

The role of nitric oxide

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When studying the impact of endothelins (ETs) on physiology and pathophysiology, this needs to be done in the context of nitric oxide (NO) synthesis and action, since these two are closely intertwined in their action. Here, we will review the work demonstrating the crosstalk between endothelin-1 (ET-1) and NO, and the recent developments regarding the role of these two mediators in inflammatory processes. Moreover, we will discuss the role of NO in pro-inflammatory diseases and the potential mechanisms of the anti-inflammatory activity of ET receptor antagonism.

Introduction

Endothelins (ETs) are known to exert pressure blood regulation in a close crosstalk with nitric oxide (NO) synthesized via the constitutively expressed endothelial NO synthase (NOS), also termed NOS-3. Since this crosstalk works under physiological conditions in the absence of inflammatory mediators, it is surprising that ET receptor antagonism should be beneficial in diseases that belong to broad range of pro-inflammatory conditions. Recent findings in animal models have started to close this gap in our understanding and highlight the importance of monitoring both ET expression and action together with NO biosynthesis to understand and probably also predict the therapeutical benefit of ET receptor antagonism in various diseases.

The crosstalk of ET-1 and NO

Blood pressure regulation

Early work on the impact of ET-1 in blood pressure regulation has demonstrated that the vasoconstrictive activity acts via the ET_A receptor located on the vascular smooth muscle cells but simultaneously addresses the ET_B receptor subtype expressed on the endothelial cells, where it leads to vasodilatation by inducing the release of NO and PGI₂ [1]. Thus, ET-1 effects were found to be different, if blood vessels were denuded or if examined in the presence of haemoglobin, which scavenges NO. In addition, *in vivo* studies have shown that an increased vasoconstriction results from inhibiting NO synthesis (as reviewed in [2]). However, more recent investigations have shown that the interaction is even more complex (Fig. 1). Binding of ET-1 induces increased NO synthesis by the endothelial NOS (NOS-3) and also increases NOS-3 expression, but in addition, ET-1-mediated signalling leads to an increased production of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA), which will potentially lower the bioavailability of NO.

Regulation of inflammatory processes

Initially, the regulatory crosstalk between ET-1 and NO was regarded as being operative in blood pressure regulation only, as outlined earlier. However, more recent data point to a role in inflammatory responses as well. Thus, it has been shown that the synthesis of both ET and NO are increased in several

inflammatory diseases, such as asthma, arthritis, inflammatory bowel disease and sepsis [2]. Inflammatory diseases in general are characterized by the expression of the inducible isotype of the NOS family, also termed NOS-2, and the diseases mentioned above are no exceptions to this (2,5). The interplay of NOS-2-derived NO with ET was not apparent until very recently, when it was reported that mice over-expressing ET-1 express NOS-2 in the kidneys (the only organ studied so far), and together with the increased presence of infiltrating immune cells, this was taken as proof for chronic inflammation [3]. In these mice, however, there is no increase in blood pressure despite the high levels of ET-1, but upon administration of a general NOS inhibitor, a marked blood pressure increase was found. In an earlier study on pulmonary inflammation [4] in the same animal model, the expression of NOS-2 was not investigated, and, unfortunately, in both studies, levels of endothelial NOS-3 were also not examined.

Inducible NO synthase as a marker and regulator of inflammation

The inducible NOS isotype (NOS-2) usually requires signals such as pro-inflammatory cytokines and/or bacterial products, as for instance endotoxin, for expression. Upon these stimuli, expression occurs in most cell types and NO is produced for a relatively long period of up to several days—also termed ‘high output NO synthesis’—in contrast to the endothelial NOS-3 or the neuronal NOS-1, both of which synthesize NO as short pulses only, mostly following a Ca⁺⁺-mediated activation. All of the NOS isotypes require the amino acid L-arginine as substrate and several cofactors for enzyme activity, and are inhibited by ADMA. Initially, high-output NO synthesis was regarded as part of the immune defence against pathogens, acting via its toxic activity at high concentrations. More recently, our understanding of the role of NOS-2-derived NO has shifted. We now know that NO has a direct impact on gene expression by altering the expression levels of several hundred genes leading to a protective ‘stress response’. It also serves the important task of down-regulating inflammation and suppressing leucocyte infiltration. In summary, at present, NOS-2-derived NO synthesis is regarded as an important regulatory and beneficial signal that is started by pro-inflammatory events and—in the mode of a classical feed back cycle—contributes to down-regulate inflammation [5].

Recently, several findings point to insufficient NO synthesis during inflammation as a factor contributing to the chronicity

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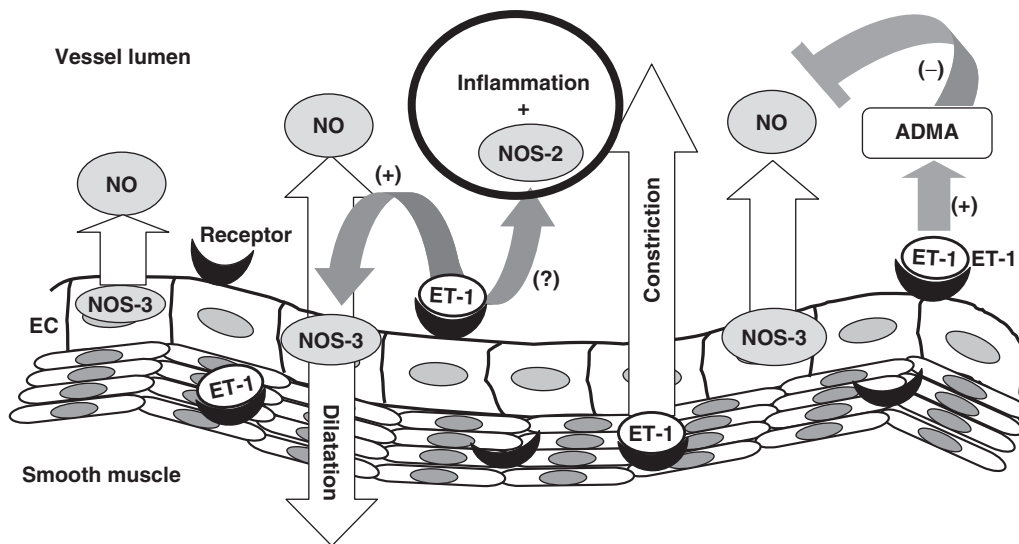


FIG. 1. ET-1 actions occur as an intense crosstalk with NO. Upon binding of ET-1 (ovals) to its receptors (black cups) on smooth muscle cells, vasoconstriction occurs. Simultaneously, binding to the endothelial receptor will induce enhanced expression and increased NO formation by endothelial NOS-3, and this increment in NO will lead to vasodilatation. The same event, however, will also induce increased formation of the endogenous NOS inhibitor ADMA, which in turn restricts NO synthesis. Recent data suggest that with excess ET-1, inflammation occurs and the inducible NOS-2 is expressed via a mechanism still unknown, and we know that beneficial effects of ET receptor antagonism in inflammatory conditions crucially depend on the synthesis of NO. EC, endothelial cells.

of diseases. Current evidence for this idea is found in three diseases, Psoriasis [6], asthma and sickle cell anaemia. All three pathological entities are characterized by NOS-2 expression and simultaneously by an over-expression of arginase-1, an enzyme that competes effectively for the common substrate L-arginine, thereby limiting NO synthesis. Indeed, it has been shown that even under physiological, non-inflammatory conditions, the L-arginine availability restricts the formation of NO, and the L-arginine supplementation increases NO output significantly [7]. In addition, there is also indirect evidence that the availability of essential cofactors—especially of tetrahydrobiopterin—may be limited in some diseases [8]. Several old and new data show convincingly that under conditions of increased NO requirement, increased or *de novo* expression of other NOS family members will occur [9]. Thus, the observation of NOS-2 expression in the ET-1 over-expressing mice may represent exactly this increased need for NO to counterbalance the ET-1 activity, or an inadequately low NO output due to restricted substrate or cofactor supply, or a combination of both. Indeed, the presence of infiltrating leucocytes argues in favour of low NO formation, otherwise the anti-adhesive activity of NO would protect from infiltration.

The molecular mechanism of endothelin receptor antagonism in inflammatory diseases: more question than answers

The role of NO synthesized via the inducible NOS-2 in inflammation has been extensively studied as outlined above. The role of ET-1 in inflammatory diseases, however, is far from being understood. Despite the knowledge of increased ET-1 levels in a number of chronic pro-inflammatory diseases, we do not

know whether this represents an inflammatory signal *per se*, or rather a bystander response during the course of these diseases. The success of ET receptor antagonists in therapy may be taken as an indication for the former mechanism. It has become evident from animal studies that the protection conferred by ET receptor antagonism during myocardial ischaemia and reperfusion absolutely depends on the synthesis of NO and is completely abrogated by simultaneous administration of a NOS inhibitor and reinstalled by administration of L-arginine [10]. Moreover, receptor antagonist treatment is ineffective in mice with a genetic defect in endothelial NOS-3. Unfortunately, in this study the expression of NOS-2 was not monitored. In addition, none of the relevant studies investigated the circulating levels of ADMA, the endogenous NOS inhibitor, which is known to increase subsequent to ET-1 signalling and which has been recognized as the number one risk predictor in cardiovascular diseases.

All of the findings published so far do not completely explain the role of ETs in inflammation. A number of open questions must be solved, especially in view of the therapeutic success of ET receptor antagonism:

- What are the signals responsible for increases in ET-1 levels in chronic pro-inflammatory diseases?
- Does ET-1 directly act in mounting an inflammatory response, or contribute to such a condition?
- Are ET receptor antagonists selective NOS-2 inhibitors?
- What is the role of ADMA formation in the action of ET receptor antagonists?

Future research will answer these questions and will, undoubtedly, thereby provide the basis for fully understanding the concept of ET receptor antagonism and its therapeutic benefits, but more importantly, this will open new therapeutical options.

<i>Rheumatology</i>	Key messages
	<ul style="list-style-type: none"> • ET-1 acts in an intensive regulatory crosstalk with NO. • ET-1 induces increased ADMA formation, an endogenous inhibitor of NO synthesis. • Some chronic inflammatory diseases are associated with increased ET-1 and NO levels. • ET over-expressing mice will develop inflammation and inducible NOS expression as an indication for ET-1 and NO crosstalk playing a role in inflammation also. • Beneficial effects of ET receptor antagonism crucially depend on NO synthesis. • Open questions on the role of ET-1 in inflammation need to be addressed in the context of NO synthesis.

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