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Short Communication

Aging Exacerbates Hydrogen Peroxide-Induced Alteration of Vascular Reactivity in Rats

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ABSTRACT

Reactive oxygen species (ROS) such as superoxide anion ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2) can be produced by vascular endothelium and smooth muscle cells under diverse physiological and pathophysiological situations. These species are known to exert various deleterious effects by which they might induce changes in vascular reactivity. The aim of the present study was to evaluate the evolution of vascular susceptibility to H_2O_2 during aging in rats. Catalase activity was assessed in aortas from young adult (4 months) and aged (24 months) Wistar rats. In parallel experiments, isolated rings from both age groups were exposed to increasing doses of H_2O_2 (0, 0.1, 1, 5, or 10 m*M*) for 20 min and the residual vascular response to phenylephrine (PE = 10^{-6} *M*) and acetylcholine (ACh = 10^{-6}*M*) was evaluated. Our results indicate that aging increases aortic catalase activity (4 months: 0.20 ± 0.02 IU/mg prot versus 24 months: 0.46 ± 0.06 IU/mg prot, $p < 0.001$) while it exacerbates vascular sensitivity to H_2O_2. These results suggest that the observed increased H_2O_2-induced alterations of vascular reactivity during aging in rats might be due to increased sensitivity of the vasculature to ROS rather than to a decrease in the defense systems against these species. Antiox. Redox Signal. 2, 363–368.

INTRODUCTION

THE IMPLICATION OF REACTIVE OXYGEN SPECIES (ROS) in numerous pathophysiological situations, including hypertension, inflammation, and reperfusion injury is now well documented (Halliwell, 1994). These species, which can be generated by vascular endothelium (Panus *et al.*, 1993) and smooth muscle cells (Gutteridge, 1995), are known to exert various deleterious effects by which they induce changes in vascular reactivity (Mian and Martin, 1997; Tanguy *et al.*, 1999). In experimental studies as well as in clinical settings, aging has been found to be associated with an increase in cardiovascular vulnerability to diverse pathophysiological situations that can directly result from the numerous modifications associated with aging (for review, see Folkow and Svanborg, 1993). In a recent study, we have shown that the increased sensitivity of isolated perfused hearts from aged rats to post-ischemic reperfusion injury can be directly linked to a decrease in cardiac ability to eliminate hydroperoxides (Boucher *et al.*, 1998). Among the hydroperoxides that are produced under pathophysiological situations, hydrogen peroxide (H_2O_2) is a powerful oxidant that is generated as a by-product of many cellular reactions (Mian and Martin, 1997). This molecule, which freely crosses cell membranes, is also produced under normal situations by diverse

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enzymatic processes. Excess of H_2O_2 is able to affect a number of known biochemical systems within the cell, including activation of the glutathione redox cycle, increased formation of oxidized sulfhydryls, DNA damage, loss of intracellular NAD^+, activation of poly ADP-ribose polymerase, a rise in intracellular free calcium, and a rapid decrease in ATP (Frimer *et al.*, 1983; Chakraborti *et al.*, 1998; Hu *et al.*, 1998; Bychkov *et al.*, 1999). All of these processes occur before loss of membrane integrity (Vroegop *et al.*, 1994).

In many of the pathophysiological situations in which H_2O_2 is potentially produced, and more especially under conditions of ischemia or during post-ischemic reperfusion, the vascular adaptation of blood flow is a crucial event, on which cell survival depends. In this context, we (Tanguy *et al.*, 1999) and others (Mian and Martin, 1997; Hu *et al.*, 1998) have examined the role of H_2O_2-induced impairment of vascular reactivity in young rats, and have determined the key role that catalase plays in limiting these effects. However, to date, no study has been devoted to examining the evolution of vascular sensitivity to H_2O_2 during aging.

Therefore, the aim of the present study was to determine whether aging is associated with an increase in vascular sensitivity to H_2O_2 in a model of rat isolated aortic rings. Moreover, because catalase activity is considered to be determinant of H_2O_2-induced alterations in vascular tissue (Mian and Martin, 1997; Hu *et al.*, 1998; Tanguy *et al.*, 1999), we have measured its evolution during aging in the aorta.

MATERIALS AND METHODS

Vascular contractility measurements

Rings of thoracic aorta from male Wistar rats from two age groups (adults = 4 months old; aged = 24 months old) were used. All animals were housed under conditions of constant temperature (22°C), humidity (55 ± 10%), and standard light-dark cycle (14 hr/10 hr). They had free access to tap water and standard food and were treated according to the guidelines of the Recommendations from the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (L358-86/609/EEC).

The animals (n = 4 per group of age) were anesthetized via an intraperitoneal injection of sodium pentobarbital (40 mg/kg, 1 ml/kg body weight) before receiving heparin (100 IU/100 grams body weight) through a saphenous vein. A portion of descending thoracic aorta was rapidly isolated and placed in a Krebs-Henseleit-type physiological buffer (K^+ = 5.9 m*M*; Ca^{2+} = 1.36 m*M*; glucose = 11 m*M*, pH 7.4) at room temperature. The aorta was delicately cut into rings of approximately 1 mm in length. The eight to nine vessel rings from a singe aorta were randomly distributed between the different experimental groups within each age group (n = 5–8 rings per group). Aortic rings were then placed in an organ bath containing oxygenated Krebs-Henseleit buffer at 37°C. In the organ bath, the rings were fixed to an isometric transducer (UF1-Harvard Biosciences, Les Ulis, France) linked to an amplifier (Freestanding-Harvard Biosciences, Les Ulis, France) and to a computerized acquisition system (MacLab AD Instruments, Castle Hill, Australia), and equilibrated for 60 min at a resting tension of 2×10^{-2} *N* (2 grams) during which the buffer was replaced every 15 min. The resting tension was then re-adjusted to 2 10^{-2} *N* before experiment. To test the integrity of the preparations, the rings were first contracted with phenylephrine (PE), an alpha-agonist, at a concentration of 10^{-6} *M*. Presence of functional endothelium in the preparation was tested by adding acetylcholine (ACh, 10^{-6} M), which induces a rapid nitric oxide (NO)-dependent relaxation of the PE-pre-contracted rings.

H_2O_2 (Sigma Chemical, France) at different concentrations (0, 0.1, 1, 5, or 10 m*M*) was then added to the organ bath for 20 min. After H_2O_2 washout, rings were submitted to a second test of integrity similar to the first one.

The contraction induced by PE after H_2O_2 washout (second test) was expressed as percent of the contraction induced by PE before addition of H_2O_2 (first test). The relaxation induced by ACh in the second test was expressed as percent inhibition of the contraction induced by PE in the second test.

Assessment of catalase activity

Catalase activity was measured on complete thoracic aorta ($n = 8$ per group) freeze-clamped at liquid nitrogen temperature immediately after excision. Frozen aortas were homogenized in 1 ml of Tris-HCl buffer [Tris HCl 50 m*M*, diethyltriaminopentaacetic acid (DTPA, 1 m*M*), phenylmethylsulfonylfluoride (PMSF, 1 m*M*), pH 7.4], using a glass Potter homogenizer. Tissue homogenates were then centrifuged for 10 min at 2,000 × *g* at 4°C to remove all nuclear debris. Catalase activity (Aebi, 1974) was determined by following H_2O_2 disappearance (at 240 nm) for 1 min in the presence of vascular supernatant (H_2O_2: ε_{240nm} = 40 $cm^2/\mu mol$).

Catalase activity was expressed both in international units per gram wet tissue weight (IU/g wwt) and in international units per milligram protein (IU/mg prot). The modified method of Lowry *et al.* (1951) was used to determine protein content of tissue homogenate supernatants, using bovine serum albumin (BSA) as standard.

Statistics

Results were expressed as mean ± SEM and compared using a Student's *t*-test modified with Bonferroni inequality following analysis of variance by Fisher's test. A difference was considered statistically significant when $p < 0.05$.

RESULTS

Vascular reactivity

Before addition of H_2O_2 to the organ bath, the basal values of vascular reactivity were first evaluated in the two age groups. Under our experimental conditions, neither the PE-induced contraction (adults 3.25 ± 0.15 m*N* versus aged 3.54 ± 0.24 m*N*, ns [not significant]) nor the rapid NO-dependent relaxation of PE-pre-contracted rings induced by ACh (adults 52 ± 3% inhibition of PE-induced contraction versus aged: 49 ± 3%, ns) were modified by aging (Table 1).

Addition of H_2O_2 to the organ bath for 20 min at doses above 0.1 m*M* led to a dose-dependent alteration of the second integrity test. This was characterized by a decrease in both the PE-induced contraction (Fig. 1) and the ACh-induced relaxation (Fig. 2) in the two age groups. However, the H_2O_2-induced alteration of vascular reactivity was significantly more severe in the group of senescent rats compared to young adults. Indeed, for doses of H_2O_2 from 1 to 10 m*M*, both the PE-induced contraction (Fig. 1) and the ACh-induced relaxation (Fig.

TABLE 1. VASCULAR REACTIVITY INDUCED BY PE (10^{-6} *M*; PE1) AND ACH (10^{-6} *M*; ACh1) IN ISOLATED RINGS OF AORTA FROM ADULT (4 MONTHS) AND AGED (24 MONTHS) RATS BEFORE ADDITION OF H_2O_2

	PE1 *(mN)*	*ACh1* *(% inhibition of PE1)*
Adults ($n = 37$)	3.25 ± 0.15	52 ± 3
Aged ($n = 28$)	3.54 ± 0.24	49 ± 3
1 vs. 2	ns	ns

Values are means ± SEM.

PE1, PE-induced contraction before addition of H_2O_2 to the organ bath. Means ± SEM. ns: not significant, aged vs. adults; modified Student's *t*-test.

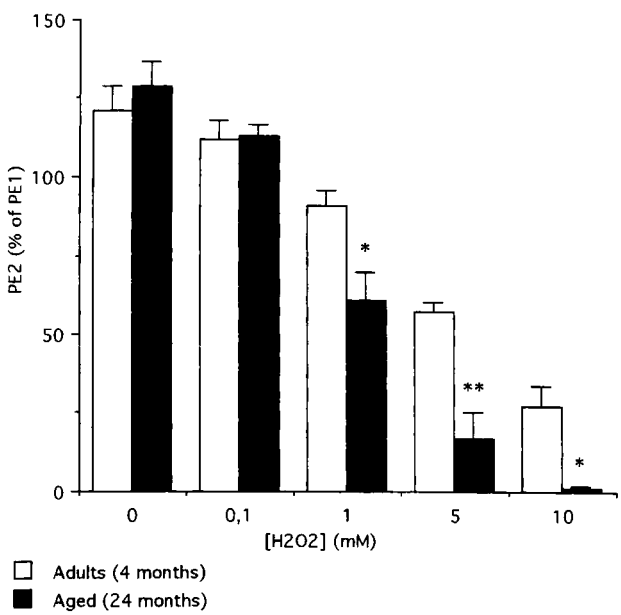

FIG. 1. Effect of aging on vascular reactivity to phenylephrine (PE = 10^{-6} *M*) after a 20-min exposure to H_2O_2 (0, 0.1, 1, 5, and 10 m*M*). The vascular response to PE (10^{-6} *M*) after H_2O_2 exposure (PE2) is expressed as a percentage of the contraction induced by PE (10^{-6} *M*) before H_2O_2 exposure (PE1). Means ± SEM. *$p < 0.05$, **$p < 0.01$ aged vs. adults; modified Student's *t*-test.

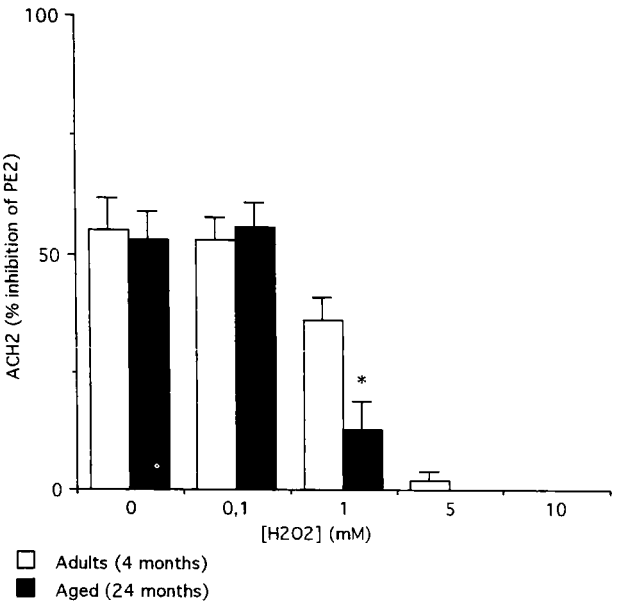

FIG. 2. Effect of aging on vascular reactivity to acetylcholine (ACh = 10^{-6} *M*) after a 20-min exposure to H_2O_2 (0, 0.1, 1, 5, and 10 m*M*). The response of PE-precontracted rings to ACh (10^{-6}*M*) after H_2O_2 exposure (ACh2) is expressed as a percentage of inhibition of the contraction induced by phenylephrine (10^{-6} *M*) after H_2O_2 exposure (PE2). Means ± SEM. *$p < 0.05$ aged *vs.* adults; modified Student's *t*-test.

2) were more depressed in the group of aortic rings senescent rat compared to young ones. Moreover, at concentrations of H_2O_2 of 5 m*M* and above, the endothelium-dependent relaxation was totally abolished in aortic rings from senescent rats while a residual activity was still measurable in rings from adult rats up to a dose of 10 m*M* H_2O_2 (Fig. 2).

Catalase activity

Aging induced an increase (130–150%) in catalase activity in the rat aortic wall (Table 2). This finding was observed when catalase activity was expressed as International Units per gram wet tissue weight (adults versus aged; $p < 0.002$) as well as when it was expressed as International Units per milligram protein (adults versus aged; $p < 0.001$).

DISCUSSION

Numerous studies have suggested that the age-dependent increased sensitivity of different biological tissues to ischemia and reperfusion could be due, at least in part, to a decrease in the cellular defense systems against ROS (Boucher *et al.*, 1998; Abete *et al.*, 1999). However, a survey of the literature shows that no consensus exists as to the evolution of these systems during aging in the different tissues and organs of mammal species (Cand and Verdetti, 1989; Rao *et al.*, 1990; Fiebig *et al.*, 1996; Boucher *et al.*, 1998; Tian *et al.*, 1998).

Several recent studies have shown that H_2O_2 is able to impair vascular reactivity measured *ex vivo* on isolated rings of young adult rats (Mian and Martin, 1997; Hu *et al.*, 1998). Moreover, the resistance of vascular tissue to H_2O_2 seems to depend on the activity of catalase (Mian and Martin, 1997), but not that of gluthatione peroxidase (Tanguy *et al.*, 1999). Paradoxically, our results show that despite the increase in catalase activity during aging, the senescent vasculature appears to be more sensitive to H_2O_2 than the vasculature of younger adults.

These results appear to be consistent with the hypothesis proposed by Remacle *et al.*, (1992). They postulated that reduction of general metabolism and free energy in old cells might be the main factors responsible for the increased susceptibility of these cells to oxidative stresses rather than any reduction of cellular defense systems against ROS.

An alternative explanation has been proposed by Zs-Nagy and Floyd (1991). These authors have suggested that the increase in cytosolic density that occurs in aged cells could represent an overall physicochemical basis for

TABLE 2. VASCULAR CATALASE ACTIVITY MEASURED IN ADULT (4 MONTHS) AND AGED (24 MONTHS) RAT AORTA

	Catalase activity	
	IU / g wwt	*IU / mg prot*
Adults ($n = 8$)	0.71 ± 0.13	0.20 ± 0.02
Aged ($n = 8$)	1.79 ± 0.20	0.46 ± 0.06
1 vs. 2	$p < 0.002$	$p < 0.001$

Means ± SEM, Modified Student's *t*-test.

a down-regulation of enzymatic defense mechanisms against ROS. Thus, according to this theory, the *in vitro* assessment of the activity of cellular enzymes involved in ROS elimination, namely catalase, superoxide dismutase, and glutathione peroxidase, might overestimate the true antioxidant potential of the tissues.

Nevertheless, the findings reported here that catalase activity in the vasculature is dramatically increased in aortas from senescent rats does not support this last hypothesis.

In conclusion, the present study shows that aging increases the vascular sensitivity to H_2O_2 while it paradoxically enhances the activity of catalase in this tissue. These observations suggest that the age-dependent increase in vascular sensitivity to H_2O_2 is not due to a decrease in the cellular defense mechanisms against ROS. Further studies are now requested to investigate the evolution of these phenomena through aging, using rats from different groups of age (*e.g.*, 16 and 36 months).

ABBREVIATIONS

ACh: acetylcholine; BSA, bovine serum albumin; Ca^{2+}, calcium; DTPA, diethyltriamino-pentaacetic acid; H_2O_2, hydrogen peroxide; NO, nitric oxide; O_2-, superoxide anion; PE, phenylephrine; PMSF, phenylmethylsulfonylfluoride; ROS, reactive oxygen species; ns, not significant.

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