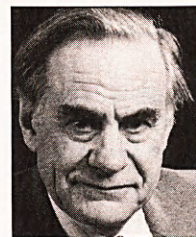


Endothelium, for example



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ABSTRACT—Endothelial nitric oxide production contributes at many levels to cardiovascular efficiency (considered as tissue perfusion relative to cardiac work). Endothelial dysfunction is found in many conditions, including all known risk factors for atheroma, and is usually generalised, when present, involving microvessels as well as large arteries, impairing cardiovascular efficiency and potentially initiating atheroma. Loss of flow-related dilation is a manifestation of endothelial dysfunction and can be measured non-invasively, thereby providing a potential marker of reversible atherogenic susceptibility.

Who would have thought that before 1980 the cardiovascular system would be controlled by an endothelial gas—and controlled so beautifully? The story, yet to be fully told, well exemplifies the fascinating unpredictability of research and the vistas it can unexpectedly open up.

Endothelium-derived relaxing factor (EDRF) was discovered by serendipity—a familiar route. Pharmacologists had long known that acetylcholine constricted their arterial preparations *in vitro* whereas it was a vasodilator *in vivo*. Zawadzki, Furchgott's technician, chanced upon the explanation—and incidentally the existence of EDRF—when he skilfully if inadvertently preserved the usually damaged endothelial layer of his arterial strip preparations and found that acetylcholine then relaxed them, as it does *in vivo* [1]. The experiment is now a classic. Our coincidental observations were not dissimilar. In developing an isolated perfused coronary artery preparation we found, contrary to expectation, that as we gained experience they contracted less well—unless they were accidentally damaged, when a reversible constrictor response re-emerged at the site of the injury. Bioassay experiments confirmed that the agent responsible was transmissible and showed that it had a half-life of only seconds [2]. A number of workers began to appreciate from its characteristics that it might be nitric oxide (NO) [3], as was subsequently confirmed [4]. This simple gas is now known to be an important and ubiquitous local intercellular signal [5–9]. It is of primitive evolutionary origin [10], being found in blood-sucking insects which cleverly use NO the better to avail themselves of

their host's blood supply; also in the horseshoe crab which has not evolved for some five million years, and even in the hydra, that most primitive of invertebrates with any pretence to a neurological system. While NO is the endogenous analogue of an established pharmacological agent, the 'lumped' response to an administered nitrovasodilator drug should in no way be equated with the integrating physiological action of locally released endogenous NO.

Physiology of EDRF

NO coordinates and fine-tunes the whole cardiovascular system and its discovery has opened a new chapter in cardiovascular physiology.

NO is generated from its substrate, L-arginine, by NO synthase, of which there are three forms—neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) (often called cNOS to distinguish its constitutive nature from iNOS). eNOS can be activated by receptor-G protein coupling in response to a wide variety of agonists, which may be broadly classified into platelet release products (eg 5-hydroxytryptamine), neurotransmitters (eg substance P, acetylcholine) or circulating hormones (eg bradykinin). Notably, it can also be stimulated by longitudinal shear stress generated by blood flow. Endothelium thus responds to changes in blood flow and also to the frequency of its pulsatility, which induces its maximal response at about 300 beats per minute (independently of species-related natural heart rate)—nicely coupling flow-related vasodilatation to the increase in heart rate usually associated with increased cardiac output [11,12]. NO has an even shorter half-life *in vivo* than *in vitro* and thus acts very locally: it is readily oxidised by oxygen and oxygen free radicals, and it is avidly bound to the haem moiety in haemoglobin and red cells [13], as well as in soluble guanylate cyclase, the enzyme responsible for mediating its main action. The consequent elevation of cytosolic cyclic GMP reduces levels of activating calcium at a number of control points as well as the responsiveness of contractile proteins to calcium [14–18]. NO thus relaxes vascular smooth muscle, particularly when it is stimulated to contract by activation of the phosphoinositol pathway [16,18], and acts as a *vasodilator*. It also mediates its own *negative feedback*, because cyclic GMP by reducing cytosolic calcium reduces the signal which activates eNOS [19,20].

NO also prevents *platelet adhesion and aggregation*

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[21] synergistically with cyclic AMP-dependent mechanisms such as are stimulated by adenosine or prostacyclin [22].

NO (from coronary microvessels and potentially from endocardial endothelium) also modulates *myocardial contraction* (Fig 1). It increases diastolic compliance and shortens the duration of contraction, with little or no depression of systolic contraction, thereby favouring diastolic filling [23–26]. Increased NO release due to increased coronary flow and heart rate thus has the potential to optimise cardiac pump function. A more stable endogenous endothelial agent with similar effects on cardiac contraction, mediated by reducing contractile protein sensitivity to calcium, has also been demonstrated though not yet identified [27]. These actions have been confirmed in the isolated intact heart [28] and are opening interesting new avenues of clinical research [29] both for potential new therapeutic agents and in elucidating effects of known agents. ACE inhibitors, for example, not only limit tissue angiotensin II production but also reduce the breakdown of bradykinin which itself stimulates NO release, thereby contributing to myocardial [28]

as well as vascular consequences for contraction and growth.

An important consequence of the short half-life of NO is that its action is limited to the site of its release, with no intravascular downstream effect. Since NO production is related to shear stress, this provides an elegant means of coordinating changes in vascular calibre throughout the bed in response to changes in flow induced, for example, by metabolic or pharmacological stimuli.

Increases in flow and pressure each have chronic as well as acute effects in large arteries. An acute increase in flow raises NO production, dilating the vessel and so normalising shear stress. A chronic increase upregulates eNOS [30] and further increases NO production; in addition, it enlarges the intravascular diameter by endothelium-dependent remodelling, so chronically normalising shear stress. An acute increase in intravascular pressure induces myogenic constriction. A chronic increase in pressure increases ACE expression [31] with consequent angiotensin II-mediated growth of vascular smooth muscle cells. These physiological adaptive mechanisms are context to the interactive processes

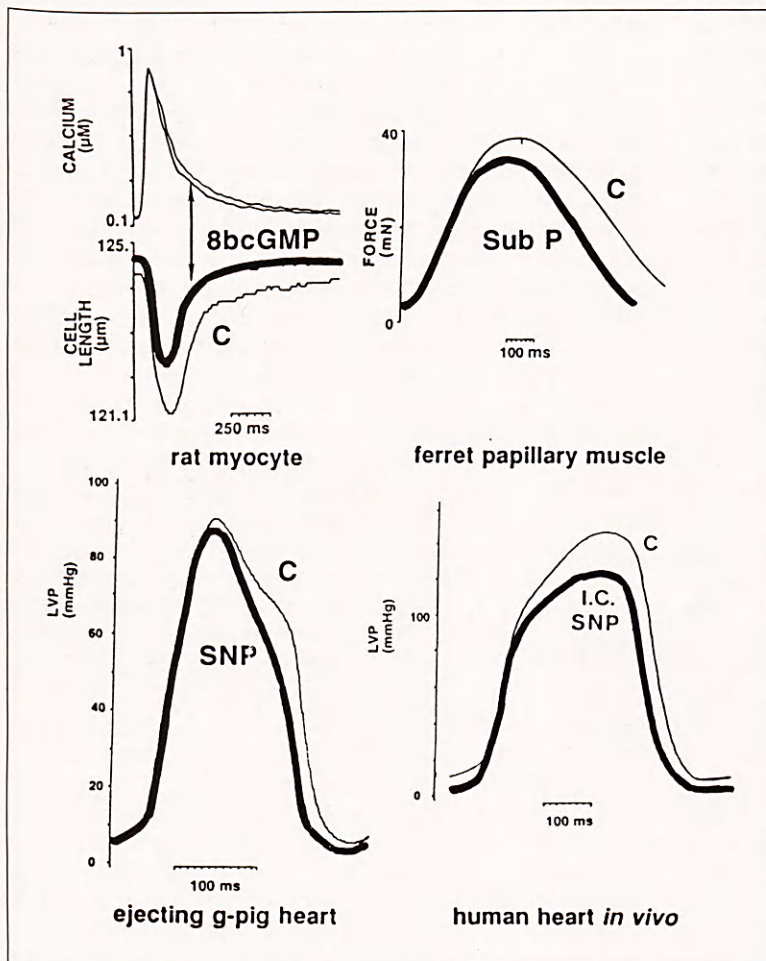


Fig 1. Effect of EDRF/NO/cyclic GMP on cardiac contraction. The effects of endogenous NO (EDRF, as stimulated by substance P (sub P)), exogenous NO (sodium nitroprusside (SNP)), and 8-bromo-cGMP ((8bcGMP), the mediator of NO effects) on cardiac contraction and cytosolic calcium in isolated myocytes, isolated heart muscle, isolated perfused hearts, and clinically. Contraction is abbreviated and diastolic compliance increased by a direct action on contractile protein response, cytosolic calcium remaining unaltered. C = control.

underlying atheroma, restenosis after angioplasty and vein graft intimal hyperplasia (see below).

Resistance arteries

Vasomotor influences are physiologically important in these vessels for they control the distribution of flow and blood pressure. Experiments with eNOS 'knock-out' mice confirm its role in maintenance of blood pressure [32]. EDRF is indeed a potent, local, flow-related vasodilator in resistance arteries. Its effects must, however, be considered interactively with the other control mechanisms *in vivo*—metabolic, myogenic and neurohumoral—with complex upstream-downstream interactions (Fig 2). The ability of a vascular bed efficiently to deliver variable flow rates in response to varying regional demand, buffered against changes in central blood pressure, and subject to neurohumorally mediated changes in the circulation is, unsurprisingly, the result of a finely integrated system of multiple controls, interacting through consequent changes in flow and pressure. The development of a novel microangiographic technique to measure the behaviour of microvessels simultaneously throughout the bed has proved useful [33,34]. Metabolic dilatation, acting through adenosine-stimulated cyclic AMP and K_{ATP} channels, acts predominantly at the smallest arterioles (< 0.1 mm diameter) [35]. EDRF acts more at slightly larger vessels, amplifying and coordinating the effect of other dilator influences throughout the vascular bed. An increase in metabolic signal can compensate for loss of EDRF if, but only if, the lowered overall ceiling of dilator reserve remains adequate to meet the metabolic demand [36]. Flow-related dilatation represents a positive feedback mechanism which opposes and thus stabilises the positive feedback of the myogenic response in which an increase in intravascular pressure induces vasoconstriction [37]. EDRF

modulates the myogenic response which mediates autoregulation of flow at different pressures [37]. EDRF activity appears from experimental studies also to be necessary for maintenance of the distribution of perfusion at different overall flow rates [33], from which it follows that loss of EDRF activity may lead to heterogeneity of perfusion.

Spontaneous vasomotion and 'chaos'

Blood vessels exhibit spontaneous vasomotion. The vasomotion appears random. Its amplitude is exaggerated in disease states. Non-linear mathematical analysis (by 'chaos' theory) of the phenomenon has shown that it is not in fact random but deterministic, or 'chaotic' in the technical sense of the term [38]. Such behaviour results from the interaction of three or more non-linear variables. It reflects here the dynamic adjustment of component contractile mechanisms in response to changing conditions. This has been viewed as parsimony of genetic content—it would be unnecessarily 'expensive' for tone to be very tightly coupled to changes in physiological need whereas, conversely, some degree of resultant temporal heterogeneity of perfusion might make for adaptability and physiological advantage. Analysis of spontaneous vasomotion along these lines gives information about the number of controlling variables, or component mechanisms responsible for the phenomenon [38]. Experiments using pharmacological probes to block specific mechanisms have identified which are responsible. Interestingly, EDRF is not one, despite the fact that it exerts a major and visible influence on the pattern and amplitude of vasomotion [39] (Fig 3). It may, however, serve to damp the amplitude of vasomotor activity and so maintain it within physiologically acceptable limits.

Fractal analysis of the geometry and branching angles of the vascular networks of small arteries, which

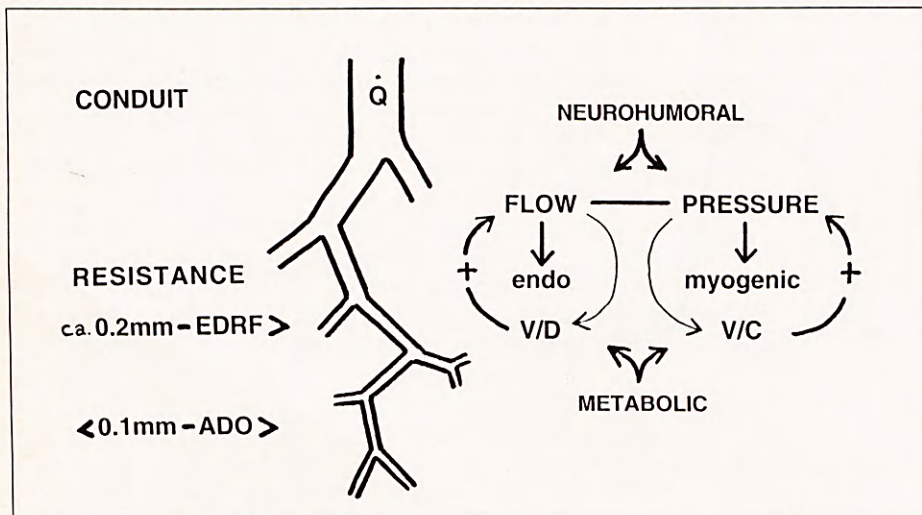


Fig 2. Interactive microvascular control. (i) Flow-related dilatation (+ feedback to flow (Q)), counter-balanced by (ii) myogenic constriction (+ feedback to pressure (P)), as influenced by (iii) metabolic dilatation (adenosine acts predominantly at < 0.1 mm diameter arterioles), under (iv) overall neurohumoral influence.

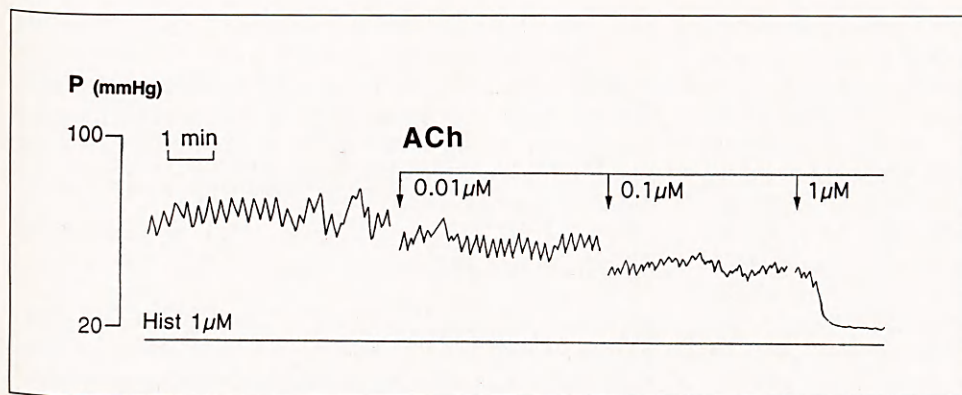


Fig 3. Spontaneous vasomotion and 'chaos'. Spontaneous vasomotion of isolated rabbit ear artery under vasoconstrictor influence by histamine (Hist), as visibly influenced by increasing EDRF activity induced by acetylcholine (ACh). Non-linear analysis shows that the vasomotion is 'chaotic', with a fractal dimension of 3, indicating ~3 contributing mechanisms responsible for the vasomotion. EDRF activity, though altering the amplitude and pattern of vasomotion, does not change the fractal dimension. EDRF thus exerts a damping influence on vasomotion without participating in its generation.

lie between the genetically determined anatomy of large arteries and the 'swamp' of cell-related capillaries, shows that their design optimises vascular volume and energy losses—a conclusion which holds true only in the presence of EDRF activity [40].

Endothelium and cardiovascular efficiency

EDRF may be seen as contributing in a number of ways to 'cardiovascular efficiency', considered in terms of tissue perfusion relative to cardiac work. We have shown in large arteries that increased flow increases distensibility by means of flow-related dilatation, with obvious physiological benefits for cardiac function [41]. Furthermore, since pulse wave velocity is inversely related to distensibility, EDRF activity results in reflected pressure waves returning more slowly to the heart, so that they do not add to systolic tension at the critical late phase of systole when increased tension can deactivate myocardial contraction. This may be particularly important in heart failure where EDRF activity is impaired, for loss of EDRF activity will then add to the load on an already prejudiced cardiac performance. In resistance arteries, loss of EDRF activity will exaggerate the pressure consequences of increased flow throughout the bed. Its coordinating and amplifying vasodilator effects will similarly be lost. The resultant heterogeneity of microvascular perfusion will lead either to patchily inadequate perfusion or to a metabolically driven increase in overall flow to meet the whole organ's needs—in either event, a loss of cardiovascular efficiency. Positive feedback from the myogenic response will be unopposed, so destabilising the system. The amplitude of spontaneous vasomotion will be unconstrained. Possible beneficial effects on myocardial diastolic compliance will be lost. The inescapable corollary is that impairment of EDRF activity will adversely affect overall cardiovascular efficiency on

many counts. The consequences of impaired EDRF activity are likely to be pervasive, albeit initially subtle. Whether they become manifest will depend on organ demands relative to perfusion reserve and on the sensitivity of the techniques used to measure them.

Pathophysiology of EDRF

EDRF activity is impaired in many conditions (hypercholesterolaemia, coronary artery disease, hypertension, smoking, diabetes, heart failure, reperfusion after ischaemia, repair after damage, renal failure, oestrogen lack, physical inactivity and ageing) [3–7]. When present, impairment usually involves both conduit and resistance arteries. Endothelium is strategically sited for signal transduction between circulating blood and the rest of the body, but it is also vulnerable to blood-borne insults. Normally, the life of an endothelial cell is some 30 years, but physical denudation removes this inhibition so that it rapidly proliferates to repair the damage, following which it remains phenotypically altered for weeks—as manifest, for example, by impaired EDRF activity. Likewise, reperfusion following ischaemia causes a burst of oxygen free radicals (OFRs) which rapidly damage endothelium and reduce EDRF activity. EDRF activity, moreover, is impaired by all known 'risk factors' for atheroma, a condition now generally accepted as initiated by endothelial dysfunction. This strongly suggests that these 'risk factors' cause endothelial dysfunction which precedes the development of atheroma (although it cannot necessarily be assumed that impairment of EDRF activity is always associated with the other phenotypic changes contributing to atherogenesis). EDRF activity is diminished also in chronic heart failure [41,42].

The mechanisms underlying impairment of EDRF activity may differ in different conditions. EDRF

activity will be determined by NO production, NO half-life and NO responsiveness. NO production may be impaired at the agonist-receptor coupling level or by limitation of substrate provision. Non-metabolised analogues of L-arginine (which are experimentally useful in measuring EDRF activity) may contribute to reduced EDRF activity in renal failure, for some are endogenous and renally excreted [43]. NO half-life will be shortened by interaction with superoxide (O_2^-). OFRs are a major determinant of NO activity in disease states. In hypercholesterolaemia, NO production is increased but EDRF activity is paradoxically reduced, due to an associated increase in O_2^- production [44]. EDRF activity can then be increased by L-arginine supplements [45,46], consistent with relative substrate depletion as NO production is increased (possibly by iNOS activation). OFRs account for the endothelial dysfunction induced by reperfusion after ischaemia [47] and possibly in heart failure [48] when elevation of angiotensin II is a component of the neurohumoral resetting. High intravascular pressure locally increases tissue ACE and angiotensin II in large arteries and reduces EDRF activity [31]. Nitrate tolerance likewise elevates tissue angiotensin II and O_2^- production and reduces EDRF activity [49]. Angiotensin II itself can reduce endothelial O_2^- production [50].

Treatment

Impairment of EDRF activity is not irreversible. Clinical trials in hypercholesterolaemic patients show that lowering serum cholesterol can, within months, improve endothelium-dependent coronary artery dilatation and flow, with some suggestion that a lipid-lowering drug with anti-oxidant activity (probucol) may be more effective than lipid lowering alone [51-53]. Fish oil [54] and exercise [55] have been shown to improve EDRF activity clinically, and so may L-arginine, ACE inhibitors and antioxidants.

EDRF activity can be measured by special investigations, eg coronary angiography and flow measurements, but it can also be measured non-invasively by ultrasound measurements of systemic conduit artery diameter. 'Wall tracking' of flow-related dilatation offers an exciting new approach to measuring generalised impairment of EDRF activity as surrogate for atherogenic potential. Precise measurements can be obtained in individual patients, and interventions to improve EDRF activity are being put to the test in relatively small and brief clinical trials. We have shown, for example, that flow-related dilatation is impaired in hypercholesterolaemia in relation to serum cholesterol levels [56], in heart failure [57], in non-insulin dependent diabetes [58] (*before* microalbuminuria appears), and in cardiac Syndrome X [59] (see below). Deanfield and colleagues, who introduced the method in principle, have similarly demonstrated loss of flow-related dilatation in a number of conditions, including hypercholesterolaemia [60] and smoking

[61]. Corroborative evidence relating such measures of endothelial function to the future development of clinical atheromatous disease will of course be needed, but the method holds promise of providing a measurable surrogate for atherogenic susceptibility. To have something to measure is a seductive stimulus to monitor management.

Endothelium and atherogenesis

In what ways may endothelium, and specifically NO, participate in the atherogenic process and its prevention? Atheroma may be considered as the consequence of a chronic inflammatory process in the intimal layers of large arteries, initiated by endothelial damage or dysfunction [62,63] (Fig 4). Endothelial dysfunction and activation can be induced by minimally oxidised low density lipoproteins (LDL) [64]. It can thus be encouraged by increased circulating levels of LDL; by increased transport of LDL through endothelium and binding to intimal glycosaminoglycans (GAGs) [65], allowing greater opportunity for oxygenation by OFRs derived from endothelial and other vascular cells; and by raised oxidant stress in the vascular wall. Once activated, endothelial cells express chemoattractants, adhesion molecules, growth factors and other cytokines [62,63]. This leads to a self-generating interactive cascade of similar phenotypic changes in other cells in the artery wall, such as smooth muscle cells and monocyte/macrophages. The consequences are that monocytes are attracted to endothelium and migrate into the intima, where they are transformed into macrocytes, and following LDL uptake, into foam cells; there also follows matrix remodelling and matrix dissolution by t-PA, u-PA and metalloproteinases [66], so freeing vascular smooth muscle cells embedded in the media to migrate into the intima [67] and there to proliferate [68]. Activated endothelial cells also show reduced EDRF activity with increased O_2^- production, each of which can contribute to this process. At every level of these interacting processes there is the potential for action and for counteraction, for regulation and counter-regulation, with much redundancy of paths. Atherogenesis may be seen as reflecting an altered balance between pro and anti-'inflammatory' components. It is becoming apparent that even vascular smooth muscle cell proliferation in the intima (a key component of atherogenesis as well as of restenosis following angioplasty) requires activation of more than one process, namely both matrix degradation and the stimulus to migrate and proliferate [68]. The cytokines, transforming growth factor- β (TGF- β) [69] and platelet derived growth factor (PDGF), together regulate extracellular matrix production and cell growth, acting via endothelial and smooth muscle cells (for editorial comment, see Ref 70). Effective therapeutic strategies will need to be focused on key common steps, such as TGF- β activation and *c-myc* induction [71], among multiple redundant mechanisms.

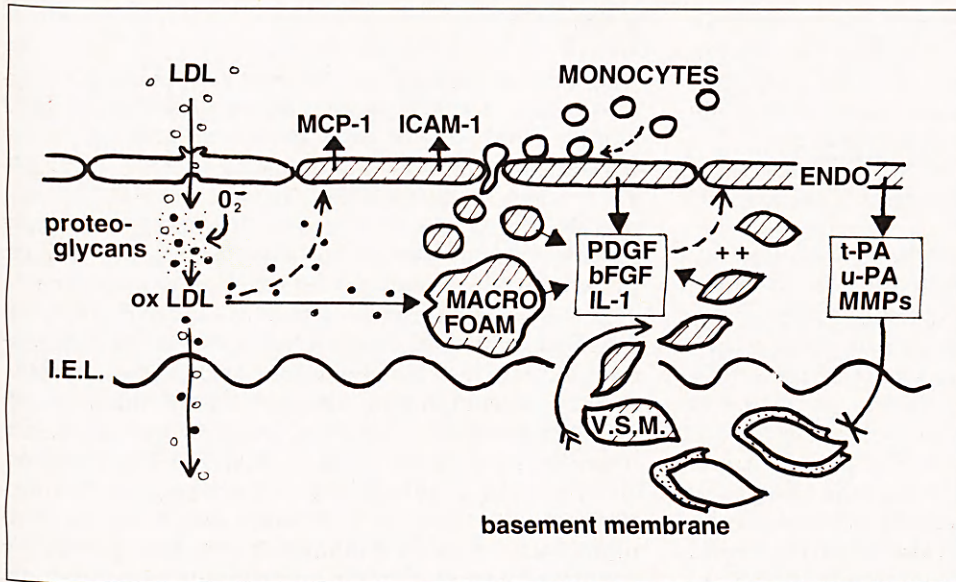


Fig 4. Main pathways in atherosclerosis. Low density lipoproteins (LDL) are oxidised by endothelium to minimally or more fully oxidised LDL (OxLDL), particularly when present in high concentration and for longer in the intima (as with high intravascular concentration, high driving pressure, and vasoconstricted state of the muscle 'sieve' in the media) where bound to intimal proteoglycans. OxLDL 'activate' (shaded) endothelial cells to express monocyte chemoattractant protein (MCP-1) and adhesion molecules (ICAM-1) which attract monocytes to adhere and migrate into the intima, there activated to macrophages which become foam cells following uptake of oxLDL via scavenger receptors. Activated endothelial cells express growth factors and cytokines (platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and interleukin-1 (IL-1)) attracting vascular smooth muscle cells to migrate from the media and proliferate in the intima. They also express tPA, uPA and metalloproteinases (MMPs) which degrade matrix, freeing smooth muscle cells to migrate. Activation (shaded) of endothelial cells, monocytes and vascular smooth muscle cells is regenerative, each expressing growth factors and cytokines. Regulatory and counter-regulatory processes exist and atherosclerosis depends on the balance between them. NO may be anti-atherogenic in that it scavenges O_2^- , can prevent monocyte attraction and intimal plaques in experimental hypercholesterolaemia, causes vasodilatation, increases cyclic GMP which is anti-proliferative, and will be present at higher concentration at sites of high shear stress where atheroma is less likely.

EDRF activity and atherosclerosis: marker or maker?

NO activity is impaired by endothelial dysfunction, and endothelial dysfunction plays a key role in initiating atherosclerosis. Loss of NO activity may be a marker of at least the potential for atheroma, or it may directly contribute to atherosclerosis. NO has a number of potential anti-atherogenic actions. Chappell and colleagues were among the first to show that EDRF activity was impaired in experimental dietary hypercholesterolaemia [72], an observation now widely confirmed. Smith and colleagues found that oxidised LDL activates protein kinase C (PKC) [73] which is now known to deactivate eNOS, adding yet another potential mechanism. The paradoxical increase in NO production with hypercholesterolaemia, despite reduced dilator activity (because of associated O_2^- production) [44,74], appears to be at least in part due to cytokine-induced activation of iNOS, as reported in hypercholesterolaemia [75] and in the comparable situation of experimental post-angioplasty restenosis [76]. NO inactivates OFRs, and anti-oxidant measures protect against experimental atheroma and vascular

smooth muscle proliferation. There is growing evidence that NO and interventions such as L-arginine which restore impaired EDRF activity, also prevent monocyte chemoattractant protein (MCP-1) expression and monocyte adhesion, O_2^- production and neo-intima formation in experimental atheroma. NO elevates cyclic GMP which is anti-proliferative *in vitro* [77]. Recently, NO has been shown also to inhibit activation of nuclear factor- κ B (NF- κ B), a transcription factor which may be involved in atherosclerosis at a number of points, including expression of MCP-1 and of NAD(P)H oxidase which increases O_2^- production [50,78]. NO vasodilates, relaxing the sieve of vascular smooth muscle in the media and so promotes faster transit of LDL through the artery wall. It is noteworthy that atheroma tends to occur at sites of low endothelial shear stress where NO production will be low. NO and O_2^- may indeed play central and opposing roles in controlling the balance between health and 'inflammation' (manifest as endothelial dysfunction, impaired EDRF activity, monocyte recruitment [79] and vascular smooth muscle cell proliferation, as in atherosclerosis if prolonged). Whereas NO can

scavenge O_2^- in appropriate relative concentrations, there is also the alternative outcome that the two can combine to form peroxynitrate ($ONOO^-$) which is more toxic though less stable and shorter lived. When OFRs are present in excess, low concentrations of NO may favour $ONOO^-$ formation and NO becomes cast as the villain, whereas in other circumstances excess NO scavenges O_2^- and NO is good news.

In more advanced atheroma, the scene changes. Relevant factors will be predominantly those which influence plaque stability and thrombosis. There may also be local endothelial denudation upon which platelets adhere and aggregate, releasing vasoactive products and growth factors: in the presence of healthy endothelium, these can induce EDRF release, vasodilatation and inhibition of further platelet aggregation (negative feedback), whereas in the absence of endothelium they have the opposite effect (positive feedback), thereby localising vasoconstriction and platelet aggregation to sites of endothelial injury. In the comparable situation of neointimal hyperplasia following experimental angioplasty exogenous NO donors inhibit platelet adhesion acutely [80] and chronically reduce the neointimal growth largely responsible for restenosis [81], suggesting that this line of potential therapy should not be ignored. Indeed, recent experiments with NOS gene transfer elegantly confirm its potential [82]. Once advanced lipid-filled atheromatous plaques have developed, they are vulnerable to fissuring at sites of weakness; thrombus then forms on the fissure, as influenced by flow and by the balance of pro- and anti-thrombotic and fibrinolytic factors. Relative proportions of macrophages (which produce O_2^-) and of vascular smooth muscle cells (which lay down fibrous matrix) are likely to determine sites of weakness and potential fissuring in the fibrous cap [83]. Whether adjacent endothelium then greatly influences subsequent thrombus formation directly is doubtful, since it is likely to be severely dysfunctional.

Microvascular endothelial dysfunction

Endothelial dysfunction, when present, is usually generalised. Microvascular endothelial dysfunction may thus commonly coexist with atheroma (clinically manifest largely as a result of late complications such as plaque rupture and thrombosis). Each, however, may be clinically silent until severe and/or complicated. The haemodynamic impact of endothelial dysfunction will be greater in microvessels than in large arteries. Haemodynamically relevant dysfunction may, however, be underestimated clinically because it is unrecognised or attributed to coexistent atheroma upstream. It is likely, for example, to contribute to the impairment of coronary perfusion reserve following successful thrombolysis. It may become clinically relevant with any of the known causes of endothelial dysfunction, including many of the manifestations of ageing itself.

Syndrome X

The term, as used by cardiologists, is applied as a diagnostic label to patients who experience unexplained angina, with positive exercise test and normal coronary angiogram [84]. The name stems from the early 1970s when the advent of catheterisation revealed that not all angina was what it seemed. Though in many cases it is due to non-cardiac chest pain, some of these patients undoubtedly do experience true angina of effort, due by inference, to microvascular dysfunction ('microvascular angina'). Efforts to incriminate the endothelium have generally been inconclusive or have failed to exclude other causes of endothelial dysfunction, but a recent report from Japan (where atheromatous coronary artery disease (CAD) is less common and the clinical substrate for microvascular dysfunction may be 'cleaner') provides good evidence of underlying coronary endothelial dysfunction [85]. We too have recently demonstrated endothelial dysfunction in a group of patients with microvascular angina in whom other known causes for endothelial dysfunction were excluded [59]. EDRF activity does indeed appear to be impaired in patients with Syndrome X, though whether this is sufficient cause for their clinical angina is another question.

Insulin resistance

Among patients with Syndrome X, moderate hypertension is relatively common though it is specifically excluded in most reported series. Diabetes, a condition known to be associated with small vessel disease, is likewise excluded from reported studies. The experimental combination of these two conditions results in foci of myocardial necrosis attributed to coronary microvascular dysfunction [86]. Clinically, both are associated with endothelial dysfunction and with atheroma. We therefore investigated whether syndrome X is associated with subclinical diabetes and found that glucose tolerance tests did indeed induce a hyperinsulinaemic response [87]. Insulin resistance has since been confirmed in patients with Syndrome X by a number of groups [88–90]. More recently we have shown that it is the levels of pro-insulin and split products which are raised, along with C-peptide (as a measure of insulin release from pancreatic β -cells), whereas true insulin levels (measured by enzyme-linked immunosorbent assay (ELISA)) remained entirely normal in response to a modified intravenous glucose tolerance test [91]. A high ratio of pro-insulin and split products to insulin is thought to reflect pancreatic β -cell dysfunction in insulin resistance and in non-insulin dependent diabetes, and may contribute to atheroma [92,93]. This observation in the absence of overt atheroma thus broadens the clinical issue of vascular dysfunction to include the microvessels, consistent with a unifying role for endothelial dysfunction. Moreover, microvascular angina is now to be added to

the growing list of conditions, which include hypercholesterolaemia, hypertension, diabetes and heart failure [94], in which endothelial dysfunction is associated with insulin resistance. Insulin resistance, like eNOS activity, is moreover influenced by physical activity [95]. The idea that endothelial dysfunction and insulin resistance may be causally related is beginning to take root. Insulin resistance is coupled to glucose uptake for which the mass of skeletal muscle is largely responsible, and increased glucose uptake appears to be due in part to an NO-mediated [96,97] increase in blood flow [98] as well as in arteriovenous glucose difference. This suggests that cellular perfusion is a limiting condition and raises the possibility that insulin resistance may be a *consequence* of inadequate tissue perfusion due to loss of EDRF activity (which may result in heterogeneity of perfusion, see above), either directly or as it mediates insulin's vasodilator effect.

Although the cardiologists's Syndrome X has totally different origins from the similarly named syndrome more recently used to label the epidemiological association between atheroma, hypertension, dyslipidaemia and insulin resistance [99], it is becoming evident that the two syndromes X have much in common. Indeed, insulin resistance is a suspiciously common participant of all conditions associated with atheroma, and its potential role in vascular disease is the subject of intense research reaching far beyond the territory of the diabetologists. Alternatively, it could be the marker of endothelial dysfunction as the real culprit—or it could both cause and effect. Pro-insulin and insulin have been reported to induce plasminogen activator inhibitor type-1 (PAI-1) [100], which in turn reduces TGF- β activation, so linking in with that line of thought in atherogenesis [69].

Now, the challenge will be to synthesise the whole from the growing number of interactive components, their redundancies, their checks and balances, and their disbalances in disease.

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