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Clinical relevance of endothelium-derived relaxing factor (EDRF)

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1 In addition to metabolic and neurohumoral factors endothelium-derived autacoids like the nitric oxide radical NO and prostacyclin are effective regulators of vascular tone and thus tissue perfusion. NO is produced in endothelial cells from L-arginine by a Ca^{2+}/calmodulin-dependent enzyme NO synthase. In addition, the NO radical is ultimately cleaved from all nitrovasodilators and resembles their vasoactive and antiaggregatory principle, which is used under pathological conditions as substitution therapy for impaired endothelial function and autacoid production. Impaired endothelium-dependent vasomotor control has been documented in hypercholesterolaemia, atheromatosis, diabetes, hypertension, and in reperfusion damage. L-arginine supplementation is effective in a few instances.

Keywords endothelium-derived relaxing factor nitric oxide

Introduction

The endothelial lining is an important factor in the regulation of vascular tone and resistance. Therefore it affects substantially tissue and organ perfusion and may contribute to the control of blood pressure (for review see Bassenge & Heusch, 1990). This control is elicited by the production and release of various local hormones (autacoids), among them endothelium-derived relaxing factor (EDRF/NO), prostacyclin (PGI_2), platelet-activating factor (PAF) and endothelin (ET). This short review analyses the essential role of endothelium-derived relaxing factor (EDRF) under clinical conditions and its relevance for the maintenance of cardiovascular homeostasis.

Identification of EDRF

EDRF is identical with nitric oxide (·NO) (Palmer *et al.*, 1987) or a closely related nitrosyl-compound. The NO radical is ultimately cleaved from all nitrovasodilators and resembles the active principle both of the vasoactive and antiaggregatory property of these widely used drugs. It specifically stimulates soluble guanylate cyclase (GC) and thus increases intracellular cGMP production. In the vasculature this results, after several steps, in vasodilatation, in platelets cGMP suppresses intracellular Ca^{2+} mobilisation, activation, adhesion and aggregation (Busse *et al.*, 1987), thus maintaining adequate blood fluidity (Bassenge *et al.*, 1989). There is a continuous, *basal* release of NO, which is modulated additionally by a second, (receptor-) *stimulated* release e.g. by acetylcholine or bradykinin, but also by mechanical factors such as blood flow-induced viscous drag or pulsatile stretching imposed on the endothelial lining (for review see Bassenge & Heusch, 1990).

NO is synthetised in a number of different cell types by the action of a recently discovered enzyme, NO synthase, present in various isoforms in different cell types as a constitutive (e.g. endothelium) or inducible (e.g. vasculature) enzyme (Bredt *et al.*, 1991; Förstermann *et al.*, 1991). In cerebral cells NO is formed and acts probably as a transmitter (Bredt *et al.*, 1990; Garthwaite *et al.*, 1988). In granulocytes and macrophages NO is synthetised and acts as a microbicidal compound (for review see Moncada *et al.*, 1991). The constitutive NO synthase in endothelial cells is Ca^{2+}, calmodulin and NADPH-dependent (Busse & Mülsch, 1990a). L-arginine acts as a precursor substance and is transformed by various oxidation steps into citrulline, cleaving the guanidino-nitrogen atom to form the NO radical (Palmer *et al.*, 1987). For experimental analyses L-arginine can be replaced by a number of biological inactive arginine analogues (L-mono-methyl-arginine L-NMMA, L-nitro-arginine L-NAG, L-amino-arginine L-NNA etc). These stereospecific blockers suppress cellular NO production mainly by inactivating NO synthase, but can also interfere with the specific membrane carrier of L-arginine (Bogle *et al.*, 1991).

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Clinical aspects of impaired EDRF formation and release into the vasculature

In various ischaemic diseases with impaired endothelial function (hypercholesterolaemia (Creager *et al.*, 1990), atherosclerosis (Harrison *et al.*, 1987a,b), hypertension-induced vascular damage (Panza *et al.*, 1990), balloon catheter-induced endothelial denudation (Fischell *et al.*, 1989), diabetes (Saenz de Tejada *et al.*, 1989), reperfusion damage (Aoki *et al.*, 1988; Lefer & Lefer, 1991; Stewart *et al.*, 1988), coronary spasm (Chesebro *et al.*, 1989; Fischell *et al.*, 1989; Nagasawa *et al.*, 1989), subarachnoid haemorrhage induced vasospasm (Hongo *et al.*, 1988; Nakagomi *et al.*, 1987) this autacoid (and EDRF) release is depressed or the released NO radical immediately inactivated by haemoglobin or by oxygen derived radicals before affecting the vasculature. The impaired EDRF release favours an enhanced vasoconstrictor tone especially under the condition of an insufficient mechanically or receptor-*stimulated* NO release in large feed arteries (e.g. coronaries). This becomes particularly obvious, when endothelial NO production is reduced or absent upon stimulation by various factors which initiate endothelial release of autacoids. Therefore the combined net vasomotor effect of circulating agonists and endothelial autacoids may shift gradually to reduced vasodilatation, or even to an excessive vasoconstrictor tone. This may result in an inadequate blood supply and thus in ischaemic damage. A diminished dilator response of resistance arteries can add to this dysregulation. Such a tendency has been observed even in the course of normal ageing in humans (Zeiher *et al.*, 1992 (submitted)) in the absence of coronary artery disease.

Effects of hypercholesterolaemia and atherosclerosis

As shown in Figure 1 the endothelium-dependent, NO-mediated dilator effect of acetylcholine (ACh) is substantially reduced in atherosclerotic vessels of test animals (cynomolgus monkeys) which were subjected to an atherogenic diet for several months. However, when the lipid profile in the plasma was improved and normalised again by feeding an appropriate regression diet for several months, the endothelium- (NO-) mediated dilator responses returned back to control (Harrison *et al.*, 1987a). Similar observations on the effect of hypercholesterolaemia-induced impairment of endothelium-mediated vasomotor function were made in hypercholesterolaemic and arteriosclerotic humans (Creager *et al.*, 1990; Zeiher *et al.*, 1991a,b, 1992). Endothelial dysfunction in coronary microvessels of hypercholesterolaemic patients can be improved by L-arginine supplementation (Drexler *et al.*, 1991).

Effects of acute and chronic hypertension

Similarly during acute and chronic hypertension in animal studies the endothelium-mediated dilator responses are severely reduced (Lamping & Dole, 1987; Lüscher *et al.*, 1987a), and this can be prevented by the initiation of an antihypertensive therapy in animal experiments (Lüscher *et al.*, 1987b). Similar observations using forearm plethysmography and intraarterial acetylcholine injections to demonstrate the reduction of a stimulated endothelial NO release have been made in hypertensive patients (Linder *et al.*, 1990; Panza *et al.*, 1990). Resting forearm blood flow was not reduced in these studies. An impaired NO-mediated vasodilatation has also been observed in hypercholesterolaemic humans by Creager *et al.* (1990) and in atherosclerosis (Zeiher *et al.*, 1992).

A sensitive parameter for the evaluation of such impaired vasomotor responses consists in the stimulation of the vessel by intravascular serotonin (5-HT): in the presence of unimpaired endothelial function, serotonin-induced endothelium (5-HT_1)-mediated dilatations predominate, yet with impaired autacoid release direct 5-HT_2-mediated constrictor responses of the vasculature are unmasked as shown in animal studies (Lamping & Dole, 1987) or in *in vitro* studies (Figure 2, Busse & Bassenge, unpublished observations). Similar sensitisations to serotonin-induced constrictions can be observed after the initiation and during the progression of experimental hypercholesterolaemia

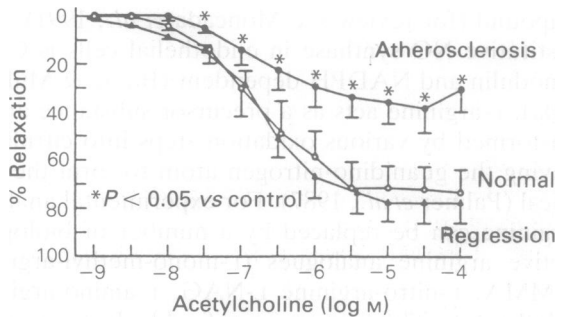

Figure 1 Effect of diet-induced atherosclerosis and of subsequent feeding of a regression diet on EDRF-mediated vasodilatation. Relaxation by EDRF release was stimulated with increasing dosages of acetylcholine (abscissa). Ordinate shows relaxation (%) of precontracted vascular rings from cynomolgus monkeys. Mean values ± s.e. mean (modified from Harrison *et al.* (1987a)).

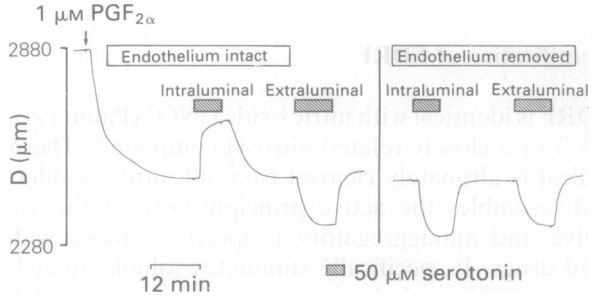

Figure 2 Endothelium (E)-dependent dilatation stimulated by intraluminal application of serotonin (HT) in E-intact vessel segment (left), compared with E-independent constriction in E-denuded segment (right), representing E-impaired vessels. Extraluminal application of HT elicits (E-independent) constrictions both in E-intact and -denuded sections. Ordinate: changes in diameter (D) of preconstricted ($PGF_{2\alpha}$), perfused canine coronary artery, paired segments (from Busse & Bassenge, unpublished observations).

and atherosclerosis (Galle *et al.*, 1990; Heistad *et al.*, 1984; Vrints *et al.*, 1988), which likewise impair endothelial vasomotor functions and limit NO-mediated dilator effects.

Experimentally impaired EDRF formation is associated with suppression of tissue perfusion and may aggravate hypertension

Obviously hypertension is associated with an impaired endothelial function and EDRF/NO release. On the other hand, the induction of experimentally impaired endothelial NO production (a) suppresses tissue perfusion, (b) results in myocardial ischaemia and augmented lactate production (Pohl *et al.*, 1990a,b) and (c) can induce hypertension as shown recently (Rees *et al.*, 1989). This emphasises the importance of a continuous basal NO production and release from the endothelial lining stimulated by (a) several circulating agonists, (b) mechanically by the viscous drag of the perfusing blood and the pulsatile stretching of the cell membranes for the adjustment of an adequate dilator tone and appropriate peripheral resistance. It is important to realise that such an endothelium-mediated dilator mechanism acts in parallel with several local humoral, hormonal and local metabolic factors, counterbalances the intrinsic myogenic constrictive tone (Pohl *et al.*, 1990a) and thus decisively modulates and controls local autoregulation.

Suppression of EDRF/NO production with stereospecific inhibitors

When endothelial NO production and the concomitant dilator effects are suppressed using stereospecific inhibitors of NO synthase, such as L-monomethylarginine (L-NMMA), L-nitroarginine (L-NAG) or L-aminoarginine (L-NAA), the continuous endothelium-mediated dilator activity is reduced, shifting the vasomotor equilibrium more to constriction. This may result in an increased peripheral resistance, diminished tissue perfusion and oxygen delivery and in an increased blood pressure. Interestingly, baroreflex-induced bradycardia cannot sufficiently counterbalance this increase in peripheral resistance and blood pressure (Bassenge, 1991). The effect of endothelium-derived nitric oxide and the significance of its suppression by these stereospecific blockers has recently been demonstrated in humans (Vallance *et al.*, 1989) and an augmented arteriolar tone and peripheral resistance has been observed.

Analysis of endothelium-mediated dilator capacity as a clinical test

The vasomotor responses elicited by acetylcholine and mediated by NO can be used to analyse the functional state of the endothelium and its autacoid release within the coronary bed. In the absence of CAD exclusively dilator responses are observed. With the progression of CAD, however, the dilator responses become continuously less, disappear subsequently and are finally dose-dependently shifted to increasing coronary constriction. A similar pattern of events can be observed, when a 'cold pressor test' (CPT) is applied instead of intracoronary ACh-stimulation in the presence or absence of CAD. During this CPT exclusively epicardial dilatations were observed angiographically in the absence, however constrictions in the presence of CAD (Zeiher *et al.*, 1989). Interventions aiming at the normalisation of the coronary vasomotor responses (e.g. antihyperlipidaemic or antihypertensive treatment) can thus be evaluated by such test protocols of endothelial function both in epicardial arteries and in resistance vessels, which control myocardial perfusion.

Substitution of EDRF by exogenous NO cleaved from nitrovasodilators

In various pathological states, such as CAD, nitrovasodilators are used to substitute for a diminished endothelial EDRF/NO production since nitrovasodilators have been shown, after being processed in a number of poorly understood metabolic steps, to ultimately yield NO (Feelisch & Noack, 1987). The subsequent NO-mediated stimulation of cGMP production, can initiate relaxation, and compensate for insufficient endothelial EDRF/NO production or for excessive NO inactivation induced by oxygen-derived radicals present in atherosclerotic vessel sections (Mügge *et al.*, 1992).

It recently became obvious that such a substitution is probably effective especially in vascular segments, which show—due to endothelial impairment or denudation—reduced EDRF production and thus inadequate cGMP production and relaxation (Bassenge & Stewart, 1988). There is both *in vitro* (Bassenge & Stewart, 1988) and *in vivo* evidence (Rafflenbeul *et al.*, 1989) that nitrovasodilator-induced dilatation is more pronounced in endothelium-impaired or denuded sections as compared with endothelium-intact segments. *In vitro* denuded sections relax as shown in Figure 3 about three times more effectively to nitrovasodilators (Bassenge, 1989a; Busse *et al.*, 1989) and similar responses have been observed in atherosclerotic, but still compliant vessel sections in CAD patients in *in vivo* studies (Rafflenbeul *et al.*, 1989).

This surprising increase in sensitivity may not only be explained by the augmentation of (the absolute) cGMP levels in the vasculature, but also by the rate of increase of cGMP levels (relative changes) upon a sudden increase in intravascular nitrovasodilator concentrations (Bassenge & Heusch, 1990).

Interactions of endogenous and exogenous nitric oxide

The continuous application of exogenous nitrovasodilators leads to 'tolerance' both in the coronary

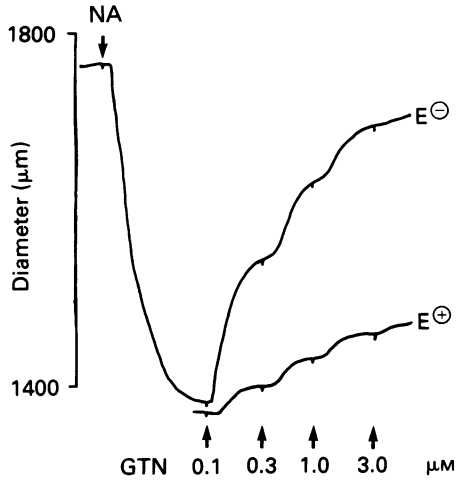

Figure 3 Augmented dilator response to nitroglycerin (GTN) in endothelium-denuded segments (top, E^-) as compared with endothelium-intact segments (bottom, E^+). Ordinate: changes in diameter D of noradrenaline (NA) preconstricted, perfused vessel segments. Abscissa: time and cumulative dosages of GTN (modified from Bassenge (1989a)).

arteries and in the venous system (Stewart *et al.*, 1986, 1987), probably by a limitation of the biotransformation from nitrovasodilators into the active compound ·NO. This results in diminished venodilatation and less reduction of preload during nitrate administration (Münzel *et al.*, 1990). Thus there is a smaller decrease of ventricular wall tension and myocardial oxygen consumption.

In such a state of tolerance, the vascular responses to the 'endogenous nitrate' EDRF, produced and released by the endothelial cell lining are not reduced. This is demonstrated in Figure 4 (Stewart *et al.*, 1987) which shows the endothelium-mediated dilator effect of acetylcholine (ACh) on large coronary arteries before and after the induction of tolerance to nitroglycerin. In the pretolerant control state ACh stimulation results in NO release from the endothelial cells and consequently in

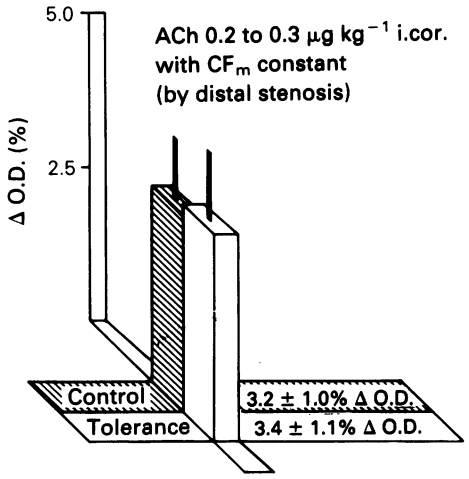

Figure 4 Acetylcholine (ACh, intracoronarily)-induced coronary dilatations in chronically instrumented conscious dogs at pretolerant control state (shaded column) and during well documented tolerance to nitroglycerin (induced by 5 day nitroglycerin-infusion). Ordinate: changes in outer diameter (% OD) of circumflex branch. No significant reduction of ACh-induced, E-mediated dilatation can be demonstrated (modified from Stewart *et al.* (1987)).

vasodilatation. Although the dilator responses to an exogenous nitrate, namely nitroglycerin are substantially suppressed during tolerance, the responses to the 'endogenous nitrate' EDRF are not affected after the induction of tolerance. Similarly the response to an endogenous stimulator of NO release, the mechanical shear stress exerted by the blood flow as viscous drag upon the endothelial lining (Bassenge, 1989b), is not suppressed during tolerance (Stewart *et al.*, 1987). Thus, a reduced sensitivity of the vasculature cannot be demonstrated, but the bioconversion of the exogenous nitrates to NO seems to be limited during tolerance (e.g. by a change in cytochrome P-450 like activity (McDonald & Bennett, 1990; Servent *et al.*, 1989)).

Clinical relevance of 'manipulating' EDRF/NO production

Recently it was shown that in cytokine and endotoxin-induced shock the specific *constitutive* enzyme NO synthase in endothelial cells is excessively expressed as *inducible* enzyme in the vasculature (Busse & Mülsch, 1990b), accounting for an uncontrolled NO release and excessive vasodilatation during various shock conditions. The therapeutical application of various stereospecific inhibitors of the arginine-dependent NO production may turn out to be a useful therapeutic tool in such states of uncontrolled and excessive vasodilator activity. The induction of NO synthase in the vasculature can be suppressed by anti-inflammatory corticosteroids (Radomski *et al.*, 1990). Endothelial dysfunction in the microvasculature of hypercholesterolaemic patients can be improved by L-arginine supplementation (Drexler *et al.*, 1991). Whether the beneficial effects of feeding fish oil to counterbalance atherosclerosis-induced vasomotor abnormalities can be attributed to improved endothelial autacoid production (Shimokawa *et al.*, 1987; Shimokawa & Vanhoutte, 1989a,b) remains to be demonstrated.

Future trends in anti-ischaemic treatment

Anti-ischaemic treatment in the next years will probably include methods for the preservation of endothelial autacoid release, e.g. by the suppression of noxious (e.g. atherogenic) factors. In addition, measures may be taken to increase intracellular arginine concentrations by supplementing exogenous L-arginine (Drexler *et al.*, 1991) and stimulating NO synthase in the endothelial lining to augment EDRF/NO formation in affected vessel sections. Under pathophysiological conditions NO synthase is excessively induced and expressed in vascular smooth muscle by endotoxins and cytokines in a variety of shock conditions, which can be suppressed and normalised by corticosteroids (Radomski *et al.*, 1990; for review see Moncada *et al.*, 1991).

Finally, the substitution therapy of endothelial autacoid production (such as nitrovasodilator admin-

istration) will probably be continued and improved. An important task for the future will be to overcome the development of tolerance during continued nitrovasodilator treatment. This may be achieved by administering agents that directly release NO (which do not need several intermediate metabolic steps in order to yield NO (Bassenge *et al.*, 1992; Bohn *et al.*, 1991)) or by the interposition of other dilator principles such as Ca^{2+}-antagonists or K^+-channel openers. With increasing extent nitrate therapy leads to activation of counterregulatory factors including stimulation of the adrenergic and of the renin-angiotensin system. Thus there may be a beneficial role for angiotensin-converting enzyme inhibitors in the prevention of amelioration of nitrate tolerance (Katz *et al.*, 1991).

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