The interaction between endothelin-1 and nitric oxide in the vasculature: new perspectives

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Abstract

Nitric oxide (NO) and endothelin-1 (ET-1) are natural counterparts in vascular function, and it is becoming increasingly clear that an imbalance between these two mediators is a characteristic of endothelial dysfunction and is important in the progression of vascular disease. Here, we review classical and more recent data that suggests that ET-1 should be regarded as an essential component of NO signaling. In particular, we review evidence of the role of ET-1 in models of acute and chronic NOS blockade. Furthermore, we discuss the possible mechanisms by which NO modulates ET-1 activity. On the basis of these studies, we suggest that NO tonically inhibits ET-1 function, and in conditions of diminished NO bioavailability, the deleterious effects of unmitigated ET-1 actions result in vasoconstriction, and eventually lead to vascular remodeling and dysfunction.
Introduction

Once thought to be an inert barrier delineating the boundaries of the circulation, the endothelium is now known to be a critical junction of vascular signaling. The endothelium produces and is acted upon by a host of mediators in what appears to be an exceedingly complex and interrelated signalome. With its capacity to release mediators involved in vasoconstriction, vasodilation, cell adhesion, growth, differentiation, proliferation and motility, normal endothelial function is a prerequisite for cardiovascular health. Indeed, endothelial dysfunction, characterized by altered production of vasoactive substances, often precedes the overt manifestation of disease, and is considered an important etiological factor in the progression of cardiovascular diseases.

Endothelin-1 (ET-1) has been known to be an important mediator of vascular function since its discovery by Yanagisawa and colleagues in the late 1980’s (106). Due to its potent and long-lasting vasoconstrictor effects, its capacity to induce vascular remodeling, fibrosis, cell proliferation, apoptosis, and its link to oxidative stress, ET-1 has been proposed to be important in the progression of numerous pathologies (51). The recognition of its importance in cardiovascular disease is perhaps best illustrated by the observation of Barton and Yanagisawa that within 4 years of ET-1’s discovery, its receptors had been cloned and pharmacological antagonists had been developed; these therapeutics were being tested in clinical trials by the early 1990’s (8). However, despite intensive study over the past two decades and the relative success of ET-1 antagonists in certain conditions (e.g. pulmonary hypertension (71), congestive heart failure (83)), the precise role of ET-1 in vascular physiology and pathophysiology has stubbornly eluded investigators. This may be due, in part, to several intricacies of the ET-1 system that makes it inherently complex (e.g. receptor localization, receptor dimerization (100)).
Notwithstanding these difficulties, ET-1, like many vascular mediators, is an intrinsically complicated system by virtue of the fact that its function in physiology and pathophysiology is inextricably linked with other vascular mediators, most notably nitric oxide (NO). As natural counterparts in vascular function, ET-1 and NO are known to interact via both direct and indirect mechanisms (3, 9, 77). Increasingly, evidence suggests that the balance between the two mediators is critical for vascular function, and disequilibrium may determine the onset and degree of certain cardiovascular diseases. However, the importance of the interaction between these two mediators is arguably still overlooked. In the present review, we highlight both classical as well as more recent evidence suggesting that NO signaling, in the systemic vasculature, should be considered a vital component of ET-1 activity, rather than as a distinct pathway. More specifically, following analysis of the evidence to date, in this review an alternative conceptual framework is proposed that focuses on the important link between NO signaling and ET-1 action in systemic vascular function. Furthermore, we highlight the differences between acute and chronic effects of ET-1 signaling that make this pathway particularly problematic to study. There is no doubt that ET-1 and NO are also important mediators of renal hemodynamics, cardiac function, as well as pulmonary vascular regulation. However, due to space limitations, these topics will not be covered; the reader is instead directed to several excellent reviews (13, 18, 46, 76, 86, 89).

Endothelin-1

The endothelins constitute a super family of peptides that are structurally similar to sarafotoxins, found in snake venom (52). There are 3 separately encoded isoforms (ET-1, ET-2, and ET-3), and although all isoforms are involved in vascular function, (reviewed in (8)) ET-1 is
the dominant isoform in the cardiovascular system, and is therefore the most often studied. ET-1 is synthesized predominantly in vascular endothelial cells, although it is also synthesized in vascular smooth muscle cells, as well as extravascular tissues such as the spleen, pancreas, lung, nervous system, as well as glomerular and epithelial cells within the kidney (16, 21, 57, 66, 96).

Expression of the endothelin gene product preproET-1 (ppET-1) is enhanced by a host of endogenous mediators, including cytokines, catecholamine, angiotensin II, arginine vasopressin, steroid hormones, and insulin. The gene is also induced by a host of noxious stimuli including mechanical and shear stress, hypoxia, oxidized lipoproteins, high levels of glucose, and lipopolysaccharide, among others. In contrast, stimuli that are generally regarded as beneficial to cells including NO, prostacyclin, atrial natriuretic peptide, epidermal growth factor, and low shear stress, inhibit ET-1 expression (8).

ppET-1 is a functionally inactive peptide of several hundred amino acids in length. It is sequentially cleaved in a multi-step process that ultimately yields vasoactive ET-1. ppET-1 is initially cleaved by a furin-like protease to generate a 39 amino acid peptide (38 amino acids in humans) called big-ET-1 (bET-1). bET-1 is subsequently cleaved by one of several enzymes to yield active ET-1 peptides of varying length and potency. Under normal physiological conditions, the predominant enzymes that carry out this function are a subgroup of zinc metalloproteinases from the neprilysin super-family, termed endothelin converting enzymes (ECE). The two isoforms of ECE, ECE-1 and ECE-2, cleave bET-1 to generate a vasoactive peptide of 21 amino acids in length (ET-1\textsubscript{1-21}). More recently, alternative pathways for ET-1 generation have been demonstrated. bET-1 can be cleaved by the matrix metalloproteinases (MMPs), specifically the gelatinase MMP-2 (and possibly MMP-9), which generate an active ET-1 peptide of 32 amino-acids in length (ET-1\textsubscript{1-32}) (27). Although active, it is presently unclear
whether ET-1_{1.32} is more or less potent and efficacious than ET-1_{1.21}, although this may depend on the vascular bed studied (27, 101) as well as its relative affinity for each endothelin receptor (see below). In addition to these pathways, chymase and calthepsin G have been shown to cleave bET-1 in a reaction that yields a third isoform of active ET-1 that is 31 residues in length (ET-1_{31}) (37, 49). Finally, the zinc metalloprotease nephrilysin, also known as neutral endopeptidase (NEP) may also play an important role in ET-1 generation. Owing to its cleavage of the amino side of hydrophobic amino acids, NEP is also capable of cleaving bET-1 to yield ET-1_{1.21}. Interestingly, unlike ECE which requires the carboxyterminus of bET-1 (105), NEP also cleaves ET-1_{1.31} to generate ET-1_{1.21} (40), albeit ET-1_{1.31} is also functional without this cleavage step (95). As both bET-1 and ET-1_{1.31} are substrates for NEP, it would seem, therefore, that ET-1_{1.32} may also be candidate for cleavage by this enzyme, although this has not yet been demonstrated.

ET-1 exerts its functions by binding to G-protein coupled ET receptors, which are expressed in several tissues, including the myocardium, lung, pancreas, spleen, nervous system, as well as in vascular tissues. Two receptors for endothelins, named ET\(_A\) and ET\(_B\), have been identified and cloned. Broadly, ETA receptors are located within the VSMC, whereas ETB receptors are located on both the endothelium as well as on VSMC. In the vasculature, ET\(_A\) receptors are made up of ET\(_{A1}\) and ET\(_{A2}\) subtypes, distinguished by their sensitivity to the endothelin receptor antagonist BQ-123; the BQ-123-sensitive ET\(_{A1}\) receptor is located in VSMC of most arteries and represents the dominant subtype (48), whereas the BQ-123-insensitive ET\(_{A2}\) receptor has been localized in human and rabbit saphenous veins (67, 91). ET\(_B\) receptors consist of ET\(_{B1}\) and ET\(_{B2}\) subtypes, and can be broadly distinguished by their endothelial and smooth muscle cell localization, respectively (20, 42, 60). Binding of ET-1 to ET\(_A\) and ET\(_B\) receptors in VSMC results in vasoconstriction, whereas the predominant effect of ET-1 binding to ET\(_B\)
receptors in the endothelium is increased NO and prostacyclin synthesis (79, 99). Whereas ET-1 binds both ET$_A$ and ET$_B$ receptors, evidence suggests ET-1$_{1-31}$ selectively binds ET$_A$ receptors (61, 81, 95), although evidence that ET1$_{1-31}$ binds ET$_B$ receptors and mediates NO release also exists (68). Whether ET-1$_{1-32}$ preferentially binds one endothelin receptor over the other is not yet known.

Vascular effects of ET-1 signaling

In healthy human subjects, systemic administration of the ECE/NEP inhibitor phosphoramidon causes a modest, albeit significant increase in forearm blood flow (43) demonstrating that ET-1 contributes to a basal vasoconstrictor tone. However, in rats as well as in dogs, administration of the ET$_{A/B}$ receptor antagonist bosentan had little effect on blood pressure (85, 94), suggesting that the contribution of ET-1 to basal tone may be species-specific, and may also depend on the vascular bed being studied. In humans, ET$_A$ receptor antagonism with BQ-123 exerted a similar increase in forearm blood flow as phosphoramidon (43), indicating that the basal vascular tone mediated by ET-1 occurs largely via ET$_A$ receptors. These findings are supported by studies in which ET-1 is administered systemically to animals and humans. Administration of ET-1 results in a biphasic response characterized by a transient depressor effect, followed by a pronounced and persistent hypertension that can be effectively inhibited with ET$_A$ receptor antagonists (22, 34, 74, 97).

The contribution of ET$_B$ receptors in the regulation of vascular tone is more complex. The aforementioned transient depressor effect associated with administered ET-1 can be inhibited by ET$_B$ receptor antagonism (47), demonstrating that ET$_B$ receptor activation can directly cause vasodilation. However, it is noteworthy that the effects of administered ET-1 may
not necessarily recapitulate the effects of endogenous endothelin, since it is not presently clear whether ET\textsubscript{B} receptors on the endothelium “see” high enough quantities of ET-1 under normal circumstances to elicit vasodilation through an NO-dependent mechanism. For this reason, studies using ET\textsubscript{B} receptor antagonists have been instrumental in elucidating the role of ET\textsubscript{B} receptors in vascular tone. In most systemic vascular beds, the effect of ET\textsubscript{B} receptor antagonism is vasoconstriction (34, 90) (with the exception of a few vascular beds, such as the coronary and pulmonary arteries as well as saphenous veins, where ET\textsubscript{B} receptors mediate vasoconstriction (5, 31, 39, 67, 91) and may constitute a significant portion of the ET-1 response in these vessels (1, 17)). Although the vasoconstriction associated with ET\textsubscript{B} antagonism could reflect, in part, diminished vasodilatory input, evidence indicates that this is not the principal mechanism. Pharmacological blockade or genetic ablation of ET\textsubscript{B} receptors results in increased circulating ET-1 (10, 34, 50, 70), and the associated increases in blood pressure can be normalized by ETA antagonism (34), suggesting this hypertensive effect more likely reflects increased ET-1 activation of ETA receptors rather than a loss of tonic vasodilatory input. The resultant increase in circulating ET-1 following ET\textsubscript{B} receptor antagonism is consistent with the role of ET\textsubscript{B} receptors in mediating systemic clearance of ET-1 (50). Additionally, it is tempting to speculate that blockade of ET\textsubscript{B} receptors may result in increased synthesis or release of ET-1 from the endothelium due to the loss of inhibitory NO production. It may be that the continual binding of ET\textsubscript{B} receptors by endothelin produces low levels of NO—below those that are required to elicit vasodilation—that inhibit ET-1 synthesis and release. The interaction between ET-1 and NO is discussed in more detail in subsequent sections.

In addition to its effects on vascular tone, prolonged ET-1 exposure causes persistent alterations in cell morphology and function. ET-1 has been shown to impact cell survival, cause
cell proliferation, cell migration, reactive oxygen and nitrogen species generation, and is known
to stimulate release of inflammatory cytokines, including TNF-α, IL-6 and mediators such as
NFκB in a number of cell types (14, 19, 58, 87). These effects are mediated largely via ET_A
receptors in most cell types, although many of these effects have also been reported in
endothelial cell cultures (24, 64, 88)—effects mediated entirely via ET_B receptor binding, since
endothelial cells do not express ET_A receptors. Thus, prolonged ET-1 binding to ET_B receptors
appears to induce deleterious effects on cell function, despite inducing production of ‘protective’
NO. In fact, Noiri et al. found that increased migration of endothelial cells mediated by ET-1
binding to ET_B receptors was dependent on NO (69); whether the other effects of ET-1 in
endothelial cells are dependent on NO is not presently clear.

Consistent with these in vitro studies, prolonged ET-1 treatment in animals has lasting
effects on vascular structure and function. Chronic administration of ET-1 to rodents promotes
vascular fibrous tissue formation (35) and stimulates cell migration and proliferation (64, 104).
However, despite clear evidence for ET-1 mediating cell specific effects, the structural and
functional consequences of prolonged ET-1 exposure in vivo have been difficult to ascertain due
to the hypertension that invariably accompanies its administration; thus, it is not clear whether
the vascular changes are mediated by ET-1 per se, or whether they stem from mechanisms
associated with increased blood pressure. To this end, Amiri et al. recently developed an
endothelial specific model of ET-1 over-expression (4). Despite showing no basal increase in
blood pressure, these animals exhibit increased wall to lumen ratios, medial thickness, as well as
altered vascular function, concomitant with increased vascular oxidative stress. These results
demonstrate direct effects of ET-1 on vascular remodeling which are relevant in
pathophysiology.
Acute NOS blockade and ET-1

Acute NOS inhibition with analogues of L-arginine results in a marked vasoconstrictor response, which manifests as a rise in blood pressure and a reduction in peripheral blood flow (82). These hemodynamic responses are entirely reversible with administration of NO donors, such as glyceryl trinitrate (GTN) or sodium nitroprusside (SNP) (12, 63), as well as L-arginine (82), demonstrating that the continued presence of NO is required to prevent vascular constriction. These observations, coupled with elegant studies in the late 1980’s that demonstrated that NO causes vasodilation (72) principally by activating sGC and increasing intracellular cGMP levels (80), has led to the prevailing concept that NO functions principally to maintain a tonic vasodilatory signal (41, 103).

However, several studies have shown that the vasoconstrictor response associated with NOS blockade has a significant component attributable to ET-1 activity. In humans, Cardillo et al. demonstrated that more than 70% of the reduction in forearm blood flow, caused by NOS inhibition with L-NMMA, could be blocked with dual ET-1 receptor antagonism (15). Similar results have been reported in animals. Using modest doses of L-NAME, Richard et al. and Filep independently demonstrated that approximately half the hypertensive response that occurred due to NOS inhibition in conscious rats could be blocked with prior treatment with ET_{A/B} receptor antagonist bosentan, or the ET_{A} receptor antagonist BQ-123 (28, 85). Consistent with these findings, in conscious rats ET_{A/B} receptor antagonism with PD14565 blocks the majority of the hypertensive response associated with L-NAME treatment (7). Interestingly, Takahashi et al. recently demonstrated that L-NAME treatment can induce release of vasoactive ET-1 from
ischemic myocardium (93), suggesting that NO inhibition may unmask ET-1 release from a
number of tissues, not just the endothelium.

Taken together, these results show that ET-1 is implicated in the hypertensive response
that accompanies acute NOS inhibition. However, there remain several important questions, not
least of which is the mechanism by which NO inhibits ET-1 mediated vasoconstriction. An
obvious question is whether NO mediated inhibition of the ET-1 pathway is a relevant
mechanism in the control of vascular tone. To answer this question, we showed in a recent, as
yet unpublished, study using conscious rats that the doses of NO-donors (SNP, GTN,
methylamine hexamethylene methylamine NONOate) required to reverse the L-NAME mediated
hypertensive effect was markedly lower than the doses required to cause direct vasodilation, and
this ability could be inhibited by the ET$_{A/B}$ receptor antagonist PD145065 (12). We also
demonstrated that the hypertensive dependence on ET-1 could not be attributed to other systemic
influences associated with NOS blockade, such as activation of the renin angiotensin system,
sympathetic nervous system, or arginine vasopressin system, since inhibition of these systems
did not alter NO’s capacity to inhibit ET-1 activity (7). We speculate that NO antagonizes ET-1
activity at lower concentrations that those required to directly elicit vasodilation, such that when
animals are treated with NOS inhibitors, the unmitigated actions of ET-1 manifest as
vasoconstriction; the subsequent administration of low doses of NO donors reverses the
unopposed actions of ET-1, thereby restoring normal blood pressure. On the basis of these
studies, a more contemporary hypothesis for a principal role of NO in normal physiological
conditions is to tonically inhibit ET-1 activity. Importantly, this countervailing action does not
preclude a role for NO as a vasodilator; rather, NO may regulate vascular tone via several
mechanisms, including those unrelated to ET-1 activity.
A point that is critical to the interpretation of the studies discussed in this section is that all of the results were obtained in intact whole animals or in conscious human subjects. In general, these data are not recapitulated *ex vivo*, likely because of the removal of local, neural and humoral factors that regulate vascular tone when vessels are isolated from an intact animal. For example, removal of nitergic innervation, as well as removal of signals that promote ET-1 expression and release are absent in isolated vessels. Therefore, the contribution of ET-1 and the importance of the interaction between NO and ET-1 may be underestimated using *ex vivo* experimental approaches.

**Chronic NOS blockade and ET-1**

In contrast to acute NOS inhibition, chronic treatment of rats with analogues of L-arginine produces a model of hypertension that has not been found to be reversed by ET-1 antagonism (6, 29, 92). Despite the apparent lack of involvement of ET-1 in this hypertensive phenotype, chronic NOS inhibition may provide more important insights into the pathophysiological mechanisms of ET-1 activity in disease progression than its acute counterpart. Importantly, in the chronic NOS inhibition model, two fundamental issues need to be considered when discussing the role of vascular mediators. The first is that the unmitigated actions of endogenous effectors will likely cause irreversible or persistent effects, in addition to adaptive changes. The second issue is that, whereas the short term setpoint of arterial blood pressure is dictated largely by cardiac output and total peripheral resistance, the long-term setpoint of arterial pressure is established principally by the kidney’s capacity to regulate sodium and water balance, in part via the pressure natriuresis mechanism (26, 36). Thus, in acute NOS inhibition the impact of NO and ET-1 on renal sodium handling is not manifested, whereas the
affected fluid regulating mechanisms become critically relevant with prolonged NOS inhibition and are not quickly reversed. The roles of ET-1 and NO in renal sodium handling have been reviewed elsewhere (18, 76).

Studies using NOS inhibition have shown that the efficacy of L-arginine, as a means to correct the resultant NO deficiency by outcompeting the NOS antagonist, is time dependent. That is, although L-arginine almost completely reverses the pressor response to L-NAME acutely, it only has a partial effect after a few days of NOS antagonism (84), and becomes altogether ineffective if treatment is delayed by several weeks (25, 65, 78, 84). These studies suggest that, after a period of NOS inhibition, the hypertension is no longer dependent on the absence of NO \textit{per se}, but rather on the resultant pathophysiological adaptive or compensatory changes in the cardiovascular system that perpetuate the hypertension and result in end organ damage (23, 65). In support of this concept, Verhagen \textit{et al} found that ET\textsubscript{A} antagonism, like L-arginine treatment, could diminish the degree of hypertension and delay its onset if treated concomitantly with L-NAME (75, 98) or even within the first two weeks (73), and yet the ET\textsubscript{A} antagonist treatment was not effective if administered 3 or more weeks following commencement of NOS inhibition (6, 30). Interestingly, ET\textsubscript{A} antagonism also partially prevented the vascular damage and remodeling associated with prolonged NOS inhibition (98). These studies suggest that ET-1 plays a particularly important role in initiating the hypertensive phenotype as well as mediating the longer term vascular remodeling effects in chronic NOS inhibition. Nevertheless, the finding that ET-1 blockade does not completely inhibit the deleterious effects of prolonged NOS inhibition suggests other mechanisms are also involved, such as activation of the renin angiotensin system and the sympathetic nervous system (6, 30). Moreover, it is presently unclear whether the pathogenic vascular remodeling occurs as a direct
consequence of ET-1, or whether it is secondary to the hemodynamic changes. The observation that endothelium-specific ET-1 over-expressing mice exhibit vascular remodeling without the hypertensive phenotype (4) is consistent with the former hypothesis, though this has yet to be validated in the chronic NOS inhibition model.

Endothelial NOS (eNOS, NOS3) knockout mice may also provide some insights into the interaction between NO and ET-1 function, however very little work has been done in this area. It is clear that eNOS knockout mice exhibit alterations in ET-1 function, although increased responsiveness to administered ET-1 appears to stem largely from increased expression of ETA receptors, and decreased expression of ETB receptors (55). Moreover, eNOS knockouts have been reported to have modest elevations in circulating ET-1 (55), although a previous study by the same group found no such differences (56). However, since ET-1 release is predominantly abluminal, circulating levels of ET-1 are likely not indicative of ET-1 involvement in this phenotype. In this regard, it is perhaps not surprising that rats treated chronically with NOS inhibitors do not exhibit marked elevations in circulating ET-1 (92), despite showing involvement of ET-1, at least initially, in this phenotype.

The potential translational implications for these concepts are substantial. Several clinical conditions characterized by endothelial dysfunction, and hence reduced NO bioavailability, exhibit the vascular and hemodynamic characteristics observed in these experimental conditions. It may be that an important step in the progression of cardiovascular disease is the loss of endothelial derived NO production resulting in increased ET-1 signaling. The findings to date would suggest that the unmitigated actions of ET-1 would therefore be reversible via ET antagonism during early stages before the induction of vascular remodeling. In
this regard, it could be that $\text{ET}_A$ receptor antagonists may be better suited for prophylactic
treatment of cardiovascular disease.

Mechanism of interaction between NO and ET-1

**ET-1 Gene Expression**

Transcription and/or translation of the endothelin gene are perhaps the most studied and
well established mechanisms by which NO, and a host of other stimuli, modulate the ET-1
pathway. Boulanger *et al.* was the first to show that inhibition of NO with L-NMMA potentiated
the release of ET-1 from cultured cells, providing direct evidence of NO-mediated inhibition of
ET-1 activity (11). Importantly, potentiation of ET-1 release was inhibited by cyclohexamide, a
protein synthesis inhibitor, suggesting *de novo* synthesis of ET-1 is involved (11). Kourembanas
and colleagues showed that administered NO could inhibit transcription of basal and stimulated
release of ET-1, as measured by nuclear run-off assay (53). In a recent study, Weng *et al*
demonstrated that very low levels of NO—concentrations as low as 20 ppm—effectively
suppressed release of ET-1 from human umbilical vein endothelial cells, corresponding with a
decreased ppET-1 mRNA (102). This *in vitro* study supports our recent findings *in vivo* that low
concentrations of NO effectively inhibit the actions of ET-1 (12). In addition to these studies,
shear stress, which is a well-established inducer of NO production, has been shown to inhibit
ET-1 release from cells (54, 59)—an effect associated with a reduction in ppET-1 mRNA
synthesis, rather than a reduction in mRNA stability (59). Kuchan *et al.* further demonstrated
that shear stress inhibition of ET-1 is mediated by NO, since this effect was abrogated by NOS
inhibition (54).
Interestingly, studies suggest that NO inhibits ET-1 release via a cGMP dependent mechanism. As discussed above, sGC represents an important intracellular receptor for NO. A study by Kuchan demonstrated that shear stress induced inhibition of ET-1 release from cultured endothelial cells is largely dependent on cGMP, but not on cGMP-dependent kinases (54).

Similarly, the research groups of Boulanger and Mitsutomi independently showed that ET-1 release could be inhibited by methylene blue or ODQ, both of which are inhibitors of cGMP (11, 62), suggesting that NO prevents ET-1 release via an endothelial cGMP-dependent mechanism. Importantly, these studies implicate cGMP signaling within the endothelium, and not within the VSMC, as a key mechanism of interaction between NO and ET-1.

**ET-1 Release**

Despite early studies contesting the role of ET-1 storage in vesicles (44, 106), subsequent studies identified ET-1 in secretory vesicles within the endothelium (38), later identified as Weibel Palade bodies (32). The identification of ET-1 in secretory vesicles is consistent with a rapid onset hypertensive effect that accompanies acute NOS inhibition. Indeed, a recent study by Goel and colleagues demonstrated that enhanced exocytosis of ET-1 is an important mechanism by which enhanced ET-1 signaling occurs in ageing vasculature (32). Interestingly, in these studies L-NAME did not affect thrombin induced ET-1 release in either young or aged vasculature (32), suggesting that NO does not play an inhibitory role on ET-1 release. However, as discussed above, it is noteworthy that the experiments performed by Goel et al. were *ex vivo*, and with the removal of tonic neural, humoral and local influences on the vasculature, the role of NO on ET-1 release could be overlooked.
Receptor Binding and Downstream Signaling

Very few studies have investigated the interaction between ET-1 and NO at the level of ET receptors. Goligorsky et al. showed that NO can diminish the duration of interaction between ET-1 and its receptors (33); this finding could be significant since ET-1 has been shown to interact with its receptors for hours under normal physiological conditions (45). Since this effect occurs extracellularly, it is perhaps not surprising that this effect was not dependent on sGC signaling (33). This study also demonstrated that NO can inhibit ET-1 signaling downstream of its receptors, namely at the level of VSMC calcium signaling. Binding of ET-1 to ETA causes a PLC mediated increase in intracellular calcium, whereas NO ultimately inhibits calcium release via both sGC-dependent and -independent mechanisms. The antagonism of NO and ET-1 at the level of calcium signaling is particularly relevant in the context of this review, since it is one of the few mechanisms whose time course is consistent with that of the hypertensive effect that occurs following acute NOS inhibition.

Significance and perspectives

It is becoming increasingly apparent that an imbalance between NO and ET-1 is important in numerous pathophysiological conditions (for review, see (2)). The reduction in NO bioavailability concomitant with increased ET-1 expression in various pathophysiological circumstances suggests an intimate link between these two mediators. Mechanistically, there has been compelling evidence that NO and ET-1 not only exert physiologically antagonistic effects within the vasculature, but may interact on several levels in a coordinated fashion (Figure 1). Taking these findings together, it may be that under normal physiological conditions, a fundamental role of NO in blood vessels is to tonically inhibit the vasoconstrictor actions of ET-
1. The constitutive synthesis of NO by endothelial cells antagonizes the ET-1 pathway via several mechanisms, including expression, release, receptor interactions, and via second messenger signaling systems. Thus, it may be that NO and ET-1 may remain in a delicate balance, without substantially contributing to overall total peripheral resistance (Figure 1A). Upon stimulation, ET-1 binding to ETB receptor located on the endothelium, coupled to NOS, may serve as an inhibitory feedback mechanism to prevent excessive and potentially damaging ET-1 signaling. However, during pathophysiologic conditions of compromised NO bioavailability, the vasoconstrictor effects of ET-1 (and other potentially deleterious effects such as VSMC proliferation and migration) become disinhibited. This may progress to a form of hypertension that eventually engages multiple mechanisms, and may lead to detrimental long-term effects on vascular structure and function (Figure 1B). Thus, the window of therapeutic intervention for ET-1 antagonism appears quite short, before irreversible changes in circulatory structure and function ensue.
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Figure Legends

Figure 1. Schematic of proposed interaction between ET-1 and NO within the vasculature. (A) Under normal physiological conditions, NO tonically inhibits ET-1 synthesis, receptor binding and potentially downstream activity. (B) In conditions of diminished NO bioavailability—due to number of possible conditions (NOS inhibition, oxidative stress, inflammation are shown as examples)—unmitigated actions of ET-1 lead to vasoconstriction and vascular remodeling. The width of arrows reflects the degree of activity. Note that NO inhibition of ET-1 release occurs via a cGMP-dependent mechanism within the endothelium. VSMC, vascular smooth muscle cells, ppET-1, preproendothelin-1; bET-1, big endothelin-1; ECE, endothelin converting enzyme; ET-1 endothelin-1; NOS, nitric oxide synthase; sGC, soluble guanylyl cyclase; ETA, endothelin receptor A; ETB, endothelin receptor B; NO, nitric oxide; PKG, protein kinase G; MMP, matrix metalloproteinase; L-Arg, L-arginine.


61. **Mazzocchi G, Rossi GP, Malendowicz LK, Champion HC and Nussdorfer GG.**


