Characterization and biological significance of endothelium-derived relaxing factor

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Introduction

The endothelial cell is a highly metabolically active organ which plays a role in metabolizing vasoactive substances and also generates factors which modulate the interactions between platelets and the vessel wall. In 1976, Moncada and colleagues discovered prostacyclin [1], one of the most potent of these factors, which inhibits platelet aggregation and induces vasodilatation by stimulating adenylate cyclase. Recently, another product of vascular endothelium, endothelium-derived relaxing factor (EDRF; [2]), has been shown to account for the vasodilator properties of some pharmacological agents and to inhibit platelet aggregation. The chemical nature of EDRF has now been identified as nitric oxide (NO; [3]). In addition to its role as regulators of vascular smooth muscle tone, prostacyclin and NO also synergize to regulate platelet–vessel wall interactions.

Biological actions of EDRF

Acetylcholine is a potent vasodilator in vivo, but is frequently inactive or occasionally produces a small contraction of vascular rings or rings in vitro. The explanation of this phenomenon was provided by Furchgott & Zawadzki [2] when they showed that acetylcholine-induced vascular relaxation was dependent on the presence of the intact endothelium. Endothelial cells stimulated with acetylcholine release a factor (later called endothelium-derived relaxing factor) which diffuses to the underlying smooth muscle to cause relaxation.

The essential role of EDRF in mediating vascular relaxation induced by a number of substances such as bradykinin (BK), substance P and adenosine nucleotides, is now well recognized. Other substances, however, including the nitrovasodilators, atrial natriuretic factor, bovine retractor-penis inhibitory factor and prostacyclin, cause vascular relaxation by endothelium-independent mechanisms.

The biological activity of EDRF was first demonstrated with a variety of donor/detector systems (for reviews see [4, 5]). Such bioassay methods revealed that EDRF was a labile substance with a half-life of between 3 and 50 s. It also became apparent that a variety of seemingly unrelated compounds could inhibit endothelium-dependent relaxation in intact vascular preparations.

Later, it was shown that superoxide anions (O$_2^-$) contribute to the instability of EDRF [6]. Superoxide dismutase (SOD), by inactivating O$_2^-$, potentiates the activity of EDRF. It was also demonstrated that compounds such as phenidone, BW755C, dithiothreitol, hydroquinone and other redox compounds inhibit the action of EDRF via the generation of O$_2^-$ [7]. Haemoglobin, however, is another inhibitor of the action of EDRF and of endothelium-dependent relaxations which probably acts through a mechanism involving binding of the EDRF molecule rather than the generation of O$_2^-$.

EDRF is also an inhibitor of platelet aggregation. We have observed EDRF-induced inhibition of platelet aggregation, in human platelet-rich plasma and human washed platelets, induced by collagen, U46619, ADP and thrombin, and have shown that EDRF is equi-active against all these aggregating agents [8]. EDRF also induces disaggregation of platelets aggregated with collagen and with U46619 [9].

The inhibitory action of EDRF on platelet aggregation can be clearly differentiated from that of prostacyclin. Unlike prostacyclin, the effect of EDRF on platelets is potentiating by SOD and M&B 22948, a selective inhibitor of the cyclic GMP phosphodiesterase, and inhibited by Fe$^{3+}$, haemoglobin and hydroquinone. These effects are consistent with those observed on vascular strips. Moreover, the inhibitory effect on platelet aggregation is accompanied by an increase in platelet cyclic GMP and is of a short duration with a half-life of 2 min, while that of prostacyclin has a half-life of 4 min.

A number of authors have demonstrated a rise in smooth muscle cyclic GMP levels associated with endothelium-dependent relaxation or with EDRF-induced vascular relaxation. EDRF, in common with the nitrovasodilators, has been shown to activate soluble guanylate cyclase in smooth muscle cells [10]. Endothelium-dependent relaxation and the action of EDRF on platelets are prevented by agents which interfere with the activation of this enzyme, such as haemoglobin and Methylene Blue [11], and are potentiated by M&B 22948. These data support the concept that stimulation of soluble guanylate cyclase underlies the vascular relaxant and anti-aggregatory actions of EDRF.

Chemical identity of EDRF

The studies carried out on EDRF since its discovery suggested that it was a very unstable molecule, sensitive to inactivation by O$_2$ and O$_2^-$ [12]. These findings, together with results of experiments with sodium nitrite (NO$_2^-$), led Furchgott [12] to suggest that EDRF may be NO. He observed that acidified NO$_2^-$, an NO generator, exhibited similar pharmacology to EDRF on vascular preparations, since it was unstable and its action was inhibited by haemoglobin and potentiated by SOD. Ignarro and colleagues [13] also showed that acetylcholine- and NO-induced relaxations and accumulation of cyclic GMP in vascular smooth muscle resemble one another, which led them to suggest that EDRF was either NO or some closely related species.

We have now shown that EDRF is NO. A comparison of the pharmacological properties of authentic NO and endothelium-released EDRF demonstrated that both substances are indistinguishable. The relaxations of isolated strips of rabbit aorta caused by both compounds were inhibited to a similar extent by Fe$^{3+}$, hydroquinone, pyrogallol and haemoglobin. Both compounds were equally unstable and their stability was increased to a similar extent by SOD or cytochrome c [14]. In platelets, the anti-aggregating activity of EDRF and NO was potentiated by SOD and M&B 22948 and was inhibited by haemoglobin and Fe$^{3+}$ [8].

Final confirmation of the identity, however, required direct measurement of the release of NO by endothelial cells. To do this, we measured the chemiluminescent product of the reaction between NO and ozone by a method based on that previously described by Downes et al. [15]. Measurements of NO in the effluent of the columns containing endothelial cells revealed its presence in sufficient quantities to account for the biological activity observed on vascular strips and on platelets. Nitric oxide was not observed in the effluent of columns containing vascular smooth muscle cells or 3T3 cells [3].

These experiments clearly demonstrated that NO is EDRF and led us to suggest that NO fulfills the criteria for the identification of a biological mediator as originally defined by Dale [16]. It is interesting that NO, a compound...
suspected to be responsible for the vascular relaxation induced by the nitrovasodilators, is produced by vascular endothelial cells. This demonstrates once again, as in other biological systems, that synthetic chemicals often produce the action of endogenous mediators. Indeed, NO can now be considered the 'endogenous nitrate'.

Some authors have suggested the possibility that there may be more than one EDRF (see [17]). More experimental evidence will be required, however, before the existence of other soluble mediators of endothelium-dependent vasodilatation can be accepted, especially in view of the fact that NO provides such a comprehensive explanation for the pharmacological findings on EDRF so far produced.

Recently discovered actions of NO

Endogenous prostacyclin and NO synergize with each other to inhibit platelet activation. Indeed, the anti-aggregating activity of both EDRF and authentic NO are potentiated by subthreshold concentrations of prostacyclin. Similarly, NO and EDRF potentiate the anti-aggregating activity of prostacyclin [9]. We have also observed synergy between prostacyclin and NO on platelet disaggregation. These findings support the notion that prostacyclin and NO released in vivo may interact at far smaller concentrations than those detectable by non-biological methods. Prostacyclin may indeed have a physiological role in the control of platelet aggregation if it acts in the presence of a background of NO close to the endothelial surface and such an interaction may represent a regulating system for maintenance of platelet homeostasis in physiological conditions. A basic role of vascular endothelial cells in vivo has been reported [6, 11]. Whether this also occurs in vivo remains to be demonstrated.

Bradykinin, which is not itself an inhibitor of platelet adhesion, inhibits adhesion of platelets to vascular endothelium when applied at concentrations which release prostacyclin and NO [18]. However, it is unlikely that prostacyclin plays a major role in preventing adhesion, for the effect of Bk is not altered by aspirin treatment. Moreover, high concentrations of exogenous prostacyclin only partially inhibit adhesion. Bradykinin-induced inhibition of adhesion to endothelial cells is potentiated by SOD and inhibited by haemoglobin, agents known to modify the activity of NO, and is mimicked by exogenous NO. Furthermore, Bk causes the release of NO in quantities sufficient to explain the inhibition of platelet adhesion.

We have also shown that platelet adhesion to collagen fibrils and to endothelial cell matrix is inhibited completely by NO, but only partially by prostacyclin [19]. Inhibition of platelet adhesion by NO and prostacyclin is potentiated by selective inhibitors of cyclic GMP phosphodiesterase, but not of cyclic AMP phosphodiesterase, indicating that elevation of cyclic GMP regulates platelet adhesion. Unlike platelet aggregation and disaggregation, synergy between NO and prostacyclin as inhibitors of adhesion was not observed.

Nitric oxide has recently been shown to prevent platelet activation during the isolation and storage of human washed platelets [20]. Platelets prepared in this way retain their normal morphological and functional characteristics for periods up to 24 h. This is a further property that NO shares with prostacyclin; however, NO is a less potent cytoprotective agent than prostacyclin and prostacyclin-washed platelets remain viable for longer periods (up to 96 h).

Physiological effects and clinical implications of EDRF

Circumstantial evidence suggests a physiological role for EDRF (for reviews see [5, 17]). Endothelium-dependent vasodilatation has been demonstrated in a number of species in vivo [21, 22] and removal of vascular endothelium enhances the responses to constrictor agents in several animal models [23, 24]. Furthermore, in humans, acetylcholine-induced vasodilatation does not occur in coronary arteries at sites of atheroma [25], suggesting that lack of generation of NO by endothelial cells could play a role in the pathogenesis of coronary vasospasm. Inhibition of NO by haemoglobin could also play a role in the vasospasm that follows subarachnoid haemorrhage. Such an inhibition in the vessel wall will not only predispose to vasoconstriction, but will also favour platelet adhesion, aggregation and the consequent release of vasoconstrictor substances that will exacerbate the tendency to vasospasm. It is also likely that under pathological conditions, not only is the ability of the endothelial cells to release EDRF reduced, but they may also generate powerful vasoconstrictor substances [26].

Endothelium-dependent relaxation is accentuated in animals with spontaneous or experimentally induced hypertension [27, 28] or with dietary-induced atherosclerosis [29]. Human tissue from subjects with coronary disease or atherosclerosis also exhibits reduced endothelium-dependent vasodilatation [25, 30]. Nitric oxide together with prostacyclin may play a role in controlling platelet and leucocyte accumulation in the vessel wall, as well as regulating smooth muscle cell proliferation and matrix metabolism [5]. The destruction of NO by oxygen radicals and/or the inhibition of prostacyclin biosynthesis by lipid peroxides may be a crucial biochemical step in the development of atherosclerosis.

The recently discovered anti-adhesive properties of NO suggest that compounds that elevate cyclic GMP levels in platelets may have potential anti-thrombotic or anti-atherosclerotic effects. The measurement of NO in vivo will provide biological significance for all the observations in this field. Such studies are not yet available.

Concluding remarks

There is considerable evidence to indicate that NO is the endogenous nitrovasodilator and that the soluble guanylate cyclase can be considered to be the receptor for NO. The exact mechanism by which NO exerts its biological actions still remains to be determined.

It is becoming evident as our knowledge increases that NO is an important mediator in the vessel wall, which may help to explain the pathophysiological basis of some diseases.

References

Endothelium-derived relaxing factor, calcium and inositol phosphates

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Abbreviations used: EDRF, endothelium-derived relaxing factor; PI, phosphatidylinositol; NA, noradrenaline; ACh, acetylcholine; DTT, dithiothreitol; Hb, haemoglobin; NP, sodium nitroprusside; InsP₃, inositol trisphosphate; InsP₄, inositol tetrakisphosphate.

Endothelium-derived relaxing factor (EDRF) is the humoral agent responsible for endothelium-dependent vascular smooth muscle relaxation (Furchgott & Zawadzki, 1980; Griffith et al., 1984). The chemical properties of EDRF have recently been shown to be similar to those of nitric oxide (Palmer et al., 1987). The vasodilator actions of EDRF, like the nitrovasodilators, are mediated through stimulation of soluble guanylate cyclase and elevation of intracellular levels of cyclic guanosine monophosphate (cyclic GMP) (Katsuki et al., 1977; Ignarro et al., 1981; Rapoport & Murad, 1983; Busse et al., 1985).

![Fig. 1. Calcium flux studies in rabbit aortae with and without endothelium](image)

(a) Calcium influx into NA-stimulated preparations with (+) and without (−) endothelium. Preparations were incubated with 

\[ ^{42}\text{Ca} \]

for 1.5 min and preincubated with agents (for times indicated below), the agent being present also during incubation with \[ ^{42}\text{Ca} \]. Abbreviations: C, control, no added agent; N, noradrenaline \[ (10^{-5} \text{M}) \], 4.5 min; A, acetylcholine \[ (10^{-5} \text{M}) \], 1.5 min; D, dithiothreitol \[ (5 \times 10^{-4} \text{M}) \], 1 min. n ≥ 12, *P < 0.05. (b) Effect of various agents on NA-stimulated \[ ^{45}\text{Ca} \] efflux rates from preparations with (+) and without (−) endothelium; agents were first added at times before addition of NA. Presented are the rates 0–5 min after NA addition. No agent significantly influenced efflux rate before or 10 min after addition of NA. Abbreviations: N, noradrenaline \[ (10^{-5} \text{M}) \]; A: acetylcholine \[ (10^{-6} \text{M}) \], 5 min; D: dithiothreitol \[ (5 \times 10^{-6} \text{M}) \], 5 min; NP, sodium nitroprusside \[ (10^{-3} \text{M}) \], 10 min. Buffer contained 1.5 mM-CaCl₂ except where shown (zero [Ca⁺]). n ≥ 6, *P < 0.05. Bars indicate mean ± S.E.M.

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