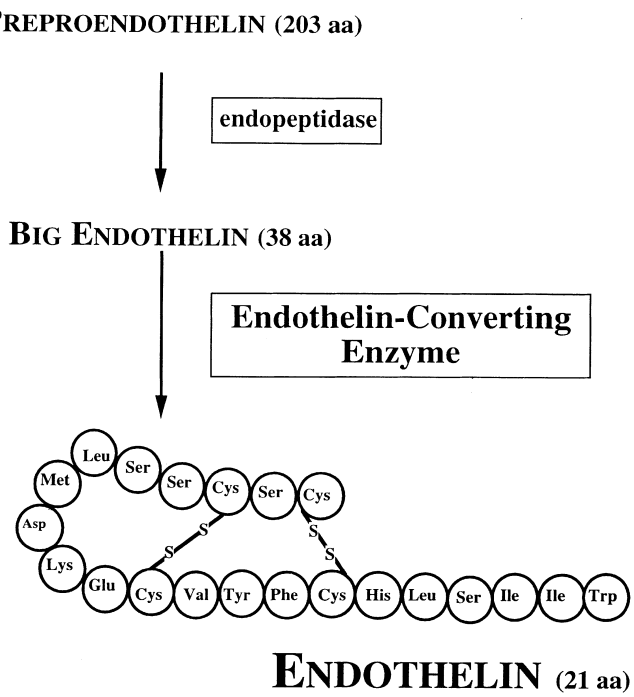

Fig. 3. Schematic representation of the formation of the 21-amino acid (aa) peptide endothelin after cleavage from a precursor.

et al., 1986; Yanigasawa *et al.*, 1988) (Fig. 1). Three isoforms of the peptide exist: endothelin-1, endothelin-2, and endothelin-3 (Masaki, 1989; Yanagisawa *et al.*, 1988a,b; Yanagisawa *et al.*, 1989), but endothelin-1 is the primary product of the endothelium (Inoue *et al.*, 1989). Each endothelin isoform is a product of separate genes that code for a precursor protein mRNA. Endothelin is generated from the precursors preproendothelin and big-endothelin (Fig. 3). The expression of mRNA and the release of the peptide are stimulated by thrombin, transforming growth factor β (TGF-β_1), interleukin-1 (IL-1), epinephrine, angiotensin II, arginine–vasopressin, calcium ionophore, and phorbol esther (Boulanger and Lüscher, 1990; Masaki, 1989; Yanagisawa *et al.*, 1988a,b, 1989).

Endothelin causes vasodilatation at lower concentrations and marked and sustained contractions at high concentrations (Kiowski *et al.,* 1991; Lippton *et al.*, 1989; Lüscher and Vanhoutte, 1990; Seo *et al.,* 1994; Warner *et al.*, 1989a,b; Wright and Fozard, 1988). The dilatator response to endothelin involves activation of endothelial receptors (ET_B-type) linked to NO and/or prostacyclin release by endothelial cells (de Nucci *et al.*, 1988; Dohi and Lüscher, 1991; Rae *et al.*, 1989; Sakurai *et al.*, 1990; Vane, 1990; Warner *et al.*, 1989a,b). The constrictive response involves the activation by endothelin of specific membrane receptors (i.e. ET_A-, and ET_B-receptors) on smooth muscle cells (Arai *et al.*, 1990; Sakurai *et al.,* 1990; Vane, 1990).

In certain blood vessels, such as in porcine coronary artery, endothelin receptors on vascular smooth muscle cells are linked to voltage operated Ca^{2+} channels via a G_i proteins (Goto *et al.*, 1989). This might explain why calcium antagonists reduce endothelin-induced vasocontriction in these vessels and are similary effective in human coronary arteries (Godfraind *et al.*, 1989). In other vessels, such as the human internal mammary artery, the contractile effects induced by endothelin-1 are mediated by a cascade activation of phospholipase C leading to the formation of diacylglycerol and inositol triphosphate (Hirata *et al.*, 1988; Resink *et al.*, 1989a). In turn, Ca^{2+} is released from the sarcoplasmic reticulum leading to an increase in intracellular Ca^{2+} concentrations and the induction of long-lasting contractions (Wallnöfer *et al.*, 1989; Yanagisawa *et al.*, 1988a,b). At concentrations where endothelin-1 exerts no direct contractile effect, it potentiates contractions to norepinephrine and serotonin (Tabuchi *et al.*, 1989; Yang *et al.*, 1990a,b). The

potentiating effects of endothelin are due to an increased Ca^{2+} sensitivity of vascular smooth muscle cells under the conditions described above and therefore can be prevented by pretreatment with calcium antagonists of the dihydropyridine type (Yang *et al.*, 1990a,b).

Under normal conditions the circulating endothelin levels are low (1.5 pg/ml), suggesting that it primarily acts as a local regulatory factor (Ando *et al.*, 1989; Hartter and Woloszczuk, 1989; Koyama *et al.*, 1989; Suzuki *et al.*, 1989); the peptide is cleared from the circulation by the lungs, the kidneys, and the liver (Aenggard *et al.*, 1989; Neuser *et al.*, 1989; Shiba *et al.*, 1989).

3.2. Cyclooxygenase products

The endothelial cyclooxygenase pathway also produces several contracting factors, such as thromboxane A_2, prostaglandin H_2 or superoxide anions, which are mainly produced in the cerebral circulation and in the veins, under physiological conditions (De Mey and Vanhoutte, 1985; Kim *et al.*, 1988a,b; Lüscher and Vanhoutte, 1990; Vanhoutte *et al.*, 1986) (Fig. 1).

4. NITRIC OXIDE, ENDOTHELIN AND THE OPHTHALMIC CIRCULATION

4.1. Basal release of nitric oxide

In the ophthalmic vascular bed, there is a constant basal release of NO, which maintains the ophthalmic circulation in a constant state of vasodilation. Indeed, in vitro, in isolated porcine or human vessels and in the porcine perfused eye, as well as, in vivo, in the miniature pig retinal circulation or in the cat optic nerve head, inhibitors of NO formation evoked vasoconstriction and decrease in blood flow (Haefliger *et al.*, 1993, 1992; Meyer *et al.*, 1993; Donati *et al.*, 1995; Buerk *et al.*, 1996).

4.2. Stimulated release of nitric oxide

In human as well as in porcine ophthalmic and ciliary arteries, and also in bovine retinal arteries, different receptor mediated-agonists, such as bradykinin, acetylcholine, and histamine, evoke endothelium-dependent relaxations (Haefliger *et al.*, 1993; Schmetterer *et al.*, 1997b; Haefliger *et al.*, 1992; Benedito *et al.*, 1991; Hoste and Andries, 1991; Zhu *et al.*, 1997). The responses to these agonists are reduced by inhibitors of NO formation, such as L-NMMA or L-NAME, demonstrating that NO is the main mediator involved in these relaxations (Fig. 4). In vitro, in porcine extraocular vessels, the sensitivity to bradykinin increases as the diameter of the vessels gets smaller, suggesting that endothelium-dependent relaxation is particularly important in small vessels and most likely also in the microcirculation (Haefliger *et al.*, 1993). Furthermore, in these vessels a duality exists, for a given agonist, when it activates endothelial or vascular smooth muscle cells. For example, as mentioned above, histamine by interacting with an H_1-histaminergic receptor evokes an endothelium-dependent relaxation mediated by NO, while, when histamine activates the same H_1-histaminergic receptor on smooth muscle cells, it induces a contraction (Haefliger *et al.*, 1992). This explains why, in the presence of an endothelial dysfunction, a given agonist, can induce in a vessel a contraction instead of dilation (Haefliger *et al.*, 1994a,b).

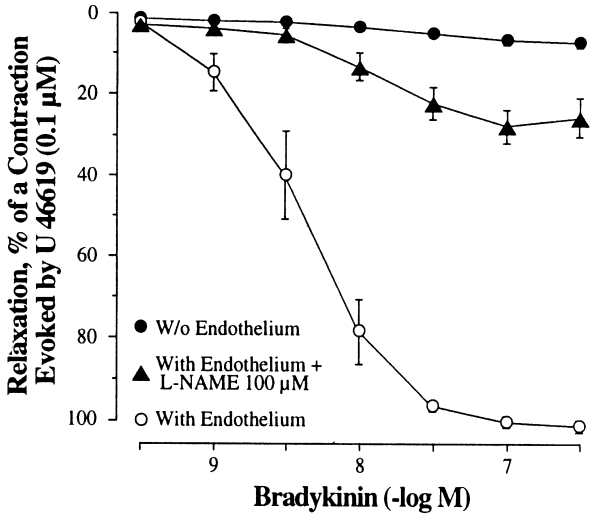

Fig. 4. Endothelium-dependent relaxation evoked by bradykinin in an isolated procine ciliary artery. In a concentration-dependent manner, bradykinin induced a relaxation of the vessel. This relaxation markedly inhibited in presence of the inhibitor of NO formation L-NAME and abolished in vessels with a non-functional (W/o) endothelium.

4.3. Endothelin-induced contractions

In isolated ophthalmic and ciliary arteries or in bovine retinal arteries, endothelin-1 evokes potent contractions (Haefliger *et al.*, 1993, 1992; Nyborg *et al.*, 1991, White *et al.*, 1996, Kulkarni *et al.*, 1994) (Fig. 5). In the perfused porcine eye, endothelin-1 and endothelin-3 increase ophthalmic flow at very low dosages and severely reduce it at higher doses for prolonged periods of time. This dual action of endothelin is best explained by the activation of an endothelilal ET_B-receptor that is already activated at very low concentrations of endothelin-1 and which evokes the endothelial release of prostacyclin, while at higher concentrations of endothelin-1, an ET_A-receptor is predominantly activated on vascular smooth muscle, evoking potent contractions (Meyer *et al.*, 1993). This observation again underlines the importance of the local endothelial-dependent regulation in the ophthalmic circulation (Haefliger *et al.*, 1994a,b). Indeed, in human subjects, after systemic injection of endothelin-1 (at doses that did not affect systemic circulation), a reduction in the pulsatile blood flow in the choroid and the optic disc could be observed (Schmetterer *et al.*, 1997a).

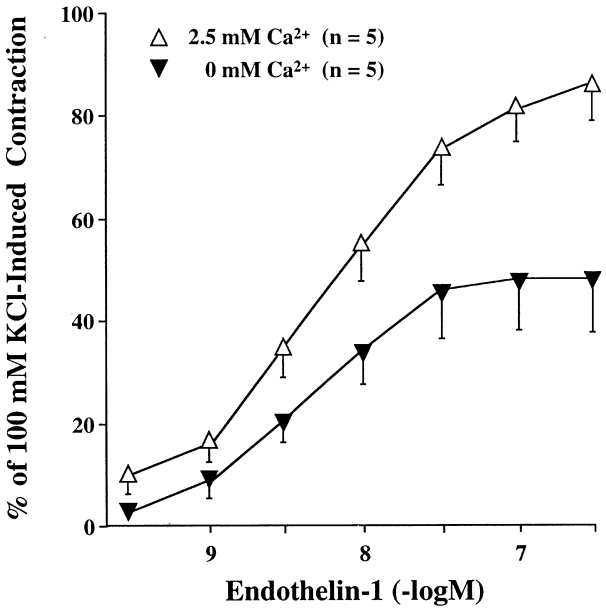

Fig. 5. Contraction evoked by endothelin-1 in an isolated porcine ciliary artery. Endothelin-1 induced in a concentration-dependent manner contraction that where only partially inhibited in the absence of extracellular calcium.

5. ENDOTHELIAL DYSFUNCTION IN PATHOLOGICAL CONDITIONS

5.1. Hypercholesterolemia and arteriosclerosis

Endothelial cells represent a well-accessible target for mechanical forces, for noxious substances, and for various cardiovascular risk factors. In isolated vessels, low-density lipoproteins (LDLs), but not high-density lipoproteins (HDLs), inhibit endothelium-dependent relaxation to acetylcholine, serotonin, and aggregating platelets (Andrews *et al.*, 1987; Kugiyama *et al.*, 1990; Tanner *et al.*, 1991). In porcine coronary artery, endothelium-dependent relaxation is moderately reduced in hyperlipidemia and markedly reduced in arteriosclerosis (Cohen *et al.*, 1988; Shimokawa *et al.*, 1987; Shimokawa and Vanhoutte, 1988, 1989). Hyperlipemia also reduces endothelium-dependent relaxation in the microcirculation (Bossaller *et al.*, 1987; Drexler *et al.*, 1989; Förstermann *et al.*, 1988; Ludmer *et al.*, 1986; Selke *et al.*, 1990; Shimokawa and Vanhoutte, 1989, Shimokawa, 1999). Furthermore, oxidized-LDL induces mRNA expression and release of endothelin from human and porcine endothelium (Boulanger *et al.*, 1992). In the isolated porcine ciliary artery, exposure to oxidized-LDL evokes endothelium-dependent contractions that is inhibited by the ET_A-receptor antagonist BQ123 or the inhibitor of protein synthesis cycloexamide, indicating that Ox-LDL affects endothelium-dependent responses through the activation of ET_A-endothelin receptor (Zhu *et al.*, 1999) (Fig. 6). It also has to be noted that, in humans, circulating and vascular endothelin is increased in arteriosclerosis (Lerman *et al.*, 1991, Shimokawa, 1999).

5.2. Hypertension

In hypertension, the balance in the endothelial production of vasodilating and vasoconstricting mediators is altered, resulting in an apparent decrease in endothelium-dependent relaxations (Lüscher, 1990, Lüscher and Vanhoutte, 1986a,b; Lüscher *et al.*, 1987; Tesfamariam and Halpern, 1988; Boulanger, 1999). In hypertensive patients and in animal models of hypertension,

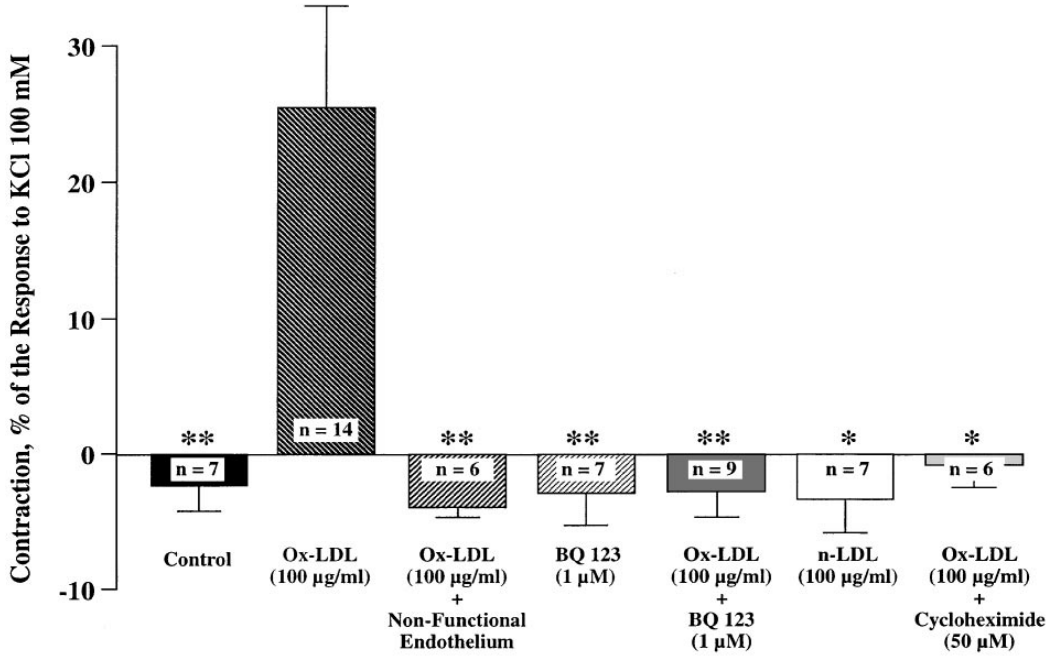

Fig. 6. Bar graph showing endothelium-dependent contractions elicited by oxidized low-density lipoprotein (Ox-LDL) in isolated quiescent porcine ciliary arteries. Incubation with Ox-LDL evoked changes in vascular tone which were significantly different from those observed in vessels incubated with either Krebs–Ringer's solution (control), native low-density lipoprotein (n-LDL), the ET_A-endothelin-receptor antagonist BQ 123, Ox-LDL co-administered with BQ 123, Ox-LDL co-administered with the protein synthesis inhibitor, cycloheximide, or Ox-LDL incubated in vessels with a non-functional endothelium (intentionally and mechanical damaged). One-way Kruskal–Wallis ($p = 0.0003$) followed by Mann–Whitney test with Bonferoni correction: *: $p < 0.05$; **: $p < 0.01$ (Zhu *et al.*, 1999).

endothelium-dependent relaxations are impaired (Lind *et al.*, 1999; Diedrich *et al.*, 1990). In Dahl-salt-sensitive rats, the decrease in endothelium-dependent relaxations is associated with impaired constitutive NOS activity as well as increased endothelin-1 plasma levels (d'Uscio *et al.*, 1997; Barton *et al.*, 1998; Barton *et al.*, 2000). In other animal models of hypertension (such as spontaneous hypertension), the contribution of the L-arginine NO pathway to endothelium-dependent responses has been reported to be impaired (Nava *et al.*, 1998). In large arteries from SHR, endothelium-dependent relaxations are impaired mainly because of the concomitant augmented release of endoperoxides activating thromboxane-endoperoxide receptors (Noll *et al.*, 1997). The endothelial dysfunction observed in hypertension is likely to be a consequence of high blood pressure, but it could facilitate the main tenance of elevated peripheral resistance at a later stage in the disease and favour the occurrence of complications, such as atherosclerosis (Noll *et al.*, 1997).

5.3. Diabetes

Diabetes mellitus is associated with accelerated atherosclerosis and an increased prevalence of cardiovascular disease (Jarrett and Keen, 1979; Kannel and McGee, 1979). Although the link between diabetes and cardiovascular disease is not fully understood, loss of the modulatory role of the endothelium could be implicated in the pathogenesis of diabetic vascular complications. There is substantial evidence that vasodilatation mediated by endothelium-derived nitric oxide is impaired in animal models of diabetes (Meraji *et al.*, 1987; Takiguchi *et al.*, 1988; Tesfamariam *et al.*, 1989) and in patients with insulin-dependent and non-insulin-dependent diabetes mellitus (Johnstone *et al.*, 1993; Calver *et al.*, 1992; Kawagishi *et al.*, 1999). It has been suggested that the pathogenesis of diabetic vascular disease may involve a reduced bioavailability of endothelium-derived NO. Although mechanisms by which diabetes contributes to endothelial dysfunction are currently unknown, it is likely that hyperglycemia,

the hallmark of diabetes mellitus, may initiate this abnormality. Hyperglycemia-induced endothelial dysfunction may result from decreased production of NO, inactivation of NO by oxygen-derived free radicals, and/or increased production of endothelium-derived contracting factors, which oppose the protective activity of NO (Cosentino *et al*., 1997; Cosentino and Lüscher, 1998; Stehouwer *et al*., 1997).

5.4. Vasospastic syndromes

In variant angina and Raynaud's disease, impaired endothelium-dependent responses are likely to be involved (Kaski *et al*., 1986; Lüscher 1991; Okumura *et al*., 1988a,b; Yasue *et al*., 1986); indeed, the local levels of endothelin are increased in both conditions (Lüscher, 1991). In experimental subarachnoidal hemorrhage of the dog, endothelium-dependent relaxations are reduced, while endothelium-dependent contractions are preserved (Kim *et al*., 1988a,b, 1989). Furthermore, cerebrospinal endothelin levels are increased and endothelin-antagonists increase vascular diameter in spastic segments. This imbalance in vascular reactivity may be an important component in the pathogenesis of cerebral vasospasm after subarachnoidal hemorrhage (Sobey and Faraci, 1998; Zimmermann and Seifert, 1998).

5.5. Ischemia and reperfusion

In the coronary artery of different species, endothelium-dependent relaxation to most agonists is attenuated after ischemia and reperfusion (Ku, 1982). The impaired endothelium-dependent relaxation to aggregating platelets and to platelet-derived substances persists, whereas, the relaxation to acetylcholine recovers (Pearson *et al*., 1990). In the heart, the injury is associated with alterations in the redistribution of blood flow (Pelc *et al*., 1990). The ischemia/reperfusion injury to the endothelium appears to be mediated by oxygen-derived free radicals (Baker *et al*., 1988; Lamb *et al*., 1987; Zweier *et al*., 1987, 1988). Indeed, superoxide anions inactivate NO and lead to toxic products that can activate vascular smooth muscle cells (Gryglewski *et al*., 1986; Katusic and Vanhoutte, 1989; Liu, 1999; Rubanyi and Vanhoutte, 1986; Vanhoutte and Rubanyi, 1988). This may explain why experimentally superoxide dismutase can prevent the endothelial dysfunction after ischemia and reperfusion (Mehta *et al*., 1988; Liu, 1999).

Furthermore, the inflammatory mediators released as a consequence of reperfusion also appear to activate endothelial cells in remote organs that are not exposed to the initial ischaemic insult. This distant response to ischaemia and reperfusion can result in leukocyte-dependent microvascular injury that is characteristic of the multiple organ dysfunction syndrome. Adaptational responses to ischaemia and reperfusion injury have been demonstrated that allow for protection of briefly ischaemic tissues against the harmful effects of subsequent, prolonged ischaemia, a phenomenon called ischaemic preconditioning. There are two temporally and mechanistically distinct types of protection afforded by this adaptational response, i.e. acute and delayed preconditioning. The factors (e.g. protein kinase C activation) that initiate the acute and delayed preconditioning responses appear to be similar; however, the protective effects of acute preconditioning are protein-synthesis-independent, while the effects of delayed preconditioning require protein synthesis (Carden and Granger, 2000).

6. THERAPEUTICAL CONSIDERATIONS

A certain number of drugs have the ability to modify endothelium-dependent responses.

6.1. Beta-adrenenoreceptor antagonists

Certain β-adrenergic blockers can affect endothelium-dependent responses. In rat aorta, non-selective β-adrenergic blockers, such as propanolol, cause relaxations that are reduced after endothelium removal (Mostaghim *et al*., 1986). In the coronary and femoral artery of the dog the non-selective β-adrenergic antagonist, carteolol, does not cause endothelium-dependent relaxation but selectively augments the abluminal release of NO to α_2-adrenergic activation (Janczewski *et al*., 1988). It also causes the intraluminal release of vasodilator prostaglandins. This effect of carteolol

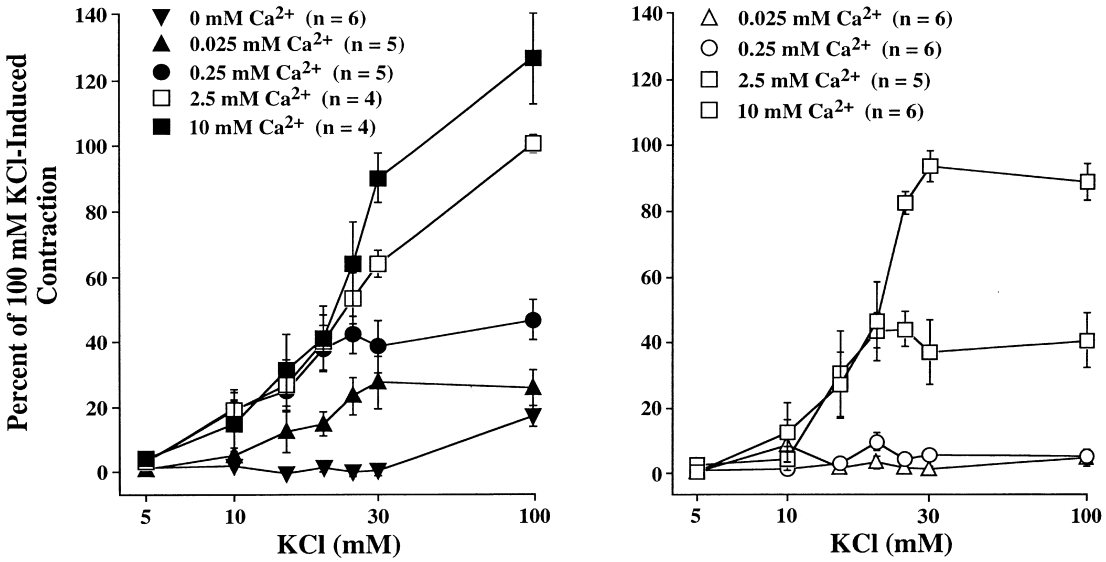

Fig. 7. In isolated porcine ciliary artery, potassium chloride induces contractions that are mediated by an influx of extracellular calcium (Ca^{2+}). Indeed, in a concentration-dependent manner, contractions evoked by KCl were abolished when decreasing extracellular calcium concentrations (left panel). In the presence of a β-blocker, such as carteolol (1 mM), despite the presence of calcium (0.25, 0.025 mM), the contractions were abolished (right panel).

is probably different from the direct endothelium-independent relaxing properties of the drug apparented to a Ca^{2+}-antagonists like effect (Brogiolo *et al.*, 2000) (Fig. 7).

6.2. Angiotensin-converting-enzyme inhibitors

Angiotensin-converting-enzyme (ACE) inhibitors do not evoke endothelium-dependent relaxations (Vanhoutte, 1989) but augment the effects of bradykinin. Angiotensin-converting enzyme, which is located at the endothelial cell membrane, is identical with kinase II, which inactivates bradykinin (Dzau, 1986; Lindsey *et al.*, 1987). This explains why angiotensin-converting-enzyme inhibitors augment endothelium-dependent relaxation to bradykinin (Vanhoutte, 1989). Even subthreshold concentrations of bradykinin can cause endothelium-dependent relaxations under these conditions (Vidal and Vanhoutte, 1988; Ruschitzka *et al.*, 1999).

In ciliary arteries, precontracted with serotonin, bradykinin causes concentration-dependent relaxations. Pre-incubation of the arteries with ACE-inhibitors, enalaprilat or benazepril, for 60 minutes significantly enhances the relaxation to bradykinin in these vessels. Similar observation could be made in the entire perfused eye. ACE inhibitors augment endothelium-dependent relaxation of vasodilatation to bradykinin via B_2-receptors linked to the formation of NO (Meyer *et al.*, 1995) (Fig. 8).

6.3. AT_1-receptor antagonists

Angiotensin II evokes in a concentration-dependent manner contraction of isolated ciliary arteries. Two angiotensin receptors have been cloned, i.e. the AT_1 and the AT_2 receptors. In porcine ciliary arteries, preincubation with valsartan, an AT_1-receptor antagonist, reduced the vasoconstrictor effect of angiotensin-II in a concentration-dependent manner. In contrast, the AT_2-receptor ligand, CGP 42112, did not reduce the response to angiotensin-II. Thus, in porcine ciliary artery, the AT_1 angiotensin receptor is exclusively responsible for the vasoconstriction evoked by angiotensin-II on vascular smooth muscle cells (Meyer *et al.*, 1995) (Fig. 9).

6.4. Ca^{2+}-channel antagonists

In porcine ciliary arteries pre-incubated with Ca^{2+}-channels antagonists, such as lacidipine or nifedipine, the maximal contraction but not the sensitivity to endothelin-1 was reduced (Meyer

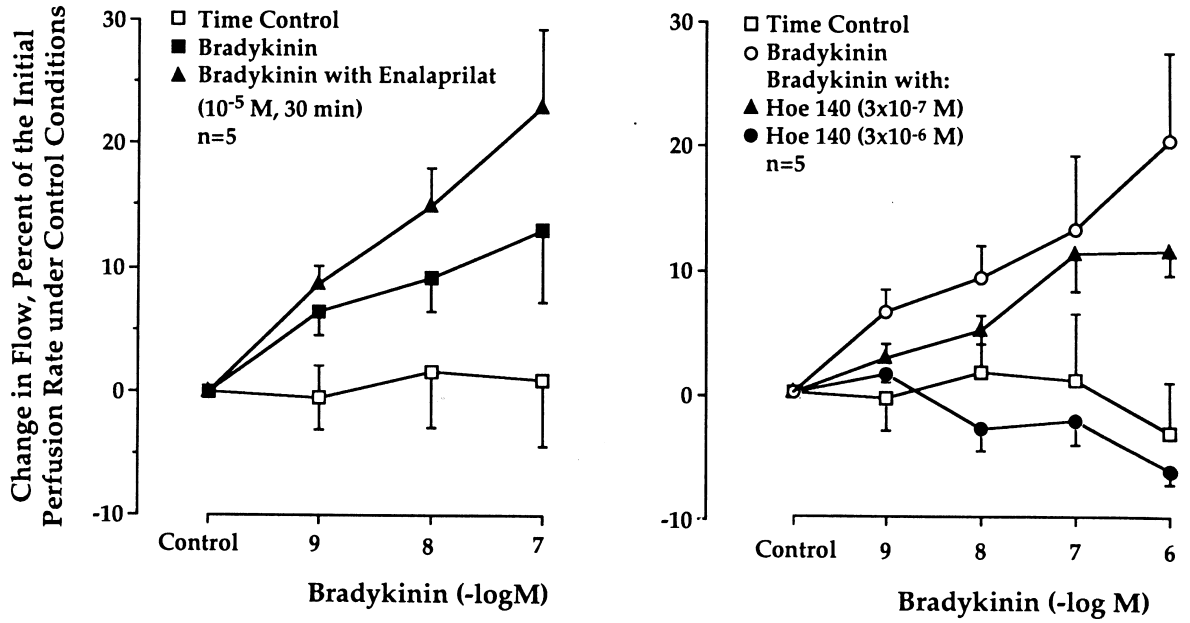

Fig. 8. Effect of the angiotensin converting enzyme (ACE) inhibitor on the endothelium-dependent vasodilatation in the perfused porcine eye. Bradykinin caused concentration-dependent increase in the ophthalmic flow that was increased in the presence of the ACE inhibitor (left panel) and decreased in the presence of increasing concentrations of the bradykinin antagonist Hoe 140 (right panel) (Meyer *et al.*, 1995a).

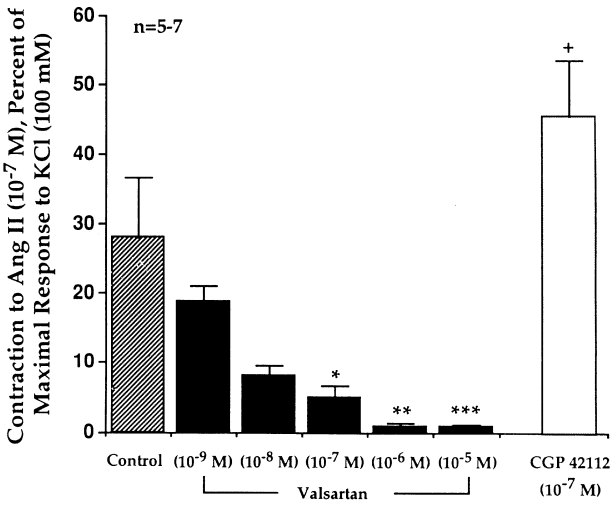

Fig. 9. Inhibitory effect of the AT_1-receptor antagonist valsartan on the contractions evoked by angiotensin II in porcine isolated ciliary arteries. In contrast, the AT_2-receptor ligand, CGP 42112, did not reduce the response to angiotensin II (Meyer *et al.*, 1995a).

et al., 1995). In contrast, in bovine retinal arteries pre-contracted with endothelin-1, calcium antagonists were very efficient in relaxing the vessels (Nyborg *et al.*, 1991). Because Ca^{2+}-antagonists, such as lacidipine and verapamil, block voltage-operated channels, these observation suggest that the development of contractions to endothelin-1 involves primarily the release of Ca^{2+} from intracellular storage, while the maintenance of the contraction evoked by endothelin-1 is essentially dependent on the activity of membrane voltage-operated Ca^{2+}-channels reperfusion (Yang *et al.*, 1990a,b). Similar observations could be reached with magnesium, considered to be a "physiological" a Ca^{2+}-antagonist, in isolated ciliary arteries (Dettmann *et al.*, 1998). Interestingly enough, it has also been reported that some β-blockers, at very high concentrations, also exhibit some kind of Ca^{2+}-antagonist-like properties (Brogiololo *et al.*, 2000; Hester *et al.*, 1994) (Fig. 7).

6.5. Platelet-inhibitors

Various platelet inhibitors, such as aspirin, prevent the formation of thromboxane A_2 by platelets and thereby inhibit platelet-vessel wall interaction (Moncada and Vane, 1978; Vane, 1971). In addition, the drugs prevent vascular prostacyclin production, which may be less favorable. This property is not shared by newer selective compounds inhibiting thromboxane A_2 synthesis or receptor (Moncada and Vane, 1978; Bochner and Lloyd, 1986). In the porcine ciliary artery,

dypiridamole, evoked an endothelium-dependent relaxation, mediated in part by NO and prostacyclin (Meyer *et al.*, 1996).

6.6. Anti-serotonergic drugs

Serotonin evokes contractions via $5HT_2$-serotonergic receptors on smooth muscle, while endothelial receptors ($5HT_1$-subtype) are linked to the release of NO. Thus, $5HT_2$-serotonergic blockers (e.g. ketanserin, naftidrofuryl) prevent the vasoconstrictor effect of platelet-derived serotonin, and often endothelium-dependent relaxations to the monoamine or aggregating platelets (Prevention of Atherosclerotic Complications with Ketanserin Trial Group, 1989; Yang *et al.*, 1991). In isolated porcine ophthalmic and ciliary artery this mechanism could not be demonstrated (Haefliger *et al.*, 1993).

7. CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, through the secretion of vasoactive substances, endothelial cells can profoundly modulate local vascular tone in response to several local hormones and platelet products. Furthermore, under pathological conditions, such as in hypertension, diabetes, arteriosclerosis, ischemia, or vasospasm, endothelial function appears to be impaired. In the ophthalmic circulation, the endothelial regulation plays a major role in the local modulation of blood flow. Furthermore, due to its strategic location within the vessels, systemic cardiovascular drugs can easily reach the endothelial regulatory system. Some modern therapeutic strategies will tend to propose to treat some ocular vascular disorders or ophthalmic complications of vascular systemic diseases by restoring or stimulating the endothelial function. Such an approach has for example already been successfully used in hypertension where angiotensin-converting enzyme inhibitors are used to diminish the inactivation of bradykinin and thus leading to an increase in the release of NO. In other words, the recognition that the vascular endothelium plays a role in both the physiology and pathophysiology of vascular regulation opens new potential therapeutic approaches for the care of our patients.

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