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Review

The importance of endothelin-1 for vascular dysfunction in cardiovascular disease

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Abstract

Endothelin (ET)-1 is a potent vasoconstrictor peptide originally isolated from endothelial cells. Its production is stimulated in a variety of different cell types under the influence of risk factors for cardiovascular disease and during the development of cardiovascular disease. Based on these observations and the biological effects induced by ET-1, including profound vasoconstriction, pro-inflammatory actions, mitogenic and proliferative effects, stimulation of free radical formation and platelet activation, ET-1 has been implicated as an important factor in the development of vascular dysfunction and cardiovascular disease. In the following the pathogenic role of ET-1, the mechanisms underlying the involvement of ET-1 for the development of vascular dysfunction and the potentially beneficial therapeutic effects of selective ET_A and dual ET_A/ET_B receptor antagonists will be discussed. In particular the changes of pathophysiological importance mediated by ET-1 in clinical studies are reviewed. These changes may be of significance for the development of various cardiovascular diseases beyond pulmonary arterial hypertension which is the currently approved indication for ET receptor antagonists.

Keywords: Atherosclerosis; Coronary disease; Endothelins; Endothelial function; Endothelial receptors

1. Introduction

1.1. Vascular function

The endothelium plays an important role in the regulation of vascular function by producing a large number of biologically active substances that participate in the regulation of vascular tone, cell growth, inflammation, and thrombosis/haemostasis. Dysfunction of the vascular endothelium is an early finding in the development of cardiovascular disease and is closely related to clinical events in patients with atherosclerosis and hypertension [1]. Therefore, knowledge regarding the mechanisms behind the development of endothelial dysfunction and pharmacological strategies targeting endothelial dysfunction is of great importance. Endothelial dysfunction often refers to a situation of reduced bioavailability and consequently impaired vasodilator effect of endothelium-derived relaxing factors such as nitric oxide (NO), prostcyclin or endothelium-derived hyperpolarizing factor. One additional important alteration in endothelial dysfunction is an increased production and biological activity of the potent vasoconstrictor and pro-inflammatory peptide endothelin (ET)-1. In the present review the pathogenic role of the altered expression and biological actions of ET-1 and its receptors in vascular dysfunction and the development of cardiovascular disease are summarized. In particular the changes of pathophysiological importance mediated by ET-1 in clinical studies and the possible mechanisms behind these changes are reviewed. These changes may be of significance for the development of various cardiovascular diseases beyond pulmonary arterial hypertension which is the currently approved indication for ET receptor antagonists.

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1.2. The family of ET peptides

Since the discovery of an endothelium-derived constricting factor in 1985 [2] and the complex description of ET performed by Yanagisawa et al. in 1988 [3], three structurally different ET isoforms [4] have been described (i.e. ET-1, ET-2, ET-3 as well as vasoactive intestinal constrictor) [4]. In addition, 31-residue ETs have been identified [5]. Amongst the three ET isopeptides, the 21-amino acid peptide ET-1 is regarded as the most prominent isoform in the cardiovascular system, accounting for the majority of pathobiological effects exerted by ETs [6].

Mature ET-1 is formed from pre-pro-ET-1 via a 39-amino acid intermediate, big ET-1 [7]. Big ET-1 is processed to ET-1 by a family of ET converting enzymes (ECEs) and other enzymes such as chymases, non-ECE metalloproteinases and endopeptidases [7,8]. Under physiological conditions, ET-1 is produced in small amounts mainly in endothelial cells, primarily acting as an autocrine/paracrine mediator. Under pathophysiological conditions however, the production is stimulated in a large number of different cell types, including endothelial cells, vascular smooth muscle cells, cardiac myocytes [9], and inflammatory cells such as macrophages [10] and leukocytes [11] (Fig. 1).

1.3. The receptors of ET peptides

The biological effects of ET-1 are transduced by two pharmacologically distinguishable receptor subtypes, ET_A and ET_B receptors, respectively [12]. In the vasculature, the ET_A receptor is mainly located on vascular smooth muscle cells and mediates potent vasoconstriction (Fig. 1). ET-1 may also induce indirect vasoconstrictor effects due to the generation of endothelium-derived thromboxane A2 [13]. The ET_B receptor is primarily located on endothelial cells, but may also be present on vascular smooth muscle cells. Stimulation of the endothelial ET_B receptor results in release of NO and prostacyclin [14] which cause vasodilatation, whereas stimulation of the vascular smooth muscle cell ET_B receptor results in vasoconstriction (Fig. 1). Thus, the net effect produced by ET-1 is determined on the receptor localisation and the balance between ET_A and ET_B receptors. Under physiological conditions, the net effect is vasoconstriction mediated by the ET_A receptor, which is partly counteracted by ET_B receptor-mediated release of NO. However, under certain pathophysiological conditions the response to ET receptor antagonists may be changed, which will be discussed below.

2. The endogenous ET system and vascular dysfunction

2.1. Changes in vascular reactivity to ET-1

In healthy humans ET-1 increases mean arterial blood pressure, reduces heart rate, cardiac output and stroke volume and causes potent and long lasting vasoconstriction in the pulmonary [15], renal, splanchnic, myocardial [16], and skeletal muscle [17] vasculature. Haynes and Webb demonstrated that the selective ET_A receptor antagonist BQ123 evokes increases in forearm blood flow in healthy men [18]. ET_B receptor antagonism may either alone or on a background of ET_A receptor antagonism cause local vasoconstriction in young healthy subjects [19]. These findings suggest that endogenous ET-1 has a physiological role in the maintenance of vascular tone in healthy humans.

Several studies have demonstrated marked changes in the vascular reactivity to ET-1 in disease (Table 1). Increased

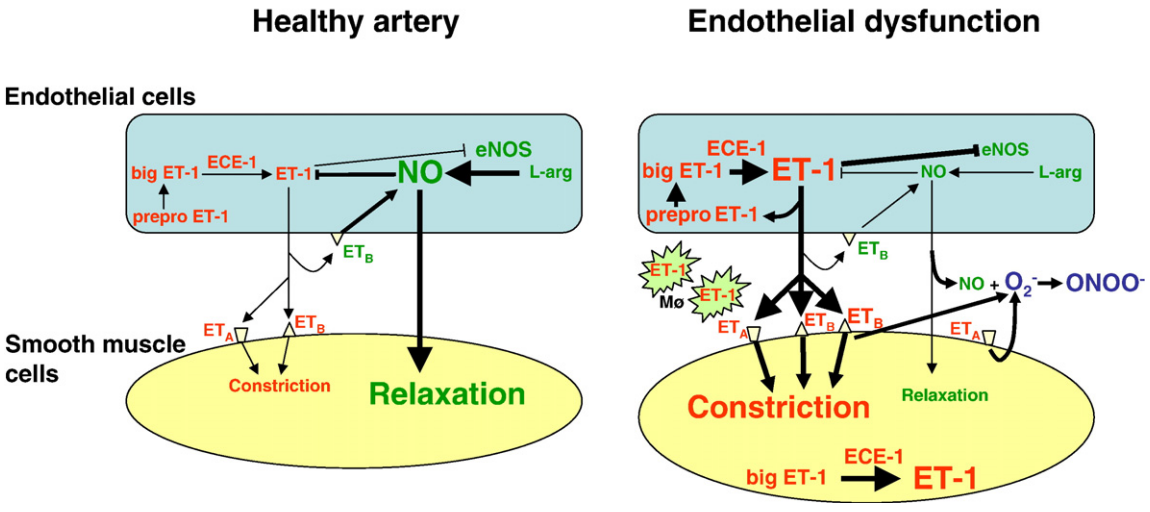

Fig. 1. Schematic figure of the arterial wall under healthy conditions (left) and in endothelial dysfunction (right). In healthy arteries the production of ET-1 is small and the bioavailability of NO is preserved. This means that the balance of effects favours vasorelaxation through increased signalling of cyclic GMP. In endothelial dysfunction there is increased expression of ET-1 in smooth muscle cells and macrophages (MØ). There is also increased expression of ET_B receptors on smooth muscle cells mediating vasoconstriction. ET-1 may decrease endothelial NO synthase (eNOS) expression, thereby reducing NO production. Both the ET_A and the ET_B receptor on smooth muscle cells may mediate formation of superoxide (O_2^-) in endothelial dysfunction. Superoxide will decrease the biological activity of NO by forming peroxynitrate ($ONOO^-$). Collectively the balance of effects is shifted towards more vasoconstriction, inflammation and oxidative stress in endothelial dysfunction.

Table 1
Hemodynamic effects of ET receptor antagonists in various clinical conditions

Clinical condition	Study parameter	ET_A	Dual ET_A/ET_B	ET_B	Comments	Reference
Hypertension ($n=11–19$)	Forearm blood flow increase	33%	63%		No effect in age-matched controls.	[20]
Hypertensives ($n=10$) and controls ($n=10$)	Forearm blood flow increase		30%		No effect in controls	[25]
Obese and lean hypertensives ($n=27$)	Forearm blood flow increase	Obese; ≈25% overweight; ≈18%			No effect in lean hypertensives or obese controls	[22]
Hypercholesterolemia ($n=12$)	Forearm blood flow increase	≈60%	Addition of ET_B blockade reversed the vasodilatation.		No effect of BQ123 in healthy controls	[24]
Atherogenic risk factors ($n=10–12$)	Forearm blood flow increase	HT; 46% Hypercholesterolemic; 24% Smokers; 0%			Healthy; 20%. No difference between smokers, healthy and hypercholesterolemic	[23]
Atherosclerosis ($n=44$)	Epicardial artery diameter change	+5.6±1.0%			Vascular resistance fell by 12±3%	[39]
Coronary artery disease ($n=8$)	Coronary blood flow increase	Atherosclerosis; 16.3% Stenosis; 21.6%			Normal arteries 7.3%	[28]
Coronary and peripheral artery disease ($n=10$)	Forearm blood flow increase	39%	102%	10%	Controls; ET_A/ET_B: 0%, no difference to patients by ET_A, and ET_B reduced flow	[27]
Coronary and peripheral artery disease ($n=37$)	Forearm blood flow increase		42%		Similar vasodilation following 3 months ACE inhibition	[40]
Insulin resistance ($n=11$)	Forearm blood flow increase	18%	0%		Neither treatment affected blood flow in controls	[43]
Coronary artery disease and diabetes ($n=44$)	Forearm blood flow increase		33%		Similar vasodilation following 3 months lipid lowering	[98]
Diabetes mellitus type 2 ($n=15$)	Forearm blood flow increase	33%	33% (addition of ET_B blockade, $n=5$)		No response by ET_A blockade in healthy controls	[29]
Diabetes and microalbumin-urea ($n=10$)	Nailbed nutrative capillary blood flow	100%+increased temperature			No effect in age-matched controls	[99]
Renal failure ($n=8$)	Renal blood flow	38% increase	No change	15% decrease	ET_A or dual blockade had minimal effects in controls	[96]
CAD and type 2 diabetes ($n=7$)	Renal blood flow	No change	24% increase		ET_A/ET_B: increased insulin sensitivity	[30]

vascular sensitivity to ET receptor stimulation is shown in patients with hypertension and atherosclerosis. Cardillo et al. found that the vasoconstrictor response to intra-arterial infusion of ET-1 in the forearm was enhanced in hypertensive as compared to normotensive individuals [20]. This response was mediated via activation of both ET_A and the ET_B receptors. In patients with atherosclerosis, the vasoconstrictor response to ET-1 was not different from that observed in age-matched controls [21]. On the other hand, the ET_B receptor agonist sarafotoxin S6c produced more pronounced reduction in forearm blood flow in patients with atherosclerosis than in the control group, indicating an upregulation of vasoconstrictor ET_B receptors. Results from studies using receptor agonists may be difficult to interpret, however. Therefore, studies in which ET receptor antagonists were administered have been performed. Administration of the selective ET_A receptor antagonist BQ123 increased forearm blood flow only in hypertensive patients but not in normotensive controls [20]. Obese hypertensives dilate more following ET_A receptor blockade than non-obese hypertensive patients [22]. In addition, BQ123 induced a greater vasodilatation in hypertensives than in subjects with hypercholesterolemia or in smokers [23]. Cardillo et al. showed that BQ123 induced a significant increase in forearm blood flow in patients with hypercholesterolemia compared to normal subjects [24] supporting the notion that risk factors for cardiovascular disease stimulate the ET system *in vivo*. The increase in forearm vasodilatation in response to BQ123 was attenuated by inhibition of NO generation [19] indicating that the effect to a major part is dependent on increased NO availability. A combination of ET_A and the ET_B receptor antagonists (BQ123 and BQ788) also evokes a more pronounced increase in forearm blood flow in patients with hypertension than in controls [20]. In accordance, Taddei et al. found that the dual ET_A/ET_B receptor antagonist TAK-044 produced a greater degree of vasodilatation in

hypertensive than in normotensive patients [25]. Collectively these observations indicate that the increased vascular tone induced by ET-1 seems to be more pronounced in hypertension than in association with other risk factors for cardiovascular disease.

The formation and activity of endogenous ET-1 has also been evaluated in patients with atherosclerosis (Table 1). Administration of big ET-1 by intra-brachial artery infusion resulted in more pronounced forearm vasoconstriction in patients with atherosclerosis than in age-matched controls [26]. This effect was accompanied by increased formation of ET-1 as well as presence of ECE immunoreactivity in atherosclerotic plaques in the radial artery, indicating increased ECE activity in patients with atherosclerosis. In another study, dual ET_A/ET_B receptor blockade evoked greater increase in forearm blood flow in patients with atherosclerosis than in controls indicating enhanced vasoconstrictor tone mediated by ET-1 [27]. Furthermore, the vasodilator response to dual ET_A/ET_B receptor blockade was greater than that induced by selective ET_A receptor blockade in patients with atherosclerosis, whereas the opposite was observed in control subjects. This suggests that antagonizing both receptors may be of greater value in achieving vasodilatation in patients with atherosclerosis. Kinlay et al. investigated the response to ET_A receptor blockade in coronary arteries of patients with coronary artery disease by intracoronary infusion of BQ123. They found that BQ123 caused coronary dilatation and that the dilator response was more pronounced in severely stenotic than in angiographically normal segments [28]. Collectively, these observations suggest that the importance of ET-1 for vascular tone becomes greater in severe atherosclerosis than under normal conditions.

Patients with type 2 diabetes also seem to have increased vasoconstrictor activity induced by endogenous ET-1. Accordingly, administration of BQ123 resulted in a significant increase in forearm blood flow in patients with type 2 diabetes whereas it had no effect in age-matched controls [29]. There was no difference between selective ET_A and dual receptor blockade in this patient group, however. Furthermore, dual ET_A/ET_B receptor blockade with BQ123 and BQ788 elicited forearm vasodilatation in patients with atherosclerosis and type 2 diabetes mellitus (Settergren et al., 2007a, manuscript in preparation; [98]). It is also of interest to note that ET_A receptor blockade increases nutritive skin capillary blood flow in patients with type 2 diabetes and microangiopathy, whereas no effect was observed in age-matched controls (Settergren et al., 2007b, manuscript in preparation; [99]). Of further importance in type 2 diabetes, we recently showed that dual ET_A/ET_B receptor blockade improved insulin sensitivity more than selective ET_A receptor blockade in obese patients with coronary artery disease and insulin resistance [30].

It is noticeable that dual ET_A/ET_B receptor antagonism seems to be more effective as vasodilators than selective ET_A receptor antagonists in various cardiovascular disorders like hypertension [20] and atherosclerosis [27] (Table 1). Even though all studies have not compared the two different ET receptor blocking strategies, available literature suggests that it is probably of importance to block both receptors to fully antagonize the vasoconstrictor actions of ET-1 in cardiovascular disease.

2.2. Mechanisms behind changed vascular activity

One explanation behind the altered response to ET receptor blockade in cardiovascular disease states may be the upregulation of ET-1 expression as described above. Another possible mechanism is related to changes in the expression and activity of the different receptor subtypes. An increased number of ET_B receptors has been demonstrated in human atherosclerotic arteries [31]. The receptors were present on inflammatory cells (i.e. macrophages, T-lymphocytes) and vascular smooth muscle cells. Moreover, intimal smooth muscle cells close to foam cells showed increased expression of ET-1 and ET_B receptors. The authors suggested that foamy macrophages and T-lymphocytes may modulate the switch from ET_A to ET_B receptors on vascular smooth muscle cells and that this switch may be of importance for the progression of atherosclerosis [31]. A recent study found both ET_A and ET_B receptor expression were increased in internal mammary arteries from patients with coronary artery disease [32]. Increased expression of ET_B receptors in relation to ET_A receptors has also been demonstrated in experimental models and patients with pulmonary arterial hypertension [33,34].

2.3. Effects of ET receptor antagonists on endothelial function

There exist important interactions between ET-1 and other endothelium-derived substances such as NO. Apart from the stimulating effect of ET-1 on NO release via the ET_B receptor as discussed above, NO is known to inhibit the production of ET-1, possibly via inhibiting superoxide [35]. Administration of ET-1 in healthy humans impairs endothelium-dependent dilatation (Fig. 2) [36,37]. Conversely, administration of ET receptor antagonists improves endothelial function in pathological situations of impaired endothelial function like atherosclerosis and hypertension. Barton et al. demonstrated in apolipoprotein E-deficient mice that ET_A antagonism improves endothelium-dependent, NO-mediated relaxation and reduces atherosclerosis, which occurred concomitantly with a reduction in tissue ET-1 concentrations [38]. This finding in experimental models has led to clinical studies in which it was demonstrated that selective ET_A receptor blockade improves forearm [36] and coronary [39] endothelium-dependent vasodilatation in patients with atherosclerosis. Interestingly, also dual ET_A/ET_B receptor antagonism improves endothelial function in the forearm of patients with atherosclerosis (Fig. 3) [40]. In internal mammary arteries, obtained from patients undergoing coronary artery bypass graft surgery, both selective ET_A and selective ET_B receptor blockade, as well as dual ET_A/ET_B receptor blockade with bosentan improved

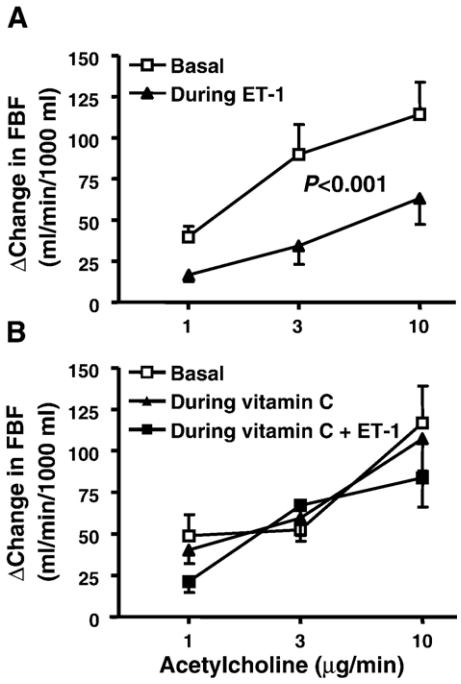

Fig. 2. Effect of acetylcholine on forearm blood flow (FBF) during saline infusion and following a 60 min infusion of ET-1 (20 pmol/min) (A), and during saline, following 15 min infusion of vitamin C and following 60 min co-infusion of vitamin C and ET-1 (B). Means±SEM; $n=12$. Significant differences are shown using two-way repeated measures ANOVA. Modified and reproduced with permission from [37]. © Elsevier.

endothelium-dependent vasodilatation [41]. Dual ET_A/ET_B receptor blockade improves forearm endothelium-dependent vasodilatation also in hypertensive patients, whereas this manoeuvre had no effect in controls [42]. These observations suggests that both selective ET_A and dual ET_A/ET_B receptor blockade improves endothelium-dependent vasodilatation in clinical studies of patients with cardiovascular disease. In a direct comparison, it was recently demonstrated that dual ET_A/ET_B but not selective ET_A receptor blockade enhanced endothelium-dependent vasodilatation in individuals with insulin resistance but without clinical signs of cardiovascular disease, however [43]. These observations imply that blockade of the ET_B receptor-mediated release of NO is not detrimental regarding endothelium-dependent vasodilatation. On the contrary, dual ET_A/ET_B blockade may have additional beneficial effects in comparison to selective ET_A receptor blockade due to the upregulation of ET_B receptors on vascular smooth muscle cells and inflammatory cells as discussed above.

As ACE inhibitors improve endothelial function in patients with coronary artery disease [44] and decrease the production of ET-1 [45], one of the mechanisms of action may be attenuation of ET-mediated vasoconstriction and endothelial dysfunction. We recently observed that ET-mediated vasoconstriction did not differ between patients with atherosclerosis treated with the ACE inhibitor ramipril or placebo [40]. However, the improvement in endothelium-dependent vasodilatation induced by dual ET receptor blockade was still evident after 3 months on ramipril [40]. This extends findings in isolated saphenous veins in which both selective ET_A receptor blockade with BQ123 and dual ET_A/ET_B receptor blockade with bosentan augmented the acetylcholine-induced vasorelaxation following ACE inhibition [46]. These observations suggest that ET receptor blockade may exert beneficial effects regarding endothelial function also when given on top of treatment with ACE inhibitors in patients with atherosclerosis.

Endothelial dysfunction is an early feature during reperfusion following an episode of ischaemia [47]. Ischaemia–reperfusion injury is at least partially related to impaired availability of endothelium-derived NO [47]. Previous studies on experimental animal models have demonstrated that ET receptor antagonists ameliorate myocardial ischaemia–reperfusion injury by reducing infarct size and improving post-ischaemic endothelium-dependent vasodilatation [48]. Selective ET_A receptor blockade (BQ123) restored endothelium-dependent vasodilatation to pre-reperfusion values in atrial microvessels harvested during coronary artery bypass surgery, with a more marked vasodilatation in vessels from patients with diabetes [49]. In a recent study, the effect of the dual ET_A/ET_B receptor antagonist bosentan was tested in a human model of ischaemia–reperfusion injury in the forearm. It was demonstrated that administration of bosentan inhibited the development of endothelial dysfunction following 20 min of forearm ischaemia (Fig. 4) [50]. Furthermore, ET-1 plasma levels were found to predict angiographic no-reflow after successful primary or rescue PCI in patients with acute myocardial infarction [51]. This indicates that ET-1 is involved in microvascular dysfunction during ischaemia–reperfusion injury, and ET receptor antagonists might be beneficial in the management of no-reflow and to prevent endothelial dysfunction following ischaemia–reperfusion in humans.

3. The molecular mechanisms linking the endogenous ET system and vascular dysfunction

3.1. Interference with NO and increased oxidative stress

Several possible molecular mechanisms may underlie the effect of ET-1 and ET receptor antagonists on endothelial

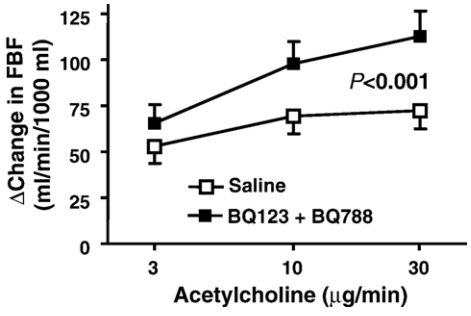

Fig. 3. Effect of acetylcholine on absolute change in forearm blood flow (FBF) during saline infusion and following a 60-min infusion of the ET_A receptor antagonist BQ123 and the ET_B receptor antagonist BQ788 in patients with atherosclerosis. Means±SEM; $n=37$. Modified and reproduced with permission from [40]. © Blackwell publishing.

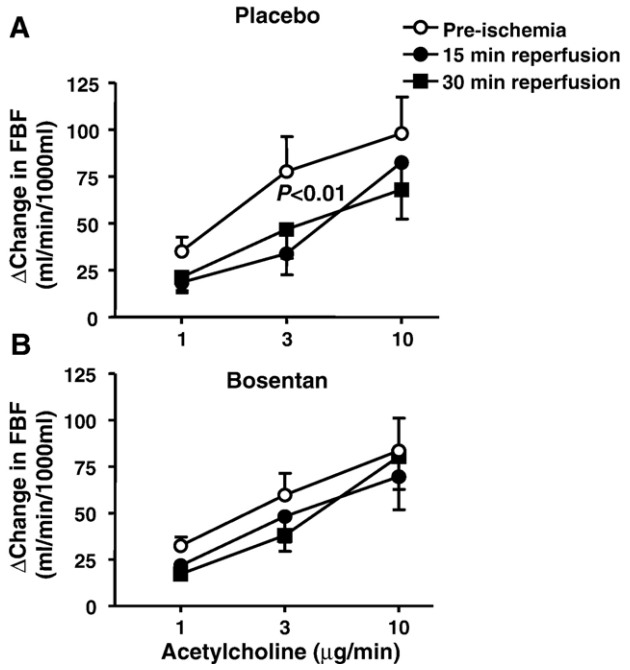

Fig. 4. Change in forearm blood flow (FBF) induced by acetylcholine before ischemia and at 15 and 30 min of reperfusion following 20 min ischaemia. Each subject was investigated after administration of (A) placebo and (B) the dual ET receptor antagonist bosentan. Significant differences from the pre-ischemic response are indicated. Means±SEM; $n=13$. Modified and reproduced with permission from [50]. © The Biochemical Society.

function and NO bioavailability (Fig. 5). The vasodilatation induced by ET_A receptor antagonism in healthy humans was reduced by 95% following inhibition of NO generation [19], whereas inhibition of prostanoid generation did not affect the response. This finding suggests that improvement of NO bioavailability plays an important role in the vasodilatation induced by ET receptor blockade. Both dual ET_A/ET_B and selective ET_A receptor blockade increase endothelial NO synthase activity in hypercholesterolemic pigs [52]. Total and calcium-dependent NO synthase activity was significantly higher in aortic endothelial cells after dual ET_A/ET_B antagonism than in those after selective ET_A blockade [52]. ET-1 impairs NO production and downregulates the expression of endothelial NO synthase in endothelial cells [53]. In addition, bosentan increased the expression of endothelial NO synthase in hearts subjected to ischaemia and reperfusion [54]. Thus, ET-1 may reduce NO bioavailability via interference with the expression and activity of endothelial NO synthase.

Another mechanism linking ET-1 to NO may be via formation of reactive oxygen species, which will result in decreased bioactivity of NO by virtue of formation of peroxynitrite (Fig. 1). The reactive oxygen species can, apart from interfering with NO, also inhibit other endothelium-dependent vasodilator pathways mediated through prostacyclin and endothelium-derived hyperpolarizing factor [55,56]. ET-1 increases superoxide production in the rat aorta *in vitro*, an effect that could be inhibited by the selective ET_A receptor antagonist BQ123 [57]. ET-1 also stimulates NADPH oxidase-derived superoxide formation in hypertensive rats, an effect that could be inhibited by ET_A receptor blockade [58]. ET-1 increased the expression of $gp91^{phox}$, the rate-limiting subunit of NADPH oxidase [59], and augmented superoxide production in endothelial cells via the ET_B receptor in human endothelial cells [60]. The stimulating effect of ET-1 on superoxide production may also be coupled to the NADPH oxidase subunit $p22^{phox}$ [61,62]. The stimulation of superoxide is linked to functional effects since ET-1 was demonstrated to impair endothelium-

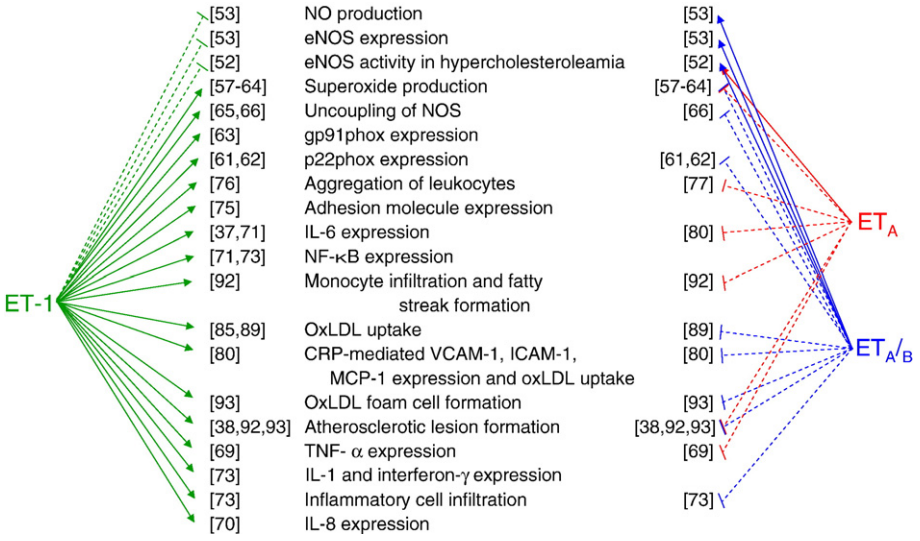

Fig. 5. Molecular mechanisms of vascular dysfunction induced by ET-1 including pro-inflammatory and pro-atherosclerotic effects. Potential benefit in cardiovascular disease states may be mediated by altering these mechanisms through dual ET_A/ET_B receptor blockade and/or selective ET_A receptor blockade. []=Reference; ⊣=inhibition; →=stimulation.

dependent relaxations of aorta from control and diabetic rats via a mechanism involving superoxide production, PI3-kinase activity and $p22^{phox}$ expression. Furthermore, chronic treatment with the dual ET_A/ET_B receptor antagonist J-104132 improved acetylcholine-mediated endothelium-dependent vasodilatation, reduced superoxide formation and prevented p22phox formation in diabetic rats [61]. These data are in agreement with *in vivo* observations in transgenic mice overexpressing ET-1 [63]. These mice exhibit endothelial dysfunction, increased NADPH oxidase activity, and increased expression of $gp91^{phox}$. The endothelial dysfunction could be restored by vitamin C, supporting the role of increased oxidative stress [63]. Furthermore, vitamin C has been shown to inhibit the formation of reactive oxygen species induced by ET-1 in isolated smooth muscle cells [64]. In addition, the effects of ET-1 on coronary vasoconstriction may be more pronounced in states of reduced bioavailability of the eNOS-co-factor tetrahydrobiopterin (BH4) [65]. Recent data demonstrate that ET-1 mediates superoxide production and vasoconstriction through activation of NADPH oxidase and uncoupled NOS in the rat aorta [66]. The uncoupling of NOS means that NOS generates superoxide instead of NO in states of BH4 deficiency. Interestingly, these effects could be inhibited by BH4 and by dual ET receptor blockade, but not by selective ET_A receptor blockade [66]. ET-1 may also promote BH4 deficiency in a rat model of hypertension via an ET_A-mediated NADPH oxidase pathway which contributes to impaired endothelium-dependent relaxation [67]. These data suggest that increased oxidative stress induced by ET-1 in the vessel wall is an important factor leading to endothelial dysfunction and enhanced susceptibility to atherosclerosis.

ET-1 has also been demonstrated to be associated with increased oxidative stress and endothelial dysfunction in humans. ET-1 stimulates superoxide formation and impairs endothelium-dependent vasodilatation in human venous bypass conduits from patients with diabetes [68]. Importantly, recent data suggest that the impairment in endothelium-dependent vasodilatation *in vivo* induced by ET-1 in healthy humans can be prevented by administration of the anti-oxidant vitamin C (Fig. 2) [37]. Taken together, these findings suggest that ET-1 may increase oxidative stress through induction of reactive oxygen species. Furthermore, ET receptor antagonists may be a therapeutic option that results in increased NO bioavailability and decreased levels of reactive oxygen species, thereby improving endothelial function in various cardiovascular disease states.

3.2. Pro-inflammatory and pro-atherosclerotic effects

Apart from its direct vasomotor activity, ET-1 has been implicated in inflammatory processes within the vascular wall (Fig. 5). Specifically, ET-1 in subnanomolar concentrations has been demonstrated to activate macrophages, resulting in the release of pro-inflammatory and chemotactic mediators, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8[69–71] which are of importance in the atherosclerotic process [72]. Cardiac overexpression of ET-1 in mice is associated with an inflammatory response involving increased activation of the pro-inflammatory transcription factor NF-κB and expression of several pro-inflammatory cytokines including TNF-α, IL-1 and IL-6 [73]. Interestingly, significant prolongation of survival was observed only with a dual ET_A/ET_B antagonist, but not with a selective ET_A antagonist [73]. In turn, transcription factors and pro-inflammatory cytokines such as NF-κB, TNF-α, and IL-6 stimulate ET-1 production [74]. ET-1 enhances the expression of adhesion molecules on TNF-α stimulated vascular endothelial cells [75] and stimulates aggregation of polymorphonuclear neutrophils [76]. Conversely, ET receptor blockade attenuates the accumulation of neutrophils and myeloperoxidase activity in the ischemic myocardium [77].

IL-6 has been implicated in the development of atherosclerosis [72] and endothelial dysfunction in humans [78]. As noted above, ET-1 stimulates IL-6 release *in vitro* [71] and *in vivo* [37]. The release of IL-6 induced by ET-1 from human vascular smooth muscle involves activation of NF-κB [71]. Possibly, release of IL-6 may further increase oxidative stress as suggested by the *in vitro* observation that IL-6 induces production of reactive oxygen species [79].

C-reactive protein (CRP) has emerged as a predictor and possible mediator of atherosclerotic cardiovascular disease. Verma et al. demonstrated that CRP stimulated the expression of adhesion molecules and monocyte chemoattractant protein-1 in endothelial cells [80]. Interestingly, this effect was inhibited by bosentan as well as an antibody against IL-6 suggesting involvement of ET-1 and IL-6 in the pro-inflammatory effect of CRP.

Hypercholesterolemia is associated with impaired endothelium-dependent vasodilation and elevated plasma and tissue ET-1 concentrations, which may account for the vasomotor dysfunction under this condition [81]. In support of this notion, inhibition of either ET_A or both ET_A and ET_B receptors restores endothelium-dependent vasodilation and NO production in hypercholesterolemic pigs [82]. The normalized NO production results from increased activity of NO synthase. The effect of dual ET_A/ET_B blockade was significantly higher than that of selective ET_A antagonism [52]. Statin therapy may further improve the beneficial effects of ET antagonism on NO-mediated vasodilation in hypercholesterolemia [83,84].

There also seems to exist important interactions between oxidized low-density lipoprotein (LDL) and ET-1 which may be of importance in atherogenesis. ET-1 augments the uptake of oxidized LDL [85], whereas oxidized and native LDL in turn stimulates the production of ET-1 [86]. Interestingly, statins have been demonstrated to decrease the expression of pre-pro ET-1 mRNA in endothelial cells [87] and the vasoconstrictor response to ET-1 *in vitro* [88]. In addition, ET-1 stimulates uptake of oxidized LDL in endothelial cells via an ET_B receptor-mediated effect [89]. ET-1 is known to be elevated in both type 2 diabetes and by high LDL cholesterol

[8,90]. Previous *in vitro* studies indicate that lipid-lowering treatment suppresses the expression of ET-1 in endothelial cells [91] thereby attenuating the negative effect of ET-1 on endothelial function. Therefore we have recently evaluated the effect of dual ET receptor blockade before and after treatment with simvastatin 80 mg od or simvastatin 10 mg plus the cholesterol absorption inhibitor ezetimibe 10 mg od in patients with dysglyceamia and coronary artery disease (Settergren et al., 2007a, manuscript in preparation; [98]). We observed a significant vasodilator effect and improvement in endothelium-dependant vasodilatation which was unaffected by aggressive cholesterol lowering, suggesting that is not through interaction with the ET system that statins exert their effect on endothelial function. On the other hand, this indicates that ET receptor blockade may exert beneficial effects on top of aggressive lipid-lowering therapy.

ET-1 may also stimulate activation and accumulation of macrophages (Fig. 1). Kowala et al. [92] demonstrated that an ET_A receptor antagonist inhibited monocyte infiltration and development of fatty streak in hypercholesterolemic hamsters. A dual ET_A/ET_B receptor antagonist reduced foam cell formation in macrophages exposed to oxidized LDL [93]. In the same study, the ET receptor antagonist significantly inhibited the development of atherosclerosis in LDL receptor knock out mice. These observations are in support of the previous observation that selective ET_A receptor blockade attenuates the development of atherosclerotic lesions in apolipoprotein E knockout mice [8,38]. Taken together, these data clearly suggest that ET receptor blockade exerts anti-atherogenic effects.

4. Selective ET_A vs. dual ET_A/ET_B receptor blockade

The changes in ET receptor expression in the vascular wall in pathological states described above may imply that blockade of both receptors is preferable to selective ET_A receptor blockade in order to fully antagonize the effects of ET-1. The changes in receptor expression are paralleled by more pronounced functional effects of dual ET_A/ET_B receptor blockade in comparison with selective ET_A receptor blockade in clinical studies. Thus, dual ET_A/ET_B receptor antagonism seems to induce more effective vasodilation than selective ET_A receptor antagonism in various cardiovascular disorders (Table 1). Additional biological effects beyond direct vascular effects of potential importance during pathophysiological conditions such as superoxide production, stimulation of pro-inflammatory cytokines and LDL uptake as well as insulin resistance seem to be mediated via the ET_B receptor in addition to the ET_A receptor (Fig. 5). Even though most studies have not compared the two different ET receptor blocking strategies, available literature suggests that it may be preferable to block both receptors to fully antagonize the pathophysiological actions of ET-1 in cardiovascular disease. On the other hand, blockade of ET_B receptors will reduce clearance of ET-1 [94] and thereby increase circulating levels of ET-1. Furthermore, the ET_B receptor may exert beneficial effects by releasing NO from endothelial cells. An additional beneficial effect mediated by the ET_B receptor is the stimulation of renal sodium and water excretion [95]. Accordingly, it has been demonstrated that only selective ET_A receptor blockade increases renal blood flow and improves renal function in patients with renal failure [96]. On the other hand, dual ET_A/ET_B receptor blockade, but not selective dual ET_A receptor blockade, increased renal blood flow in patients with coronary artery disease and type 2 diabetes but with normal renal function [30]. These apparently conflicting results illustrate the need for carefully designed larger randomised clinical studies to clarify the potentially beneficial clinical effects of dual ET_A/ET_B receptor blockade over selective ET_A receptor blockade in different patient groups. Moreover, since the expression of ET receptors differs between healthy subjects and patients with cardiovascular disease as well as between various types and states of cardiovascular disease, it is of importance to characterize the response to receptor blockade in each population.

5. Conclusion and future perspectives

At present ET receptor antagonists are approved for the treatment of pulmonary arterial hypertension. A large body of evidence has accumulated, indicating that the ET system is causally involved in vascular dysfunction during a large number of additional cardiovascular diseases. ET-1, the ET receptors and the biological effects mediated by ET-1 are markedly altered and become substantially more pronounced during development of cardiovascular disease. ET receptor antagonists have been shown to be effective in several animal models, and initial clinical studies indicate that they also improve vascular function in patients with cardiovascular disease. The ET_B receptor becomes of functional greater importance in several disorders like hypertension [20], atherosclerosis [27] and insulin resistance [30,43]. This suggests that dual ET_A/ET_B receptor blockade may be superior to selective ET_A receptor blockade in certain conditions by inducing vasodilatation, improving endothelial function and insulin sensitivity in humans, however further studies with head to head comparisons in various cardiovascular disorders are warranted. Larger clinical studies have so far mainly been performed in pulmonary hypertension with favourable clinical results. Liver toxicity, as a main side effect of ET receptor antagonists has to some extent limited their use but was not considered a major problem in clinical trials [97]. However, transaminase levels should be monitored in future clinical studies, since this may be a limitation if ET receptor antagonists are to be considered in larger cohorts of patients with cardiovascular disease. Considering the potentially important role of ET-1 in the development of vascular dysfunction reviewed in the present article, conditions with increased inflammatory activity, oxidative stress and vascular tone such as atherosclerosis, hypertension and vascular complications in diabetes may be of interest to explore in larger clinical trials using ET receptor antagonists.

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