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Review article

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SECRETORY FUNCTIONS OF THE VASCULAR ENDOTHELIUM

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The endothelial cells which line the blood vessels as a monolayer exert a remarkable control over the vascular system. Indeed, the endothelium can be regarded as a highly active metabolic and endocrine organ in its own right. On the hand, vasoactive substances such as serotonin and bradykinin are inactivated and on the other the cells can enzymatically produce the vasoconstrictor, angiotensin II and secrete endothelin-1 ((ET-1). Perhaps more importantly, the cells also produce two unstable vasodilator substances, which potently inhibit platelet clumping: prostacyclin and endothelium-derived relaxing factor (EDRF) which has been identified as nitric oxide (NO; 1). Both substances seem well designated as local hormones, released to influence adjacent cells. The endothelial cell, therefore, exerts control over the cardiovascular system by elaborating dilator substances as well as vasconstrictors.

K e y w o r d s: *endothelium, endothelin-1, prostacyclin, nitric oxide*

This account deals with the functions of prostacyclin, NO and the powerful vasconstrictor, ET-1 as well as the interactions between these mediators.

VASORELAXANT FACTORS

Physical or chemical perturbation of, or damage to the cell membrane, results in the formation of prostacyclin (2), which was discovered in 1976 by Vane and his colleagues. Thus, pulsatile pressure, as well as a number of endogenous mediators and some drugs, stimulate its generation. Some endogenous stimulators include substances derived from plasma such as bradykinin or thrombin, or those liberated from activated platelets such as serotonin, platelet-derived growth factor, interleukin-1 or adenine nucleotides. Largely the same stimuli lead to the release of NO from endothelial cells (3).

It is important to understand that psysiologically, prostacyclin is a local hormone rather than a circulating one. In this context, prostacyclin release by endothelial cells affects the local environment, on the abluminal side causing relaxation of the underlying smooth muscle and in the lumen, preventing platelets and perhaps other blood cells from clumping onto the endothelium. When assay systems for prostacyclin and its breakdown product, 6-keto-$PGF_{1\alpha}$ were in their infancy, high blood levels were reported but as the assays have become more refined, it is clear that blood levels (in the region of 3pg/ml) are too low to have a generalised effect (4). Thus, any prostacyclin in the circulation most likely represents an „overflow" after it has had its local action.

NO (or EDRF) is also secreted from endothelial cells (5). It too is a typical local hormone, only effective in the immediate vicinity of the cell which releases it. Any that escapes into the bloodstream would decay chemically within a few seconds, were it not immediately inactivated by haemoglobin. The inactivation of NO by haemoglobin suggests that the vasoconstriction associated with subarachnoid haemorrhage may be due to removal by haemoglobin of a basal NO release (6).

Prostacyclin is generated from arachidonic acid which is converted into prostaglandin (PG) endoperoxides by the enzyme cyclooxygenase. Prostacyclin synthase subsequently forms prostacyclin from the endoperoxide, PGH_2. NO is formed from L-arginine by a constitutive enzyme in endothelial cells which needs cofactors such as NADPH, tetrahydrobiopterin and calcium/calmodulin (7). An isoform of this synthase from rat cerebellum has been cloned and expressed (8) and it is likely that there is a whole family of NO synthases. Interestingly, endothelial cells have mechanisms for maintaining their concentrations of free arginine including intracellular generation (9), by recycling L-citrulline (10).

A better understanding of the physiological importance of prostacyclin and NO comes from the actions of substances which prevent their release. For instance, aspirin and similar substances prevent prostacyclin formation, but have little effect on normal blood pressure. This allows us to conclude that endothelial production of prostacyclin plays little part in normal contol of the blood pressure. However, inhibitors of NO production, such as NG-monomethyl-L-arginine (MeArg) or N^{ω}-nitro-L-arginine give an immediate increase in blood pressure (11), strongly suggesting that a basal production of NO is a physiological mechanism to actively dilate the normal circulation. The rise in blood pressure lasts for an hour or more, but is dramatically reversed by the injection of the natural substrate for NO synthase, L-arginine. Clearly, a failure of this continuous NO release could contribute to hypertension. Indeed, there are many experimental results showing that vessels from hypertensive animals release less NO than those from normal ones. In patients

with essential hypertension the release of NO from the brachial artery by acetylcholine is reduced (12). There is also strong evidence that the vessels of healthy humans are continuously dilated by NO released from endothelial cells for injection of MeArg intra- arterially into the human forearm causes a substantial vasoconstriction which lasts for 45—60 min unless it is reversed by L-arginine (13).

NO relaxes vascular smooth muscle and potently inhibits aggregation and adhesion of platelets by raising intracellular cyclic GMP. The vasodilator and anti-platelet actions of prostacyclin on the other hand are mediated by an increase in the concentrations of cyclic AMP in smooth muscle cells and platelets. Importantly, there is clear synergism between the anti-aggregatory effects of prostacyclin and NO on platelets (14).

That activation of the same receptors, or a change in membrane conformation induced by shear stress, will lead to the release of both NO and prostacyclin suggests that these substances act in concord a common mechanism of defence for the endothelium (15). The synergism between NO and prostacyclin in preventing platelet activation then takes on a new significance, particularly as some circulating blood cells also release NO and can interact with the endothelium (16).

Another clue to the physiological role of prostacyclin and NO comes from the well recognised fact that damage to or perturbation of cell membranes leads to increased production of prostaglandins. This, taken together with the ability of smooth muscle cells to synthesize prostacyclin suggests that the prostacyclin system is a mechanism held in reserve to re-inforce the NO system locally when endothelial damage occurs. For example, when part of an artery becomes denuded of endothelial cells, platelets stick to the damaged area to begin the repair process. The loss of NO production allows platelet adhesion as a monolayer or carpet, but the generation of prostacyclin by the subendothelium will prevent clumps from forming, for prostacyclin potently inhibits aggregation but not adhesion.

With major damage to a vessel, by rupture or by a cut, NO and prostacyclin will work together to prevent intraluminal aggregation of platelets but allow extra-luminal adhesion. It is also possible that they work separately or in concord to provide an important defence against intravascular thrombosis and atherosclerosis.

The capacity of vascular tissue to generate prostacyclin decreases with age, in dabetes and in atherosclerosis (3), suggesting a direct link between prostacyclin biosynthesis in the vessel wall and its susceptibility to thrombotic or atherosclerotic episodes. Prostacyclin increases the activity of enzymes which metabolize cholesterol esters in smooth muscle cells, suppresses the accumulation of cholesterol esters by macrophages and prevents release of growth factors which cause thickening of the vascular wall (17). In this

context, some of the cardiovascular consequences of heavy smoking may be linked to inhibition of prostacyclin synthesis by nicotine (18), although tobacco smoke may contain other substances toxic to the cardiovascular system.

There is a substantial body of evidence from animal studies that lack of NO can also contribute to the aetiology of a number of diseases including hypertension, atheroslerosis and diabetes (for review, see ref. 3).

In human subjects acetylcholine by releasing NO dilated healthy coronary arteries but not atherosclerotic ones (19). Thus, decreased formation of NO may be a contributory factor to atherosclerosis although the thickening of the vessel wal could also form a barrier for the penetration of NO to the smooth muscle (20). Indeed, oxidised low density lipoproteins, which are abundant in atherosclerotic states, block endothelium-dependent relaxations of rabbit aortic strips (21). Interestingly, nitric oxide (22) and sodium nitroprusside (23) inhibit mitogenesis of fibroblasts (22) and cultured smooth muscle cells (23). An anti-proliferative action of NO produced by the endothelial cell could prevent the hypertrophy of smooth muscle which takes place during the development of atherosclerosis.

NO produced by an inducible synthase enzyme acts as the mediator of some pathological processes. For example, in endotoxin shock, escherichia coli lipopolysaccharide or tumour necrosis factor cause a fall in blood pressure by inducing an endothelial NO synthase (24). Similarly, induced NO production in macrophages is responsible for their cytotoxic and bactericidal functions (25). An enzyme induced by cytokines in endothelial cells generates NO which destroys by lysis cultured tumour cells (26).

The instability of prostacyclin makes it cumbersome to administer therapeutically. However, it has a limited clinical indication for preventing blood coagulation and preserving platelets in extracorporeal circulations (27). Of greater therapeutic import is the beneficial effect seen in blinded clinical trials after intravenous infusions for several hours over several days in peripheral vascular diseases including Reynaud's phenomenon. A constant infusion has also controlled primary pulmonary hypertension (28). Promising results have also been obtained in severe congestive heart failure (29).

Stable analogues such as iloprost are in late clinical trial (30, 31) for peripheral vascular disorders. An important blinded study (32) compared intravenous daily 6h infusions of iloprost for up to 28 days with oral aspirin in thromboangitis obliterans. After 21—28 days 58 out of 68 iloprost-treated patients showed ulcer healing or relief from ischaemic pain compared with 11 of 65 in an aspirin-treated group. Six months after start of treatment the response rate was 88% in patients treated with iloprost compared with 21% of those on aspirin. Orally active analogues such as cicaprostand beraprost are also progressing to the clinic (33).

The discovery of the arginine-NO-cyclic GMP system will lead to new therapeutic agents specifically designed to release NO at sites where its generation may be failing. Similarly, as did the forging of the aspirin-prostaglandin link in 1971, the new knowledge will highlight new uses for the present nitrovasodilators (34).

VASOCONSTRICTOR FACTORS

In addition to the dilator factors prostacyclin and NO, the vascular endothelium generates at least three vasoconstrictor substances (35). One is angiotensin II and the others have been postulated variously to be cyclooxygenase products or the superoxide anion. One has been properly identified by the discovery of endothelin-1 (ET-1) by Masaki et al at Tsukuba University (36). It is a 21 amino acid peptide bound together by two disulfide bridges (Cys1-Cys15 and Cys3-Cys11) and resembles closely the structure of the sarafotoxins found in the venom of the Isreali burrowing asp, Atractaspis engaddensis (37).

There are three structurally and pharmacologically different isopeptides in human and other mammalian species, called ET-1, ET-2 and ET-3. ET-1 is the only endothelin to be made by endothelial cells. We do not yet know where ET-2 is made, unless it is in the kidneys. ET-3 is mainly associated with nervous tissue.

Like many other biologically active peptides, ET-1 is produced from a prepropeptide. The production of the active peptide involves the formation of a 38 or 39 amino acid „big" ET and then an unusual proteolytic cleavage between a Trp and Val residue by a putative endopeptidase called "endothelin-converting enzyme". This converting enzyme may well prove to be an important pharmaceutical target for control of ET-1 release, especially if ET-1 is involved in hypertension or vasospastic disorders (38). Several non-specific peptidases which can be inhibited with phosphoramidon convert big ET to ET-1. The enzymes in 10 polymorphonuclear leukocytes are particularly active and provide rapid conversion (39). Interestingly, phosphoramidon lowers the blood pressure of spontaneously hypertensive rats (40).

Most vasoactive hormones can be released almost explosively by different types of stimuli but no such rapid release of ET-1 has been demonstrated from cells or organs. Many substances including thrombin, adrenaline or calcium ionophore A23187 cause the slow release of immunoreactive ET-1 from cultured endothelial cells (36). Some of these releasers act by receptor-mediated mechanisms.

Plasma levels of ET-1 in healthy humans as measured by radioimmunoassay have been estimated at 0.26 to 5 pg/ml (38). However, the concentration of

ET-1 at the endothelium/smooth muscle interface is likely to be much higher (because of the small volume distribution) than in the blood stream. ET-1 is, therefore, more likely to be a local rather than systemic regulating factor.

Plasma concentrations of "big" ET-1 ranged from 3.2 to 14 pg/ml and were doubled in acute myocardial infarction (41). It was unexpected to find "big" ET-1 in the circulation for although it has little of the activity of ET-1 its release by the endothelial cells may still have effects on the underlying vascular smooth muscle. Furthermore, it raises the possibility that "big" ET-1 could be converted to ET-1 in cells other than endothelial cells (42).

The most striking property of ET-1 is the long lasting hypertensive action. ET-1 is the most active pressor substance yet discovered, with a potency some ten times that of angiotensin II. After a single intravenous injection into pithed or chemically-denervated rats, the blood pressure was elevated for more than one hour (36). In other species, intravenous infusions of ET-1 also produced intense vasoconstriction ad rises in mean arterial blood pressure (43). In contrast to this prolonged hypertensive activity, ET-1 was quickly eliminated from the bloodstream of the rat, over 60% disappearing after 1 minute, with high uptake in the lung, kidney and liver (44). Intense vasoconstriction and decrease in blood flow were also recorded in human vessels when ET-1 was infused into the brachial artery or injected intradermally (45).

The vasculature of the kidney is about ten times more sensitive than other organs to the vasoconstrictor effects of ET-1 (43). As a result of the increased vascular resistance and contraction of mesangial cells, glomerular filtration rate and urine volume were substantially reduced. ET-1 also inhibited renin relase from isolated kidney preparations (46), but increased release in anaesthetised dogs (47).

Of the other actions of ET-1, one of the most interesting is the ability to stimulate mitogenesis. The mitogenic effects of ET-1 have been shown in cardiovascular smooth muscle cells, in fibroblasts and in mesangial cells (48). Since NO and ET-1 exert opposite effect on the glomerulus and mesangial cells, they may provide a regulatory role for glomerular function.

The potent vasconstrictor action taken together with the raised plasma levels detected in myocardial infarction and pulmonary and essential hypertension may argue for involvement of ET-1 in hypertensive states (49). Release by ET-1 aldosterone (50) and catecholamines (51) from the adrenal glands would contribute to the hypertension as also would the possible release of renin. It is interesting that the sensitivity of renal artery segments to ET-1 was greater in spontaneously hypertensive than in normal rats (52).

ET-1 may have a role in acute renal failure (53), for it is a powerful renal vasoconstrictor and high plasma levels (20 pg/ml) have been reported in hemodialysis or kidney transplant patients (54). This may reflect reduced elimination of the peptide or increased synthesis by diseased kidney tissues.

Immunoreactive ET-1 is secreted by cultured kidney cells (55) and has also been reported in the urine of healthy human volunteers in concentrations six times higher than those detected in plasma (56).

The discovery of ET-1 adds a new dimension to the role of endothelial cells in disease states, and raises many important questions as to its patho-physiological function. Does secretion of ET-1 contribute to some forms of hypertension? The answer will surely come when inhibitors of the "converting" enzyme become available, or when antagonists for the ET-1 receptor are made.

Does secretion of ET-1 influence the genesis or progression of atherosclerosis? Studies with cultured rat aortic smooth muscle cells have shown that ET-1 stimulates their proliferation (48). This trophic effect could account for the vascular wall hypertophy typical of atherosclerosis and hypertension as would the proliferation of mesangial cells.

Finally, does ET-1 have any physiological role? Our working hypothesis is that ET-1 is a local hormone, released by the endothelial cells to constrict the underlying smooth muscle. The pressor activity of any ET-1 released luminally into the circulation would be strongly ameliorated by removal in the lungs and by release of the vasodilators prostacyclin and NO (see later). Interestingly, suppression of NO formation in isolated arteries by MeArg leads to an increase in ET-1 release (57), suggesting a further intracellular feedback between the systems.

The widespread distribution of ET-1 receptors and the potent pharmacological actions of ET-1 strongly argue for a regulatory function in the cardiovascular system. Because of its slow release and long action it is likely that ET-1 would be involved in long-term (hours or days) regulation of this system. Alternatively, ET-1 could be produced locally in an emergency or in defensive events such as haemostasis or wound repair. The indications that mRNA for preproendothelin is induced by factors such as thrombin and transforming growth factor-p, known to be produced at the site of injury, would lend support to this idea. It is interesting that immunoreactive-endothelin is present in higher concentrations in the fetal circulation than in maternal blood and may produce potent and long-lasting constriction of the umbilical blood vessels just after delivery (58).

INTERACTIONS BETWEEN VASOACTIVE FACTORS

When administered intravenously into rats, the pressor activity of ET-1 is limited by the release of prostacyclin and NO (59). For instance, in pithed rat preparations (60) and in rabbits anaesthetised with sodium pentobarbitone (61) inhibition of cyclooxygenase with indomethacin caused significant augmentation of pressor responses to ET-1.

Additionally, when the resting blood pressure is high, the prolonged pressor effect of a bolus injection of ET-1 is preceded by a transient, dose-related vasodepressor response (36). The abolition of this depressor effects by the specific inhibitor of NO synthesis, MeArg and reversal of the block by L-arginine confirmed NO release (62). However, in less well controlled experiments, MeArg did not inhibit the depressor action of ET-1 in conscious rats (63).

The generation of eicosanoids (59, 64) may, therefore, contribute importantly to the overall haemodynamic effects of ET-1. Certainly, prostacyclin, thromboxane (TX) A_2 and PGE_2 are released from perfused isolated organs of seaeral species by ET-1 or ET-3. For example, both ET-1 and ET-3 released prostacyclin and PGE_2 from perfused rabbit and rat kidneys (65). However, prostacyclin is the only prostanoid consistently released from all isolated organs tested.

The vasoconstrictor action of ET-1 can be counterbalanced in many systems by the release of NO. Thus, ET-1 elicited release of NO from endothelial cells lining the rabbit perfused isolated aorta and from rat mesentery preparations (66) clearly demonstrating that the release of NO can substantially limit the pressor action of ET-1 in these vascular beds. Fukuda et al. (67) subsequently showed that the vasodilating effects of low doses of ET-3 in rat perfused mesenteric arteries were abolished by an infusion of MeArg, thus confirming the release of NO by ET-3.

The anti-platelet effects of ET-1 on *ex vivo* and *in vivo* platelet aggregation in anaesthetized rabbits were also mediated by the release of an anti-aggregatory prostaglandin, most likely prostacyclin as well as of tPA into the circulation. Indomethacin abolished both the ET-1 induced inhibition of platelet aggregation and the corresponding rise in platelet cyclic AMP (68). That these effects were most likely due to the release of prostacyclin was confirmed by raised levels of 6-oxo-PGF_{1a} in plasma (69). They were unlikely to be due to release of NO, which also inhibits platelet function (70) for NO does not survive in the bloodstream.

Thiemermann et al., (1990) (71) showed that ET-1 inhibited ADP-stimulated aggregation of ^{111}indium-labelled platelet *in vivo* measured by scintillation probes in the anaesthetized rabbit. This was abolished by indomethacin which also significantly potentiated and prolonged the pressor response brought about by ET-1. Thus, ET-1 potently inhibited platelet aggregation in the anaesthetised rabbit *in vivo* by releasing a hypotensive and anti-aggregatory cyclo-oxygenase product, presumably prostacyclin, into the circulation.

It has been suggested that ET-1 and ET-3 release prostacyclin and NO by activating different membrane receptors from those which mediate contraction of smooth muscle. In fact, two distinct ET-1 receptors, each coupled to

a G protein, have been described. One (the ETA receptor) shows high specificity for ET-1 and the messenger RNA is widely distributed in vascular smooth muscle, the central nervous system, the heart and the lungs (72). The other (ETB) equally accepts all three endothelins, as well as sarafotoxins and is coupled through a G protein to phospholipase C, leading to transient increases in intracellular free Ca^{2+} (73).

The fibrinolytic system in rabbits is activated by intra-arterial injections of ET-1. When applied directly to euglobulin clots in vitro the peptide did not induce fibrinolysis, but blood samples taken from rabbits injected with ET-1 showed a significant, biphasic reduction of the euglobulin clot lysis time. One minute after administration of ET-1 there was a transient but significant increase in blood tissue plasminogen (tPA) antigen levels which returned to basal values during the course of the experiment (74). A late onset fibrinolytic effect which occurred 30—60 min after injection of ET-3 matched the fibrinolysis observed after an injection of prostacyclin and was prevented by pretreatment of the rabbits with indomethacin. Thus, the second phase of fibrinolysis after ET-1 injection was probably due to the release of prostacyclin (75).

The competition between ET-1 and NO is further emphasised since NO released from endothelial cells can inhibit production of ET-1. Preincubation of intact pig aortae with the inhibitor of NO synthesis, MeArg, potentiated the thrombin-stimulated but not the basal release of ET-1 (57). Thus, under normal physiological conditions NO may suppress the elaboration of ET-1. However, impaired production of NO as for example in hyperlipidaemia, hypertension or atherosclerosis could result in the unopposed release of ET-1 and pathological constriction of diseased blood vessels (3).

It is clear that much more remains to be learnt about the interactions between the three major vasoactive mediators of the vascular endohelium, prostacyclin, NO and ET-1.

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REFERENCES

1. Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327:524-526.
2. Piper P, Vane JR. The release of prostaglandins from lung and other tissues. *Ann NY Acad Sci* 1971; 180:363-385.
3. Gryglewski RJ, Botting RM, Vane JR. Mediators produced by the endothelial cell. *Hypertension* 1988; 12:530—548.

4. Blair IA, Barrow SE, Waddell KA, Lewis PJ, Dollery CT. Prostacyclin is not a circulating hormone in man. *Prostaglandins* 1982; 23:579—589.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288:373—376.
6. Kanamaru K, Waga S, Kojuma T, Fujimoto K, Niwa S. Endothelium-dependent relaxation of canine basilar arteries, Part 2. Inhibition by hemoglobin and cerebrospinal fluid from patients with aneurysmal subarachnoid hemorrhage. *Stroke* 1987; 18:938—943.
7. Bredt DS, Snyder SH. Isolation of nitric oxide synthetase, a calmodulin requiring enzyme.*Proc Natl Acad Sci USA* 1990; 87:682—685.
8. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome. *Nature* 1991; 351:714—718.
9. Mitchell JA, Hecker M, Vane JR. The generation of L-arginine in endothelial cells is linked to the release of endothelium-derived relaxing factor. *Eur J Pharmacol* 1990; 176:253—254.
10. Hecker M, Mitchell JA, Harris HJ, Katsura M, Thiemermann C, Vane JR. Endothelial cells metabolize NG-monomethyl-L-arginine to L-citrulline and subsequently to L-arginine. *Biochem Biophys Res Commun* 1990; 167:1037—1043.
11. Moncada S, Palmer RMJ, Higgs EA. Biosynthesis of nitic oxide from L-arginine. A pathway for the regulation of cell function and communication. *Biochem Pharmacol* 1989, 38:1709—1715.
12. Panza JA, Quyyumi AA, Brush Jr JE, Epstein SE. Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N. Engl J. Med* 1990; 323:22—27.
13. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 1989; 2:997—1000.
14. Radomski MW, Palmer RMJ, Moncada S. The anti-aggregatory properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987; 92:639—646.
15. De Nucci G, Gryglewski RJ, Warner TD, Vane JR. Receptor-mediated release of endothelium-derived relaxing factor and prostacyclin from bovine aortic endothelial cells is coupled. *Proc Natl Acad Sci USA* 1988; 85:2334—2338.
16. Salvemini D, de Nucci G, Gryglewski RJ, Vane JR. Human neutrophils and mononuclear cells inhibit platelet aggregation by releasing a nitric oxide-like factor. *Proc Natl Sci USA* 1989; 86:6328—6332.
17. Willis AL, Smith DL, Vigo C, Kluge AF. Effects of prostacyclin and orally active stable mimetic agent RS-93427-007 on basic mechanisms of atherogenesis. *Lancet* 1986; 2:682—683.
18. Dadak CH, Leithner C, Sinzinger H, Silberbauer K. Diminished prostacyclin formation in umbilical arteries of babies born to women who smoke. *Lancet* 1981; 1:94.
19. Ludmer PL, Selwyn AP, Shook PL. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986; 315:1046—1051.
20. Fostermann U, Mugge A, Alheid U, Haverich U, Frolich JC. Selective attenuation of endothelium mediated vasodilation in atherosclerotic human coronary arteries. *Circ Res* 1988; 62:185—190.
21. Jacobs M, Plane F, Bruckdorger KR. Native and oxidized low-density lipoproteins have different inhibitory effects on endothelium-derived relaxing factor in the rabbit aorta. *Br J Pharmacol* 1990; 100:21—26.
22. Barret ML, Willis AL, Vane JR. Inhibition of platelet-derived mitogen release by nitric oxide (*EDRF*). *Agents Actions* 1989; 27:488—491.
23. Garg UC, Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989; 83:1774—1777.

24. Kilbourn RG, Gross SS, Jubran A et al. N^G-methyl-Larginine inhibits tumour necrosis factor-induced hypotension: implications for the involvement of nitric oxide. *Proc Aatl Acad Sci USA* 1990; 87:3629—3632.
25. Adams LB, Hibbs JB, Taintor RR, Krahenbuhll JL. Microbiostatic effect of murine-activated macrophages for Toxoplasma gondii. *J Immunol* 1990; 144:2725—2729.
26. Li L, Kilbourn RG, Adams J, Fidler IJ. Role of nitric oxide in lysis of tumour cells by cytokine-activated endothelial cells. *Cancer Res* 1991; 51:2531—2535.
27. Longmore DB, Hoyle PM, Gregory A, et al. Prostacyclin administration during cardiopulmonary bypass in man. *Lancet* 1981; 2:800—804.
28. Jones DK, Higenbottam TW, Wallwork J. Treatment of primary hypertension with intavenous epoprostenol (prostacyclin). *Br Heart J* 1987; 57: 270—278.
29. Yui Y, Nakajima H, Kawai H, Kawai C, Murakami T. Prostacyclin therapy in patients with congestive heart failure. *Am J Cardiol* 1982; 50:320—324.
30. Yardumian DA, Isenberg DA, et al. Successful treatment of Raynaud's syndrome with iloprost,a chemically stable prostacyclin analogue. *Br J Rheumatol* 1988; 27:220—226.
31. Rademaker M, Cooke ED, Almond NE, et al. Comparison of intravenous infusions of iloprost and oral nitedipine in treatment of Raynaud's phenomenon in patients with systemic sclerosis: double blind randomised study. *Br J Med* 1989; 298:561—564.
32. Feissinger JN, Schafer M, Angelides NS. For the TAO Studa. Trial of iloprost versus aspirin treatment for critical limb ischaemia of thromboangitis obliterans. *Lancet* 1990; 335:55—557.
33. Sturzebecher S, Hildebrand M, Schobel C, Seifert W, Staks T. Platelet inhibitory and haemodynamic effects of a new stable PGI_2 analogue, cicaprost (ZK 96480), in different animal species and in man. *Biomed Biochim Acta* 1988; 47:S45—S47.
34. Korbut R, Lidbury PS, Vane JR. Prolongation of fibrinolytic ativity of tissue plasminogen activator by nitrovasodilators. *Lancet* 1990; 335:669.
35. Rubanyi GM. Endothelium-derived vasconstictor factor. In Endothelial cells, Vol 3, US Ryan (ed.) Florida, CRC Press, *Boca Raton,* 1988, pp. 61—74.
36. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; 332:411—415.
37. Landan G, Bdolah A, Wollberg Z, Kochva E, Graur D. Evolution of the sarafotoxin/endothelin superfamily of proteins. *Toxicon* 1987; 29:237—244.
38. Cernacek P, Stewart DJ. Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. *Biochem Biophys Res Commun* 1989; 161:562—567.
39. Sessa WC, Kaw S, Hecker M, Vane JR. The biosynthesis of endothelin-1 by human polymorphonuclear leukocytes. *Biochem Biophys Res Commun* 1991; 174:613—618.
40. McMahon EG, Palomo MA, Moore WM. Phosphoramidon blocks the pressor activity of big endothelin [1-39] and lowers blood pressure in spontaneously hypertensive rats. *J Cardiovasc Pharmacol* 1991; 17 (Suppl. 7) 529—533.
41. Miyauchi T, Yanagisawa M, Tomizawa T, et al. Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1989; 2:53—54.
42. Kashiwabara T, Inagaki Y, Ohta H, et al. Putative precursors of endothelin have less vasoconstrictor activity in vitro but a potent pressor effect in vivo. *FEBS Lett* 1989; 247:73—746.
43. Pernow J, Franco-Cereda A, Matran R, Lundberg JM. Effect of endothelin-1 on regional vascular resistances in the pig. *J Cardiovasc Pharmacol* 1989; 13 (Suppl 5):S205—S206.
44. Anggard E, Galton S, Rae G, et al. The fate of radioiodinated endothelin-1 and endothelin-3 in the rat. *J Cardiovasc Pharmacol* 1989; 13 (Suppl 5):S46—S49.
45. Brain SD, Crossman DC, Buckley TL, Williams TJ. Endothelin-1: demonstration of potent effects on the microcirculation of human and other species. *J Cardiovasc Pharmacol* 1989; 13 (Suppl 5):S147—S149.

46. Matsumura Y, Makase K, Ikegawa R, Hayashi K, Ohyama T, Morimoto S. The endothelium-derived vasoconstrictor peptide endothelin inhibits renin release in vitro. *Life Sci* 1989; 44:149—157.
47. Miller WL, Redfield MM, Burnett Jr JC. Integrated cardiac, renal, and endocrine actions of endothelin. *J Clin Invest* 1989; 83:317—320.
48. Kohno M, Yasunari K, Murakawa K-I et al. Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 1990; 88:614—618.
49. Saito Y, Nakao K, Yamada T et al. Plasma endothelin1-like immunoreactivity level (ET-1-LI) in healthy subjects and patients with hypertension. *Hypertension* 1989; 14:335—336 (abstract).
50. Cozza EN, Gomez-Sanchez CE, Foecking MF, Chiou S. Endothelin binding to cultured calf adrenal zona glomerulosa cells and stimulation of aldosterone secretion. *J Clin Invest* 1989; 84:1032—1035.
51. Boarder MR, Marriott DB. Characterization of endothelin-1 stimulation of catecholamine release from adrenal chromaffin cells. *J Cardiovasc Pharmacol* 1989; 13 (Suppl. 5): S223—S224.
52. Tomobe Y, Miyauchi T, Saito A et al. Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. *Eur J Pharmacol* 1988; 152:373—374.
53. Firth JD, Raine AEG, Ratcliffe, PJ Ledingham JGG. Endothelin: an important factor in renal failure? *Lancet* 1988, 2:1179—1182.
54. Tomita K, Ujlie, K, Nakanishi T et al. Plasma endothelin levels in patients with acute renal failure. *N Engl J Med* 1989; 321:1127.
55. Kosaka T, Suzuki N, Matsumoto H et al. Synthesis of the vasconstrictor peptide endothelin in kidney cells. *FEBS Lett* 1989; 249:42—46.
56. Berbinschi A, Ketelslegers J. Endothelin in urine. *Lancet* 1989; 2:46.
57. Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990; 85:587—590.
58. Nakamura T, Kasai K, Konuma S et al. Immunoreactive endothelin concentrations in maternal and fetal blood. *Life Sci* 1990; 46:1045—1050.
59. De Nucci G, Thomas R, D'Orleans-Juste P et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. *Proc Natl Acad Sci USA* 1988; 85:9797—9800.
60. Walder C, Thomas R, Thiemermann C, Vane JR. The haemodynamic effects of endothelin-1 in the pithed rat. *J Cardiovasc Pharmacol* 1989; 13 (Suppl. 5):S93—S97.
61. Thiemermann C, Lidbury PS, Thomas GR, Vane JR. Comparison of the haemodynamic and platelet inhibitory effects of endothelin-1 and endothelin-3 in the anaesthetized rabbit. In *Endothelium-derived Contracting Factors* Rubanyi GM, Vanhoutte PM (eds) Basel, Kargel 1990; pp. 128—134.
62. Whittle BJR, Lopez-Belmonte J, Rees DD. Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *Br J Pharmacol* 1989; 98:646—652.
63. Gardiner SM, Compton AM, Bennett T, Palmer RMJ, Moncada S. The effects of N^G-monomethyl-Larginine (L-NMMA) on the haemodynamic actions of endothelin-1 in conscious Long Evans rats. *Br J Pharmacol.* 1989; 98:626P.
64. Karwatowska-Prokopczuk E, Wennmalm A. Effects of endothelin on coronary flow, mechanical performance, oxygen uptake and formation of purines and on outflow of prostacyclin in isolated rabbit heart *Circ Res* 66:46—54.
65. Trybulec M, Dudek RR, Gryglewski RJ. Effects of endothelin-1 and endothelin-3 on the release of prostanoids from isolated perfused rat kidney. *J Cardiovasc Pharmacol* 1991; 17 (Suppl. 7):S229—S232.

66. Warner TD, Mitchell JA, de Nucci G, Vane JR. Endothelin-1 and endothelin-3 release EDRF from isolated perfused arterial vessels of the rat and rabbit. *J Cardiovasc Pharmacol* 1989; 13 (Suppl. 5):S85—S88.
67. Fukuda N, Izumi Y, Soma M et al. L-N^G-monomethyl arginine inhibits the vasodilating effects of low dose of endothelin-3 on rat mesenteric arteries. *Biochem Biophys Res Commun* 1990; 167:739—745.
68. Thiemermann C, LidburyPS, Thomas GR, Vane JR. Endothelin-1 releases prostacyclin and inhibits ex vivo platelet aggregation in the anaesthetized rabbit. *J Cardiovasc Pharmacol* 1989; 13 (Suppl. 5):S138—S141.
69. Herman F, Magyar K, Chabrier P-E, Braquet P, Filep J. Prostacyclin mediates antiaggregatory and hypotensive actions of endothelin in anaesthetized beagle dogs. *Br J Pharmacol* 1989; 98:38—40.
79. Azuma H, Ishikawa M, Sekizaki S. Endothelium-dependent inhibition of platelet aggregation. *Br J Pharmacol* 1986; 88:411—415.
71. Thiemermann C, May GR, Page CP, Vane JR. Endothelin-1 inhibits platelet aggregation in vivo: a study with ^{111}lundium-labelled platelets. *Br J Pharmacol* 1990; 99:303—308.
72. Lin HY, Kaji EH, Winkel GK, Ives HE, Lodish HF. Cloning and functional expression of a vascular smooth muscle endothelin v1 receptor. *Proc Natl Acad Sci USA* 1991; 88:3185—3189.
73. Sakurai T, Yanagisawa M, Takuwa Y et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. *Nature* 1990; 348:732—735.
75. Lidbury PS, Thiemermann C, Korbut R, Vane JR. Endothelins release tissue plasminogen activator and prostanoids. *Eur J Pharmacol* 1990; 186:205—212.
75. Korbut R, Lidbury PS, Thomas GR, Vane JR. Fibrinolytic activity of endothelin-3. *Thromb Res* 1989; 55:797—799.

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