European Heart Journal (1991) 12 *(Supplement E),* 2-11

Endothelium-derived nitric oxide: the endogenous nitrovasodilator in the human cardiovascular system

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KEY WORDS: Cyclic 3',5'-guanosine monophosphate, endothelium derived relaxing factor, nitrates, 3-morpholino sydnonimine (SIN-1), platelets.

The L-arginine pathway within endothelial cells in the blood vessel wall is the source of production of the endogenous nitrovasodilator, nitric oxide (NO). NO is released under basal conditions and in response to various stimuli such as shear stress and in response to platelet-derived products, coagulation factors and hormones. NO is the mediator of endothelium-dependent relaxation in the circulation and exerts its effects by activating soluble guanylyl cyclase in vascular smooth muscle, which in turn leads to the formation of cyclic guanosine monophosphate (cGMP) and to relaxation. In addition to its effects on vascular smooth muscle, NO is also released abluminally to interact with circulating platelets. Increases in cGMP in platelets are associated with a decreased adhesion and aggregation of cells. Thus, endothelium-derived NO, through its vasodilator and anti-aggregatory properties, prevents vasospasm and thrombus formation in the circulation and thereby helps to maintain blood flow to vital organs such as the heart. Therapeutic nitrates also exert their effects by releasing NO from their molecules and activating soluble guanylyl cyclase. Their effects are particularly pronounced in arteries without endothelium and are reduced in the presence of the basal formation of endothelium-derived NO in intact arteries. The lower basal formation of endothelium-derived NO in veins, as compared to arteries, contributes to the greater sensitivity of venous circulation to nitrates.

Thus, the endothelial L-arginine pathway plays an important protective role in the local regulation of blood flow and through its vasodilator and antiplatelet properties.

Introduction

Ever since Thomas Lauder-Brunton first introduced amylnitrate into clinical medicine[11], nitrovasodilators of different classes have been extensively used in the medical treatment of angina pectoris, myocardial infarction and heart failure![2,41]. Understanding the mechanisms of action of nitrates has been greatly expanded in the last decade, particularly since the discovery of endothelium-dependent relaxation and its mediator, the endogenous nitrovasodilator, NO[15,6]. This review summarizes current knowledge of the endothelial L-arginine/NO pathway in the human circulation and updates previous articles[7].

Endothelium-dependent vasodilation

In isolated blood vessels, as well as in the intact organism, endothelium-dependent relaxation or vasodilatation occurs respectively in response to physical, chemical and hormonal stimuli (Fig. I)![6,8,12]. Indeed, acetylcholine relaxes or dilates conduit arteries and increases local blood flow when infused intra-arterial]y|i3.i4], jn t"ne circulation of the human forearm, intra-arterial infusion of acetylcholine causes a pronounced increase in blood flow, unaffected by acetylsalicylic acid (which inhibits the formation of prostacyclin) or by phentolamine (which excludes a contribution by the prejunctional inhibitory effects of the muscarinic agonist on adrenergic neurotransmission)"[4]. Similarly, in sympathectomized animals, the vasodilator response to intra-arterial acetylcholine is maintained[1151]. Thus, the vasodilator effects of acetylcholine are not mediated by prostacyclin and are independent of the sympathetic nervous system, but are reduced or prevented by removal of the endothelium or by inhibitors of the formation or action of endothelium-derived NO[15].

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Nature of endothelium-derived relaxing factor (EDRF)

Interaction between the endothelium and vascular smooth muscle cells could occur either by direct cell-to-cell contact[116,17] or by local mediators. In conduit arteries, the release of an endothelium-derived relaxing factor(s) (EDRF) has been demonstrated using a 'sandwich' preparation of the rabbit aorta[18] and confirmed with cascade-bioassay techniques using perfused blood vessels with endothelium or endothelial cells in culture as donor tissues[18,23]. Since endothelium-dependent relaxation induced by acetylcholine is unaffected by inhibitors of cyclo-oxygenase and purinergic antagonists, prostaglandins or adenosine[5-8] can be excluded as mediators.

Experiments in which the transit time between the donor segment with endothelium and the bioassay tissue without endothelium can be varied, allowed the bio-

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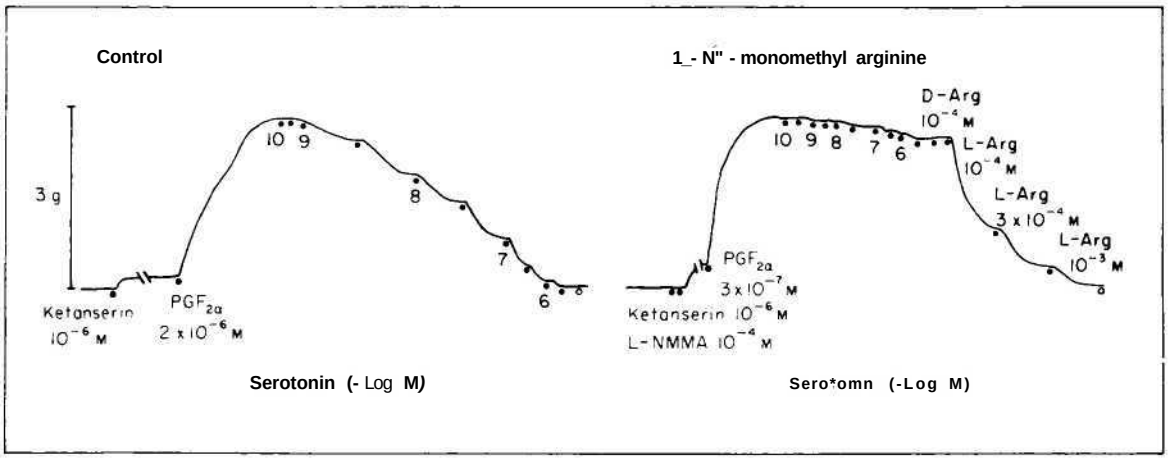


Figure 1 Endothelium-dependent relaxation with serotonin in the porcine coronary artery. In isolated rings (pretreated with the 5-HT; serotonergic antagonist, ketanserin. to inhibit the direct contractile effects of serotonin), serotonin causes a concentration-dependent and complete relaxation. After pretreatment of the blood vessel with L-N°-monomethyl arginine (L-NMMA; the inhibitor of NO formation), the response to serotonin is markedly reduced. While D-argine (D-Arg) does not restore the response. L-arginine (L-Arg; the precursor for the formation of NO) fully restores the response. (Reproduced with permission[1"*1]).

logical half-life of EDRF to be estimated, which is in the range of a few seconds[151]. The scavenger of superoxide anions, superoxide dismutase, markedly stabilizes EDRFl[21;4'] indicating that the oxygen-derived free radical, superoxide anion, inactivates the factor. This observation and the stimulatory effect of the factor on soluble guanylyl cyclase (with concomitant formation of cGMP) led to the proposal[1252"1] that EDRF is the radical NO (Fig. 2). Similar to EDRF, NO is labile with a half-life of a few seconds, its biological activity (relaxing effect) is blocked by haemoglobin and superoxide anions, but enhanced by superoxide dismutase. The chemical and biological similarities between EDRF and NO in a variety of isolated blood vessels supports the concept that EDRF is identical to NO[1271]. In line with that interpretation, cultured endothelial cells exposed to bradykinin release NO and the amount of the radical liberated (as measured by chemiluminescence) can explain the biological activity of EDRF[|:7]L Release of NO in response to acetylcholine and the Ca·* ionophore A23187 has also been demonstrated in the intact rabbit aorta[12"1]. Debate continues on whether NO is released as such or together with a carrier molecule (for instance the amino acid L-cysteine to yield L-nitrosocysteine[12M1]).

Formation of endothelium-derived NO

In porcine endothelial cells in culture, NO is formed from the amino acid L-arginine (Fig. 2;[13"-1,1]). Cultured endothelial cells deprived of L-arginine lose their ability to release NO, while administration of L-arginine restores this response[13"1]. In arteries obtained from experimental animals and humans, endothelium-dependent relaxation by acetylcholine is inhibited by the analogue of the amino acid L-N^G-monomethyl arginine (L-NMMA) and restored by the addition of L-arginine (Figs 1 and 3:[1<2"']). L-NMMA. but not its enantiomer D-NMM A, induced endothelium-dependent contractions in intramyocardial coronary arteries (Fig. 4;[33r]) and markedly increases arterial blood pressure in rabbits[341]; in addition, the analogue of L-arginine inhibits the hypotensive effect of acetylcholine[1,41]. Blood vessels obtained from animals treated with L-NMMA have a

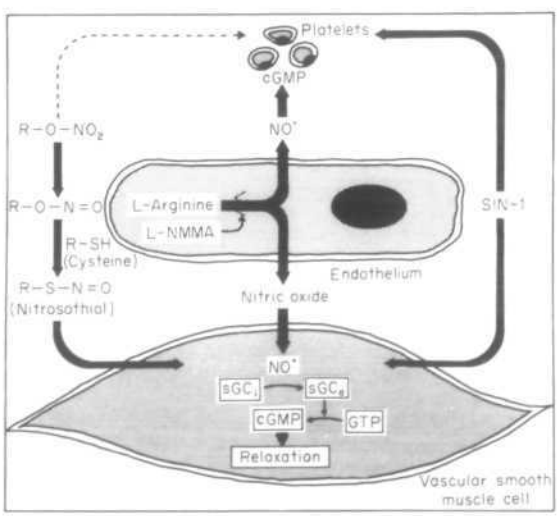


Figure 2 The endothelial L-arginine/NO pathway. The endogenous nitrovasodilator. NO. is formed from L-arginine within endothelial cells and released both luminally and abluminally. In vascular smooth muscle cells. NO activates soluble guanylyl cyclase (sGC) and in turn leads to the formation of cyclic GMP. the second messenger mediating relaxation. In platelets, increased levels of cyclic GMP are associated with a decreased adhesion and aggregation of the cells. Therapeutic nitrates such as organic nitrates (R-O-NO;) or sydnonimines (SIN-1: the active metabolite of molsidomine) directly activate sGC by releasing NO from their molecules. In contrast to SIN-1. organic nitrates have to undergo a biotransformation requiring thiol groups. (Reproduced with permission[1^1]).

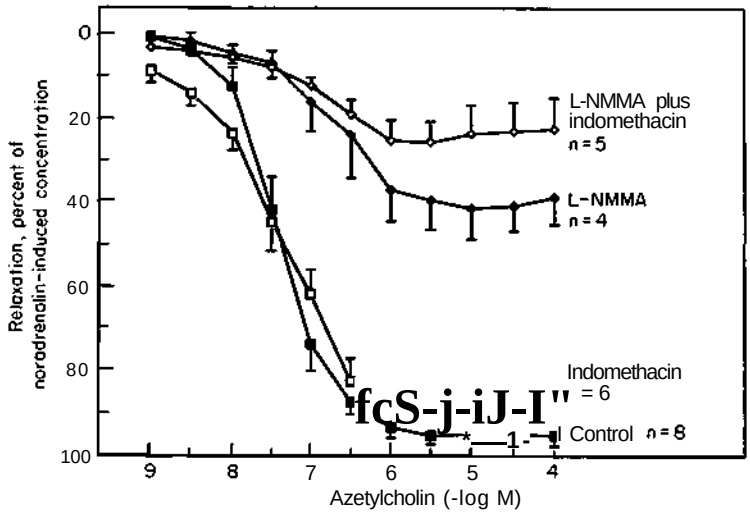


Figure 3 Endothelium-dependent relaxation of acetylcholine in the human internal mammary artery. Under control conditions, acetylcholine causes full relaxation, but is inhibited by the formation of prostaglandins indomethacin. The inhibitor of NO formation L-N^G-monomethyl arginine (L-NMMA; 10^{-4} M) markedly reduces the response. Under these conditions, inhibition of prostaglandin formation has a weak additional inhibitory effect. (Reproduced with permission[321]).

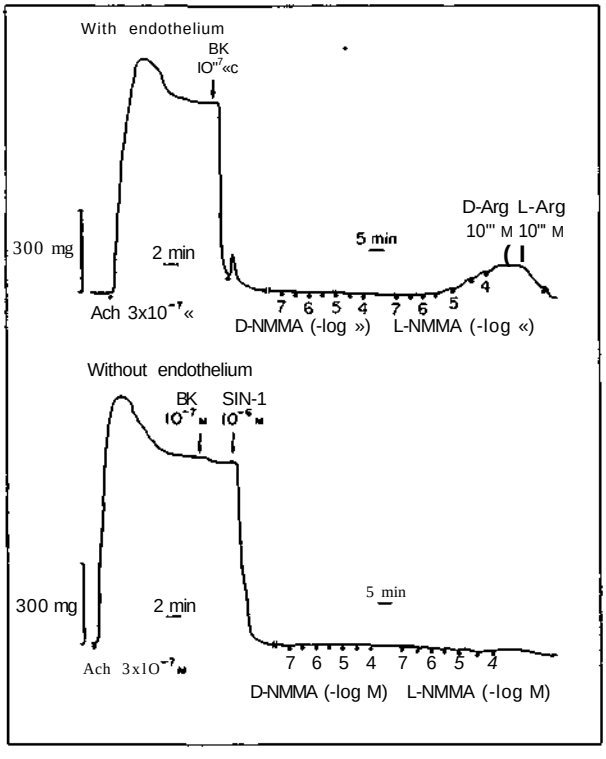


Figure 4 Basal formation of endothelium-derived NO in intramyocardial porcine coronary arteries. In an isolated artery with endothelium contracted with acetylcholine (Ach), bradykinin (BK) causes full relaxation. The inhibitor of NO formation L-N^G monomethyl arginine (L-NMMA) causes endothelium-dependent contractions (which are not mimicked by its stereoisomer D-NMM A). Reintroduction of L-arginine (L-Arg) but not of D-arginine (D-Arg) reverses the contraction. In contrast, in a preparation without endothelium (lower panel) L-NMMA is without effect. (Reproduced with permission[13;1]).

blunted ability to form NO[1341]. Thus, formation of endothelium-derived NO from L-arginine occurs under basal conditions and after stimulation with acetylcholine. The enzyme(s) involved (NO synthase) is Ca^{2+}-dependent, requires NADPH and leads to the formation of NO and L-citrulline from L-arginine[135,137].

Mechanism of release

Neurohumoral mediators stimulate the release of EDRF by activating specific endothelial receptors (see[5]). However, the signal transduction from the receptors to the release of the relaxing factors differ. In the porcine and canine coronary artery, endothelium-dependent relaxation evoked by the $alpha_2$-adrenergic agonist UK 14,304, 5-hydroxytryptamine (Fig. 1) and leukotriene C_4 are abolished or reduced by pertussis toxin, which irreversibly ribosylates d proteins[138,140]. In contrast, endothelium-dependent relaxation by bradykinin, adenosine diphosphate (ADP) and particularly by the ionophore A23187 remain unaffected by the toxin[138-40]. In intramyocardial coronary arteries, neither of the receptors is linked to a G| protein[133]. Thus, at least two distinct biochemical pathways are involved in the release of EDRF. Loss of functional Gi proteins in regenerated endothelial cells causes a selective dysfunction of the serotonin-induced release of NO and porcine coronary arteries[141].

In freshly harvested endothelial cells of the rabbit aorta, acetylcholine induces a transient hyperpolarization[142]. The importance of this change in membrane potential for the release of EDRF remains uncertain, however, as cultured endothelial cells preserve electrophysiological responses, in particular hyperpolarization

to acetylcholine[141441]. while they lose their ability to release EDRF in response to the muscarinic agonist.

In endothelial cells in culture, bradykinin and histamine, but not the Ca^{2}~ ionophore A23187, stimulate the metabolism of phosphoinositol, leading to the formation of inositol trisphosphate which induces intracellular Ca^{2}* mobilization'[45^191]. Phorbol esters (which down-regulate protein kinase C) inhibit the release of EDRF evoked by acetylcholine or substance P. but not that caused by the Ca^{2}* ionophore A23187, indicating that diacylglycerol (which is concomitantly formed after activation of phospholipase C) is important in contributing to the release of the factor stimulated by certain receptor-operated agonists[1501].

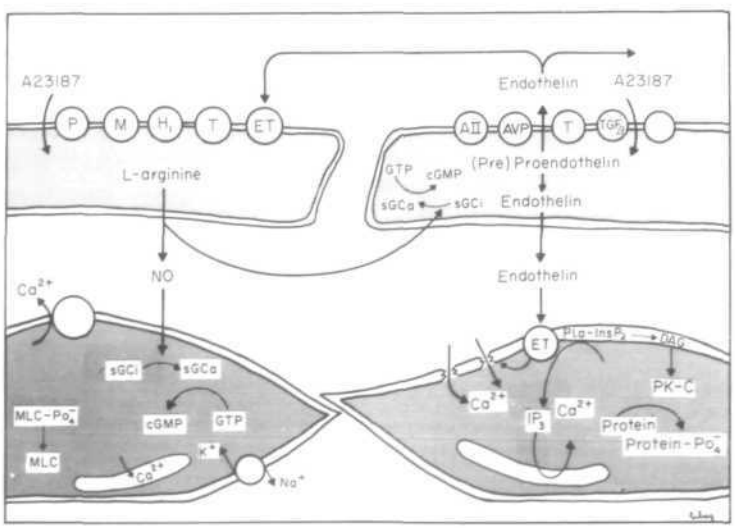
Increases in intracellular Ca^{2+} in endothelial cells must play a crucial role in either the production and/or release of EDRF. Indeed, depletion of extracellular Ca^{2+} inhibits endothelium-dependent relaxation of muscarinic agonists, but not those of the endothelium-independent vasodilator, sodium nitroprusside'[51„531]. In addition, in cultured endothelial cells, stimulation of the release of EDRF by bradykinin, adenosine trisphosphate (ATP) and ADP, thrombin and mellitin is accompanied by an increase in cytoplasmic Ca^{2+} concentrations[1421]. The contribution of the intracellular release of Ca^{2+} to the augmentation of the cytosolic level of the ion varies; however, the sustained release of EDRF requires the influx of extracellular Ca^{2+}, most likely through a receptor-operated Ca^{2+} channel[154„591]. Indeed, in isolated blood vessels, Ca^{2+} antagonists such as verapamil or dihydropyridines do not prevent endothelium-dependent relaxation by acetylcholinel[60-641].

The production and/or release of EDRF requires oxygen, since anoxia prevents relaxation induced by acetylcholine. but not that caused by endothelium-independent vasodilators[165^1„1]. In the rabbit aorta, endothelium-dependent relaxation is reduced by agents that inhibit mitochondrial electron transport or uncouple oxidative phosphorylation, suggesting that the production and/or release of the factor depends on mitochondrial synthesis of ATP'[67'].

Endothelial effects of NO

Endothelial cells contain soluble and paniculate guanylyl cyclase and can form cGMP (Fig. 5;[681]). A number of stimuli which cause the release of EDRF as well as nitrovasodilators and exogenous NO augment the accumulation of cGMP by cultured endothelial cells'[68„701]. The accumulation is inhibited by haemoglobin and methylene blue and augmented by superoxide dismutase[1691]. The presence of the analogue of the cyclic nucleotide, 8-bromo cyclic cGMP, blunts relaxation evoked by acetylcholine and substance P, whereas ATP, A23187 and nitrovasodilators are unaffected[17'1]. One possible reason for the increase in cGMP is that EDRF feeds back in a negative fashion on its own release by activating the soluble guanylyl cyclase of the endothelial cells[168-69711].

Endothelium-derived NO can interact with endothelin production (Fig. 5;'[721]). Indeed, production of the peptide from the endothelium of intact porcine aorta upon stimulation with thrombin is augmented in the presence of L-NMMA and methylene blue (Fig. 6;[1721])- Since thrombin is known to cause endothelium-dependent relaxation in a variety of blood vessels, this indicates that the enzyme concomitantly activates the formation of NO and endothelin in the intima of intact blood vessels and that the former inhibits the produc-



FigureS Interaction between endothelium-derived NO and endothelin. The two substances can interact at the level of vascular smooth muscle where NO causes relaxation and ET, contraction, as well as at the level of the endothelium. where NO activates soluble guanylyl cyclase (sGC) and in turn reduces thrombin-induced endothelin production. (Reproduced with permission'[•]).

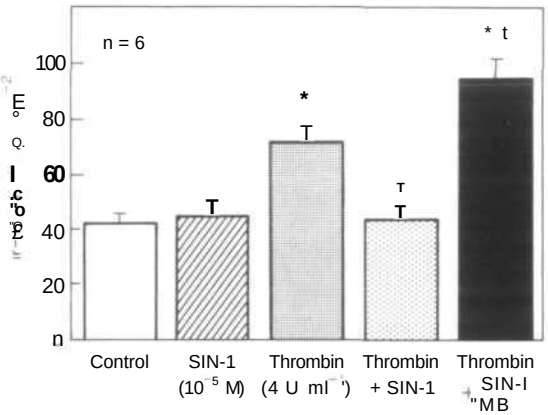


Figure 6 Modulation of the production of immunoreactive endothelin (ir-endothelin) by the nitrovasodilator SIN-1. The thrombin-induced production of the peptide can be prevented by the NO donor SIN-1 and augmented by the inhibitor of soluble guanylyl cyclase methylene blue (MB). These experiments demonstrate that the formation of endothelin induced by thrombin can be inhibited via the cGMP-dependent mechanism. (Reproduced with permission[1,11]).

tion of the latter (Fig. 5;[173-73]). In line with that observation, superoxide dismutase (which inhibits the breakdown of NO) as well as the non-hydrolysable analogue of cGMP, 8-bromo cGMP, prevent the thrombin-induced formation of endothelin[1721]. Similarly, nitrates such as SIN-1 and nitroglycerin inhibit the production of endothelin induced by the enzyme (Fig. 6;[174]).

Release of NO by non-endothelial cells

Rat peritoneal neutrophils[17S7*]l, cytotoxic activated macrophages[1771] and cerebellar cell suspensions obtained from newborn rats are also able to synthesize and release NO[7,11].

Vascular effects of endothelium-derived NO

In blood vessels with endothelium, the relaxation induced by acetylcholine, histamine and the Ca[:'] ionophore A23187 are associated with an increase in the intracellular concentration of cGMP in *smooth muscle celts* (Fig. 2:[71]-[,i]). The rise in cGMP in the cells slightly precedes vascular relaxation. Removal of the endothelium prevents formation of the nucleotide induced by acetylcholine. but not that evoked by sodium nitroprusside. nitroglycerin or exogenous NO[1*1]. The inhibitor of soluble guanylyl cylase. methylene blue, prevents formation of cGMP and prevents or reverses endothelium-dependent relaxation by acetylcholine (see[5-12-2]). suggesting that the cyclic nucleotide mediates the vascular actionof EDRF.

In quiescent aortas of rat and rabbit, the inhibitors of cGMP phosphodiesterase induce endothelium-dependent relaxation, suggesting that in intact blood vessels guanylyl cyclase is continuously activated[1,1,]. Indeed, basal levels of cGMP are higher in preparations with. than in those without, endothelium and are higher in cultured vascular smooth muscle grown in co-culture with endothelial cells than in smooth muscle grown alone[81-83-84]!.

Several mechanisms have been proposed to explain why cGMP induces vascular relaxation, including decreases in intracellular calcium, inhibitory effects on phosphoinositol metabolism and on protein kinases (Fig. 5). Rat aortas with endothelium have a lower $^{45}Ca^{2}$* content than those without endothelium, suggesting that EDRF released under basal conditions either reduces Ca^{2}* influx, inhibits Ca^{2}* mobilization from intracellular stores or augments the efflux of the ion[851]. In the rabbit aorta, endothelium-dependent relaxation evoked by acetylcholine is associated with a reduced Ca^{2}~ influx[86]. Inhibitors of EDRF, such as phenidone, prevent the response, while sodium nitroprusside and 8-bromo cGMP mimic it. Thus, EDRF induces relaxation in part by decreasing Ca^{2+} entry. Furthermore, cGMP, by activating a protein kinase, stimulates cyclic 3',5'-adenosine monophosphate-dependent Ca^{2+} extrusion across the sarcolemma of vascular smooth muscle[87].

Cyclic GMP inhibits the breakdown of phosphatidylinositol in vascular smooth muscle and in platelets[88,90], but removal of the endothelium increases the hydrolysis of phosphatidylinositide with increased accumulation of inositol monophosphate in the aorta of the rat and rabbit[89]. Similar effects can be observed after lysis of the endothelium or after inhibition of guanylyl cyclase[89]!.

Finally, in the rat aorta, acetylcholine and sodium nitroprusside decrease the incorporation of labelled phosphor into myosin light chains[79-91-92]. Removal of the endothelium abolishes the effect of the muscarinic agonist, but not that of the nitrovasodilator. Thus, EDRF may act through a cGMP-dependent protein kinase which controls the phosphorylation and dephosphorylation of myosin light chains.

Antiplatelet effects of NO

Platelets also contain enzyme-soluble guanylyl cyclase and can form cGMP (Fig. 2:[93 **]), but increased production of cyclic nucleotide is associated with reduced platelet adhesion and aggregation. EDRF. as well as exogenous NO inhibit platelet adhesion to the endothelium and platelet aggregation in vitro and in vivo (Fig. 7:[9,11,5]). Both also increase the content of cGMP, and reduce the thrombin-induced rise in intracellular Ca^{2}*•*.

The potency of EDRF as an anti-aggregant and disaggregant substance is comparable to that of prostacyclin[1,1]. Prostacyclin and NO potentiate each other even at subthreshold concentrations in their anti-aggregatory action[1-1,]. In the rabbit, intravenous infusions of the muscarinic agonist carbachol increase the platelet content in cGMP and inhibit their aggregation induced by ADP[IMV]. Since both effects can be prevented by the simultaneous administration of either methylene blue or haemoglobin. EDRF is the most likely mediator.

Interestingly, aggregating platelets release enough

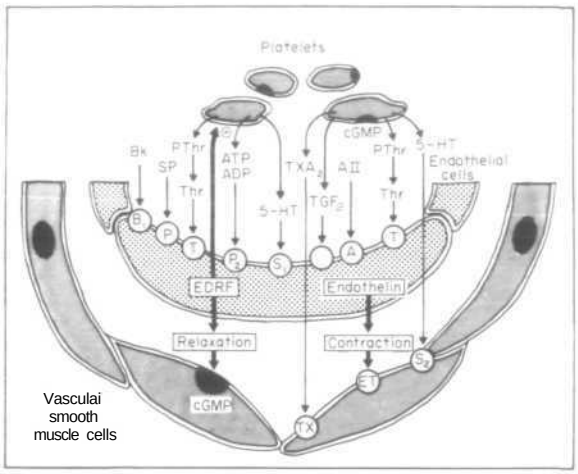


Figure 7 Endothelium and platelet-vessel wall interaction. Platelets (top) release a variety of mediators such as adenosine diphosphate (ADP). adenosine trisphosphate (ATP) and, 5-hydroxy-tryptamin (serotonin: 5-HT). In addition, thromboplastin leads to the formation of thrombin. All these platelet-derived mediators and products of the coagulation cascade can interfere with the endothelium to evoke the release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGN;not shown). Both substances, if released into the lumen of the blood vessel wall can inhibit platelet function and reduce platelet adhesion and aggregation. EDRF and. in certain but not all blood vessels, PGI_2 can also evoke relaxation of vascular smooth muscle. Thus, by releasing EDRF and PGK the endothelium plays a protective role in the circulation by preventing platelet adhesion, aggregation and platelet-induced vasospasm. while endothelin (GT) causes profound contraction. (Reproduced with permission[7'].)

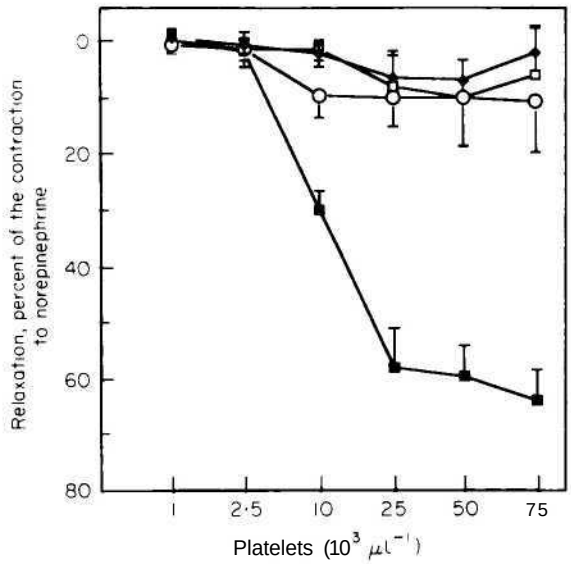


Figure 8 Endothelium-dependent (•. n = 5) relaxation of aggregating platelets in the normal human internal mammary artery. Platelets activated by Krebs-Ringer Henseleit solution cause marked relaxation in isolated arteries contracted with norepinephrine. As these relaxations are prevented by endothelium removal (•. n = 5), apyrase (O, n = 4) (to break down ATP and ADP to AMP) and the inhibitor of NO formation L-N^G-monomethyl arginine (L-NMMA, •, n = 4), adenine nucleotides released by the platelets must activate purinergic receptors on the endothelium linked to the release of endothelium-derived nitric oxide, which in turn causes vascular relaxation. (Reproduced with permission' '*!.)

ATP and ADP to stimulate the release of endothelium-derived NO and cause endothelium-dependent relaxation in normal human arteries (Fig. 8;I[106]I). Thus, release of EDRF in response to platelet-derived products (Fig. 7) may provide a negative feed-back mechanism inhibiting adhesion and aggregation of the platelets at sites where they are activated.

Humoral effects of NO

EDRF released from isolated blood vessels in the dog reduces the production of renin (demonstrated in slices of canine kidneys)[1"171]. Anatomically, endothelial cells and juxtaglomerular cells are in close proximity in the wall of the preglomerular arterioles. Since endothelial cells can respond to shear stress, with an increased release of EDRF, modulation of renin release by the factor could link changes in perfusion pressure in the afferent arterioles with the release of renin enzyme. Hence, endothelial cells may act as the intra-renal baroreceptor.

In the atrium of the rat, endocardium (and endothelium) removal with saponin augments the basal release of atrial natriuretic peptide[1""1]. A similar effect can be obtained with inhibitors of EDRF such as haemoglobin, methylene blue and hydroquinone. This suggests that EDRF released from either the endocardium or from endothelial cells of the coronary vasculature inhibits the release of the natriuretic peptide.

Endothelium-derived NO and nitrates

The vascular effects of exogenous nitrates are modulated by the presence of endothelium[11"9-1121]. Indeed, in arteries with intact endothelial cells, the relaxing effects of sodium nitroprusside, nitroglycerin or SIN-1 (the active metabolite of molsidomine) are reduced, compared with preparations without endothelium (Fig. 9;l"l). As the inhibitor of NO formation, L-NMMA also augments the relaxing effects of nitrates, and the activity of the endothelial L-arginine NO pathway appears to reduce the responsiveness of blood vessels to nitrates[1""21]. As the basal formation of endothelium-derived NO is smaller in veins as compared to arteries, this may also explain why nitrates have more pronounced effects in the venous as compared to the arterial circulation. One interesting clinical implication of this phenomenon may be the fact that nitrates preferentially dilate those vascular segments with dysfunctional endothelial cells.

Nitrates can also interfere with endothelin production stimulated by thrombin (Fig. *6f^*) which may provide a new vascular mechanism of action of nitrovasodilators such as SIN-1 and nitroglycerin[1741].

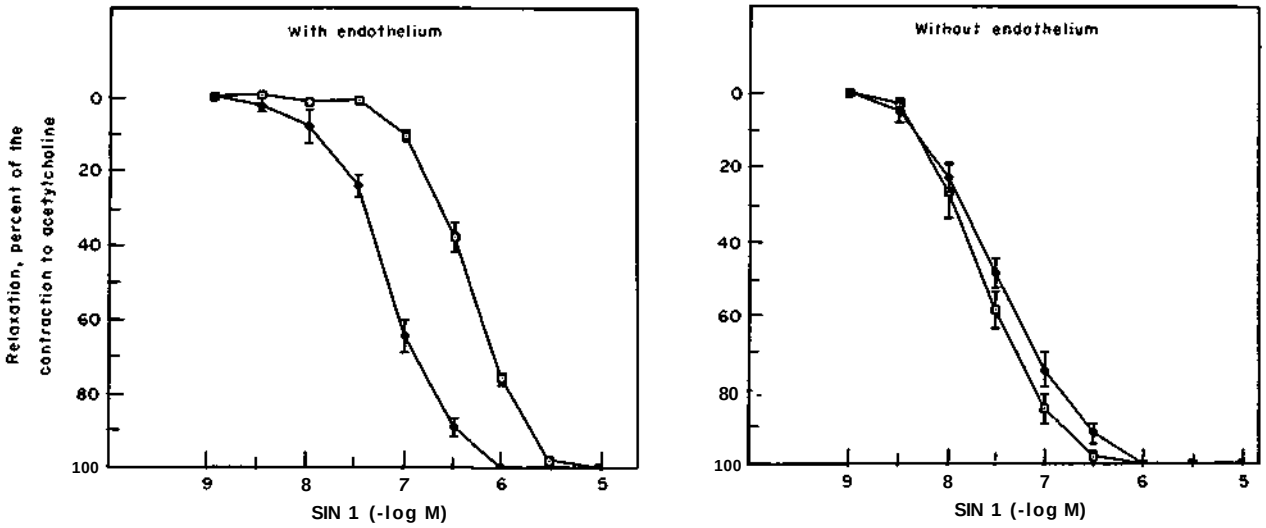


Figure 9 Basal formation of endothelium-derived NO and the effects of therapeutic NO donor. The sydnonimine, SIN-1, causes marked relaxation in intramyocardial porcine coronary arteries. The sensitivity to SIN-1 is more pronounced in arteries without endothelium than in those with it. In arteries with endothelium, the inhibitor of NO formation, L-N^G-monomethyl arginine (L-NMM A), markedly augments the response, while in preparations without endothelium, the inhibitor is without effect —D—, control; —•—, L-NMMA 10^{-4} M; n = 4. (Reproduced with permission[1321].)

Conclusion

The endothelium is thus a source of a number of substances which can evoke a relaxation of the underlying smooth muscle. The most important EDRF is NO which is formed from L-arginine. Its action is mediated by increases in cGMP in vascular smooth muscle cells, the endothelium, platelets and certain other tissues. As a vasodilator and inhibitor of platelet function, endothelium-derived NO plays a protective role in the circulation. Besides NO and prostacyclin, endothelium releases other EDRF(s) such as endothelium-derived hyperpolarizing factor. The biochemical nature and physiological importance of these substance(s) remains to be denned.

The authors thank Bernadette Libsig and Sabine Bohnert for technical assistance. Original research of the authors reported in this manuscript was made possible by grants of the Swiss National Research Foundation (grant No. 32-25468.88; SCORE-grant No. 3231-025150), the Swiss Cardiology Foundation and the support of Hoechst Pharmaceutica, Paris, France.

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