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Invited Comment

Oxygen species in the microvascular environment: regulation of vascular tone and the development of hypertension

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Abstract
Derangements of the three endothelium-related vasodilator systems (prostaglandins, endothelium-derived hyperpolarizing factor(s) and nitric oxide) cause the endothelial dysfunction observed in hypertension. Free radical-induced nitric oxide degradation plays a crucial role in hypertension. An increase in superoxide producing enzymes such as NAD(P)H oxidase and xanthine oxidase has been demonstrated. Superoxide dismutase may correct endothelial dysfunction *in vitro* and superoxide dismutase mimetics can lower blood pressure in experimental animals. Antioxidant agents and xanthine oxidase-inhibiting compounds have been used in humans. In addition, the synthesis of vasoconstrictor peroxides derived from the activity of cyclooxygenase in the endothelium and the vascular smooth muscle is stimulated by the $OH^{\bullet}$ radical. Hydrogen peroxide levels are augmented in hypertension, but its role is unclear because recent investigations have shown that this substance may act as a hyperpolarizing factor. It is thought that the therapeutic benefit of antihypertensive drugs, such as calcium antagonists and angiotensin-converting enzyme inhibitors, could be in part due to an inhibition of free radical production. A role of superoxide in the endothelial dysfunction and hypertension of chronic renal failure has also been suggested by recent animal experiments.

Keywords: endothelium; hydrogen peroxide; hypertension; nitric oxide; oxydril radical; superoxide

Introduction

The control of circulatory homeostasis depends on vascular endothelium responses to chemical, hormonal and haemodynamic changes. The endothelium releases mediators which affect vascular smooth muscle cell activity. Imbalance between vasodilators and vasoconstrictors, inducing endothelial dysfunction, has been described in a variety of conditions [1]. Depressed endothelium-dependent vascular relaxation was first demonstrated in spontaneously hypertensive rats (SHR) by Konishi and Su in 1983 [2]. This phenomenon occurs progressively as arterial pressure increases with age [3]. By contrast, the response to sodium nitroprusside or nitroglycerine [4] is maintained. Endothelial dysfunction is similarly present in patients with essential hypertension [5–8], and even in their normotensive descendants [9]. However, it is still unclear whether endothelial dysfunction causes the hypertensive process or, in contrast, high arterial pressure damages the endothelium. Whatever the sequence of events, endothelial dysfunction may maintain increased vascular resistance, leading to hypertension and its complications [10].

Locally released endothelium-derived vasodilators in hypertension

The term endothelial dysfunction refers to alterations of endothelial properties, such as anticoagulant and anti-inflammatory functions, modulation of vascular growth and remodeling. However, in the context of tone control (and hypertension), this term indicates deranged vasodilator activity. Three main vasodilator pathways have been recognized. The first is cyclooxygenase dependent [11,12]. Two mechanisms which have received much attention recently are the nitric oxide (NO)-dependent mechanism [13] and those related to a heterogeneous group of substances, defined as endothelial-derived hyperpolarizing factor(s) (EDHF) [14]. EDHF is involved in shear stress-induced endothelium-dependent relaxation via Ca-activated K channels [15], and is thought to modulate vasoconstriction [16], and to mediate the response to contractions induced by α1-adrenoceptor stimulation of smooth muscle, at least in the mesenteric artery [17].

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All these systems are altered in hypertension. Abnormal synthesis of prostaglandins has been described in human and animal hypertension [11,12]. There is a decrease in the basal production of NO and expression of endothelial NO synthase in SHR [18,19], although in the salt-sensitive hypertensive Dahl rat, no alteration of NO synthase genes was found [20]. Hypertensive patients are not deficient in L-arginine and its administration fails to improve acetylcholine response [5,6,8]. However, NO synthesis inhibitors provoke less vasoconstriction in the forearm of hypertensive patients than normotensive controls [21], suggesting decreased NO bioavailability. No association was found between NO synthase gene markers and hypertension in humans [22]. In SHR rats, decreased EDHF-related endothelial function is associated with decreased NO system activity [23]. These changes are reversible in response to antihypertensive treatment [24], administration of cholecalciferol, which normalizes blood pressure [25], and endothelin receptor antagonists [26].

Superoxide-NO relationship in the control of vascular tone

Recent research suggests that NO degradation is increased in the presence of excessive production of free radicals such as superoxide anion ($O_2^{-\bullet}$) [27,28]. In several pathological conditions, in which endothelial dysfunction is related to decreased NO bioactivity (diabetes mellitus, arteriosclerosis, cigarette smoking, hypercholesterolaemia, hypertension), the vascular production of superoxide is increased. Because $O_2^{-\bullet}$ and NO both contain unpaired electrons in their outer orbitals, they undergo an extremely rapid, diffusion-limited radical/radical reaction, leading to formation of peroxynitrite anion ($ONOO^{-\bullet}$), a strong oxidant which is rapidly protonated at physiological pH to yield the highly reactive peroxynitrous acid, which in turn generates the hydroxyl radical $OH^{-\bullet}$ [29]. Production of $O_2^{-\bullet}$ in the vessel wall inactivates NO and blunts endothelium-dependent vasodilation [30]. Thus, controlling the amount of $O_2^{-\bullet}$ is critically important for preserving NO bioactivity at the level of the vessel wall.

NAD(P)H oxidases are major sources of superoxide in vascular cells. The NAD(P)H oxidases of the cardiovascular system are membrane-associated enzymes that catalyze the 1-electron reduction of oxygen using NADH or NADPH as the electron donor [31]. Upon stimulation by various agents, $O_2^{-\bullet}$ is produced within minutes to hours by endothelial cells and vascular smooth muscle cells [32,33], in contrast to the almost instantaneous release seen in neutrophils. One of the most important attributes of the cardiovascular oxidase is its responsiveness to hormones and vasoactive substances (angiotensin II, thrombin, PDGF, TNF-α), haemodynamic forces and local metabolic changes. Activation of the oxidase can be mediated by intracellular second messengers, including calcium, protein kinase C and lipoxygenase metabolites of arachidonic acid [31]. A second enzymatic pathway generating superoxide in the vessel wall is xanthine-oxidase (XO). Because the measurement of the activity of this enzyme remains difficult, its precise role in vascular physiology is still unclear and rests mainly on indirect evidence derived from studies of the effects of antagonists of enzyme activity such as oxypurinol [34]. Recently a molybdenum-deficient form of XO has been identified, which uses NADH as its substrate [35]. It is thus possible that some of the evidence gathered on NADH-oxidase reflects in fact the activity of this modified form of XO. A third enzyme capable of producing $O_2^{-\bullet}$ is endothelial nitric oxide synthase (eNOS). This enzyme uses L-arginine as its substrate and needs tetrahydrobiopterin (BH_4) bound near its heme group in order to produce NO. In absence of substrate or BH_4 (eNOS uncoupling), eNOS produces $O_2^{-\bullet}$ [36–38]. There is evidence that this occurs in some forms of hypertension (see below). In addition, it has recently been demonstrated that eNOS may produce $O_2^{-\bullet}$ and H_2O_2 even in normal conditions [39].

The superoxide dismutases (SOD) represent a major cellular defense against $O_2^{-\bullet}$ and formation of peroxynitrite [40]. Three isoenzymes have been identified, including a cytosolic copper/zinc-containing form (Cu/ZnSOD), a mitochondrial manganese-containing form (MnSOD), and an extracellular isoenzyme (ecSOD), which is also a copper/zinc-containing enzyme. In the vessel wall, one third to one half of total SOD activity is made up by ecSOD [41]. Immunohistochemical studies have shown that vascular ecSOD is localized in high concentrations between the endothelium and the smooth muscle layer, which endothelium-derived NO must cross to stimulate smooth muscle relaxation [42]. Upregulation of ecSOD in response to NO has been demonstrated. This can reduce reactions of NO with $O_2^{-\bullet}$, thereby enhancing the beneficial biologic effects of NO released by the endothelium.

Vascular superoxide in hypertension

Evidence for microvascular oxidative stress in animal hypertension has accumulated recently [43–45]. Blood SOD activity is lower in ISIAH (stress-sensitive) rats compared with that of Wistar rats [46]. Moreover, the functional importance of reactive oxygen species (ROS) in hypertension has been suggested by the fact that the administration of tempol (a stable membrane-permeable SOD mimetic) or heparin-binding SOD, which localizes within the vessel wall, normalized blood pressure in SHR [27,47]. Similar findings have been demonstrated in DOCA-salt hypertension [48] and in renovascular hypertensive rats [49]. In the model of chronic infusion of angiotensin II in rats, the NAD(P)H subunit p22phox mRNA is upregulated and

NAD(P)H oxidase-derived $O_2^{-\bullet}$ production increases [31]. Both hypertension and the increase in p22phox mRNA were prevented by pretreatment with SOD [50]. Aortic NAD(P)H oxidase activity and expression of p22phox messenger RNA are elevated in SHR, and both are decreased after antihypertensive treatment [51]. Superoxide anions may be involved in the development of hypertension-related cardiac hypertrophy [52], stroke [53] and renal damage [54] observed in SHR. It should also be mentioned that Na loading decreases endothelium-dependent vasodilation in normotensive [55], spontaneously hypertensive [56] and salt-sensitive Dahl rats [57] as well as in the DOCA-salt model of hypertension [58]. A recent study has shown that salt loading stimulates the production of reactive oxygen species in resistance vessels from normotensive rats [59]. Erythrocyte SOD activity is reduced in patients with essential hypertension [60] and in hypertensive pregnant women [61].

Other reactive oxygen species ($OH^{-\bullet}$ and H_2O_2)

In essential hypertension, the role played by vasoconstrictor cyclooxygenase (COX) products, such as PGH_2 and thromboxane [62] is probably important. COX expression is elevated in SHR rats [62] and acetylcholine paradoxically produces endothelium-dependent contractions. These can be inhibited by selective COX-1 inhibitors, which also reduce blood pressure [63]. Recent evidence shows that $OH^{-\bullet}$, originating from endothelial $O_2^{-\bullet}$ produced through the XO pathway, stimulates COX [14].

Plasma concentrations of H_2O_2 are elevated in salt-sensitive Dahl rats [64], and H_2O_2 produces PGH_2/TXA_2-mediated contraction in aortic segments from SHR. On the other hand, H_2O_2 impairs KCl-induced contraction, acting as a K channel opener (EDHF) [39,65], and induces relaxation via ATP-sensitive K channels in endothelium-denuded aortic rings taken from SHR. Relaxation in response to levcromakalim (an ATP-channel opener) is augmented in SHR aorta. Catalase, but not SOD or desferrioxamine reduced them. These results suggest that in chronic hypertension, vasorelaxation to an ATP-sensitive K channel opener is augmented and that H_2O_2 produced in smooth muscle cells may partly contribute to these relaxations [66]. H_2O_2 levels are elevated in humans with essential hypertension. Hydrogen peroxide production was correlated positively with plasma rennin activity (PRA), and negatively with cardiac contractility and renal function [67].

Free radical production inhibitors in hypertension

Antioxidant agents have been used to treat endothelial dysfunction, as found in arterial hypertension [68]. In hypertensive patients, vitamin C improves coronary artery responses to acetylcholine and the endothelium-dependent vasomotor capacity [69] and intravenous infusion of ascorbic acid at high-dose ameliorated the impaired endothelium-dependent vasodilation to methacholine [70]. Administration of xanthine oxidase inhibitors, such as allopurinol, given intravenously to SHR, produces a temporary drop in arterial blood pressure [71]. Oxypurinol, with a longer half-life, was effective in reversing endothelial dysfunction in double transgenic hypertensive rats, carrying the human renin and angiotensinogen genes [72]. However, oxypurinol did not affect blood pressure in human hypertension [73].

Effect of antihypertensive drugs on free radical production

Angiotensin-converting enzyme inhibitors [74] and calcium antagonists [75] improve endothelium-dependent vasodilation in hypertensive patients, and facilitate the vasoconstrictor responses of NO antagonists [76]. This may be, at least in part, the consequence of their antioxidant action, inhibiting free radical production [77,78]. A similar action has been reported with losartan, an angiotensin II receptor antagonist, which depressed the angiotensin II-induced production of superoxide radicals [79].

Oxygen free radicals in endothelial dysfunction and hypertension of chronic renal failure

Hypertension, common in patients with chronic renal failure (CRF), is a major determinant of the rate of deterioration of renal function, and of morbidity and mortality of dialysis patients [80]. Its pathogenesis is not well understood. Endothelial dysfunction due to reduced NO bioactivity [81], has been demonstrated by experimental [82] and human [83,84] studies. There is evidence that oxidative stress occurs early during the evolution of CRF. Parameters of oxidative stress and antioxidant enzyme activities are altered in CRF patients [85–87]. The activity of antioxidant enzymes decreases in the renal cortex of rats after subtotal nephrectomy [88,89], and the associated increase in oxidative stress seems to play a role in the development of renal fibrosis, since antioxidant treatments (vitamin E [90] or magnesium lithospermate B [88]) may hinder its progression. Very little is known of the role played by superoxide in endothelial dysfunction and hypertension in CRF. In one study, the antioxidant substance lazaroid has decreased blood pressure in rats after subtotal nephrectomy [91]. We have recently shown that exogenous SOD restores the decreased response to acetylcholine in isolated mesenteric arteries from rats after 5/6 nephrectomy and the SOD-mimetic tempol prevents the increase of blood pressure in these animals [92]. These studies suggest that increased superoxide production in the vascular

wall is an important pathogenetic factor in the development of endothelial dysfunction and high blood pressure in CRF.

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