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Coronary and aortic vasoreactivity protection with endothelin receptor antagonist, bosentan, after ischemia and hypoxia in aged rats

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Abstract

This study investigated the effects of bosentan, a dual endothelin ET_A and ET_B receptor antagonist, during hypoxia–reoxygenation of senescent aorta and during ischemia–reperfusion of senescent heart. Isolated aortic rings and isolated hearts from adult and senescent rats were submitted, respectively, to hypoxia/reoxygenation (20/30 min) and to low-flow ischemia/reperfusion (45/30 min), without or with bosentan (10^{-5} M). In the aorta, bosentan treatment prevented the impairment of relaxation in response to acetylcholine after hypoxia–reoxygenation at both ages. In the heart, coronary flow recovery during reperfusion, which is lower in senescents than in adults (48% vs. 76% of baseline value, respectively; $P<0.05$) was fully prevented by bosentan. Prevention of endothelial dysfunction during reoxygenation of hypoxic aorta and of coronary vasoconstriction during reperfusion of ischemic heart with a dual endothelin ET_A and ET_B receptor antagonist suggests a role of endothelin in the vulnerability of aorta to hypoxia–reoxygenation, and of coronary arteries to ischemia–reperfusion, especially during aging.

Keywords: Aging; Endothelin; Ischemia, reperfusion; Coronary vasculature; Hypoxia, reoxygenation; Aorta

1. Introduction

Endothelin is increased in different pathological states such as ischemia–reperfusion and hypoxia–reoxygenation, in animals (Vitola et al., 1996; Brunner et al., 1997) as in humans (Krüger et al., 1997). Endothelin exerts a vasoconstrictor effect mediated by both ET_A and ET_B receptors (Balwierczak, 1993; Hercule and Oyekan, 2000). Gene expression and release of endothelin are increased after myocardial ischemia (Brunner et al., 1997; Vitola et al., 1996), leading to oxidative stress (Ishida et al., 1990) and coronary vasoconstriction (Hiller et al., 1997), as well as after hypoxia in endothelial cells (Li et al., 1994). In addition, both hypoxia and ischemia potentiate vasoconstriction in response to exogenous endothelin (Douglas et al., 1991; Neubauer et al., 1991). Bosentan, a potent orally active non-peptide antagonist of endothelin ET_A and ET_B receptors (Clozel et al., 1994) reduces myocardial and coronary injury in both in vivo and ex vivo models of ischemia–reperfusion (Wang et al., 1995a,b; Li et al., 1995; Fraccarollo et al., 1997), limits hypoxic vasoconstriction in rat aorta (Pape et al., 1997) and has vasodilator effects in humans with coronary artery disease (Wenzel et al., 1998).

During aging, the vasomotricity of blood vessels is decreased and the endothelial layer is altered (Marin, 1995), leading to a decreased endothelium-dependent relaxation in response to acetylcholine in humans (Egashira et al., 1993) as well as in animals (Barton et al., 1997). In

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addition, the aged heart, characterized by a reduced coronary flow and perfusion reserve, has a higher vulnerability to myocardial ischemia and reperfusion (Assayag et al., 1998; Hachamovitch et al., 1989). Finally, vasoconstriction, in response to endothelin, is potentiated during aging, especially after ischemia–reperfusion (Goodwin et al., 1999). However, beneficial effects of endothelin receptor antagonists on such coronary vasoconstriction during ischemia–reperfusion, as well as on blood vessels' vasoreactivity during hypoxia–reoxygenation, have never been investigated during aging.

The objective of the present study was to investigate whether, in senescence, bosentan exerts beneficial effects on (1) endothelial and smooth muscle injury of rat aorta after hypoxia–reoxygenation, using an isolated blood vessel preparation and on (2) coronary injury of rat heart after ischemia–reperfusion, using an isolated perfused heart model.

2. Materials and methods

2.1. Animals and experimental groups

Adult (4-month-old) and senescent (24-month-old) male Wistar rats were obtained from IFFA CREDO (Lyon, France). The spontaneous mortality rate of the 24-month-old population was 50% (Assayag et al., 1997, 1998).

For isolated heart studies, 4- and 24-month-old rats were subdivided into two randomized subgroups submitted to ischemia–reperfusion without (4-mo-control, $n=11$- and 24-mo-control, $n=9$, respectively) or with (4-mo-bosentan, $n=8$ and 24-mo-bosentan $n=11$, respectively) bosentan treatment. For isolated aortic ring studies, 4- and 24-month-old rats were subdivided into two randomized subgroups to investigate hypoxia–reoxygenation without (4-mo-control, $n=12$- and 24-mo-control, $n=13$, respectively) or with (4-mo-bosentan, $n=7$- and 24-mo-bosentan, $n=8$, respectively) bosentan treatment.

Anesthesia was induced by i.p. injection of thiopental sodium (Nesdonal, Specia, Rhône-Poulenc Rorer, Paris, France) (Assayag et al., 1998). The experiments were conducted in accordance with the recommendations of the European Community for the use of experimental animals (L358-86/609/EEC).

2.2. Isolated heart preparation and protocol for ischemia–reperfusion

During anesthesia, the hearts were rapidly excised and Langendorff perfusion under a constant hydrostatic pressure of 75 mm Hg was initiated as previously described (Assayag et al., 1997, 1998). During all the experiments, the hearts were perfused with a Krebs–Henseleit bicarbonate buffer (composition in mM: NaCl 118, $NaHCO_3$ 25, KCl 4.8, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.2, glucose 11) at 37 °C, which was continuously bubbled with 95% O_2 (partial oxygen pressure at the aortic cannula: 620–680 mm Hg) and 5% CO_2 to maintain a pH = 7.4 (Assayag et al., 1997, 1998). After removal of the right atrium, adult and senescent hearts were paced at 240 beats min^{-1} at 200% of threshold and 2 ms stimulation duration (SD9 stimulator, Grass instrument, Quincy, MA) (Assayag et al., 1997, 1998), to prevent arrhythmias during control perfusion, as well as during reperfusion because severe arrhythmias, mainly of supraventricular origin, occur spontaneously in vivo in the senescent heart (Carré et al., 1992). After 10 min of equilibration in this Langendorff perfusion with Krebs–Henseleit bicarbonate buffer (composition, temperature, bubbling and partial oxygen pressure at the aortic cannula as described above) under a constant hydrostatic pressure of 75 mm Hg, baseline values of coronary flow and active tension were measured. Active force was measured with a hook attached to a force transducer (type 351, Hugo Sachs Electronik, March-Hugstetten, Germany) connected to a Gould recorder (2000 model, Gould Electronic, Cleveland, OH), as previously described (Assayag et al., 1997, 1998).

Then, after switching the system to flow-controlled perfusion for 10 min, each heart being constantly perfused with Krebs–Henseleit bicarbonate buffer (composition, temperature, bubbling and partial oxygen pressure at the aortic cannula similar to those described above during Langendorff perfusion) at its own initial coronary flow with a peristaltic pump (Minipulse 3, Gilson, Villiers le bel, France), the hearts were submitted to 45 min of low-flow ischemia by reducing the coronary flow to 15% of its initial value (Assayag et al., 1998). The use of flow-controlled perfusion, which did not modify the baseline parameters of coronary perfusion or of force development in either adult or aged heart as compared to Langendorff perfusion (Assayag et al., 1998), allowed quick adjustment of coronary flow reduction during ischemia, with the calibrating peristaltic pump, at exactly 15% of the initial coronary flow for each heart. Under these conditions, the same degree of ischemia was induced in all hearts, whatever their initial coronary flow, which differs between adult and senescent hearts but also among the senescent heart group. During ischemia, pacing was stopped and an acrylic cover was placed over the perfusion chamber to maintain the temperature at 37 °C. In 4-mo-bosentan and 24-mo-bosentan groups, bosentan was added 5 min before starting ischemia and was maintained throughout the perfusion.

After 45 min of ischemia, the hearts were reperfused according to Langendorff under a constant hydrostatic pressure of 75 mm Hg, as described above for the measurement of baseline parameters. Coronary flow and active force were measured after 1, 3, 5, 10, 15, 20, 25 and 30 min of reperfusion. At the end of perfusion, the hearts were blotted and weighed quickly.

2.3. Aortic ring preparation and protocol for hypoxia and reoxygenation

During anesthesia, the thoracic aorta was quickly removed and placed in Krebs–Henseleit bicarbonate buffer (composition in mM: NaCl 118, $NaHCO_3$ 25, KCl 4.8, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11). After removal of adherent tissue, the vessels were cut into 3–4 mm rings. Aortic rings were mounted onto two stainless steel supports, suspended in the tissue bath containing Krebs–Henseleit bicarbonate buffer at 37 °C, continuously bubbled with 95% O_2 to maintain a partial oxygen pressure of 670–680 mm Hg in the incubation bath and 5% CO_2 to maintain a pH of 7.4. The rings were connected to an isometric force transducer (UF1-Harvard Biosciences, Les Ulis, France), linked to an amplifier (Freestanding, Harvard Biosciences) and a computerized acquisition system (MacLab AD Instruments, Castle Hill, Australia), to record changes in isometric force. The resting tension was adjusted to 2 g since preliminary studies in our laboratory had indicated that a preload of 2 g corresponds to the optimal length for tension development in aorta from 4- and 24-month-old rats. Then, the rings were equilibrated for 60 min.

After equilibration, test doses of phenylephrine (10^{-6} M), acetylcholine (10^{-6} M) or sodium nitroprusside (10^{-5} M) were added to the rings, to ensure reproducibility of contraction, relaxation and endothelial integrity. Each vessel ring was precontracted with phenylephrine (10^{-6} M). After the precontraction reached a plateau, endothelium-independent relaxation was produced with sodium nitroprusside (10^{-6} M). After rinsing, precontraction with phenylephrine (10^{-6} M) was repeated and endothelium-dependent relaxation was produced with acetylcholine (10^{-6} M). Then after rinsing and recovery of the resting tension, precontraction was induced with phenylephrine (10^{-6} M) and the aortic rings were submitted to 20 min of hypoxia by changing the gas mixture to 95% N_2–5%CO_2 (partial oxygen pressure in the bath: 36–38 mm Hg after 2 min) followed by 30 min of reoxygenation (bubbling with 95% O_2–5%CO_2; partial oxygen pressure in the bath: 670–680 mm Hg). At the end of the protocol and after rinsing, the responses to phenylephrine, acetylcholine and sodium nitroprusside were tested in all groups. In the 4- and 24-mo-bosentan groups, bosentan was added to the perfusion bath 5 min before the induction of hypoxia and maintained throughout hypoxia and reoxygenation. After each experiment, the rings were blotted and weighed. Preliminary experiments, performed under normoxia to test the stability of the preparation and to make sure that hypoxia-induced changes were not due to repeated exposure to the vasoactive drugs, showed that neither vasoconstriction with phenylephrine and vasorelaxations with acetylcholine and sodium nitroprusside nor basal vascular tone were significantly modified by 50 min of normoxia with or without bosentan (10^{-5} M) whatever the age of the animals.

2.4. Drugs

All concentrations of the drugs used in isolated heart and aortic ring experiments are expressed as final molar concentration in Krebs–Henseleit solution. Acetylcholine hydrochloride and phenylephrine were purchased from Sigma (St. Quentin-Fallavier, France) and sodium nitroprusside was from SERB laboratories (Nitriate, SERB, Paris, France).

Bosentan (Ro 47-0203, 4-*tert*-butyl-*N*-[6-(2hydroxyethoxy)-5-(2-methoxy-phenoxy)-2,2′ -bipyrimidin-4-yl] benzenesulfonamide), a dual endothelin ET_A and ET_B receptor antagonist (Clozel et al., 1994), was kindly provided by

Table 1
Baseline parameters of coronary flow and active tension in isolated perfused hearts and of contraction with phenylephrine (PE) and relaxation with acetylcholine (Ach) and sodium nitroprusside (SNP) in aortic rings from 4- and 24-month-old groups

	4-mo-control	24-mo-control	4-mo-bosentan	24-mo-bosentan
Isolated perfused hearts				
Coronary flow ($ml \cdot g^{-1}$ of heart weight)	10.0 ± 0.6	8.3 ± 0.4[a]	9.8 ± 0.6	7.8 ± 0.3[a]
Active tension ($g \cdot g^{-1}$ of heart weight)	10.8 ± 0.5	6.7 ± 0.3[b]	10.2 ± 0.7	6.6 ± 0.3[b]
Isolated aortic rings				
Contraction with PE ($mg \cdot mg^{-1}$ of wet weight)	307.4 ± 14.7	230.8 ± 8.9[c]	295.6 ± 16.6	214.3 ± 10.8[b]
Relaxation with Ach (% of contraction to PE)	47.90 ± 5.18	32.07 ± 2.07[b]	47.00 ± 3.25	33.50 ± 1.79[b]
Relaxation with SNP (% of contraction to PE)	91.39 ± 1.28	84.43 ± 1.64[b]	92.76 ± 0.84	84.02 ± 1.76[b]

[a] $P < 0.05$ vs. 4-month-matched group.
[b] $P < 0.01$ vs. 4-month-matched group.
[c] $P < 0.001$ vs. 4-month-matched group.

Dr. Martine Clozel (Actelion, Allschwil, Switzerland). Bosentan was used at 10^{-5} M, a concentration sufficient for endothelin ET_A and ET_B receptor blockade, as previously reported from different investigations of the role of endothelin during ischemia–reperfusion (Wang et al., 1995b; Li et al., 1995; Zhang et al., 1998) or the vasoconstrictor effects of endothelin in rat isolated heart (Li et al., 1995) as well as in rat aortic rings (Küng and Lüscher, 1995; Blandin et al., 2000; Talbodec et al., 2000). At this concentration of 10^{-5} M, the inhibitory effect of bosentan is specific for endothelin-1 and bosentan does not inhibit binding of other vasoactive compounds (Clozel et al., 1994).

2.5. Expression of results and statistical analysis

Coronary flow and active force of isolated hearts were divided by the wet heart weight to obtain, respectively, the coronary flow in milliliters per gram of heart weight and the active tension in gram per gram of heart weight. Force generated by the aortic rings was divided by the wet weight of the ring to obtain the isometric tension in milligram per milligram of ring wet weight. The initial transient hypoxic contraction was expressed as net increase in tension and the hypoxic relaxation as net decrease in tension. The late contraction and contraction of reoxygenation were expressed as absolute tension developed. Relaxation in response to acetylcholine and sodium nitroprusside were expressed as the percentage of contraction in response to phenylephrine.

All the data are expressed as means ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) with a Scheffe test. Group-to-group comparison was performed with an unpaired Student's *t*-test while comparisons among the same group were performed with a paired Student's *t*-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Baseline parameters of isolated heart and aortic rings

In isolated hearts, baseline parameters of coronary flow and active tension are reduced in 24-month-old groups as compared to those in 4-month-old groups (Table 1). In isolated aortic rings, the maximum contraction with phenylephrine was lower in 24-month-old than in 4-month-old groups, as well as maximum relaxation with acetylcholine and sodium nitroprusside (Table 1).

3.2. Coronary and myocardial function during ischemia–reperfusion: effects of bosentan

Reperfusion induced an impairment of coronary flow in ischemic hearts at both ages (Fig. 1) but this impairment was greater in 24-mo-control than in 4-mo-control groups (Fig. 1). Similarly, active tension recovery after 10 min of reperfusion was strongly reduced in 24-month-old hearts as compared to 4-month-old ones (Fig. 1).

In 4-month-old as in 24-month-old rats, coronary vasoconstriction was fully prevented by bosentan treatment since ischemic hearts returned to a coronary flow similar to that measured before ischemia, as early as in the first minutes of reperfusion (Fig. 1). In 24-month-old hearts, the coronary protective effect of bosentan is associated with an improvement of active tension recovery similar to that observed in 4-mo-control hearts (Fig. 1).

3.3. Aortic vasoreactivity during hypoxia–reoxygenation: effects of bosentan

During hypoxia, the aortic rings showed a rapid and short-lasting increase (1–2 min following hypoxia) in tension of short duration (initial hypoxic contraction), fol-

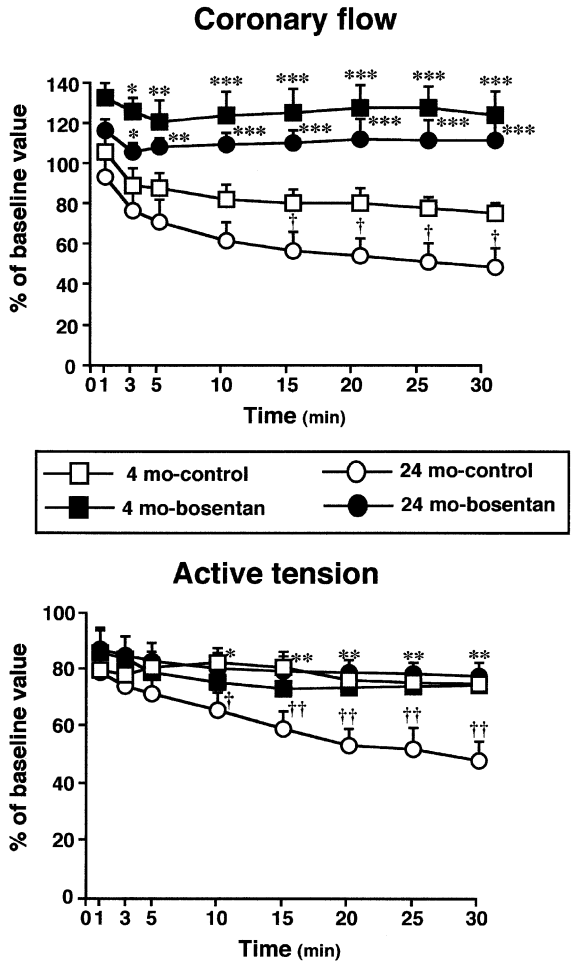

Fig. 1. Coronary flow and active tension recoveries during 30 min of reperfusion following 45 min of low-flow ischemia in 4- and 24-month-old isolated hearts not treated (4-mo-control and 24-mo-control) or treated with (4-mo-bosentan and 24-mo-bosentan) bosentan. $*P<0.05$, $**P<0.01$, $***P<0.001$ vs. age-matched control group; $\dagger P<0.05$ vs. 4-mo-control group.

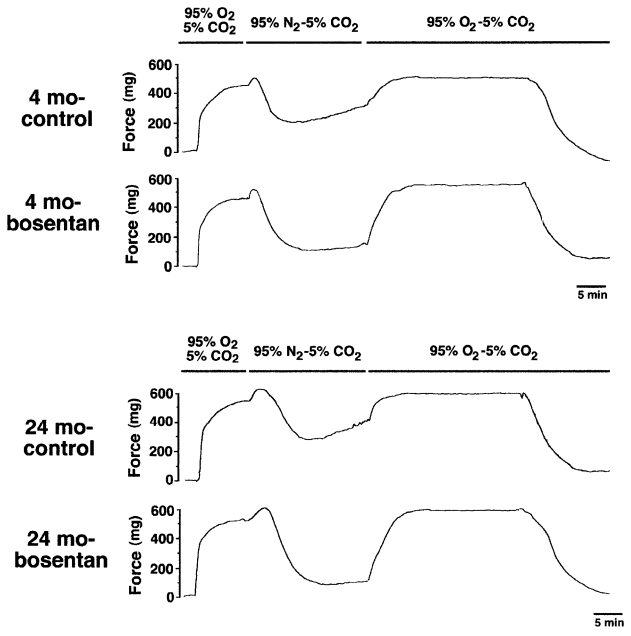

Fig. 2. Isometric tension recordings showing effects of 30 min of reoxygenation (95% O_2–5% CO_2 bubbling) following 20 min of hypoxia (95% N_2–5% CO_2 bubbling) on vascular tone of 4-month-old and 24-month-old aortic rings not treated (4-mo-control and 24-mo-control) or treated with (4-mo-bosentan and 24-mo-bosentan) bosentan.

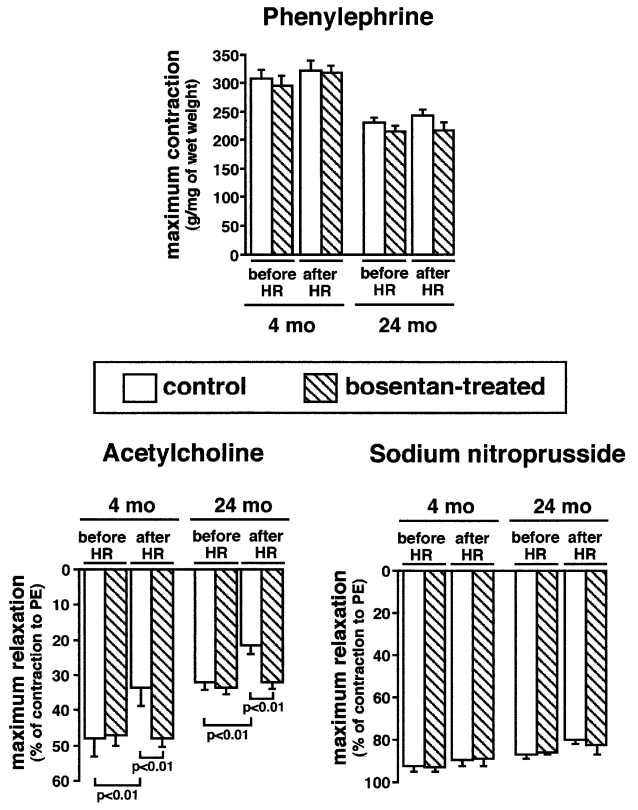

Fig. 3. Phenylephrine-induced contraction and acetylcholine- and sodium nitroprusside-induced relaxations of 4- and 24-month-old (mo) aortic rings not treated (control) or treated with (bosentan-treated) bosentan before and after hypoxia–reoxygenation (HR). All 24-month-old groups were significantly different from their 4-month-old matched group, $P<0.01$. Relaxation in response to acetylcholine and sodium nitroprusside was expressed as the percentage of contraction with phenylephrine (PE).

lowed by a partial relaxation (hypoxic relaxation) and then a gradual and sustained contraction (delayed hypoxic contraction) (Fig. 2). The initial hypoxic contraction was not modified by bosentan treatment in either 4- or 24-month-old rats (Table 2). Bosentan increased the maximum hypoxic vasorelaxation in 24-mo- but not in 4-mo-bosentan groups. Its administration also limited the delayed hypoxic contraction at both ages (Table 2) but this bosentan effect was greater in the 24- than in the 4-month-old groups (-17.1% vs. -5.9% of contraction with phenylephrine, respectively, $P<0.05$). During reoxygenation, bosentan did not modify the maximum contraction in either 4- or the 24-month-old rings (Fig. 2, Table 2).

After 30 min of reoxygenation, contraction in response to phenylephrine as well as endothelium-independent relaxation in response to sodium nitroprusside were not modified in any of the groups, whatever the age of the animals and the treatment (Fig. 3). In contrast, endothelium-dependent relaxation with acetylcholine was impaired to a similar extent in 4-mo-control and in 24-mo-control groups ($-76.0 \pm 15.7\%$ and $-78.1 \pm 10.1\%$ of contraction with phenylephrine, respectively) (Fig. 3). Bosentan treatment fully prevented these endothelium-dependent alterations in both groups since the relaxations with acetylcholine in 4-mo-bosentan and in 24-mo-bosentan groups were similar to those observed before hypoxia–reoxygenation (Fig. 3).

Table 2
Effects of Bosentan on initial hypoxic contraction, hypoxic relaxation, delayed hypoxic contraction and contraction during reoxygenation, expressed in milligram per milligram of wet weight, in aortic rings of 4- and 24-month-old groups

	4-mo-control	4-mo-bosentan	24-mo-control	24-mo-bosentan
Peak of initial hypoxic contraction	42.9 ± 11.1	43.5 ± 5.0	38.1 ± 3.7	46.6 ± 9.7
Peak of hypoxic relaxation	195.1 ± 14.8	219.1 ± 23.8	158.3 ± 12.4	213.3 ± 14.1[a]
Maximum delayed hypoxic contraction	255.8 ± 28.0	156.2 ± 32.0[a]	184.9 ± 20.6	53.0 ± 4.8[b]
Peak of contraction during reoxygenation	425.8 ± 36.2	379.0 ± 26.7	308.3 ± 14.0	248.6 ± 19.6

The initial transient hypoxic contraction was expressed as the net increase in tension and the hypoxic relaxation as net decrease in tension. The late contraction and contraction of reoxygenation were expressed as absolute tension developed.

[a] $P<0.05$ vs. age-matched group.

[b] $P<0.001$ vs. age-matched group.

4. Discussion

This study showed that coronary flow recovery during reperfusion following low-flow ischemia was less in senescent than in adult heart. This post-ischemic impairment of coronary perfusion was fully prevented by the administration of the endothelin receptor antagonist, bosentan, in adult as well as in senescent rats. In the aorta, bosentan treatment limited the hypoxic delayed contraction and, although it did not affect the reoxygenation-induced contraction, it fully prevented aortic endothelial dysfunction after hypoxia–reoxygenation in adult and senescent rings.

4.1. Vasoreactivity during aging

During aging, coronary flow and reserve are reduced (Assayag et al., 1997, 1998; Hachamovitch et al., 1989) while arteriolar density is decreased (Engelmann et al., 1987) and these alterations are associated with a contractile dysfunction (Assayag et al., 1997, 1998). Similarly, aortic vasoreactivity is impaired since both contraction in response to an $\alpha 1$ agonist and relaxation in response to sodium nitroprusside are reduced during aging (review in Marin, 1995). Such alterations of vascular tone in different species and vessels have been attributed to the age-related alterations of the arterial wall structure (Marin, 1995). In the aorta, endothelium-dependent relaxation in response to acetylcholine varies with both the age and the strain of rats used (Koga et al., 1988; Barton et al., 1997), but all reports, including ours, indicate a decrease in endothelium-dependent relaxation during aging, mainly through a lower NO release and expression (Barton et al., 1997; Czernadas et al., 1998), a decreased NO-dependent activation of soluble guanylate cyclase (Klöß et al., 2000) and/or an increased generation of vasoconstrictor prostanoids (Matz et al., 2000).

4.2. Coronary vasoreactivity during ischemia–reperfusion: effect of bosentan

Reperfusion of senescent ischemic myocardium induces major coronary vasoconstriction, associated with poor recovery of both coronary flow and active developed tension, which indicates a specific impairment of coronary vasoreactivity during low-flow ischemia–reperfusion in senescence. Such an impairment of coronary vasoreactivity with aging has also been observed during prolonged low-flow ischemia (Assayag et al., 1998), as well as after reperfusion following no-flow ischemia (Tani et al., 1997). In addition, recovery of coronary endothelial function is impaired in aged rats since the basal release of NO is reduced (Amrani et al., 1996) while vasoconstriction in response to endothelin is markedly increased after ischemia–reperfusion (Goodwin et al., 1999).

Gene expression, biosynthesis and release of endothelin are enhanced in different animal models of myocardial ischemia and reperfusion (Vitola et al., 1996; Brunner et al., 1997) as well as during myocardial infarction in humans (Krüger et al., 1997). Endothelin has numerous deleterious cardiac effects (Brunner et al., 1997; Sharif et al., 1998), including major coronary vasoconstriction (Hiller et al., 1997; Goodwin et al., 1999), which is potentiated during aging in isolated perfused heart (Goodwin et al., 1999) but not in isolated arteries (Tschudi and Lüscher, 1995). In the adult heart, endothelin receptor antagonists reduce this coronary injury in both in vivo and ex vivo models of no-flow ischemia and reperfusion (Li et al., 1995; Wang et al., 1995a,b; Hiller et al., 1997; Gonon et al., 1998). However, the effects of such receptor antagonists have not yet been investigated in the senescent heart. Bosentan, a dual endothelin ET_A and ET_B receptor antagonist (Clozel et al., 1994), was chosen because both subtypes of receptors are present in the smooth muscle of rat coronary bed (Balwierczak, 1993). The present study showed that bosentan administration fully prevents the coronary vasoconstriction induced by reperfusion following low-flow ischemia, in adult, but also in senescent hearts in which coronary post-ischemic recovery is particularly impaired. The cardioprotective effects of bosentan were previously reported for adult heart. Indeed, its administration limits the no-reflow area, preserves endothelium-dependent vasodilatation and enhances myocardial recovery after no-flow ischemia and reperfusion in rats (Li et al., 1995; Wang et al., 1995a) but also limits infarct size (Wang et al., 1995b) and improves survival (Fraccarollo et al., 1997). In addition, endothelin receptor antagonists inhibit polymorphonuclear leukocyte activation by endothelin (Gomez-Garre et al., 1992), which leads to anion superoxide production (Ishida et al., 1990) and consequently limits endothelial and myocardial oxidative injury during reperfusion (Gonon et al., 1998; Maczewski and Beresewicz, 2000). Our study is the first to report such a beneficial effect of bosentan on coronary flow recovery during reperfusion following low-flow ischemia, especially in the senescent heart in which this coronary protection is associated with improvement of contractile function recovery. These results support the view that endothelin is strongly involved in the events leading to major low-flow ischemia and reperfusion injury during aging. The cardioprotective effects of bosentan cannot result from the prevention of endothelin-induced arrhythmias (Sharif et al., 1998) in our model of paced isolated heart. However, they could be related to the limitation of oxidative injury since the vulnerability to oxidative stress is increased during aging (Abete et al., 1999).

4.3. Aortic vasoreactivity during hypoxia–reoxygenation: effects of bosentan

Senescent as well as adult aorta (Yang and Mehta, 1995), develops a transient contraction followed by relaxation during the first minutes of hypoxia, and then a sustained

contraction. The mechanisms implicated in this initial transient contraction have been well examined in a variety of adult blood vessels (Jin et al., 1992; Muramatsu et al., 1992; Yang and Mehta, 1995) and the results of our study suggest, as do others (Yang and Mehta, 1995), that endothelin is not involved. In senescent aorta, this contraction is also unmodified by bosentan treatment, suggesting that the decrease in basal NO synthesis and release during aging (Barton et al., 1997) is implicated rather than endothelin production, as in adult aorta (Muramatsu et al., 1992; Yang and Mehta, 1995).

Although hypoxic relaxation has been observed in different blood vessels, the mechanisms involved remain unclear and vary between studies (Gräser et al., 1992; Jin et al., 1992). In adult rat aorta, hypoxic dilatation is potentiated after de-endothelization. We found, as had others (Yang and Mehta, 1995; Pape et al., 1997), that endothelin is not implicated in such a relaxation since endothelin receptor antagonists have no effect. However, this hypoxic relaxation is improved by bosentan treatment during aging, suggesting that endothelin production limits vasorelaxation during hypoxia in senescent aorta, in sharp contrast to adult aorta.

Delayed hypoxic vasoconstriction is prevented by de-endothelization, indicating that the presence of intact functional endothelium is critical for its development. The involvement of an endothelium-derived constrictor factor (EDCF), which is endothelin according to some authors (Pape et al., 1997), but is related to NO synthesis according to others (Yang and Mehta, 1995), has been proposed. We found that bosentan treatment limits this hypoxic vasoconstriction in adult, but also and to a greater extent, in senescent aorta, suggesting that endothelin plays a significant role in the development of this delayed vasoconstriction, especially during aging.

Reoxygenation following hypoxia induces a rapid and major contraction of similar amplitude with or without bosentan treatment at both ages, indicating that endothelin is not implicated in this phenomenon. Responses to phenylephrine and sodium nitroprusside are not impaired after reoxygenation, while relaxation in response to acetylcholine is reduced in adult and senescent vessels. Our results indicate, for the first time, that bosentan treatment fully prevents the impairment of endothelium-dependent relaxation at both ages. Oxidative stress is involved in this endothelium injury (Gao et al., 1996; Yokoyama et al., 1996) since numerous studies pointed to the role of oxygen-derived free-radicals and neutrophils in reoxygenation/reperfusion injury by their deleterious action on endothelial cells (Wang et al., 1998; Yokoyama et al., 1996). As in the coronary vasculature, the prevention by bosentan of endothelin-induced anion superoxide production and its deleterious effects, may be partly responsible for the prevention of endothelium injury observed in this study.

In conclusion, antagonism of both endothelin ET_A and ET_B receptors with bosentan fully prevents coronary vasoconstriction during low-flow ischemia–reperfusion while it limits endothelial dysfunction in senescent aorta after hypoxia–reoxygenation. These results indicate that endothelin is involved in the vasoreactive changes of both coronary arteries and aorta during aging, and contributes especially to the alteration of coronary flow recovery during reperfusion of adult but also of senescent ischemic hearts. In humans, endothelin levels are increased during myocardial ischemia (Krüger et al., 1997) and are highly predictive of cardiac death (Omland et al., 1994). The persisting high mortality for myocardial infarction during aging, in spite of revascularization therapy (Lesnefsky et al., 1996), could be related to the increased microvascular dysfunction, as suggested by the major coronary vasoconstriction during ischemia (Assayag et al., 1998) as well as the impairment of flow recovery during reperfusion that we have observed. Our results suggest that endothelin receptor antagonists could have cardioprotective effects during myocardial ischemia in senescence.

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