CURRENT RESEARCH

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The Vascular Endothelium as a Regulator of the Ocular Circulation: A New Concept in Ophthalmology?

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Abstract. The endothelium influences local vascular tone by releasing endothelium-derived relaxing factors such as nitric oxide, prostacyclin and a putative hyperpolarizing factor. In isolated ophthalmic arteries and the perfused eye, all endothelial factors importantly contribute to vascular regulation. In larger ophthalmic vessels, this is due to their effects on vascular smooth muscle cells; in smaller vessels, pericytes can be influenced as well. Contracting factors formed include peptide endothelin-1 and cyclooxygenase products, such as thromboxane A_2 and prostaglandin H_2. In the peripheral circulation endothelial dysfunction occurs under pathological conditions, both in conduit arteries and the microcirculation. An imbalance of endothelium-derived relaxing and contracting factors could be important for the development of vascular ophthalmic complications like hypertension, diabetes, arteriolosclerosis and retinal ischemia. Endothelial dysfunction may also contribute to vasospastic events in retinal migraine and some forms of low tension glaucoma associated with Raynaud phenomenon and migraine. **(Surv Ophthalmol 39:**123–132, 1994)

Key words. blood flow regulation • ciliary artery • endothelium-derived contracting factor (EDCF) • endothelium-derived relaxing factor (EDRF) • ocular circulation • ophthalmic artery • retinal artery

Disturbances of ocular blood flow are involved in many ophthalmic diseases and therefore are of utmost clinical relevance. The eye is one of the best-perfused organs in the body.[1] In humans and in many experimental animals, the eye has two separate systems of blood vessels, which anatomically and physiologically differ: the retinal vessels, which supply part of the retina, and the uveal or ciliary blood vessels, which supply the rest of the eye. There is autonomic innervation of the extraocular vessels as well as of choroidal vessels. In primates, neural innervation of the

central artery occurs only as far as to the lamina cribrosa. There is no neural innervation of vessels of the retina and optic nerve head, although alpha-, beta-adrenergic and cholinergic receptors are present.[1]

As in other tissues, the factors that determine local blood flow through the eye are 1) perfusion pressure, 2) tone of resistance vessels, and 3) vascular blood viscosity. Tone depends on the contractile state of smooth muscle cells and pericytes, which is regulated by neurotransmitters, circulating hormones, myogenic and metabolic factors (pO_2, phosphate, potassium), as well as by endothelium-derived factors.[71]

Within the cardiovascular system, the endothelium lies in a stragegic anatomical position between blood components and smooth muscle cells. It long had been known that the endothelium regulates permeability, activates and inactivates hormones,[71] affects coagulation, platelet function[9,96,97] and fibrinolysis, as well as vascular tone (Fig. 1).[71] Indeed, in the last decade, numerous factors released by endothelial cells have been characterized, such as nitric oxide and prostacyclin, which inhibit vascular contraction and proliferation, as well as platelet function. Another factor, endothelin-1, causes contraction and facilitates smooth muscle cell proliferation. Finally, endothelial cells are a source of growth factors, such as platelet-derived growth factor, basic fibroblast growth factor and transforming growth factor beta-1, and growth inhibitors such as heparin-like substances.

I. Local Mediators of Vasoactivity

A. L-ARGININE/NITRIC OXIDE PATHWAY

Nitric oxide is a powerful vasodilator and inhibitor of platelet function.[9,29,30,90,96,97] Nitric oxide is formed from L-arginine by constitutive nitric oxide synthase.[8,89] Nitric oxide synthase is also found in nerves and platelets and an inducible form (by endotoxin, tumor necrosis factor and interleukin-1) exists in macrophages, neutrophils and smooth muscle cells.[122] Nitric oxide acts as the neurotransmitter of nonadrenergic, noncholinergic nerves, which are physiologically important regulators of blood flow to the corpora cavernosa.[19,43]

In endothelial cells, the formation of nitric oxide is stimulated in response to products released from platelets (i.e., ATP/ADP, serotonin, thrombin),[11–14,17,27,56,69,71,126] hormones and autacoids (acetylcholine, bradykinin histamine, noradrenaline, substance P and vasopressin) (Fig. 1).[11,30,46,104] Shear stress (i.e., the viscous drag exerted by the circulating blood to which endothelial cells are exposed) activates potassium current across the endothelial cell membrane[15,24,59,94,102]; this, in turn, causes hyperpolarization, which triggers the release of nitric oxide and prostacyclin and mediates flow-dependent vasodilation.

In vascular smooth muscle and platelets, nitric oxide activates soluble guanylyl cyclase which leads to the formation of cyclic 3′,5′-guanosine monophosphate (cGMP) and, in turn, to relaxation via a decrease in intracellular Ca^{2+} and dephophorylation of myosine light chains (Fig. 1).[6,9,98] Nitric oxide production can be inhibited by analogues of L-arginine (L-N^G-monomethylarginine; L-NMMA; nitro-L-arginine methyl ester: L-NAME).[23,90,101,111] Circulating methyl- and dimethylarginines in plasma act as endogenous inhibitors of NO.[113] Furthermore, hemoglobin and oxygen-derived free radicals inactivate nitric oxide.[29,30,103]

B. NON-NITRIC OXIDE RELAXING FACTORS

In addition to nitric oxide endothelial cells produce prostacyclin from arachidonic acid (via cyclooxygenase)[81]; it acts as a vasodilatator and inhibitor of platelet function, but through stimulation 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, which provide local protection against vasospasm, ischemia and thrombus formation.

In addition, an endothelium-derived hyperpolarizing factor (EDHF)[26,71,101,115] has been proposed, based on the fact that endothelial cells are able to increase the membrane potential of vascular smooth muscle via activation of ATP-dependent potassium channels or possibly sodium-potassium ATPase (Fig. 1). Its chemical nature remains elusive. EDHF contributes to endothelium-dependent relaxation particularly with certain agonists such as bradykinin.[101]

C. ENDOTHELIN

Endothelial cells produce the 21 aminoacid peptide endothelin,[7,44,71,72,74,124,125] of which three isoforms exist: endothelin-1, endothelin-2 and endothelin-3 (Fig. 1). Endothelin-1 is the only isoform produced by the endothelium; it is generated from preproendothelin and proendothelin and its production is stimulated by thrombin, transforming growth factor β, interleukin-1, an-

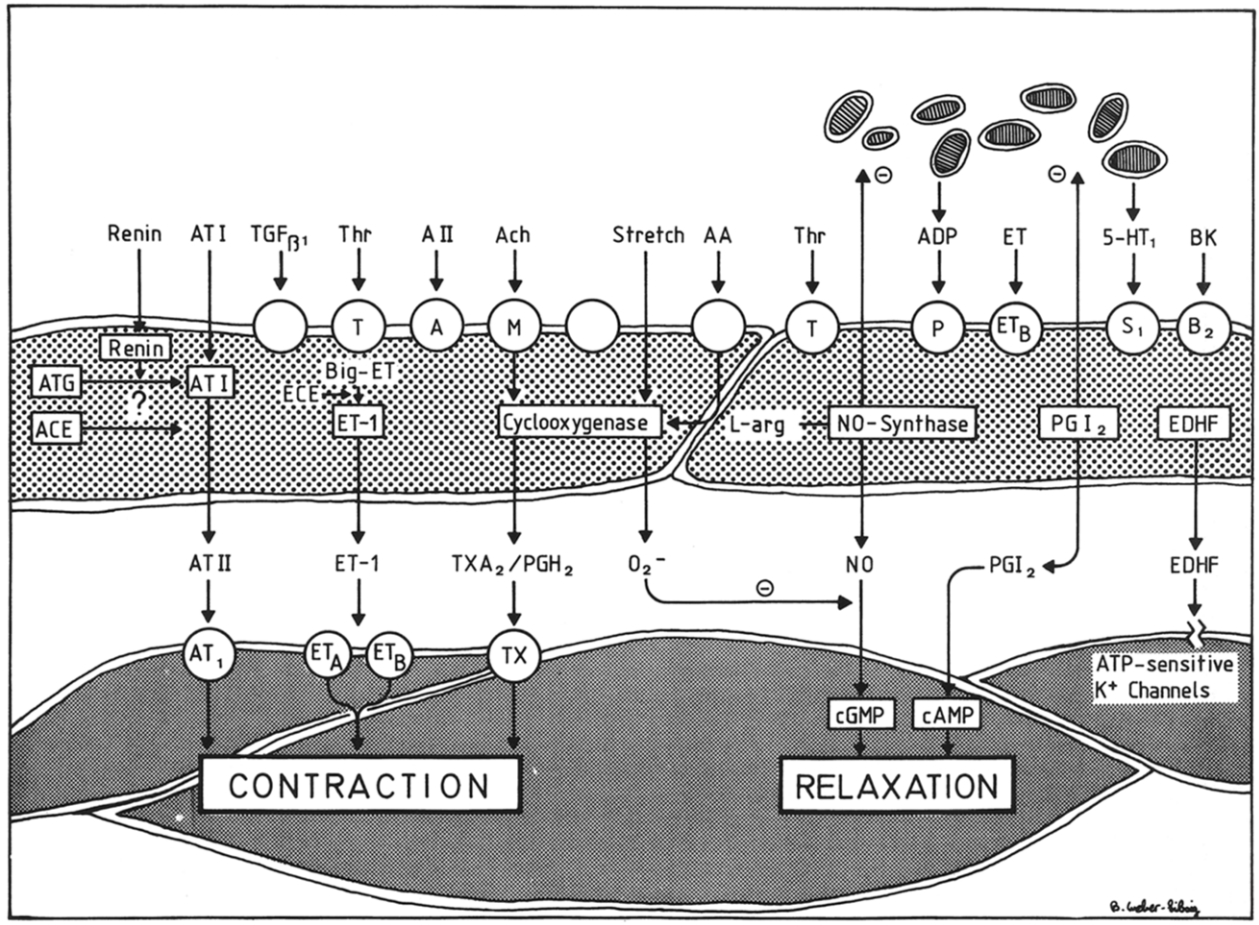

Fig. 1. Schematic representation of endothelium-derived vasoactive substances. A = angiotensin receptor; AA = arachidonic acid; ACE = angiotensin converting enzyme; Ach = acetylcholine; ADP = adenosine diphosphate; AT I/II = angiotensin I/II; ATG = angiotensinogen; cAMP/cGMP = cyclic adenosine/guanosine monophosphate; EDHF = endothelium-derived hyperpolarizing factor; ET_A/ET_B = endothelin receptor on smooth muscle cell/on endothelial cell; M = muscarinic receptor; NO = nitric oxide; O_2 = superoxide radicals; P = purinergic receptor; PGH_2 = prostaglandin H_2; PGI_2 = prostacyclin; T = thrombin receptor; TXA_2 = thromboxane A_2; S = serotonin receptor; 5-HT = serotonin.

giotensin II, arginine-vasopressin and epinephrine.[72,74] Endothelin production is inhibited by nitric oxide, prostacyclin and a putative inhibitory factor produced by vascular smooth muscle cells.

In vascular smooth muscle, endothelin binds to ET_A-, but also ET_B-receptors, linked to phospholipase C[66,68]; the formation of inositol triphosphate and diacylglycerol[99] increases intracellular Ca^{2+} [118] and evokes long-lasting contractions. In certain vessels, endothelin activates voltage-operated Ca^{2+}-channels via its receptor and a G_i-protein. At concentrations at which endothelin-1 exerts no direct contractile effect, it potentiates contractions to norepinephrine and serotonin.[110,125] In vivo, endothelin causes a transient vasodilation which precedes its pressor effect.[124] The dilatator response to endothelin involves activation of endothelial ET_B-receptors linked to nitric oxide and prostacyclin release.[18,22,100,121]

Under normal conditions the circulating endothelin levels are very low,[109] suggesting that it primarily acts as a local regulatory factor.[72,74] Indeed, two-thirds of the endothelin formed by endothelial cells is released abluminally towards vascular smooth muscle.

D. CYCLOOXYGENASE-DERIVED CONTRACTING FACTORS

The endothelial cyclooxygenase pathway produces several contracting factors like thromboxane A_2, prostaglandin H_2 or superoxide anions, particularly in the cerebral circulation and in veins (Fig. 1).[47–50,80] Prostaglandin H2 and thromboxane A2 cause contraction and activate platelets. Oxygen-derived free radicals can cause direct contraction, and they inactivate nitric oxide.

II. Mediators of Vascular Tone in the Ophthalmic Circulation

Although endothelial regulatory systems are expressed in the entire cardiovascular system, a great heterogeneity exists from one vascular bed to another, between species, between agonists stimulating endothelium-derived factors and with respect to their relative potency.[71] Certain agonists only cause endothelium-dependent responses in one vascular bed, but not in another or cause endothelium-dependent contractions in the cerebral circulation and endothelium-dependent relaxations in other organs.[17,46] Endothelial responses are more pronounced in arteries than in veins and with decreasing vascular diameter in the same vascular bed.[17,34,71,88]

A. BASAL RELEASE OF NITRIC OXIDE

In isolated porcine and human ophthalmic and bovine retinal arteries, inhibitors of nitric oxide formation induce endothelium-dependent contractions.[33,34,128] In the perfused porcine eye, L-NAME reduces ophthalmic flow by 40%.[78] Hence, the ophthalmic circulation is in a constant state of vasodilation due to the basal release of nitric oxide. The basal release of nitric oxide assures local blood flow, protects against vasoconstrictor stimuli and prevents platelet activation and other blood cells.

B. STIMULATED RELEASE OF NITRIC OXIDE

In the human as well as porcine and bovine ophthalmic artery, acetylcholine, bradykinin and histamine evoke endothelium-dependent relaxations through the release of nitric oxide.[4,5,33,34,42,83,128] In extraocular porcine vessels, the sensitivity to bradykinin increase in vessels with a smaller diameter.[34] In the perfused porcine eye, bradykinin increases flow and reverses the decrease in flow induced by thromboxane analogue.[78] The response to bradykinin is inhibited by L-NAME, suggesting that nitric oxide is the mediator. In contrast, in the porcine ophthalmic artery, L-NMMA reduces the response to bradykinin, but a considerable relaxation persists. This indicates that in large ophthalmic arteries bradykinin most likely releases both nitric oxide and EDHF.[34]

C. ENDOTHELIN

In isolated ophthalmic and retinal arteries, endothelin-1 evokes potent contractions.[33,34,84,128] After exposure to the peptide, marked tachyphylaxis occurs in most blood vessels, reflecting agonist-induced ET-receptor down-regulation.[33,34] Even more strikingly than with nitric oxide, the sensitivity to endothelin increases with decreasing vascular diameter.[34] In the perfused porcine eye, endothelin-1 increases ophthalmic flow at very low dosages and severely reduces it at higher doses for prolonged periods of time.[78] Endothelin-3 evokes similar increases in flow, but it is less potent as a vasoconstrictor. This duel action of endothelins is due to the activation of endothelial ET_B-receptors releasing prostacyclin (mediating transient vasodilation) and by the activation of ET_B-receptors on vascular smooth muscle at higher concentrations of endothelin.

D. CYCLOOXYGENASE PRODUCTS

In porcine ophthalmic arteries with endothelium, quick stretch evokes a transient increase in tension.[128] As the endothelium-dependent response requires cyclooxygenase,[128] the contracting factor most likely is thromboxane A_2 or prostaglandin H_2[72] (Fig. 1). Stretch-activated ion channels in endothelial cells[59] are permeable to Ca^{2+} and their opening frequency increases with stretch. They may act as mechanotransducers and allow the endothelium to sense mechanical forces and respond to them. An endothelium-derived contracting factor may also be involved in the vasoconstriction induced by increases in transmural pressure in the isolated cerebral artery of the cat.[37–40] Both responses may modulate the autoregulation of local blood flow.

E. ENDOTHELIUM-DERIVED FACTORS AND PERICYTES

Pericytes are contractile cells adjacent to the endothelial lining of capillaries.[1] Pericytes are likely to modulate blood flow in very small ophthalmic arteries and capillaries. Cultured pericytes relax to sodium nitroprusside (a nitric oxide donor), which stimulates guanylyl cyclase

and increases cGMP.[35] In addition, the cells have been shown to respond to endothelin-1[10,60] and prostacyclin.[21,51]

III. Endothelial Dysfunction

Endothelial cells are a target for mechanical factors, noxious circulating substances and cardiovascular risk factors.[71] Hence, endothelial dysfunction may importantly mediate the vascular effects of various cardiovascular risk factors in the blood vessel wall. Under most conditions, endothelial dysfunction is likely to be a consequence of the effects of risk factors, but it remains possible that primary endothelial dysfunction also is involved in vascular disease. Little is known about endothelial cell dysfunction in the ophthalmic circulation, but defects occuring in other vascular beds may be relevant for the eye as well.

A. HYPERCHOLESTEROLEMA AND ARTERIOSCLEROSIS

In isolated vessels, low-density lipoproteins (LDL), but not high-density lipoproteins (HDL), inhibit endothelium-dependent relaxation to acetylcholine, serotonin and aggregating platelets.[2,57,111] Chronic hyperlipidemia moderately reduces endothelium-dependent relaxations in the coronary circulation, including the human forearm and coronary circulation.[6,27,106] Furthermore, oxidized LDLs induce mRNA expression and release of endothelin.[7] In arteriosclerosis, circulating and vascular endothelin levels are increased.[61]

B. HYPERTENSION

In peripheral arteries of experimental animals[16,20,22,66-68,112] and the human forearm and coronary circulation, hypertension is associated with an impaired basal formation of nitric oxide[113] and reduced endothelium-dependent vasodilation.[62] In contrast, the circulating levels of endothelin are not increased except in the presence of atherosclerosis and renal failure.[72,73]

C. DIABETES

Endothelial dysfunction also occurs in diabetes. In isolated penile corpora cavernosa of diabetic men with impotence, endothelium-dependent relaxations to acetylcholine, but not those to direct vasodilators such as sodium nitroprusside or papaverine, are decreased, indicating a reduced nitric oxide release in diabetes.[19] Similar conclusions have been reached regarding the mesenteric and cerebral microcirculation of diabetic rats.[28,75]

D. VASOSPASTIC SYNDROMES

In variant angina, Raynaud's disease and ocular migraine[31,32,54] altered endothelium-dependent responses could well be involved. Indeed, the local levels of endothelin are increased in both conditions.[72,73] In experimental subarachnoidal hemorrhage of the dog, endothelium-dependent relaxations are reduced, while endothelium-dependent contractions are preserved.[52,53] Cerebrospinal endothelin levels are up and endothelin-antagonists increase vascular diameter of spastic segments.[72,74] This imbalance in vascular reactivity may be an important component in the pathogenesis of cerebral vasospasm after subarachnoidal hemorrhage.

E. ISCHEMIA AND REPERFUSION

In the coronary artery of different species, endothelium-dependent relaxations to most agonists, including platelet-derived substances, are attenuated after ischemia and reperfusion.[56,91,92,114] The ischemia/reperfusion injury to the endothelium appears to be mediated at least in part by oxygen-derived free radicals.[58,108,129] Indeed, superoxid anions inactivate nitric oxide and lead to toxic products which can activate vascular smooth muscle cells.[3]

F. ENDOTHELIAL INJURY AND REGENERATION

In vivo endothelial denudation is followed by the regeneration of endothelial cells,[104] which exhibit an impaired endothelium-dependent relaxation to aggregating platelets most probably due to a dysfunction of the G_i-protein linked to the $5HT_1$-serotonergic receptors.[101,105] Furthermore, contractions evoked by serotonin are enhanced, due to the formation of acyclooxygenase-derived contracting factor by regenerated endothelium.[104]

G. IMPLICATIONS FOR THE OPHTHALMIC CIRCULATION

In the majority of systemic vascular diseases endothelium function is impaired, and similar alterations are likely to occur in the ocular circulation, especially in hypertension, diabetes, atherosclerosis or cerebrovascular ischemia. Endothelial dysfunction may also participate in vasospastic phenomena, particularly ocular or ret-

inal migraine[93] and in some forms of low tension glaucoma associated with Raynaud phenomenon and migraine.[31,32,54,120]

IV. Therapeutic Considerations

A certain number of drugs which are currently used in clinical ophthalmology have the ability to modify endothelium-dependent responses.

A. LOCAL ANESTHETICS

In porcine ciliary arteries, lidocaine, bupivacaine or mepivacaine impair the formation of nitric oxide from L-arginine induced by bradykinin, which may importantly contribute to the reduction in blood flow to the eye during retrobulbar anesthesia.[77] Interestingly, exogenous substitution by L-arginine restores the response to bradykinin even in the presence of the local anasthetics, suggesting a reduced intracellular availability of the precursor amino acid.

B. β-ADRENERGIC BLOCKERS

Certain β-adrenergic blockers exhibit mild endothelium-dependent effects. In rat aorta, relaxations to propranolol are reduced after endothelium removal.[82] In the coronary and femoral arteries of the dog, carteolol selectivity augments the abluminal release of nitric oxide in response to α_2-adrenergic activation and the intraluminal release of vasodilator prostaglandins.[45]

C. ANGIOTENSIN-CONVERTING ENZYME INHIBITORS (ACE)

ACE is located in the endothelial cell membrane and is identical with the kinase II which inactivates bradykinin.[25,63,107] This explains why ACE-inhibitors augment endothelium-dependent relaxation to bradykinin in isolated arteries[116,117,127] and increase ophthalmic flow induced by bradykinin in the ophthalmic microcirculation.[79] Hence, ACE inhibitors not only inhibit the formation of angiotensin II, but promote the effects of the L-arginine nitric oxide pathway activated by bradykinin.

D. Ca^{2+} ANTAGONISTS

Ca^{2+} antagonists have no direct endothelial effects, but facilitate the response of vascular smooth muscle to the relaxing factors and inhibit that to endothelin-1, at least in small blood vessels. In the human ophthalmic artery, nifedipine does not prevent contractions to endothelin-1,[33] whereas contractions are abolished by nitredipine in bovine small retinal arteries.[84] Possibly, influx of extracellular Ca^{2+} after exposure to endothelin-1 is particularly important in smaller arteries, while in larger ones endothelin releases intracellular Ca^{2+}. In vivo, nicardipine increases blood flow to the optic nerve head, but not to the retina of the cat.[41]

E. SEROTONERGIC ANTAGONISTS

$5HT_2$-serotonergic receptors on smooth muscle mediate contractions, while endothelial receptors ($5HT_1$-subtype) are linked to the release of NO. $5HT_2$-serotonergic blockers (e.g., ketanserin,[95] naftidrofuryl) prevent the vasoconstrictor effects of serotonin and augment endothelium-dependent relaxations to the monoamine or aggregating platelets.[104] This therapeutical approach has already been successfully used in low tension glaucoma.[76]

F. ENDOTHELIN RECEPTOR ANTAGONISTS

Specific antagonists for ET_A- and ET_B-receptors have recently been developed.[73] In the perfused porcine eye, FR139317, a specific ET_A-antagonist, markedly inhibits the decrease in ophthalmic flow induced by endothelin-1.[78] As endothelin is increased in several disease states in which the eye is involved (i.e., certain forms of hypertension, diabetes, Raynaud's syndrome, migraine, etc.[72]), these newly developed drugs provide an important tool to delineate the role of locally formed endothelin in these disease states and, potentially, a new therapeutic approach.

V. Conclusion

Through the local secretion of vasoactive substances, endothelial cells profoundly modulate local vascular tone under basal conditions and in response to mechanical forces and local hormones and platelet products. In the ophthalmic circulation, nitric oxide and endothelin-1 are important regulators of blood flow. A dysfunction of these local regulatory systems may importantly contribute to alterations in the ophthalmic circulation occurring in hypertension, diabetes, atherosclerosis, ischemia or vasospasm. Hence, in the ophthalmic circulation, endothelium-dependent regulatory mechanisms expand our knowledge of the physiology and pathophysiology of eye diseases and may offer new therapeutic approaches in the future.

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