

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

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*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 amyl nitrate into clinical medicine<sup>11</sup>, nitrovasodilators of different classes have been extensively used in the medical treatment of angina pectoris, myocardial infarction and heart failure<sup>12,41</sup>. 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<sup>15,61</sup>. This review summarizes current knowledge of the endothelial L-arginine/NO pathway in the human circulation and updates previous articles<sup>71</sup>.

## 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. 1)<sup>6,8,121</sup>. Indeed, acetylcholine relaxes or dilates conduit arteries and increases local blood flow when infused intra-arterially<sup>13,14</sup>. In the 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)<sup>41</sup>. Similarly, in sympathectomized animals, the vasodilator response to intra-arterial acetylcholine is maintained<sup>151</sup>. 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<sup>151</sup>.

## 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<sup>116,171</sup> 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<sup>181</sup> and confirmed with cascade-bioassay techniques using perfused blood vessels with endothelium or endothelial cells in culture as donor tissues<sup>18,231</sup>. Since endothelium-dependent relaxation induced by acetylcholine is unaffected by inhibitors of cyclo-oxygenase and purinergic antagonists, prostaglandins or adenosine<sup>5,6,31</sup>, 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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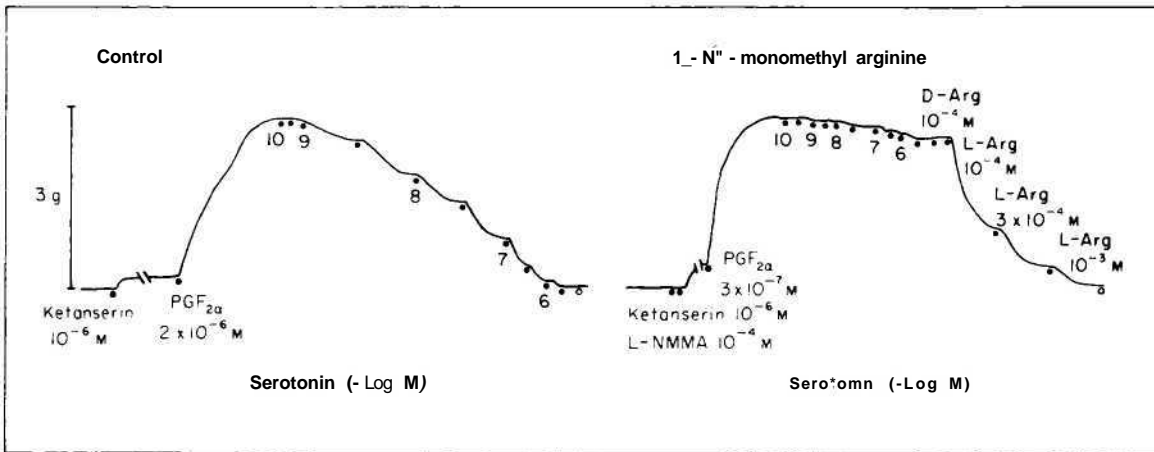


Figure 1 Endothelium-dependent relaxation with serotonin in the porcine coronary artery. In isolated rings (pretreated with the 5-HT; serotonergic antagonist, ketanserin, to inhibit the direct contractile effects of serotonin), serotonin causes a concentration-dependent and complete relaxation. After pretreatment of the blood vessel with L-N<sup>G</sup>-monomethyl arginine (L-NMMA; the inhibitor of NO formation), the response to serotonin is markedly reduced. While D-arginine (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<sup>114</sup>).

logical half-life of EDRF to be estimated, which is in the range of a few seconds<sup>151</sup>. The scavenger of superoxide anions, superoxide dismutase, markedly stabilizes EDRF<sup>21,41</sup> 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<sup>125,211</sup> 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<sup>127</sup>. 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<sup>17</sup>. Release of NO in response to acetylcholine and the Ca<sup>2+</sup> ionophore A23187 has also been demonstrated in the intact rabbit aorta<sup>121</sup>. 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<sup>12M1</sup>).

### Formation of endothelium-derived NO

In porcine endothelial cells in culture, NO is formed from the amino acid L-arginine (Fig. 2,<sup>13n-11</sup>). Cultured endothelial cells deprived of L-arginine lose their ability to release NO, while administration of L-arginine restores this response<sup>13n1</sup>. In arteries obtained from experimental animals and humans, endothelium-dependent relaxation by acetylcholine is inhibited by the analogue of the amino acid L-N<sup>G</sup>-monomethyl arginine (L-NMMA) and restored by the addition of L-arginine (Figs 1 and 3:<sup>12n1</sup>). L-NMMA, but not its enantiomer

D-NMMA, induced endothelium-dependent contractions in intramyocardial coronary arteries (Fig. 4;<sup>133</sup>) and markedly increases arterial blood pressure in rabbits<sup>134</sup>; in addition, the analogue of L-arginine inhibits the hypotensive effect of acetylcholine<sup>141</sup>. Blood vessels obtained from animals treated with L-NMMA have a

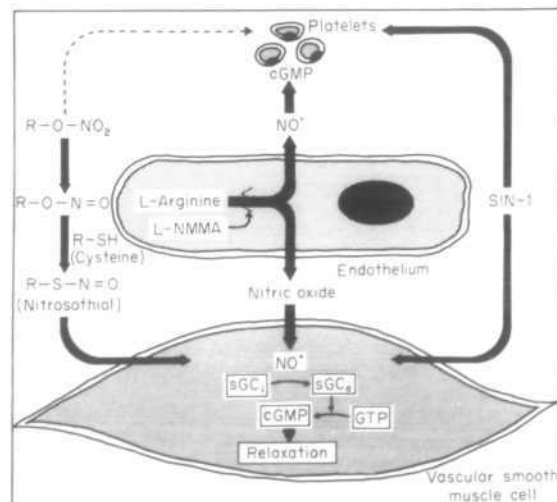
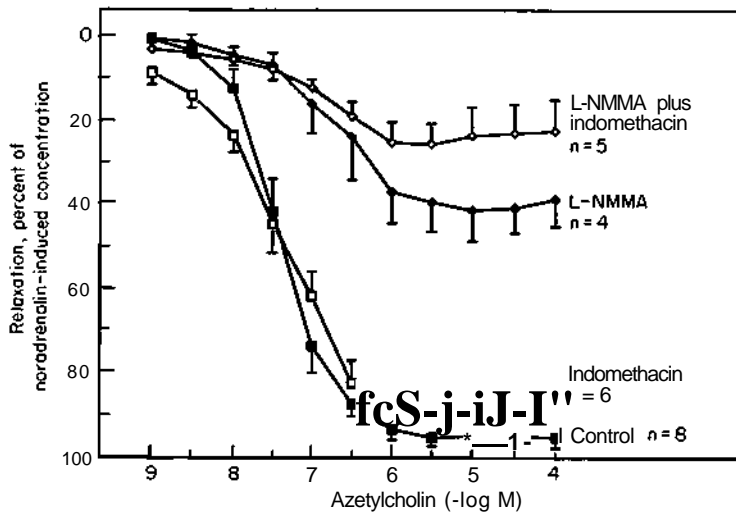
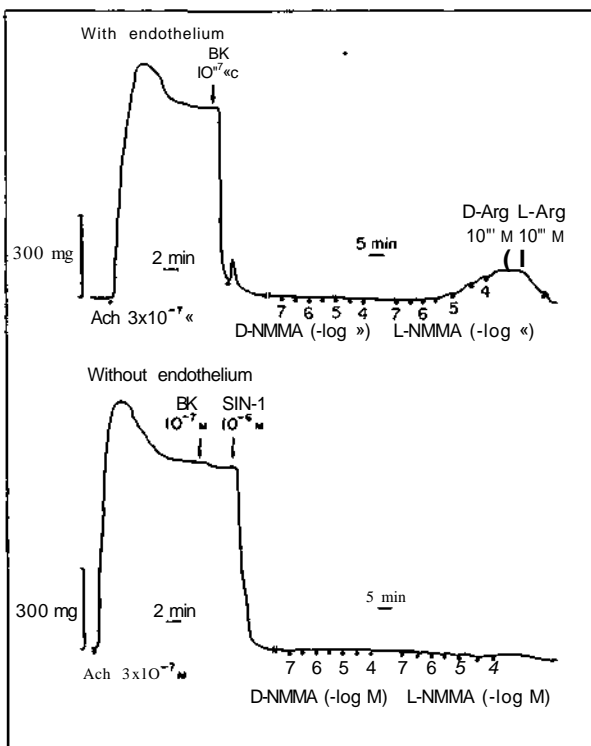


Figure 2 The endothelial L-arginine/NO pathway. The endogenous nitrovasodilator, NO, is formed from L-arginine within endothelial cells and released both lumenally and ablumenally. In vascular smooth muscle cells, NO activates soluble guanylyl cyclase (sGC) and in turn leads to the formation of cyclic 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<sub>2</sub>) 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<sup>141</sup>).



**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<sup>G</sup>-monomethyl arginine (L-NMMA; 10<sup>-4</sup> M) markedly reduces the response. Under these conditions, inhibition of prostaglandin formation has a weak additional inhibitory effect. (Reproduced with permission<sup>321</sup>).



**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<sup>G</sup>-monomethyl arginine (L-NMMA) causes endothelium-dependent contractions (which are not mimicked by its stereoisomer D-NMMA). 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<sup>131</sup>).

blunted ability to form NO<sup>1341</sup>. 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<sup>2+</sup>-dependent, requires NADPH and leads to the formation of NO and L-citrulline from L-arginine<sup>135,371</sup>.

### Mechanism of release

Neurohumoral mediators stimulate the release of EDRF by activating specific endothelial receptors (see<sup>51</sup>). 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<sub>2</sub>-adrenergic agonist UK 14,304, 5-hydroxytryptamine (Fig. 1) and leukotriene C<sub>4</sub> are abolished or reduced by pertussis toxin, which irreversibly ribosylates d proteins<sup>138,401</sup>. In contrast, endothelium-dependent relaxation by bradykinin, adenosine diphosphate (ADP) and particularly by the ionophore A23187 remain unaffected by the toxin<sup>38-401</sup>. In intramyocardial coronary arteries, neither of the receptors is linked to a G<sub>i</sub> protein<sup>1331</sup>. Thus, at least two distinct biochemical pathways are involved in the release of EDRF. Loss of functional G<sub>i</sub> proteins in regenerated endothelial cells causes a selective dysfunction of the serotonin-induced release of NO and porcine coronary arteries<sup>411</sup>.

In freshly harvested endothelial cells of the rabbit aorta, acetylcholine induces a transient hyperpolarization<sup>421</sup>. 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<sup>141,441</sup>, 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  $\text{Ca}^{2+}$  ionophore A23187, stimulate the metabolism of phosphoinositol, leading to the formation of inositol trisphosphate which induces intracellular  $\text{Ca}^{2+}$  mobilization<sup>45,191</sup>. 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  $\text{Ca}^{2+}$  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<sup>1501</sup>.

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

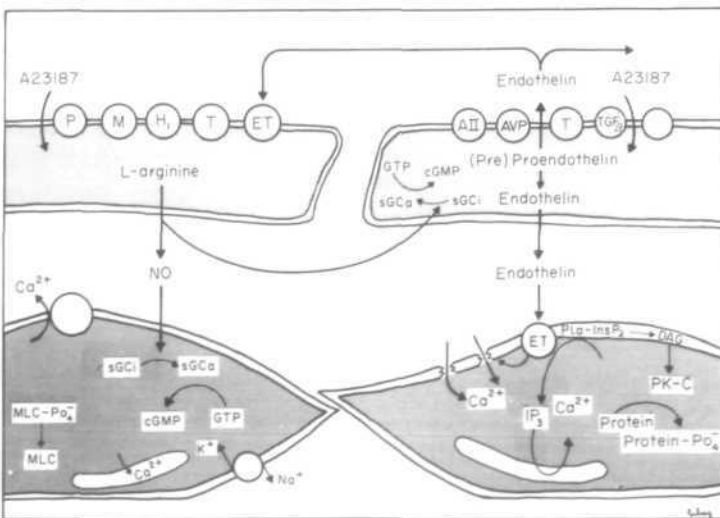
The production and/or release of EDRF requires oxygen, since anoxia prevents relaxation induced by

acetylcholine, but not that caused by endothelium-independent vasodilators<sup>165,171</sup>. 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<sup>671</sup>.

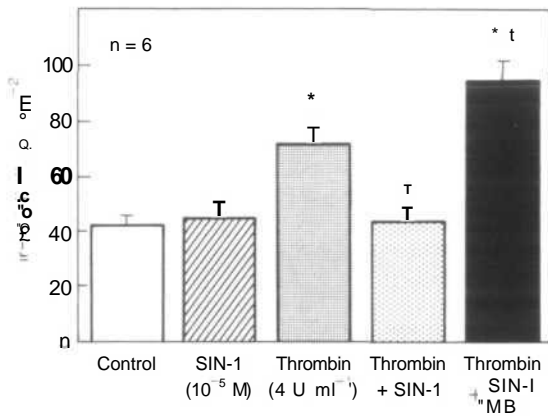
### Endothelial effects of NO

Endothelial cells contain soluble and particulate guanylyl cyclase and can form cGMP (Fig. 5;<sup>1681</sup>). 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<sup>68,701</sup>. The accumulation is inhibited by haemoglobin and methylene blue and augmented by superoxide dismutase<sup>1691</sup>. 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<sup>171</sup>. 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<sup>168,69,711</sup>.

Endothelium-derived NO can interact with endothelin production (Fig. 5;<sup>721</sup>). 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;<sup>1721</sup>). 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-



**Figure 5** 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<sup>173</sup>).



**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<sup>171</sup>).

tion of the latter (Fig. 5;<sup>73,73r</sup>). 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<sup>172</sup>. Similarly, nitrates such as SIN-1 and nitroglycerin inhibit the production of endothelin induced by the enzyme (Fig. 6;<sup>74</sup>).

### Release of NO by non-endothelial cells

Rat peritoneal neutrophils<sup>175,1</sup>, cytotoxic activated macrophages<sup>177</sup> and cerebellar cell suspensions obtained from newborn rats are also able to synthesize and release NO<sup>171</sup>.

### Vascular effects of endothelium-derived NO

In blood vessels with endothelium, the relaxation induced by acetylcholine, histamine and the Ca<sup>2+</sup> ionophore A23187 are associated with an increase in the intracellular concentration of cGMP in *smooth muscle cells* (Fig. 2;<sup>71</sup>). 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<sup>181</sup>. The inhibitor of soluble guanylyl cyclase, methylene blue, prevents formation of cGMP and prevents or reverses endothelium-dependent relaxation by acetylcholine (see<sup>5,12,12r</sup>), suggesting that the cyclic nucleotide mediates the vascular action of 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<sup>111</sup>. 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<sup>81,83,84</sup>.

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 <sup>45</sup>Ca<sup>2+</sup> content than those without endothelium, suggesting that EDRF released under basal conditions either reduces Ca<sup>2+</sup> influx, inhibits Ca<sup>2+</sup> mobilization from intracellular stores or augments the efflux of the ion<sup>85</sup>. In the rabbit aorta, endothelium-dependent relaxation evoked by acetylcholine is associated with a reduced Ca<sup>2+</sup> influx<sup>86</sup>. 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<sup>2+</sup> entry. Furthermore, cGMP, by activating a protein kinase, stimulates cyclic 3',5'-adenosine monophosphate-dependent Ca<sup>2+</sup> extrusion across the sarcolemma of vascular smooth muscle<sup>87</sup>.

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

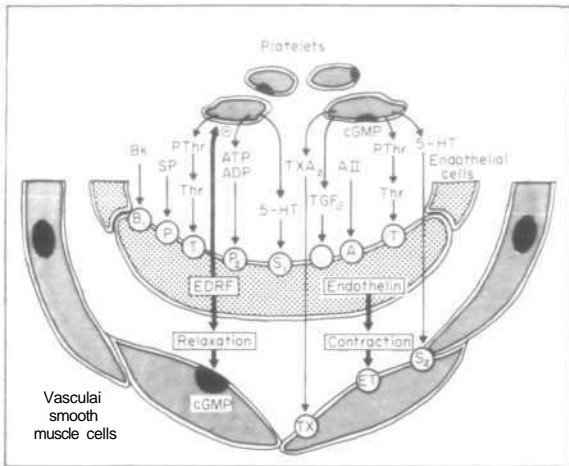
Finally, in the rat aorta, acetylcholine and sodium nitroprusside decrease the incorporation of labelled phosphorus into myosin light chains<sup>79,91,92</sup>. Removal of the endothelium abolishes the effect of the muscarinic agonist, but not that of the nitrovasodilator. Thus, EDRF may act through 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;<sup>93</sup>), 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;<sup>91,95</sup>). Both also increase the content of cGMP, and reduce the thrombin-induced rise in intracellular Ca<sup>2+</sup><sup>96,97</sup>.

The potency of EDRF as an anti-aggregant and disaggregant substance is comparable to that of prostacyclin<sup>111</sup>. Prostacyclin and NO potentiate each other even at subthreshold concentrations in their anti-aggregatory action<sup>111</sup>. In the rabbit, intravenous infusions of the muscarinic agonist carbachol increase the platelet content in cGMP and inhibit their aggregation induced by ADP<sup>112</sup>. 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



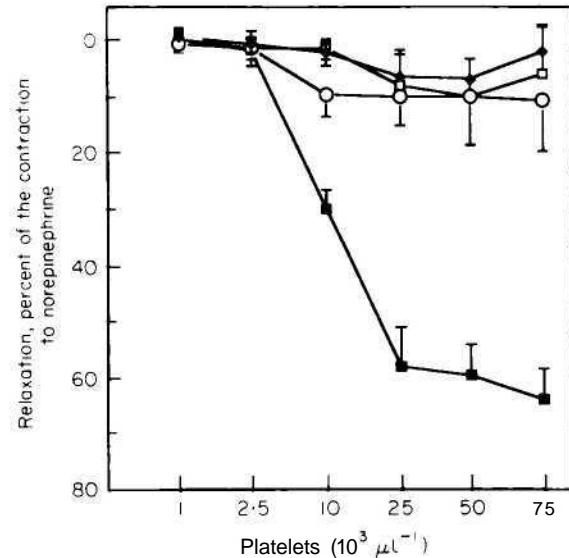
**Figure 7** Endothelium and platelet-vessel wall interaction. Platelets (top) release a variety of mediators such as adenosine diphosphate (ADP), adenosine triphosphate (ATP) and 5-hydroxy-tryptamin (serotonin: 5-HT). In addition, thromboplastin leads to the formation of thrombin. All these platelet-derived mediators and products of the coagulation cascade can interfere with the endothelium to evoke the release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI<sub>2</sub>; 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<sub>2</sub> can also evoke relaxation of vascular smooth muscle. Thus, by releasing EDRF and PGI<sub>2</sub> the endothelium plays a protective role in the circulation by preventing platelet adhesion, aggregation and platelet-induced vasospasm, while endothelin (ET) causes profound contraction. (Reproduced with permission<sup>71</sup>.)

ATP and ADP to stimulate the release of endothelium-derived NO and cause endothelium-dependent relaxation in normal human arteries (Fig. 8; <sup>106</sup>). Thus, release of EDRF in response to platelet-derived products (Fig. 7) may provide a negative feedback 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)<sup>1171</sup>. 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<sup>1111</sup>. 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



**Figure 8** Endothelium-dependent ( $\bullet$ ,  $n = 5$ ) 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 ( $\bullet$ ,  $n = 5$ ), apyrase (O,  $n = 4$ ) (to break down ATP and ADP to AMP) and the inhibitor of NO formation L-N<sup>G</sup>-monomethyl arginine (L-NMMA,  $\bullet$ ,  $n = 4$ ), 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<sup>111</sup>.)

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<sup>1109-1121</sup>. 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; <sup>111</sup>). 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<sup>11121</sup>. 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<sup>1</sup>) which may provide a new vascular mechanism of action of nitrovasodilators such as SIN-1 and nitroglycerin<sup>1741</sup>.

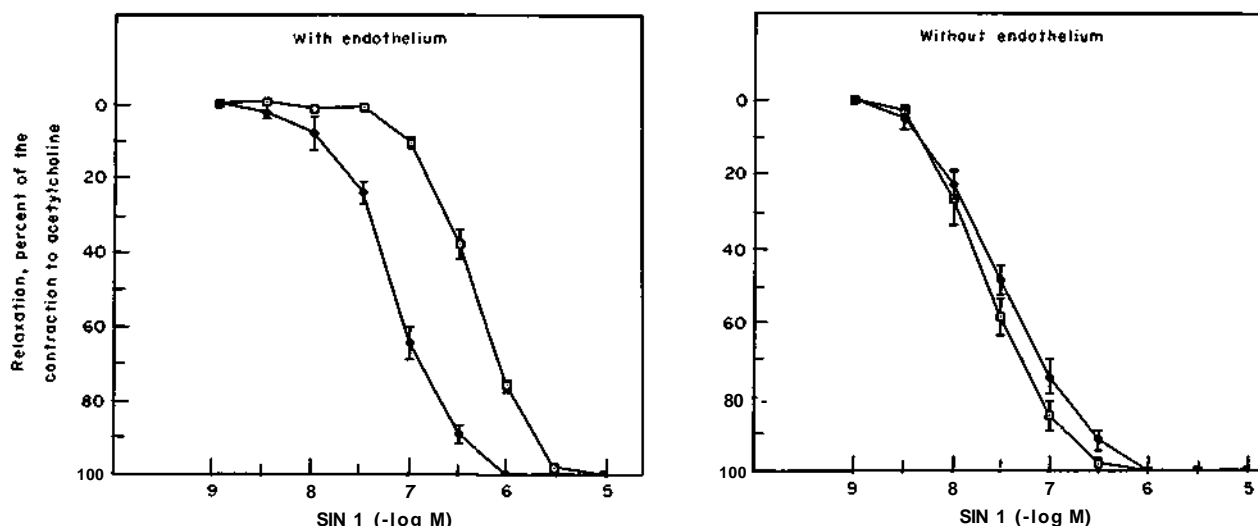


Figure 9 Basal formation of endothelium-derived NO and the effects of therapeutic NO donor. The sydnonimine, SIN-1, causes marked relaxation in intramyocardial porcine coronary arteries. The sensitivity to SIN-1 is more pronounced in arteries without endothelium than in those with it. In arteries with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

## 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.

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## References

- [1] Brunton L. Use of nitrite of amylin in angina pectoris. *Lancet* 1867; ii: 97-8.
- [2] Parker J A. Nitrate therapy in stable angina pectoris. *N Engl J Med* 1987; 316:1635-42.
- [3] Jugdutt BJ, Warnica JW. Intravenous nitroglycerin therapy to limit myocardial infarct size, expansion, and complications. *Circulation* 1988; 88: 906-19.
- [4] Cohn JN, Archibald DG, Ziesche S *et al.* Effect of vasodilator therapy on mortality in chronic congestive heart failure. *N Engl J Med* 1986; 314:1547-52.
- [5] Lüscher TF, Vanhoutte PM. The endothelium: Modulator of cardiovascular function. Boca Raton, USA: CRC Press, 1991:1-215.
- [6] Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989; 3: 2007-18.
- [7] Lüscher TF, Richard V, Tschudi M, Yang Z, Boulanger C. Endothelial control of vascular tone in large and small coronary arteries. *J Am Coll Cardiol* 1990; 15: 519-27.
- [8] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 299: 373-6.
- [9] Bossaller C, Habib GB, Yamamoto H, Williams C, Wells S, Henry PD. Impaired muscarinic endothelium-dependent relaxation and cyclic guanosine 5'-monophosphate formation in atherosclerotic human coronary artery and rabbit aorta. *J Clin Invest* 1987; 79: 170-4.
- [10] Lüscher TF, Cooke JP, Houston DS, Neves R, Vanhoutte PM. Endothelium-dependent relaxations in human peripheral and renal arteries. *Mayo Clin Proc* 1987; 62: 601-6.
- [11] Förstermann U, Mügge A, Bode SM, Frölich JC. Response of human coronary arteries to aggregating platelets: Importance of endothelium-derived relaxing factor and prostanooids. *Circ Res* 1988; 63: 306-12.
- [12] Lüscher TF, Diederich D, Siebenmann R *et al.* Difference between endothelium-dependent relaxations in arterial and in venous coronary bypass grafts. *N Engl J Med* 1988; 319: 462-7.
- [13] Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986; 315:1046-51.
- [14] Linder L, Kiowski W, Bühler FR, Lüscher TF. Indirect evidence for the release of endothelium-derived relaxing factor in the human forearm circulation in vivo: Blunted response in essential hypertension. *Circulation* 1990; 81: 1762-7.
- [15] Webb RC, Vander AJ, Henry JP. Increased vasodilator responses to acetylcholine in psychosocial hypertensive mice. *Hypertension* 1987; 9: 268-76.
- [16] Davies PF, Ganz D, Diel PS. Reversible microcarrier-mediated junctional communication between endothelial cells and smooth muscle cell monolayers: An in vitro model of vascular cell interactions. *Lab Invest* 1985; 85: 710-18.
- [17] Davies PF, Olesen S-P, Clapham DE, Morrel EM, Schoen FJ. Endothelial communication. *Hypertension* 1988; 11: 563-72.
- [18] Boulanger C, Hendrickson H, Lorenz RR, Vanhoutte PM. Release of different relaxing factors by cultured porcine endothelial cells. *Circ Res* 1989; 64:1070-8.

- [19] Cocks TM, Angus JA, Campbell JH, Campbell GR. Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. *J Cell Physiol* 1985; 123: 310-20.
- [20] Griffith TM, Edwards DH, Lewis MJ, Newby AC, Henderson AH. The nature of endothelium-derived vascular relaxant factor. *Nature* 1984; 308: 645-7.
- [21] Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 1986; 320: 454-6.
- [22] Lückhoff A, Busse R, Winter I, Bassenge E. Characterization of vascular relaxant factor released from cultured endothelial cells. *Hypertension* 1987; 9: 295-303.
- [23] Rubani GM, Lorenz RR, Vanhoutte PM. Bioassay of endothelium-derived relaxing factor(s): inactivation by catecholamines. *Am J Physiol* 1985; 249: H95-H101.
- [24] Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am J Physiol* 1986; 250: H822-H827.
- [25] Furchgott RF. Studies on relaxation of rabbit aorta by sodium nitrite: the basis for the proposal that acid-activatable inhibitory factor from bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor in nitric oxide. In: Vanhoutte PM, ed. *Vasodilation: vascular smooth muscle, peptides, autonomic nerves and endothelium*. New York, NY: Raven Press, 1988: 401-14.
- [26] Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacological and chemical properties identical to those of nitric oxide radical. *Circ Res* 1987; 61: 866-79.
- [27] Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524-6.
- [28] Chen W, Palmer RMJ, Moncada S. Release of nitric oxide from rabbit aorta. *J Vase Med Biol* 1989; 1: 2-6.
- [29] Myers PR, Minor RL, Guerra R Jr, Bates JN, Harrison DG. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* 1990; 345: 161-3.
- [30] Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; 333: 664-6.
- [31] Rees DD, Palmer RMJ, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *Br J Pharmacol* 1989; 96: 418-24.
- [32] Yang Z, von Segesser L, Bauer E, Stulz P, Tschudi M, Lüscher TF. Differential activation of the endothelial L-arginine and cyclooxygenase pathway in the human internal mammary artery and saphenous vein. *Circ Res* 1991; 68: 52-60.
- [33] Tschudi M, Richard V, Biihler FR, Lüscher TF. Importance of endothelium-derived nitric oxide in intramyocardial porcine coronary arteries. *Am J Physiol* 1990; 260: H13-H20.
- [34] Rees DD, Palmer RMJ, Moncada S. The role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proc Natl Acad Sci USA* 1989; 86: 3375-8.
- [35] Marietta MA. Nitric oxide: Biosynthesis and biological significance. *Trends Pharmacol Sci* 1989; 14: 488-92.
- [36] Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. *Proc Natl Acad Sci USA* 1990; 87: 682-5.
- [37] Palmer RMJ, Moncada S. A novel citrulline-forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. *Biochem Biophys Res Commun* 1989; 158: 384-52.
- [38] Flavahan NA, Shimokawa H, Vanhoutte PM. Pertussis toxin inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries. *J Physiol* 1989; 408: 549-60.
- [39] Richard V, Tanner FC, Tschudi M, Lüscher TF. Differential activation of the endothelial L-arginine pathway by bradykinin, serotonin and clonidine in porcine coronary arteries. *Am J Physiol* 1990; 259: H1433-H1439.
- [40] Flavahan NA, Vanhoutte PM. Pertussis toxin inhibits the basal and leukotriene C<sub>4</sub>-stimulated release of endothelium-derived relaxing factor(s) (Abstract). *FASEB J* 1989; 3/3: A533.
- [41] Shimokawa H, Flavahan NA, Vanhoutte PM. Natural course of the impairment of endothelium-dependent relaxations after balloon endothelium-removal in porcine coronary arteries. *Circ Res* 1989; 63: 740-53.
- [42] Busse R, Fichtner H, Lückhoff A, Kohlhardt M. Hyperpolarization and increased free calcium in acetylcholine-stimulated endothelial cells. *Am J Physiol* 1988; 255: H965-H969.
- [43] Olesen S-P, Clapham DE, Davies PF. Haemodynamic shear stress activates a K<sup>+</sup> current in vascular endothelial cells. *Nature* 1988; 331: 168-70.
- [44] Olesen S-P, Davies PF, Clapham DE. Muscarinic-activated K<sup>+</sup> current in bovine aortic endothelial cells. *Circ Res* 1988; 62: 1059-64.
- [45] Lambert TL, Kent RS, Whorton AR. Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. *J Biol Chem* 1986; 261: 15288-93.
- [46] Loeb AL, Izzo NJ, Johnson RM, Garrison JC, Peach MJ. Endothelium-derived relaxing factor release associated with increased endothelial cell inositol trisphosphate and intracellular calcium. *Am J Cardiol* 1988; 62: 36G-10G.
- [47] Rapoport RM. Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidyl-inositol hydrolysis in rat aorta. *Circ Res* 1986; 58: 407-10.
- [48] Rapoport RM. Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidyl-inositol hydrolysis in rat aorta. *Circ Res* 1986; 58: 407-10.
- [49] Resink TJ, Grigorian GY, Moldabaeva AK, Danilov SM, Biihler FR. Histamine-induced phosphoinositide metabolism in cultured human umbilical vein endothelial cells. Association with thromboxane and prostacyclin release. *Biochem Biophys Res Commun* 1987; 144: 438-46.
- [50] Lewis MJ, Henderson AH. A phorbol ester inhibits the release of endothelium-derived relaxing factor. *Eur J Pharmacol* 1987; 137: 167-71.
- [51] Long CJ, Stone TW. The release of endothelium-derived relaxant factor is calcium dependent. *Blood Vessels* 1985; 22: 205-8.
- [52] Singer HA, Peach MJ. Calcium- and endothelial-mediated vascular smooth muscle relaxation in rabbit aorta. *Hypertension* 1982; 4 (Suppl II): 19-25.
- [53] Winquist RJ, Bunting PB, Schofield TL. Blockade of endothelium-dependent relaxation by the amiloride analog dichorobenzamil: Possible role of NA<sup>+</sup>/Ca<sup>++</sup> exchange in the release of endothelium-derived relaxing factor. *J Pharm Exp Ther* 1985; 235: 644-50.
- [54] Johns A, Lategan TW, Lodge NJ, Ryan US, van Breemen C, Adams DJ. Calcium entry through receptor-operated channels in bovine pulmonary artery endothelial cells. *Tissue Cell* 1987; 19: 733-45.
- [55] Lodge NJ, Adams DJ, Johns A, Ryan US, van Breemen C. Calcium activation of endothelial cells. In: the proceedings of the second international symposium on resistance arteries. Halpern W, Pegram BL, Brayden JE, Mackey K, McLaughlin MK, Osol G, eds. Ithaca, NY: Perinatology Press, 1988; 152-61.
- [56] Loeb AL, Izzo NJ, Johnson RM, Garrison JC, Peach MJ. Endothelium-derived relaxing factor release associated with increased endothelial cell inositol trisphosphate and intracellular calcium. *Am J Cardiol* 1988; 62: 36G-10G.
- [57] Lückhoff A, Busse R. Increased free calcium in endothelial cells under stimulation with adenine nucleotides. *J Cell Physiol* 1986; 126: 414-20.
- [58] Lückhoff A, Pohl U, Miilsch A, Busse R. Differential role of extra- and intracellular calcium in the release of EDRF and



- prostacyclin from cultured endothelial cells. *Br J Pharmacol* 1988; 95:189-96.
- [59] Schilling WP, Ritchie AK, Navarro LT, Eskin SG. Bradykinin-stimulated calcium influx in cultured bovine aortic endothelial cells. *Am J Physiol* 1988; 255: H219-H227.
- [60] Rubanyi GM, Schwartz A, Vanhoutte PM. The calcium agonist Bay K 8644 and (+) 202,791 stimulate the release of endothelial relaxing factor from canine femoral arteries. *Eur J Pharmacol* 1985; 117:143-4.
- [61] Rubanyi GM, Schwartz A, Vanhoutte PM. The effect of diltiazem and verapamil on endothelium-dependent responses in canine blood vessels (Abstract). *Pharmacologist* 1985; 27: 290.
- [62] Rubanyi GM, Schwartz A, Vanhoutte PM. Calcium transport mechanisms in endothelial cells regulating the synthesis and release of endothelium-derived relaxing factor. In: Vanhoutte PM, ed. *Relaxing and contracting factors: biological and clinical research*. Clifton, NJ: Humana Press, 1988:179-88.
- [63] Spedding M, Schini V, Schoeffter P, Miller RC. Calcium channel activation does not increase release of endothelium-derived relaxant factors (EDRF) in rat aorta although release of EDRF may modulate calcium channel activity in smooth muscle. *J Cardiovasc Pharm* 1986; 8: 1130-7.
- [64] De Mey J, Vanhoutte PM. Interaction between Na<sup>+</sup>, K<sup>+</sup> exchanges and the direct inhibitory effect of acetylcholine on canine femoral arteries. *Circ Res* 1980; 46: 826-36.
- [65] De Mey JG, Vanhoutte PM. Anoxia and endothelium-dependent reactivity in canine femoral artery. *J Physiol (London)* 1983; 335: 65-74.
- [66] Furchgott RF: Role of the endothelium in responses of vascular smooth muscle. *Circ Res* 1983; 53: 557-73.
- [67] Griffith TM, Edwards DH, Lewis MJ, Newby AC, Henderson AH. Production of endothelium-derived relaxant factor is dependent on oxidative phosphorylation and extracellular calcium. *Cardiovasc Res* 1986; 20: 7-12.
- [68] Martin W, White DG, Henderson AH. Endothelium-derived relaxing factor and atriopeptin II elevate cyclic GMP levels in pig aortic endothelial cells. *Br J Pharmacol* 1988; 93: 229-39.
- [69] Schini VB, Boulanger C, Regoli D, Vanhoutte PM. Production of cyclic GMP by kinins in cultured porcine aortic endothelial cells (Abstract). *FASEB J* 1989; 3/3: A533.
- [70] Schini V, Grant NJ, Miller RC, Takeda K. Morphological characterization of cultured bovine aortic endothelial cells and the effects of atriopeptin II and sodium nitroprusside on cellular and extracellular accumulation of cyclic GMP. *Eur J Cell Biol* 1988; 47: 53-61.
- [71] Evans HG, Smith JA, Lewis MJ. Release of endothelium-derived relaxing factor is inhibited by 8-bromo-cyclic guanosine monophosphate. *J Cardiovasc Pharmacol* 1988; 12: 672-77.
- [72] Boulanger C, Lüscher TF. Release of endothelium-derived relaxing factor in porcine resistance arteries: Inhibition by nitric oxide. *J Clin Invest* 1990; 85: 587-90.
- [73] Lüscher TF, Boulanger C, Yang Z, Dohi Y. Interaction between endothelin and endothelium-derived relaxing factors. In: Rubanyi, GM, ed. *Endothelin*. Oxford, UK: Oxford University Press: 1991 (in press).
- [74] Boulanger CM, Lüscher TF. Heparin and nitric oxide donors inhibit the thrombin-induced release of endothelin from the intact porcine aorta. *Circ Res* 1991; 68:1768-72.
- [75] McCall TB, Boughton-Smith NK, Palmer RMJ, Whittle BJR, Moncada S. Synthesis of nitric oxide from L-arginine by neutrophils. *Biochem J* 1989; 261: 293-6.
- [76] Rimele TJ, Sturm RJ, Adams LM, Henry DE, Heaslip RJ, Weichman BM, Grimes D. Interaction of neutrophils with vascular smooth muscle: Identification as a neutrophil-derived relaxing factor. *J Pharmacol Exp Ther* 1988; 245: 102-11.
- [77] Hibbs Jr JB, Taintor RR, Vavrin Z. Macrophage cytotoxicity: Role for L-arginine deiminase and imino nitrogen oxidation to nitrite. *Science* 1987; 235:473-6.
- [78] Garthwaite J, Charles SL, Chess-Williams R: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature* 1988; 336: 385-7.
- [79] Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. *Nature* 1983; 306:174-6.
- [80] Förstermann U, Mielsch A, Böhme E, Busse R. Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived factor from rabbit and canine arteries. *Circ Res* 1986; 58: 531-8.
- [81] Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ Res* 1983; 52: 352-7.
- [82] Martin W, Villani GM, Jothianandan D, Furchgott RF. Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. *J Pharmacol Exp Ther* 1985; 232: 708-16.
- [83] Martin W, Furchgott RF, Villani GM, Jothianandan D. Phosphodiesterase inhibitors induce endothelium-dependent relaxation of rat and rabbit aorta by potentiating the effects of spontaneously released endothelium-derived relaxing factor (EDRF). *J Pharmacol Exp Ther* 1986; 237: 539-7.
- [84] Ganz P, Davies PF, Leopold JA, Gimbrone MA, Alexander RW. Short- and long-term interactions of endothelium and vascular smooth muscle in coculture: Effects of cyclic GMP production. *Proc Natl Acad Sci USA* 1986; 83: 3552-6.
- [85] Godfraind T, Egleme C, Osachie IA. Role of endothelium in the contractile response of rat aorta a-adrenoceptor agonists. *Clin Sci* 1985; 68 (Suppl 10): 65-71.
- [86] Collins P, Lewis MJ, Henderson AH. Endothelium-derived factor relaxes vascular smooth muscle by cyclic GMP-mediated effects on calcium movements. In: Vanhoutte PM, ed. *Relaxing and contracting factors: biological and clinical research*. Clifton, NJ: Humana Press, 1988: 267-83.
- [87] Popescu LM, Panoiu C, Hinescu M, Nutu O. The mechanism of cGMP-induced relaxation in vascular smooth muscle. *Eur J Pharmacol* 1985; 107: 393-4.
- [88] McNicol A, MacIntyre DE. Reversal of agonist-induced phosphoinositide hydrolysis in human platelets by cAMP, cGMP and 1,2-diacylglycerol. *Br J Pharmacol* 1980; 61:159-65.
- [89] Rapoport RM. Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphatidylinositol hydrolysis in rat aorta. *Circ Res* 1986; 58: 407-10.
- [90] Takai Y, Karbuchi K, Matsubara T, Mishiyuka Y. Inhibitory action of guanosine 3',5'-monophosphate on thrombin-induced phosphatidylinositol turnover and protein phosphorylation in human platelets. *Biochem Biophys Res Comm* 1981; 101: 61-7.
- [91] Fiscus RR: Molecular mechanisms of endothelium-mediated vasodilation. *Semin Thromb Hemost* 1988; 14 (Suppl 1): 12-22.
- [92] Fiscus RR, Rapoport RM, Murad F. Endothelium-dependent and nitrovasodilator-induced activation of cyclic GMP-dependent protein kinase in rat aorta. *J Cycl Nucl Prot Phosphor Res* 1983-84; 9: 415-25.
- [93] Radomski MW, Palmer RMJ, Moncada S. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochem Biophys Res Comm* 1987; 148: 1482-9.
- [94] Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; 92: 639-46.
- [95] Alheid U, Frölich JC, Förstermann U. Endothelium-derived relaxing factor from cultured human endothelial

- cells inhibits aggregation of human platelets. *Thrombos Res* 1987;47:561-71.
- [96] Busse R, Lückhoff A, Bassenge E. Endothelium-derived relaxant factor inhibits platelet activation. *Naunyn-Schmiedeberg's Arch Pharmacol* 1987; 336: 566-71.
- [97] Azuma H, Ishikawa M, Sekizaki S. Endothelium-dependent inhibition of platelet aggregation. *Brit J Pharmacol* 1986; 88: 441-5.
- [98] Hogan JC, Lewis MJ, Henderson AH. In vivo EDRF activity influences platelet function. *Br J Pharmacol* 1988; 94: 1020-22.
- [99] Macdonald PS, Read MA, Dusting GJ. Synergistic inhibition of platelet aggregation by endothelium-derived relaxing factor and prostacyclin. *Thromb Res* 1988; 49: 437-46.
- [100] Radomski MW, Palmer RMJ, Moncada S. Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. *Br J Pharmacol* 1987; 92: 181-7.
- [101] Radomski MW, Palmer RMJ, Read NG, Moncada S. Isolation and washing of human platelets with nitric oxide. *Thrombos Res* 1988; 50: 537-46.
- [102] Rae GA, Trybulec M, de Nucci G, Vane Jr. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; 92: 639-46.
- [103] Sneddon JM, Vane JR. Endothelium-derived relaxing factor reduces platelet adhesion to bovine endothelial cells. *Proc Natl Acad Sci USA* 1988; 85: 2800-4.
- [104] Furlong B, Henderson AH, Lewis MJ, Smith JA. Endothelium-derived relaxing factor inhibits in vitro platelet aggregation. *Br J Pharmacol* 1987; 90: 687-92.
- [105] Mellion BT, Ignarro LJ, Ohlstein EH, Pontecorvo EG, Hyman AL, Kadowitz PJ. Evidence for the inhibitory role of guanosine 3', 5'-monophosphate in ADP-induced human platelet aggregation in the presence of nitric oxide and related vasodilators. *Blood* 1981; 57: 946-55.
- [106] Yang Z, Stulz P, von Segesser L, Bauer E, Turina M, Liischer TF. Platelets differently interact with arterial and venous coronary bypass grafts: Role of endothelium-derived nitric oxide and antiplatelet drugs. *Lancet* 1991; 337: 939-43.
- [107] Vidal-Ragout MJ, Romero JC, Vanhoutte PM. Endothelium-derived relaxing factor inhibits renin release. *Eur J Pharmacol* 1988; 149:401-2.
- [108] Lorenz RR, Sanchez-Ferrer CF, Burnett JC, Vanhoutte PM. Influence of endocardial-derived factor(s) on the release of atrial natriuretic factor (Abstract). *FASEB Journal* 1988; 2: 1293.
- [109] Shirasaki T, Su C, Lee TJ-F, Kolm P, Cline Jr WH, Nickols GA. Endothelial modulation of vascular relaxation to nitrovasodilators in aging and hypertension. *J Pharmacol Exp Ther* 1986; 239: 861-866.
- [110] Pohl U, Busse R. Endothelium-derived relaxant factor inhibits effects of nitrocompounds in isolated arteries. *Am J Physiol* 1987; 252: H307-H313.
- [111] Diederich D, Yang Z, Böhler FR, Liischer TF. Impaired endothelium-dependent relaxations in hypertensive resistance arteries involve the cyclooxygenase pathway. *Am J Physiol* 1990; 258: H445-H451.
- [112] Liischer TF, Richard V, Yang Z. Interaction between endothelium-derived nitric oxide and SIN-1 in human and porcine blood vessels. *J Cardiovasc Pharmacol* 1990; 14 (Suppl. 11); 76-80.