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MINIREVIEW

Age-Related Endothelial Dysfunction with Respect to Nitric Oxide, Endothelium-Derived Hyperpolarizing Factor and Cyclooxygenase Products

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Summary

Vascular aging is associated with both structural and functional changes that can take place at the level of the endothelium, vascular smooth muscle cells and the extracellular matrix of blood vessels. With regard to the endothelium, reduced vasodilatation in response to agonists occurs in large conduit arteries as well as in resistance arteries with aging. Reviews concerning the different hypotheses that may account for this endothelial dysfunction have pointed out alterations in the equilibrium between endothelium-derived relaxing and constricting factors. Thus, a decreased vasorelaxation due to nitric oxide and, in some arteries, endothelium-derived hyperpolarizing factor as well as an increased vasoconstriction mediated by cyclooxygenase products such as thromboxane A_2 are likely to occur in age-induced impairment of endothelial vasodilatation. Furthermore, enhanced oxidative stress plays a critical role in the deleterious effect of aging on the endothelium by means of nitric oxide breakdown due to reactive oxygen species. The relative contribution of the above phenomenon in age-related endothelial dysfunction is highly dependent on the species and type of vascular bed.

Key words

Vascular aging • Endothelial dysfunction • NO • EDHF • Cyclooxygenase

Introduction

Aging is associated with marked changes in the cardiovascular system, especially at the level of the vascular wall. Despite the possible role of intrinsic sympathetic nerves, abnormalities of the vascular wall are accompanied by both structural and functional changes that can take place at the level of the endothelium, vascular smooth muscle cells and the extracellular matrix of blood vessels. Arteries exhibit an increase in media thickness, wall collagen content and the size or number of smooth muscle cells, leading to arterial stiffening (Guyton *et al.* 1983, Michel *et al.* 1994, Gaballa *et al.* 1998, Moreau *et al.* 1998). With regard to the endothelium, endothelial thickening and the presence of mononuclear cells have been reported (Guyton *et al.* 1983). At the functional level, several studies have shown

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changes of vasoconstrictor mechanisms in response to agonists such as noradrenaline (Barton *et al.* 1997, McAdams and Waterfall 1986, Owen 1986) and endothelin-1 (Barton *et al.* 1997, Dohi and Lüscher 1990). The changes in vascular reactivity to vasoconstrictor agents may be due to a combination of alterations of smooth muscle cells and a reduced vasodilatory function of the endothelium. Indeed, the endothelium plays a critical role in the regulation of smooth muscle tone by releasing several endothelium-derived relaxing and contracting factors (Lüscher and Tanner 1993) under basal conditions and in response to agonists or physical stimuli. Endothelial dysfunction with aging has been described in clinical and experimental reports. Age-related endothelial dysfunctions with respect to agonist-induced relaxation such as acetylcholine will be reviewed below. The balance between endothelium-derived relaxing and contracting factors will be discussed.

Mechanisms of endothelium-dependent relaxation to acetylcholine

Acetylcholine causes endothelium-dependent vasodilatation through activation of the muscarinic receptor. The stimulation of the latter leads to an increase in cytosolic Ca^{2+} which is a prerequisite step for the production and release of nitric oxide (NO), cyclooxygenase (COX) products and an unidentified factor named endothelium-derived hyperpolarizing factor (EDHF). These factors once released by the endothelium can trigger smooth muscle cell relaxation. In large conduit arteries, such as the aorta, NO appears to be the predominant factor, whereas participation of EDHF is more important in resistance arteries (Chen *et al.* 1988, Feletou and Vanhoutte 1988, Mombouli and Vanhoutte 1997, Bobadilla *et al.* 1997).

Endothelium-dependent relaxation to acetylcholine is altered with aging

The concept of generalized endothelial dysfunction in terms of relaxation is now well recognized both in large conduit arteries and in small resistance vessels from different species including man. With respect to large conduit arteries, relaxation to acetylcholine is decreased with age in the aorta of Wistar-Kyoto (WKY) rats. The maximal effect is reduced from 100 % in young (4-6 weeks old), to 50 % in adult (3-6 months old) and 25 % in old (12-25 months) rats (Koga *et al.* 1988). A similar observation has been reported in the same type of artery from 72 to 74-week-old animals (Küng and Lüscher 1995). Hongo *et al.* (1988) have shown a decrease in the maximal responses to acetylcholine of the carotid artery in 11-month-old WKY rats. The mesenteric (Shimizu and Toda 1986) and femoral (Haidet *et al.* 1995) arteries taken from beagles also displayed reduced endothelial relaxation with age. Finally, reduced acetylcholine-induced vasodilatation has been found in brachial (Taddei *et al.* 1995) and coronary (Egashira *et al.* 1993) arteries from aged humans. With respect to resistance arteries, the effect of age on endothelium-dependent relaxation has not been extensively studied. Nevertheless, an age-dependent reduction of endothelial vasodilator function induced by carbachol has been reported in perfused mesenteric arterial bed (Atkinson *et al.* 1994). Furthermore, our data from branch II or III of the superior mesenteric artery in Wistar rats showed that endothelial relaxation in response to acetylcholine is impaired in this resistance artery (Fig. 1A). Thus, there appears to be no doubt concerning endothelial dysfunction with aging independently of either the type of artery or the species. However, a certain anatomical heterogeneity has been reported in some cases between the aorta and femoral artery of the rat (Barton *et al.* 1997). However, the nature of age-related endothelial dysfunction is complex and not fully understood. Different hypotheses have been advanced in order to elucidate the underlying mechanisms.

A decrease in the number of agonist receptors (i.e. muscarinic receptors for acetylcholine) or agonist affinity to its receptor may account for the age-related endothelial dysfunction. However, no direct demonstration of a defect of any specific endothelial membrane receptor yet has been reported. In addition, endothelial dysfunction is not limited to a default in the muscarinic receptor signal transduction, because the impairment of endothelium-dependent relaxation is also found with other agonists such as ATP (Hongo *et al.* 1988) and histamine (Moritoki *et al.* 1986). Furthermore, endothelial dysfunction *via* a change in Ca^{2+} handling independently of the production of second messenger can be ruled out as alterations of endothelial vasodilatation produced by the calcium ionophore, A23187, have also been reported (Barton *et al.* 1997). Taken together, it is likely that the generation (i.e. synthesis or release) of an endothelium-derived relaxing factor or the response to it account for the age-related endothelial dysfunction.

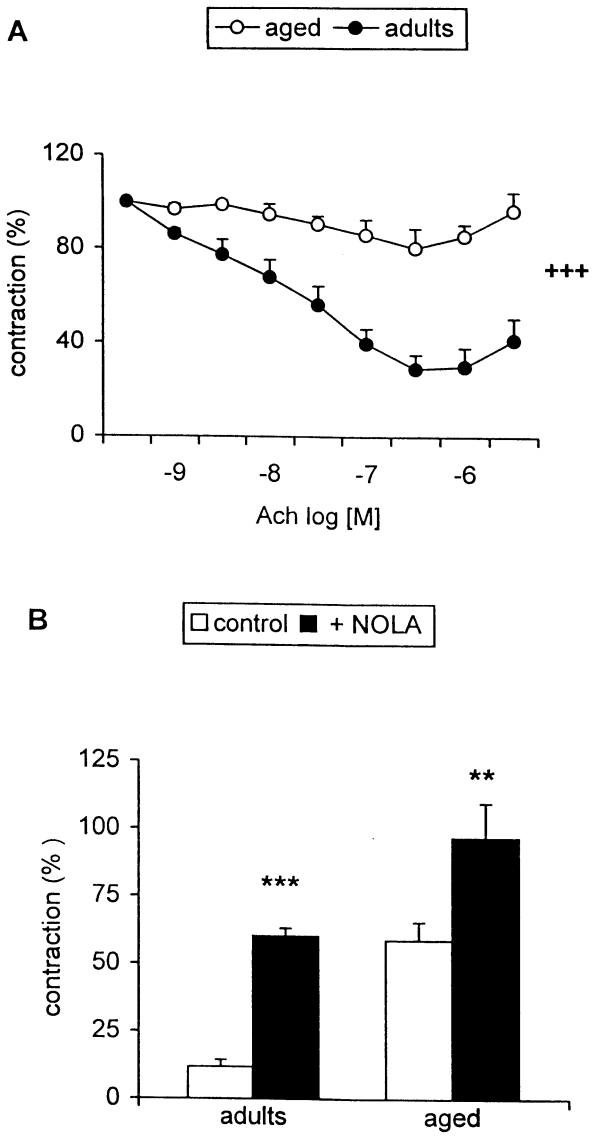

Fig. 1. *[A]. Influence of age on relaxant responses to acetylcholine (ACh) in the small mesenteric artery from adult (12-14 weeks old) and aged (70-100 weeks old) rats.* ***[B]*** *Effects of N^{ω}-nitro-L-arginine (NOLA, 30μM) on maximal relaxation induced by acetylcholine (0.3 μM) in aortic rings (precontracted with 1 μM noradrenaline) of adult (12-14 weeks old) and aged (70-100 weeks old) male Wistar rats. Results are expressed as a percentage of the previous contraction to noradrenaline (1 μM). Values are mean ± S.E.M. of 7-10 experiments. Analysis of variance or Student's t-test were used for statistical analysis. $^{+++}p<0.001$, significantly different with age; $^{**}p<0.01$, $^{***}p<0.001$, significantly different from acetylcholine response in the absence of NOLA.*

One of the major endothelial vasodilators, NO, is formed by endothelial NO synthase (eNOS) by conversion of the amino acid L-arginine the availability of which has been reported to be reduced in the circulation of aged rats (Reckelhoff *et al.* 1994). NO-induced relaxation may involve different mechanisms. However, it acts primarily *via* activation of the soluble guanylyl cyclase in the underlying smooth muscle cells with a resulting increase in the content of cyclic GMP (Rapoport and Murad 1983, Stoclet *et al.* 1998).

The decrease in endothelial vasodilatation could be explained, at least partly, by an age-related attenuation of smooth muscle sensitivity to NO. Hence, aging is associated with a reduced vasodilatation response to NO donors in the perfused mesenteric bed without functional endothelium (Atkinson *et al.* 1994). However, no evidence for an impaired responsiveness of vascular smooth muscle to NO donors with age has been found in the rat aorta (Barton *et al.* 1997). Furthermore, we did not observe any age-related change in the relaxation produced by another NO donor, S-nitrosopenicillamine, both in the aorta and the small mesenteric artery of the rat. The differential results might be attributed to anatomic heterogeneity of vascular preparations. Further studies are needed to clarify this point.

Reduced endothelium-dependent relaxation in aged rats may be due to a decreased release of endothelium-derived NO. In our study, the use of NO synthase inhibitor, N^{ω}-nitro-L-arginine (NOLA), showed that the contribution of endothelial NO in the response to acetylcholine appears to be little affected by aging in the aorta (Fig. 1B), but it is dramatically reduced in the small mesenteric artery (Alvarez de Sotomayor *et al.* 1999). These results are in accordance with observations suggesting a reduced participation of the NO pathway with aging (Dohi *et al.* 1990). Indeed, direct measurements using porphyrinic NO microsensor showed a decreased endothelial NO release with aging (Tschudi *et al.* 1996). This could be a consequence of increased NO breakdown due to an augmented production of superoxide anions (Gryglewski *et al.* 1986, Rubanyi and Vanhoutte 1986), an alteration of antioxidant defense systems or an increased production of reactive oxygen species different from superoxide anions (Beckman and Ames 1998) (discussed below).

At the level of eNOS, a reduced expression of the eNOS mRNA with aging has been reported (Barton *et al.* 1997). However, no change or even an increase in eNOS protein expression has been observed in arteries from aged rats (Cernadas *et al.* 1998, Chou *et al.* 1998). There is no discussion about the fact that eNOS activity is reduced in arteries from aged rats (Cernadas *et al.* 1998, Chou *et al.* 1998). The latter may account for age-related

decrease of endothelial NO vasodilatation independently of NO breakdown by reactive oxygen species.

In conclusion, with regard to NO, the decrease of endothelial NO with aging is far from being clear. The defect may occur at different levels from eNOS mRNA up to the sensitivity of smooth muscle cells to endothelial NO. Nevertheless, one can postulate that the reduced contribution of NO in age-related endothelial dysfunction mainly concerns the activity of eNOS, NO breakdown by reactive oxygen species, or both. Furthermore, endothelial cells can release other factors such as EDHF or cyclooxygenase vasodilatator products, the contribution of which might be altered with aging.

Endothelium-derived hyperpolarizing factor is an as yet unidentified substance(s) that cause(s) endothelial relaxation by hyperpolarization of the underlying vascular smooth muscle cells (Chen *et al.* 1988, Feletou and Vanhoutte 1988, Mombouli and Vanhoutte 1997). In blood vessels from various species this hyperpolarization is resistant to inhibitors of NOS and cyclooxygenase. As mentioned above, EDHF greatly contributes to the endothelial-dependent relaxation in resistance arteries as compared to large conduit arteries. Since an age-related reduced endothelial function is also observed in resistance arteries, the EDHF-component of the relaxation might be altered in these vessels independently or in addition to NO.

In the superior mesenteric artery of the rat, Fujii *et al.* (1993) found that hyperpolarization and relaxation to acetylcholine decline with age. The authors advanced the hypothesis that these alterations might be due to reduced synthesis, release or diffusion of EDHF. Recently, it has been reported that EDHF plays an important role in human gastroepiploic distal arteries in which aging significantly impairs EDHF-mediated relaxation (Urakami-Harasawa *et al.* 1997). The evolution of the relative contribution of EDHF in the acetylcholine-mediated vasodilatation with age was studied in resistance arteries (i.e. branch II or III of he mesenteric artery) (Alvarez de Sotomayor *et al.* 1999). The effects of the combination of a NO-synthase inhibitor, N^{ω}-nitro-L-arginine (NOLA, 30 μM), and a COX inhibitor, indomethacin (10 μM), on the maximal relaxation induced by acetylcholine (1 μM) were evaluated in small mesenteric arteries from adult (14 weeks old) and aged (70-90 weeks old) male Wistar rats. The NOLA plus indomethacin-insensitive component of acetylcholine-induced relaxation, that reflects the EDHF-component of the response, was not significantly different in arteries from adult and old rats (Fig. 2). These results suggest that EDHF relaxation may be preserved with aging by contrast to NO in these resistance arteries. Possible reasons for the conflicting results could concern either species differences or an anatomical heterogeneity of vascular regulation with aging.

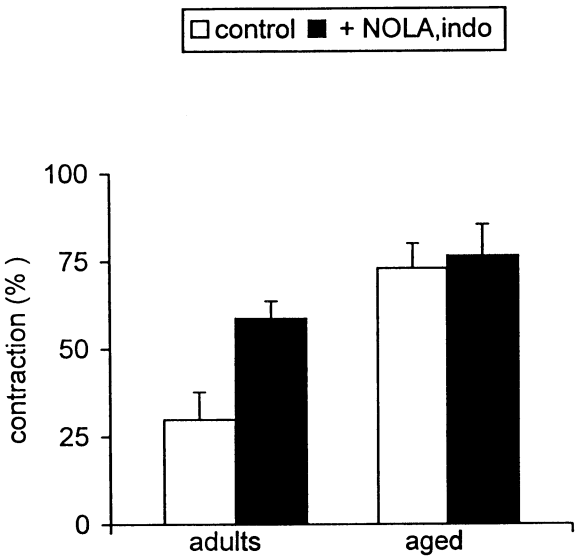

Fig. 2. *Effects of indomethacin (indo, 10 μM) and N^{ω}-nitro-L-arginine (NOLA, 30 μM) on maximal relaxation induced by acetylcholine (1 μM) in small mesenteric arterial rings (precontracted with 1 μM noradrenaline) of adult (12-14 weeks old) and aged (70-90 weeks old) male Wistar. Results are expressed as a percentage of the previous contraction to noradrenaline (1 μM). Values are mean ± S.E.M. of 7-10 experiments.*

The age-related reduced endothelium-dependent relaxation, independently of the decreased contribution of either endothelial NO or EDHF, might be due to an increased production of endothelium-derived constricting factors. The most likely candidates are the vasoconstrictor products from endothelial COX such as thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2) (Maclouf *et al.* 1998, Vane *et al.* 1998). Once released, these COX-derived contracting factors activate the PGH_2/TXA_2 receptor (TP receptor) on smooth muscle cells leading to vascular contraction. In our study, the relative contribution of the COX-derived contracting metabolites in acetylcholine-mediated vasodilation was investigated. The effect of indomethacin (10 μM) on the maximal relaxation induced by acetylcholine was evaluated in aortic rings from adult (12-14 weeks old)

and aged (70-100 weeks old) male Wistar rats. The acetylcholine response was significantly enhanced in the presence of indomethacin in arteries from aged but not adult rats (Fig. 3). Thus, aging is associated with an increased participation of endothelial vasoconstrictor products from COX the nature of which remains to be determined. Also, whether the endothelial production of vasoconstrictor prostaglandins or their efficacy is enhanced needs further studies.

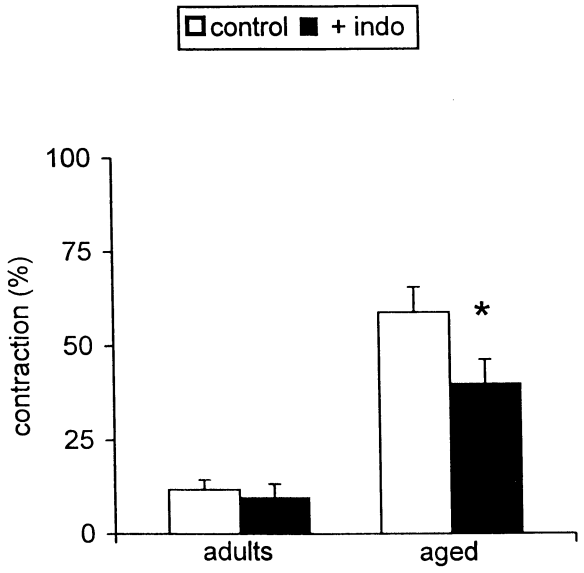

Fig. 3. *Effects of indomethacin (indo, 10 μM) on maximal relaxation induced by acetylcholine (1 μM) in aortic rings (precontracted with 0.3 μM noradrenaline) of adult (12-14 weeks old) and aged (70-100 weeks old) male Wistar rats. Results are expressed as a percentage of the previous contraction to noradrenaline (1 μM). Values are mean ± S.E.M. of 7-10 experiments. Student's t-test was used for statistical analysis. *$p<0.05$, significantly different from acetylcholine response in the absence of indomethacin.*

Controversial data have been reported in the literature on the involvement of vasocontrictor products from COX in age-related endothelial dysfunction. In the senescent rat aorta, the endothelium releases contracting factors that may be TXA_2 and other prostanoids (Koga *et al.* 1989). In contrast, Küng and Lüscher (1995) reported that the reduced endothelial relaxation in the same artery of senescent WKY is not due to formation of PGH_2, but it involves impaired formation or increased inactivation of NO. Furthermore, impaired vasodilatation in aged rats does not appear to be related to the production of a cyclooxygenase constrictor substance in rat cerebral arterioles (Mayhan *et al.* 1990).

Oxidative stress is an important factor contributing to vascular dysfunction with aging (Gryglewski *et al.* 1986, Beckman and Ames 1998). To protect against the deleterious effects of oxiradicals and to prevent the lipid peroxidation process, both non-enzymatic and enzymatic antioxidant defense systems exist. The first one includes compounds such as α-tocopherol, ascorbate, carotenoids (Beckman and Ames 1998), and the second concerns enzymes such as catalase, superoxide dismutase (SOD) and the glutathione-dependent enzymatic system (Beckman and Ames 1998). The organism possesses a delicate equilibrium between free radical production and anti-oxidant systems that can be altered in pathophysiological processes such as atherosclerosis and aging. Endothelial cells are vulnerable to oxidative stress due to their low antioxidant capacity and elevated metabolism of arachidonic acid (Marin and Rodriguez-Martinez 1995).

Age-related endothelial dysfunction may involve mechanisms such as alterations of antioxidant defense systems, increased oxidative injury or both. Rodriguez-Martinez *et al.* (1998) has recently shown that aging enhanced the lipid peroxidation process, as indicated by an increase in the malondialdehyde (MDA) plasma levels in rats. MDA is a lipid peroxidation derivative resulting from oxidation of fatty acids such as arachidonic acid. The increase in plasma MDA levels is accompanied by an induction of lipid peroxide detoxification enzymes. The effects of MDA appeared to be mediated by superoxide anions. The changes in the blood prooxidant-antioxidant equilibrium with age contribute, at least partly, to the impairment of the relaxant responses evoked by acetylcholine in the rat tail artery.

The effect of aging on vascular SOD activity, an important antioxidant enzyme that determines the release of biologically active NO and that is abundant in the vascular wall, is not well documented. Alterations in vascular SOD activity may be particularly important for endothelial function, since increased oxidative stress contributes to endothelial dysfunction. Barton *et al.* (1997) reported that vascular SOD activity was higher in femoral arteries than the aorta, but no effect of aging on SOD activity in the aorta or the femoral artery was observed. These authors proposed that the differential regulation of SOD in different vascular beds may predispose to age-related impairment of endothelial functions, as aging reduced relaxation to acetylcholine in the aorta but not in the femoral artery. Along with this hypothesis, the authors found a decrease of plasma SOD activity. However, it cannot be excluded that antioxidant

systems distinct from SOD are also altered by the aging process. Increased production of reactive oxygen species observed during aging may not only impair the transduction pathways leading to the release of relaxant factors by endothelial cells, but it can also increase the breakdown of endothelial NO. Thus, increased oxidative stress may partly participate in age-related endothelial dysfunction.

Conclusions

In this review, we have made an attempt to describe the different hypotheses that may be involved in the reduced endothelial vasodilator function with aging. It can be concluded that the balance between endothelial relaxant and constricting factors is shifted towards the latter with aging. Thus, endothelial dysfunction is associated with both a decreased NO and, in some arteries, EDHF-related relaxation and an increase in vasoconstriction by cyclooxygenase products, mainly thromboxane A_2. The increase in oxidative stress plays a role in the deleterious effect of aging on the endothelium. This results not only in impairment of the pathways leading to the production of relaxant factors in the endothelium, but also to the destruction of the biologically active NO. Furthermore, it cannot be excluded that the observed alteration of endothelial vasodilatation may be the consequence of functional antagonism produced by reactive oxygen species at the level of smooth musculature. Indeed, reactive oxygen species *per se* can produce vasoconstriction. Finally, endothelial relaxant or constricting factors can interact with each other. This point has not been discussed, but should be considered. Nevertheless, Auch-Schwelk *et al.* (1992) highlighted a chemical neutralization of PGH_2 by NO resulting in endothelial dysfunction with age. One should keep in mind that all of the above phenomena may be differentially regulated depending on the species and the anatomical heterogeneity of vascular beds.

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