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THEMED SECTION: ENDOTHELIUM IN PHARMACOLOGY

REVIEW

Endothelium-dependent contractions and endothelial dysfunction in human hypertension

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The endothelium is a crucial regulator of vascular physiology, producing in healthy conditions several substances with a potent antiatherosclerotic properties. Accordingly, the presence of endothelial dysfunction is associated with subclinical atherosclerosis and with an increased future risk of cardiovascular events. A large body of evidence supports the fundamental role of nitric oxide (NO) as the main endothelium-derived relaxing factor. However, in the presence of pathological conditions, such as hypertension, endothelial cells, in response to a number of agents and physical stimuli, become also a source of endothelium-derived contracting factors (EDCFs), including endothelins and angiotensin II and particularly cyclooxygenase-derived prostanoids and superoxide anions. These latter were at first identified as responsible for impaired endothelium-dependent vasodilation in patients with essential hypertension. However, cyclooxygenase-dependent EDCFs production is characteristic of the aging process, and essential hypertension seems to only anticipate the phenomenon. It is worth noting that both in aging and hypertension EDCF production is associated with a parallel decrease in NO availability, suggesting that this substance could be oxygen free radicals themselves. Accordingly, in hypertension both indomethacin, a cyclooxygenase inhibitor, and vitamin C, an antioxidant, increase the vasodilation to acetylcholine by restoring NO availability. In conclusion, hypertension is characterized by a decline in endothelial function, associated with a progressive decrease in NO bioavailability and increase in the production of EDCF. The mechanisms that regulate the balance between NO and EDCF, and the processes transforming the endothelium from a protective organ to a source of vasoconstrictor, proaggregatory and promitogenic mediators remain to be determined.
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Keywords: endothelium; hypertension; vasodilation; endothelium-derived contractions; EDCF; cyclooxygenase; oxidative stress

Abbreviations: COX, cyclooxygenase; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarizing factor; eNOS, nitric oxide synthase; ET, endothelin; FBF, forearm blood flow; L-NMMA, monomethyl-L-arginine; NO, nitric oxide; ROS, reactive oxygen species

Introduction

The endothelium, considered for years a mere selectively permeable barrier between the bloodstream and the vascular wall, is now recognized to be a fundamental homeostatic organ for the regulation of the vascular tone and structure. Under physiologic conditions, endothelial cells are able to synthesize and secrete a large spectrum of antiatherosclerotic substances, the most characterized of which is nitric oxide (NO), a gas generated from the metabolism of L-arginine by constitutive endothelial NO synthase (eNOS) (Vanhoutte, 1989). In normal conditions, endothelial stimulation induces the production and release of NO, which diffusing to surrounding tissues and cells, exerts its cardiovascular protective role by relaxing media-smooth muscle cells, preventing leukocyte adhesion and migration into the arterial wall, muscle cell proliferation, platelet adhesion and aggregation, and adhesion molecule expression (Vanhoutte, 1989; Taddei *et al.*, 2003). In disease conditions, including the presence of cardiovascular risk factors, the endothelium undergoes functional and structural alterations and loses its protective role, becoming a pro-atherosclerotic structure (Vanhoutte, 1989). The loss of the normal endothelial function is referred to as 'endothelial dysfunction', which is characterized by impaired NO bioavailability. This can follow either a reduced production of NO by eNOS, or, more frequently, an increased breakdown by reactive oxygen species (ROS) (Vanhoutte, 1989; Taddei *et al.*, 2003). When NO availability is significantly reduced, the endothelium

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activates various compensatory physiological pathways. In this setting, the endothelium-dependent vasodilation is partly maintained, although impaired, by the production and release of endothelium-derived vasodilators other than NO, such as prostanoids (prostacyclin) and other endothelium-derived hyperpolarizing factors (EDHFs). Of importance, a dysfunctioning endothelium also becomes a source of other substances and mediators which are detrimental to the arterial wall, including endothelin-1 (ET-1), thromboxane A_2, prostaglandin H_2 and ROS (Taddei *et al.*, 2003), with various pro-atherosclerotic features, including a vasoconstricting action. Accordingly, the presence of endothelial dysfunction, characterized by both NO deficiency and activation of endothelium-dependent vasoconstriction, has been implicated in the pathogenesis of atherosclerosis and thrombosis (Taddei *et al.*, 2003; Brunner *et al.*, 2005).

How to evaluate endothelial function

The regulation of the endothelial physiology is largely district-specific and differs in various organs and tissues, and within the same vascular district it largely varies in relation to vessel size – that is, large arteries (macrocirculation) versus arterioles (microcirculation). It is therefore conceivable that the use of systemic circulating markers of endothelial function is unreliable. In addition, NO is a gas with a very short half-life and its moment-by-moment quantification in a specific vascular district is almost impossible. For these reason, NO bioavailability in humans is indirectly estimated from its local vasodilating effect after endothelia stimulation with specific external mechanical and pharmacological stimuli, that is, through vascular reactivity tests (Deanfield *et al.*, 2005). Specifically, endothelium-dependent vasodilation can be evaluated by the use of either receptor-operated (acetylcholine, bradykinin, substance P), mechanical (increase in shear stress) or mixed (dynamic exercise and cold pressor test) stimuli and in different vascular beds (John and Schmieder, 2000; Deanfield *et al.*, 2005). In the heart, endothelial function can be assessed in the macrocirculation by quantitative angiography, evaluating the change in coronary artery diameter after local infusion of agonists (e.g. acetylcholine), and in the microcirculation as changes in flow by intravascular ultrasound (Deanfield *et al.*, 2005). This central coronary approach has conceivably the highest clinical value, as it explores the vascular bed more often involved by atherosclerosis and responsible for cardiac events. However, its invasiveness highly limits its applicability (Deanfield *et al.*, 2005). For this reason, several other techniques have been developed to assess peripheral circulation endothelial function. In particular, peripheral microcirculation can be thoughtfully studied by venous plethysmography to evaluate forearm blood flow (FBF) changes to intraarterial infusion of various substances. This approach is very useful as it allows to study the mechanisms underlying endothelial dysfunction by administering endothelial agonist and antagonist (Deanfield *et al.*, 2005). However, again FBF is still invasive and requires brachial artery cannulation. Therefore, in recent years flow-mediated dilation of the brachial artery has been widely used among researchers; indeed, although its reproducibility is limited, it has the advantage of being non-invasive as it uses ultrasound analysis of brachial artery diameter after local increase in shear stress, induced by 5 min of forearm ischemia (Deanfield *et al.*, 2005) and additionally the stimulus used (increase in flow) is more physiological than the local infusion of endothelial agonists at pharmacological concentrations (e.g. muscarinic agents). Finally, it is noteworthy that vascular responses obtained in different vascular districts and using different stimuli and techniques are poorly related and give therefore different information (Anderson *et al.*, 1995). Considering this aspect and the autocrine-paracrine nature of endothelial physiology, high caution should be paid in the interpretation of experimental studies and mostly in considering data obtained in a vascular district as completely indicative endothelial function in other district (e.g. coronary circulation).

Clinical significance of endothelial dysfunction

Endothelial dysfunction, defined as reduced vasodilating response to endothelial stimuli, is observed in the presence of major cardiovascular risk factors, including aging (Brunner *et al.*, 2005), menopause (Brunner *et al.*, 2005), smoking (Brunner *et al.*, 2005), diabetes mellitus (Brunner *et al.*, 2005), hypercholesterolemia (Brunner *et al.*, 2005) and hypertension (Brunner *et al.*, 2005). Notably, the presence of multiple risk factors is able to determine a progressive worsening of endothelial function (Vita *et al.*, 1990; Benjamin *et al.*, 2004). Conversely, the presence of endothelial dysfunction is also suggested to increase the susceptibility to develop hypertension (Rossi *et al.*, 2004) and diabetes (Rossi *et al.*, 2005), thus being not only a collateral feature of established risk factors, but also a possible pathogenetic mechanism for their onset.

The hypothesis that endothelial dysfunction is clinically relevant in the progression of the atherosclerotic process is supported by several evidences. Thus, the presence of subclinical and clinical target organ damage, an intermediate stage in the continuum of vascular disease, is associated to the presence of endothelial dysfunction. In particular, increased intima-media thickness of the common carotid artery, a non-invasive marker of atherosclerosis is directly related to the impairment of endothelial dysfunction in the peripheral circulation (Ghiadoni *et al.*, 1998; Juonala *et al.*, 2004). Moreover, in patients with coronary artery stenosis, an impairment of endothelium-dependent vasodilation in coronary arteries is present, not only in a diseased vessel but also in a non-diseased pre-stenotic arterial segments (Ludmer *et al.*, 1986) or vessels (Quyyumi *et al.*, 1997), and in the coronary microcirculation (Egashira *et al.*, 1993; Quyyumi *et al.*, 1997). Interestingly, an inverse relation between the presence of intramural plaques as detected by intravascular ultrasound and vasodilation to intracoronary acetylcholine is present in patients without angiographic evidence of coronary atherosclerosis (Zeiher *et al.*, 1994). These data are supported also by longitudinal studies, showing a significant augmented risk of developing arteriolosclerosis and plaques in heart transplanted patients with coronary endothelial dysfunction (Davis *et al.*, 1996; Hollenberg *et al.*, 2001). Overall, these data support the presence of a link between endothelial dysfunction and the probability of developing or worsening structural changes in the coronary and carotid circulation.

In recent years a large number prospective of studies has been conducted in several groups of patients to prove the prognostic significance of the association between endothelial dysfunction and cardiovascular disease (Taddei and Salvetti, 2002; Lerman and Zeiher, 2005). As previously noted, the endothelium-dependent vasodilating responses in different vascular districts of the same subject are poorly related (Anderson *et al.*, 1995; Eskurza *et al.*, 2001; Park *et al.*, 2001), both because of the different techniques and stimuli used and because of the highly region-specific regulation of endothelial physiology. Despite this district specificity, the presence of endothelial dysfunction almost invariably results to be an independent predictor of future clinical events wherever detected. Accordingly, this prognostic role has been demonstrated in peripheral (Neunteufl *et al.*, 2000; Heitzer *et al.*, 2001; Gokce *et al.*, 2002; Brevetti *et al.*, 2003) and central circulation (Schachinger *et al.*, 2000; Suwaidi *et al.*, 2000; Halcox *et al.*, 2002; Schindler *et al.*, 2003; Targonski *et al.*, 2003), in microcirculation (Heitzer *et al.*, 2000; Suwaidi *et al.*, 2000; Targonski *et al.*, 2003) and large arteries (Neunteufl *et al.*, 2000; Schachinger *et al.*, 2000; Suwaidi *et al.*, 2000; Gokce *et al.*, 2002; Halcox *et al.*, 2002; Brevetti *et al.*, 2003; Schindler *et al.*, 2003; Targonski *et al.*, 2003) and independently from the used endothelial stimulus. Caution must, however, be paid in interpreting the data, as so far the total number of clinical events investigated is limited and not conclusive to define the presence of endothelial dysfunction as an independent risk factor for cardiovascular events or as an integrated marker for the global risk.

Endothelial dysfunction in essential hypertension

Most of studies investigating endothelium-derived contracting factors (EDCFs) and relaxing factors in humans have been performed in the peripheral microcirculation using the perfused forearm technique, which providing the possibility of local intra-arterial infusion of vascular active agents, allows to investigate fine biochemical mechanisms (Figure 1). With this technique, the detection of increase or decrease in FBF by venous plethysmography is a highly reliable index of local vasodilation or vasoconstriction respectively.

A large body of evidence invariably demonstrates that the presence of endothelial dysfunction is a hallmark of the hypertensive patient (Panza *et al.*, 1994; 1995; Taddei *et al.*, 1998b; John and Schmieder, 2000). A reduction in the net production of NO does not seem to be importantly implicated in impairing NO bioavailability in hypertension, although a lack of the enzyme substrate L-arginine by enhanced activity of vascular arginase has been recently suggested in some forms of experimental hypertension (Zhang *et al.*, 2004). Nonetheless, so far the main cause of hypertension-related endothelial dysfunction in humans has been identified with an increased NO breakdown. In particular, hpertension-related endothelial dysfunction has been demonstrated to be the consequence of increased oxidative stress production (Taddei *et al.*, 1998a). ROS, mainly superoxide anions, are highly reactive and destroy NO, thus reducing its bioavailability and producing peroxynitrites (Vanhoutte, 1989), which have several negative effects on vascular function and structure (Szabo *et al.*, 2007). Supporting this hypothesis, the intra-arterial administration of high doses of the antioxidant vitamin C in the forearm of essential hypertensive patients, is able to acutely restore a normal endothelium-dependent vasodilation and to restore NO bioavailability (Taddei *et al.*, 1998a). Various enzymatic and non-enzymatic sources of ROS have been described to be activated in endothelial cells, smooth muscle cells and inflammatory cells within the arterial wall of hypertensive patients, including NAD(P)H-oxidase, xanthine oxidase, cyclooxygenase (COX) and

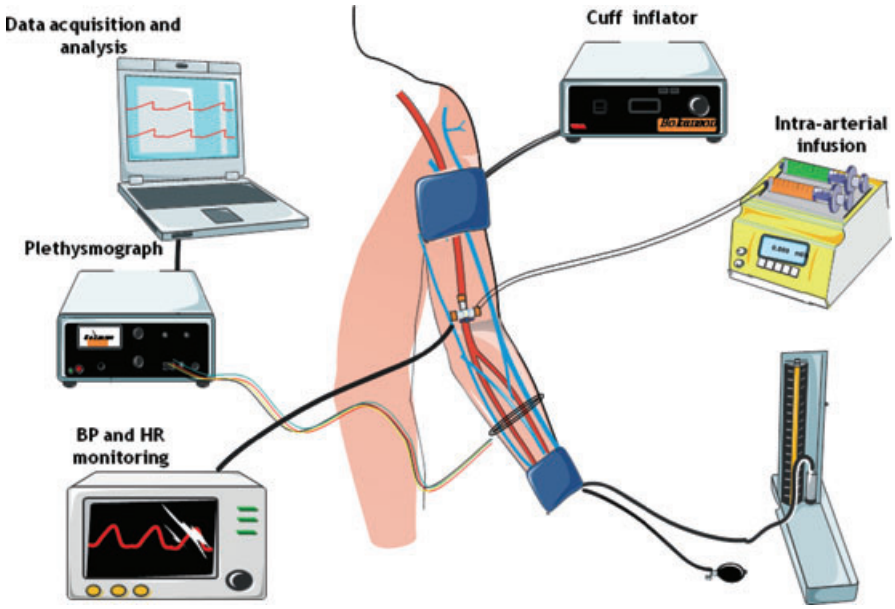

Figure 1 Schematic representation of the perfused forearm technique to evaluate endothelial function in human peripheral microcirculation. The brachial artery of the non-dominant forearm is cannulated for drug infusion at systemically ineffective rates, intra-arterial blood pressure (BP) and heart rate (HR) monitoring. Forearm blood flow is measured by strain-gauge venous plethysmography.

uncoupled eNOS (John and Schmieder, 2000). In the presence of hypertension, the reduced NO availability is partially compensated by the activation of alternative pathways, including the production and release of EDHFs which contribute to maintain endothelium-dependent vasodilation (Taddei *et al*., 1999a). In this condition, a complex interplay between NO and ET-1 can contribute to the establishment of endothelial dysfunction. Indeed, despite having normal circulating levels of ET-1, hypertensive patients show an augmented vasoconstrictor activity of the peptide in the peripheral circulation which parallels the diminished NO availability (Schiffrin, 1999). At the vascular level, ET-1 binds to and exerts its effects through its specific receptors ET_B and ET_A. In particular, ET_A are mainly localized in the smooth muscle cell and stimulate vascular contraction and hypertrophy (Penna *et al*., 2006). On the contrary, ET_B are scantly represented on smooth muscle cells but they are abundant on endothelial cells and mediate NO release thus inhibiting vasoconstriction and cell proliferation (Penna *et al*., 2006). In the presence of endothelial dysfunction, activation of endothelial ETB receptors is not able to increase NO-mediated vasodilation and the resulting contracting effect of ET-1 is enhanced (Penna *et al*., 2006). This phenomenon is further fostered by the reduced inhibitory effect of NO on ET-1 production and activity (Taddei *et al*., 1999b). The overall altered equilibrium between the two systems can lead to an increased vasoconstricting and proliferative activity of endotelin-1.

It is worth noting that hypertension-related endothelial dysfunction does not seem to represent a pathogenetic mechanism for the increased blood pressure values, as there is no association between the degree of endothelial dysfunction and blood pressure values (John and Schmieder, 2000). The condition seems rather to be partly genetically determined and accordingly offspring of hypertensive patients, although normotensive, show impaired endothelial function (Taddei *et al*., 1996b). Finally, endothelial dysfunction is not a specific feature of hypertension, but it is common to the majority of cardiovascular risk factors (Deanfield *et al*., 2005).

EDCFs

In the years following the discovery of the obligatory role of endothelial cells to induce a vasodilating response to acetylcholine in rabbit isolated arteries (Furchgott and Zawadzki, 1980), De Mey and Vanhoutte (De Mey and Vanhoutte, 1982; 1983) also observed that the endothelium can induce contractions of isolated canine arteries and veins. Since then, the pathophysiology of endothelium-dependent contraction has been deeply investigated. Several agents and physical stimuli induce such contractions through the activation of various biochemical pathways leading to the production and secretion of different EDCFs in different animal species and vascular districts. Although relaxing factors play a crucial role in the regulation of circulatory vasomotion, experimental evidence support the concept that also contracting factors have a significant role, which becomes particularly important in aging, in the hypertensive process or under other pathological conditions such as diabetes, vasospasm and reperfusion injury (Katusic and Shepherd, 1991; Luscher *et al*., 1992).

Among proteic mediators, ET represent a potent vasoconstricting agent released by the endothelium, particularly in pathological conditions (Vanhoutte, 1989). Additionally, also angiotensin II, beyond being produced systemically, can be released by endothelial cell and induce local vascular constriction (Vanhoutte, 1989). However, COX-dependent EDCFs are at the moment considered to play a primary role (Figure 2). It was demonstrated that arachidonic acid, a precursor of COX-derived products, is able to induce endothelium-dependent contractions in arteries and veins and that this phenomenon is inhibited by COX blockers (Miller and Vanhoutte, 1985; Katusic *et al*., 1988). Under pathological conditions, such as experimental diabetes and hypertension, COX-dependent EDCFs can be released following endothelial stimulation with acetylcholine or the calcium ionophore A23187 (Konishi and Su, 1983; Luscher and Vanhoutte, 1986; Katusic *et al*., 1988), as well as physical stimuli such as shear stress (Huang *et al*., 2000). Experimental studies have identified two main COX-derived products which can behave as EDCFs, namely thromboxane A2 or prostaglandin H2 (Vanhoutte, 1989; Luscher *et al*., 1992). However, although considered a vasodilator, also derived prostacyclin is able to behave like an EDCF under disease conditions, such as hypertension (Rapoport and Williams, 1996; Gluais *et al*., 2005). These substances, once produced diffuse to the underlying vascular smooth muscle cells and through the activation of specific receptors (TP receptors) (Vanhoutte *et al*., 2005) induce contraction. Accordingly, most COX-mediated endothelium-dependent contractions are inhibited by smooth muscle cell TP-receptor antagonists (Tesfamariam *et al*., 1989; Yang *et al*., 2002; Virdis *et al*., 2007). Interestingly, in the setting of hypertension a hyperresponsiveness of vessels to TP-mediated contraction is present despite the lack of TP gene overexpression (Tang *et al*., 2008).

Initial data showed that in the aorta of spontaneously hypertensive rats, preferential inhibitors of COX-1 rather than those of COX-2 prevented the endothelium-dependent contractions to acetylcholine (Ge *et al*., 1995; Yang *et al*., 2002). Similarly, in vessels from angiotensin II-infused mice selective, COX-1 but not COX-2 inhibition is able to improve endothelium-dependent vasodilation, and this phenomenon is accompanied by increased expression of COX-1 and decreased expression of COX-2 gene (Virdis *et al*., 2007). It is to note that also the activation of COX, and particularly of membrane-bound COX-1, is able to produce superoxide anions. It was demonstrated that increased vascular levels of oxidative stress, either COX-derived or produced by other sources, in both spontaneously hypertensive rats and angiotensin-II-infused mice is able enhance membrane-bound COX transformation of arachidonic acids into endoperoxides (Ge *et al*., 1995; Vanhoutte *et al*., 2005; Virdis *et al*., 2007). The increased activity of COX-1 is related to both an enhanced gene expression and to a direct activation of the enzyme. However, interestingly, the angiotensin-II-dependent COX-1enzymatic activation but not expression seems to be ROS-mediated (Virdis *et al*., 2007).

Finally, it is to note that beside the role of COX-1 in modulating endothelium-dependent contractions, COX-2 is also able to produce EDCFs, particularly in those vascular district

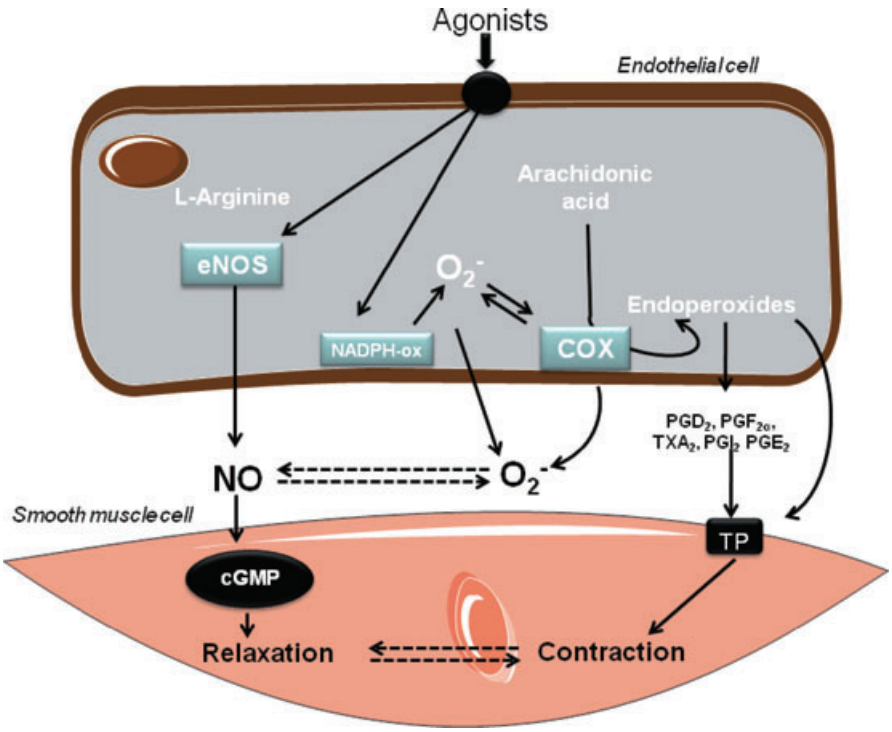

Figure 2 Schematic representation of the interplay between endothelium-derived relaxing (nitric oxide – NO) and contracting factors. Under endothelial stimulation nitric oxide synthase (eNOS) is stimulated to generate NO from L-arginine and NO, diffusing to the underlying smooth muscle cells, induces relaxation by increasing the production of cyclic-GMP. In pathologic conditions, such as hypertension, endothelial stimulation also leads to an increased production of superoxide anions (O_2^-) by NADPH-oxidase (NADPH-ox) and cyclooxygenase (COX). Released superoxide is able to scavenge NO, thus reducing its bioavailability and impairing endothelium-dependent vasodilation. Additionally, stimulated COX also produces endoperoxides and consequently thromboxane-A_2, prostaglandin-E_2, prostaglandin-$F_{2\alpha}$ prostaglandin-D_2 and prostacyclin, which binding to a specific receptor (TP) on smooth muscle cells, cause vasoconstriction.

in which it is expressed at a higher level (Camacho *et al.*, 1998; Shi and Vanhoutte, 2008).

COX-dependent contractions in human hypertension

As already underscored, hypertension is associated to a reduced vasodilating response to acetylcholine (Panza *et al.*, 1990; Taddei *et al.*, 1993); interestingly in hypertensive patients, but not in healthy controls, intraarteial administration of the COX inhibitor indomethacin at high doses is able to improve the vasodilation to acetylcholine (Taddei *et al.*, 1993), suggesting the possible production of COX-dependent EDCFs contributing to the onset endothelial dysfunction in human hypertension (Figure 3). Notably, COX-inhibition does not influence endothelial function in secondary forms of hypertension, indicating that COX-derived EDCFs conceivably do not play a significant role in determining endothelial dysfunction in these conditions (Taddei *et al.*, 1993) (Figure 3). Taken together, these results suggest that EDCF production is not a consequence of the simple increase in blood pressure values, but is likely genetically related to essential hypertension. At this regard, the possibility that EDCFs could be related to the pathogenesis of essential hypertension is excluded by data derived from young normotensive offspring of essential hypertensive patients. These subjects show an impaired response to acetylcholine as compared with matched offspring of normotensive subjects, but not to the endothelium-independent vasodilator sodium nitroprusside (Taddei *et al.*, 1996b), suggesting the presence of endothelial dysfunction. However, in contrast to frank hypertensive patients, this alteration is not improved by indomethacin infusion; on the contrary, the administration of the eNOS substrate L-arginine is effective in enhancing the vasodilating response to acetylcholine indicating that a primary defect in the L-arginine-NO pathway, rather than the production of COX-derived EDCFs, is responsible for the endothelial dysfunction in subjects with a familial predisposition to develop hypertension (Taddei *et al.*, 1996b).

A strong cross-relation is present between the hypertensive progress and ageing in terms of endothelium-dependent vasodilation and contraction. It is well-known that the ageing process is accompanied by a progressive worsening of NO availability and of endothelium-dependent vasodilation, both in large arteries and in small vessels of the peripheral and coronary circulation (Vita *et al.*, 1990; Egashira *et al.*, 1993; Zeiher *et al.*, 1993; Taddei *et al.*, 1995a; 1997a,b), and increasing age is therefore one of the main determinants of endothelial dysfunction. The main mechanism responsible for age-related endothelial dysfunction in the adults, at least in the peripheral microcirculation, seems to be a primary defect in the L-arginine-NO pathway, as the administration of L-arginine is able to restore the forearm vasodilation to intrabrachial acetylcholine. In contrast, after the age of 60 years, along with a further impairment of the L-arginine-NO-

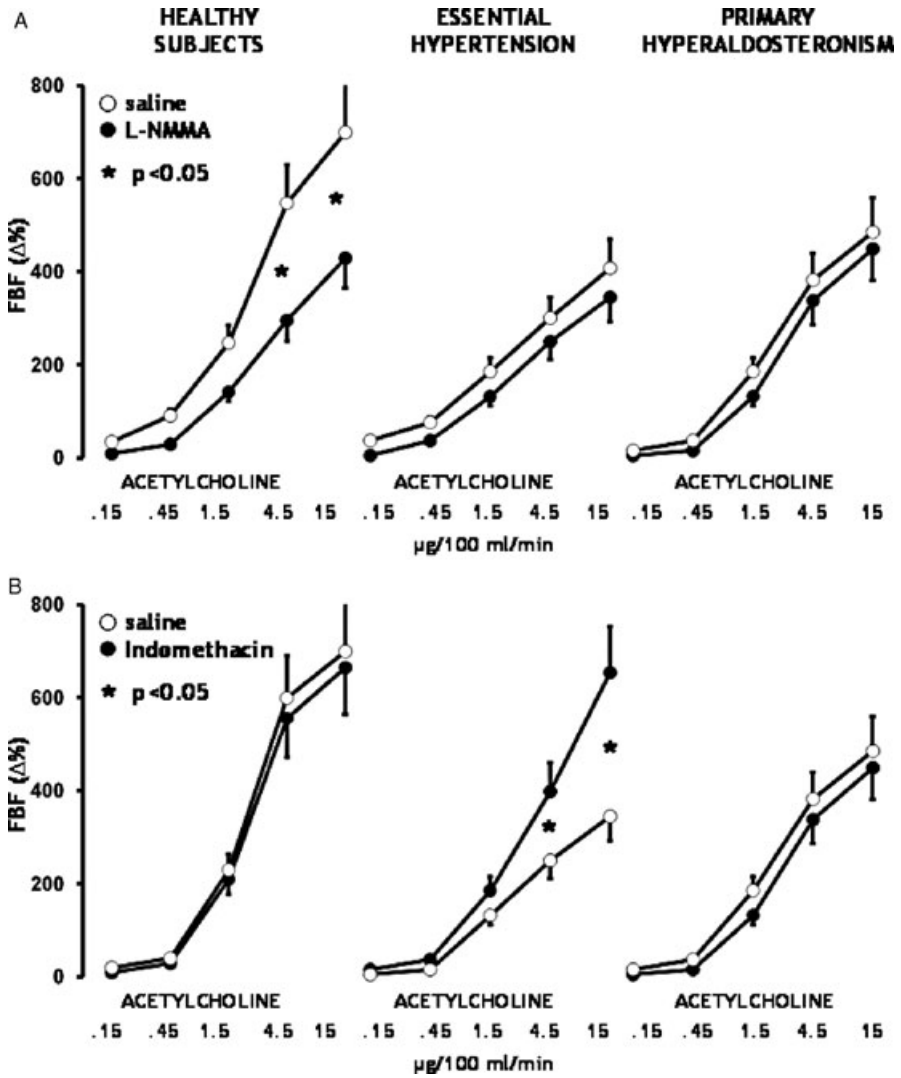

Figure 3 Change in forearm blood flow (FBF%) in the forearm microcirculation in response to increasing doses of the endothelium-dependent vasodilator acetylcholine in healthy subjects and hypertensive patients. (A) Both essential and secondary hypertensive patients clearly show a reduced maximal vasodilation to acetylcholine. Moreover, only in healthy subjects this response is inhibited by the co-administration of the eNOS inhibitor L-NMMA, demonstrating the presence of endothelial dysfunction in hypertensive patients (role of NO). (B) The vasodilation to acetylcholine is not influenced by the co-administration of the COX-inhibitor indomethacin in healthy subjects and in secondary hypertension, while it is improved in essential hypertensives, demonstrating the significant role of COX-derived contracting factors in this patients. Reproduced using data from Taddei *et al.* (1997b). COX, cyclooxygenase; eNOS, nitric oxide synthase; L-NMMA, monomethyl-L-arginine.

pathway, COX-dependent EDCF production becomes evident and significant (Taddei *et al.*, 1997b) (Figure 4). This natural alteration of the equilibrium in the vascular reactivity is anticipated by essential hypertension, which therefore represents a condition of premature vascular ageing. Indeed, in hypertensive patients, the production of COX-dependent EDCFs starts in the age range of 31–45 years and in patients older than 45 years the potentiating effect of indomethacin is increased in parallel with increasing age (Taddei *et al.*, 1997b) (Figure 4). The overall data indicate that COX-dependent EDCFs production is a phenomenon naturally occurring in the vascular ageing process and can be anticipated in the presence of hypertension.

It is worth noting that COX-derived EDCFs do not seem to play a significant role in the regulation of basal vascular tone of essential hypertensive patients, as intrabrachial indomethacin infusion does not influence forearm basal flow (Taddei *et al.*, 1993). Indeed, COX-derived vasoconstricting factors are not produced in baseline conditions and therefore do not modulate tonic NO release. Indeed, intrabrachial monomethyl-L-arginine (L-NMMA) infusion causes a dose-dependent vasoconstriction, which is related to basal NO production (Vallance *et al.*, 1989). Conversely, in essential hypertension the vascular response to L-NMMA is reduced, indicating a decrease in basal NO release (Calver *et al.*, 1992; Taddei *et al.*, 1995b). Interestingly, when L-NMMA is co-infused with indomethacin in the brachial artery of essential hypertensive patients, COX inhibition does not improve the blunted vasoconstrictor response to the eNOS inhibitor, suggesting that COX-derived EDCFs production is not respon-

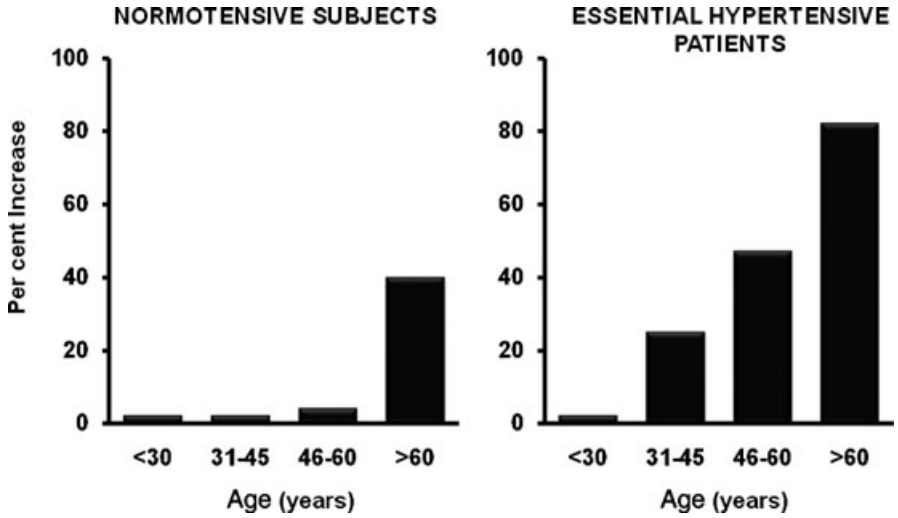

Figure 4 Bars show the potentiating effect induced by indomethacin infusion (50 μg·100 mL^{-1} forearm tissue per minute) on the vasodilating response to acetylcholine in normotensive subjects and patients with essential hypertension divided into subgroups according to age.

sible for the impaired basal release of NO (Taddei *et al.*, 1997a). Similarly, antioxidant vitamin C infusion is also devoid of effect on vasoconstriction to L-NMMA in essential hypertension, ruling out a possible role for oxidative stress in determining this impairment (Taddei *et al.*, 1998a). These data suggest that although essential hypertensive patients show impaired NO availability, involving both basal and agonist-stimulated release, the mechanisms responsible for these two specific defects are different and COX activity seems to be implicated only in the pathophysiology of the latter.

COX-derived EDCFs in other clinical conditions

Estrogens are known to protect the vessel wall both indirectly, by improving metabolic profile (Bush *et al.*, 1987), and by a direct beneficial action on endothelial cells as demonstrated by the negative effects of acute endogenous estrogen deprivation following ovariectomy (Gisclard *et al.*, 1988; Williams *et al.*, 1990). The progressive estrogen levels decrease, as occurring in menopausal women is characterized by endothelial dysfunction, in both normotensive and hypertensive females (Celermajer *et al.*, 1994; Taddei *et al.*, 1996a).

Ovariectomy/hysterectomy for uterine leiomyoma represents a useful model to investigate the role of acute estrogen deprivation on vascular reactivity. In these patients acute estrogen deprivation induces a decrease in the forearm vasodilation to acetylcholine, but not to the endothelium-independent vasodilator within 1 month after surgery (Pinto *et al.*, 1997), and this alteration is corrected by estrogen replacement therapy (Pinto *et al.*, 1997). As expected, in normal adult women the vasodilatory response to acetylcholine is blunted by the co-administration of an eNOS inhibitor, while COX-inhibitors have no effect. However, after ovariectomy, indomethacin becomes able to enhance the response to acetylcholine, with no effect of the eNOS inhibitor, suggesting that acute endogenous estrogen deprivation fosters COX-derived EDCFs production and blunts NO availability. This scenario is completed reverted by estrogen replacement therapy (Pinto *et al.*, 1997). Taken together, these results suggest that estrogen protects endothelial function by maintaining a physiological NO availability and preventing the formation of COX-derived EDCFs.

Also in patients with congestive heart failure, systemic COX inhibition with oral indomethacin is able to partially enhance the blunted vasodilating response to acetylcholine, although the nature of the COX-derived vasoconstrictor is not elucidated, yet (Katz *et al.*, 1993).

Conclusions

Overall, available data clearly indicate that essential hypertension is characterized by an impaired vascular NO bioavailability and endothelium-dependent vasodilation in the coronary and peripheral circulation. In parallel with this alteration, an increased production of EDCFs is present and contributes to the condition of endothelial dysfunction of these patients. Among EDCFs, COX-derived prostanoids and superoxide anions seem to be the most important determinants for the impaired agonist-stimulated vasodilation, but not for basal vascular tone. These processes do not appear to be characteristic of hypertension, but rather the acceleration by the hypertensive process of the para-physiologic age-related vascular changes. The mechanisms that regulate the balance between endothelium-derived relaxing and contracting factors and the processes transforming the endothelium from a protective organ to a source of vasoconstrictor, proaggregatory and promitogenic mediators, such as COX-dependent EDCFs remain to be determined.

Conflict of interest

None.

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