Oxidative Stress Induced by tert-Butyl Hydroperoxide Causes Vasoconstriction in the Aorta from Hypertensive and Aged Rats: Role of Cyclooxygenase-2 Isoform

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ABSTRACT

We analyzed the mechanisms involved in the effect of tert-butyl hydroperoxide (t-BOOH) in isolated aortic rings with and without endothelium from normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) at 6, 18, and 24 months of age. t-BOOH (1 μM-10 mM) induced concentration-dependent contractions that were scarcely modified by aging and potentiated in SHR and by endothelium removal. The nitric oxide synthase and prostacyclin synthase inhibitors L-NAME, N\(^\text{G}\)-nitro-L-arginine methyl ester (100 μM) and tranylcypromine (100 μM), respectively, increased both basal tone and the t-BOOH-induced contractions in intact segments from WKY, with these effects not observed in SHR. Indomethacin (10 μM), a nonspecific cyclooxygenase inhibitor, and SQ 29,548 (10 μM), a prostaglandin H\(_2\)/thromboxane A\(_2\) receptor blocker, abolished the t-BOOH-induced vasoconstriction, independent of age and hypertension. In both strains, these contractile responses were unaltered by the thromboxane synthase inhibitor imidazole (10 μM). The cyclooxygenase-2 inhibitor NS-398 (10 μM) abolished or markedly reduced the t-BOOH-induced contractions in segments with or without endothelium, respectively. In addition, expression of cyclooxygenase-2 protein was detected in aorta from WKY and SHR in either basal condition or after stimulation with t-BOOH. These results suggest that (1) t-BOOH-induced vasoconstriction in the aorta from WKY and SHR is essentially mediated by cyclooxygenase-2 metabolites, different from thromboxane-A\(_2\), probably prostaglandin-H\(_2\), and/or isoprostanes; (2) aging scarcely modifies, whereas endothelium negatively modulates, these contractions in both strains; and (3) nitric oxide and prostacyclin exert a negative modulator role on the t-BOOH-induced vasoconstriction in WKY, with this modulator role lost in SHR.

Lipid peroxidation is a free radical-dependent process that is involved in the modification of structure and permeability of membranes, age pigment formation, oxidative modification of low-density lipoproteins, and alteration of vasomotor tone regulation (Hicks and Gebicki, 1978; Kikugawa et al., 1981; Esterbauer et al., 1992; Rodríguez-Martínez et al., 1996, 1998a). Of special clinical interest is the role played by lipid peroxidation in the pathogenesis of cardiovascular diseases, such as congestive heart failure, atherosclerosis, or preclampsia (Holvoet et al., 1995; Wang and Walsh, 1995; Díaz-Velez et al., 1996), as well as in the vascular damage associated with aging (Rodríguez-Martínez et al., 1998a). Thus, enhancement of lipid peroxidation derivatives in plasma or vascular tissues (Rodríguez-Martínez and Ruiz-Torres, 1992; Cester et al., 1994; Rodríguez-Martínez et al., 1998a), along with a certain degree of endothelial alteration (Marín, 1993, 1995; Marín and Rodríguez-Martínez, 1997), appears to be common findings in hypertension and aging.

Oxidative stress induced by lipid peroxidation derivatives can affect the vascular reactivity modifying basal tone, the responses to agonists, Ca\(^2+\)-signaling mechanisms, and the production, release, or effect of endothelium-derived paracrine factors (Gurtner and Burke-Wolin, 1991; Schilling and Elliott, 1992; Hubel et al., 1993; Rodríguez-Martínez et al., 1996). In this regard, we recently described that the classic marker of lipid peroxidation processes, malondialdehyde, produces an impairment of the relaxant responses evoked by acetylcholine and that the imbalance between the glutathione-dependent antioxidant system and malondialdehyde levels in blood is implicated in the dysfunction of endothelium-dependent relaxations with age (Rodríguez-Martínez et al., 1996, 1998a).

ABBREVIATIONS: WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; MAP, mean arterial pressure; KHS, Krebs-Henseleit solution; COX, cyclooxygenase; H\(_2\)O\(_2\), hydrogen peroxide; l-NAME, N\(^\text{G}\)-nitro-L-arginine methyl ester; NO, nitric oxide; PG, prostaglandin; t-BOOH, tert-butyl hydroperoxide; TX, thromboxane.
A precursor in the formation of malondialdehyde is, among other hydroperoxides, tert-butyl hydroperoxide (t-BOOH), a membrane-permeant oxidant that has been extensively used as a model of oxidative stress in different systems. In the past years, considerable evidence has been accumulated to suggest that t-BOOH is able to induce changes in either Ca$^{2+}$-signaling mechanisms (Schilling and Elliott, 1992; Elliott and Doan, 1993; Elliott et al., 1995) or monovalent cation homeostasis (Elliott and Schilling, 1992; Cutaia and Parks, 1994; Koliwad et al., 1996a,b) in vascular endothelial cells. Vascular reactive can also be altered by t-BOOH. Thus, vasoconstriction can be induced by perfusing isolated rabbit lung (Gurtner and Burke-Wolin, 1991) or isolated human placental cotyledons (Walsh et al., 1993) with t-BOOH. This oxidant can also potentiate the contractile responses to potassium or norepinephrine in rat mesenteric artery (Hube et al., 1993) or reduce the relaxant responses to acetycholine in rat coronary vessels (Yaghi and Watts, 1993). Nevertheless, the vascular effect and the mechanism of action of t-BOOH in hypertension and aging have been scarcely studied, although in both processes oxidative stress could play a causative role (Rodrı́guez-Martı́nez and Ruiz-Torres, 1992; Boulimouı́et al., 1997). Therefore, the objective of this study was to assess the influence of hypertension, age, and endothelium on the modulation of the responses to t-BOOH on vasomotor tone in aortic rings from normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) at 6, 18, and 24 months of age, as well as the possible mechanisms involved.

**Materials and Methods**

This study was performed in 36 WKY and 36 SHR male rats at ages 6, 18, and 24 months, which were born and fed with regular chow at the facilities of the Facultad de Medicina of the Universidad Autónoma of Madrid. The procedures were in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. At the ages studied, hypertension is well established in SHR as indicated by the values of mean arterial pressure (MAP) measured in a randomly selected group of six animals of each age. These animals were anesthetized with diethyl ether (Panreac, Barcelona, Spain), and the right carotid artery was cannulated to measure and record MAP by means of a pressure transducer (Letica, Barcelona, Spain) connected to a polygraph (Letica Polygraph 2006). MAP values (mean ± S.E.) in 6-, 18-, and 24-month-old rats were 101 ± 5, 111 ± 20, and 103 ± 17 mm Hg for WKY and 170 ± 10, 179 ± 7, and 181 ± 6 mm Hg for SHR (P < .05 SHR versus WKY at all ages studied). Reactivity Experiments. Aortas from WKY and SHR were carefully dissected out, cleaned of connective tissue, divided into segments of 3 mm in length, and placed in an organ bath containing 5 ml of Krebs-Henseleit solution (KHS) at 37°C as previously described (Rodrı́guez-Martı́nez et al., 1998b). KHS was continuously bubbled with a 95% O$_2$, 5% CO$_2$ mixture, which gave pH 7.4. The isometric contraction was recorded through a force-displacement transducer (FT03C; Grass, Quincy, MA) connected to a polygraph (Grass model 7D). After a 60-min equilibration period, segments were exposed to 75 mM KCl to check their functional integrity. After a washout period, the functionality of vascular endothelium was confirmed by the ability of 10 µM acetylcholine to relax segments contracted with 0.01 µM norepinephrine. To remove vascular endothelium, the segments were incubated for 20 min with saponin (0.3 mg/ml KHS); the inability of acetylcholine to relax these segments confirmed the success of this procedure. The responses to 75 mM KCl were unaltered by endothelium removal.

**Experimental Protocol.** To analyze the influence of age, endothelium, and hypertension on the effect elicited by t-BOOH on basal tone, cumulative concentrations of this oxidant (1 µM to 10 mM) were added to segments with and without endothelium from WKY and SHR at 6, 18, and 24 months of age. Only one concentration-response curve to t-BOOH was performed in each aortic segment, because a slow and maintained increase in basal tone was observed 30 min after the first concentration-response curve to t-BOOH was carried out.

The modulation of the responses to t-BOOH by nitric oxide (NO) and prostacyclin was assessed in intact segments from both strains. For this, segments were incubated for 20 min with the NO synthase inhibitor N$^\text{G}$-nitro-L-arginine methyl ester (L-NAME; 100 µM) or with the prostacyclin synthase inhibitor trans-2-phenylecyclcopropylamine (tranylcypromine, 100 µM) before the performance of the concentration-response curve to t-BOOH.

To assess whether prostanooids could be implicated in the mechanism of action of t-BOOH, segments with and without endothelium from 6-, 18-, and 24-month-old WKY and SHR were incubated for 30 min with either indomethacin (10 µM), a nonspecific cyclooxygenase (COX) inhibitor, or SQ 29,548 (10 µM), a prostaglandin (PG)H$_2$/thromboxane (TX)A$_2$ receptor antagonist before a concentration-response curve to t-BOOH was done. In another set of experiments, segments with and without endothelium from 6-month-old WKY and SHR were incubated for 30 min with either 1-(7-carboxyheptyl)imidazole (imidazole, 10 µM), a TX synthase inhibitor, or NS-398 (10 µM), a specific COX-2 inhibitor, before the performance of a concentration-response curve to t-BOOH.

**Western Blotting.** Basal or stimulated (1 mM t-BOOH, 45 min) aortas from WKY and SHR were homogenized in a buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM sodium vanadate, and 1% SDS. Homogenates were centrifuged at 13,000 rpm for 5 min, and supernatant samples were stored at −70°C until used. Supernatant samples (15 µg protein/lane) were applied to 7.5% SDS-polyacrylamide gels and electrophoretically transferred to hydrophobic polyvinylidene difluoride membranes (Amersham International plc, Little Chalfont, Buckinghamshire, UK) in Tris-glycine transfer buffer (20% methanol, 0.05% SDS, 25 mM Tris, and 190 mM glycine) in a Trans-Blot Cell (Bio-Rad Laboratories, Hercules, CA). Membranes were blocked for 90 min at room temperature with 5% nonfat dry milk in Tris-buffered saline containing 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 0.1% Tween 20 and incubated with a monoclonal primary mouse antibody against COX-2 (1:250; Transduction Laboratories, Lexington, UK) for 75 min at room temperature. The membranes were washed thoroughly and incubated with horseradish peroxidase-coupled anti-mouse IgG antibody (1:2000; Transduction Laboratories) for 1 h. After successive washes, bound antibodies were visualized by enhanced chemiluminescence (ECL Kit; Amersham International plc) and exposure to Kodak X-OMAT film. A densitometric scan was made of the Western blots using NIH Image 1.56 software. To calculate the relative density of the bands of COX-2 expression in WKY and SHR, the density of the band representing COX-2 positive control was taken as 100%.

**Solutions and Drugs.** The composition of KHS was 115 mM NaCl, 2.5 mM CaCl$_2$, 4.6 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.2 mM MgSO$_4$, 7H$_2$O, 25 mM NaHCO$_3$, 11.1 mM glucose, and 0.03 mM disodium EDTA. L-Norepinephrine hydrochloride, acetycholine chloride, t-BOOH, hydrogen peroxide (H$_2$O$_2$), saponin, sodium azide, indomethacin, glycine, sodium vanadate, L-NAME, and tranylcypromine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). SQ 29,548 and 1-(7-carboxyheptyl)imidazole hydrochloride were obtained from ICN Ibérica, S.A. (Barcelona, Spain). NS-398 was obtained from Calbiochem (La Jolla, CA). In addition, Tween 20, Tris, and acrylamide were purchased from Bio-Rad Laboratories.

Stock solutions (10 mM) of acetylcholine, tranylcypromine, and imidazole were made in distilled water, whereas those of norepinephrine, indomethacin, SQ 29,548, and NS-398 were prepared in...
saline [0.9% NaCl (w/v)-ascorbic acid (0.01% w/v)] solution, NaHCO₃ (0.5% w/v), absolute ethanol, and DMSO, respectively. These solutions were kept at ~20°C, and appropriate dilutions were made in distilled water on the day of the experiment. Daily solutions of saponin and t-BOOH were prepared in oxygenated KHS. Final concentrations of ethanol (0.001%) and DMSO (10 μM) in the organ bath did not modify basal tone. Experiments with SQ 29,548 and tranylcypromine were carried out under sodium vapor light.

**Statistical Analysis.** Results are expressed as mean ± S.E. In the reactivity experiments, a two-factor ANOVA was used to determine significant differences between strains or groups of treatment. A value of \( P < .05 \) was considered statistically significant.

More than three rats were used in each set of vascular reactivity experiments. The contractile responses to t-BOOH were expressed in percentage of the previous contraction induced by 75 mM KCl.

## Results

T-BOOH (1 μM to 10 mM) produced concentration-dependent contractions in segments with and without endothelium from 6-, 18-, and 24-month-old WKY and SHR (Fig. 1). These contractions were greater in segments from SHR than in those from WKY rats at the three ages. Aging scarcely modified the t-BOOH-induced contractions (Fig. 1), and only a slight decrease of these responses was observed in segments without endothelium from WKY of 18-month-olds compared with 6-month-olds (ANOVA, \( P < .05 \)). The contractile responses induced by 10 mM t-BOOH were compared with those elicited by 10 mM H₂O₂, another membrane-permeant oxidant that induces vasoconstriction. The results from the 10 to 14 aortic segments used in each set of experiments were for t-BOOH and H₂O₂, respectively, 588 ± 83 and 599 ± 65 mg for intact segments from WKY, 1170 ± 122 and 830 ± 109 mg (\( P < .05 \)) for intact segments from SHR, 1220 ± 137 and 1428 ± 113 mg for endothelium-denuded segments from WKY, and 1425 ± 114 and 1345 ± 119 mg for endothelium-denuded segments from SHR. In addition, the responses induced by the receptor-mediated vasoconstrictor norepinephrine (30 nM) in both strains were also analyzed. The results obtained from 10 to 14 aortic segments used in each set of experiments were for WKY and SHR, respectively, 1180 ± 66 and 1225 ± 37 mg for intact segments and 1140 ± 56 and 1150 ± 37 mg for endothelium-denuded segments.

At the three ages, and in both strains, endothelium removal potentiated the contractile responses induced by t-BOOH (Fig. 1). Curiously, the contractions elicited by t-BOOH in segments without endothelium from WKY were similar to those observed in segments with endothelium from SHR (Fig. 1). In analyzing the negative modulator role of endothelium on the t-BOOH-induced contractions, we observed that the inhibition of NO synthase with L-NAME (100 μM) increased either basal tone (273 ± 45 mg, \( n = 10 \)) or the contractile responses elicited by t-BOOH in intact segments from normotensive rats (Fig. 2). Similar results were obtained with the prostacyclin synthase inhibitor tranylcypromine (100 μM), which increased basal tone (114 ± 58 mg, \( n = 12 \)) and the contractions induced by t-BOOH (Fig. 2). In addition, L-NAME or tranylcypromine did not modify the t-BOOH-induced contractions in the hypertensive rats (Fig. 2).

The involvement of COX products in the contractions elicited by t-BOOH was analyzed. Indomethacin (10 μM, a non-specific COX inhibitor) practically abolished the contractions elicited by t-BOOH in segments with and without endothelium from WKY and SHR of 6-, 18-, and 24-month-olds (Figs.

**Fig. 1.** Effect of age, hypertension, and endothelium on the contractile responses elicited by t-BOOH in segments with (E+) and without (E−) endothelium from WKY and SHR of 6-, 18-, and 24-month-olds. Results (mean ± S.E.) from 8 to 12 arterial segments used in each set of experiments are expressed in percentage of the contraction elicited by 75 mM KCl.

**Fig. 2.** Effect of the inhibitors of NO synthase and prostacyclin synthase, L-NAME (100 μM) and tranylcypromine (100 μM), respectively, on the t-BOOH-induced contractions in segments with endothelium (E+) from WKY and SHR of 6 months. The contractile responses to t-BOOH in segments without endothelium (E−) are shown for comparison. Results (mean ± S.E.) from 10 to 12 arterial segments used in each set of experiments are expressed in percentage of the contraction elicited by 75 mM KCl.
3 and 4). A similar effect produced the incubation of segments with SQ 29,548 (10 μM, a PGH₂/TXA₂ receptor blocker) (Figs. 3 and 4).

In WKY and SHR of 6 months, the inhibition of TX synthase with imidazole (10 μM) did not modify the contractile responses induced by t-BOOH (Fig. 5). The COX-2 inhibitor NS-398 (10 μM) blocked the responses induced by t-BOOH in the same way as indomethacin in segments with endothelium from both strains (Fig. 6B). Nevertheless, in endothelium-denuded segments, NS-398 reduced the t-BOOH-induced contractions, whereas indomethacin abolished them (ANOVA, P < .001 NS-398 versus indomethacin, for both strains, Fig. 6B). In addition, the expression of COX-2 isoform was evaluated by Western blotting in either basal or t-BOOH-stimulated (1 mM, 45 min) aortas from both strains (Fig. 6A). In lysates of mouse macrophages (positive control), the anti-COX-2 antibody reacted strongly with COX-2 isoform; however, no protein of the expected size (i.e., 70 kDa) was detected in lysates of rat erythrocytes (negative control). Expression of COX-2 protein was detected in aorta from WKY and SHR in either basal situation or after stimulation with t-BOOH (Fig. 6A). The relative density of the bands of COX-2 expression in the aorta from WKY and SHR in basal condition was around 30 and 60% versus COX-2 positive control (100%), respectively. After exposure to 1 mM t-BOOH, an increase of relative densities was observed in both strains.

Table 1 shows that age or the removal of endothelium did not modify the contractions elicited by 75 mM KCl in both strains. In hypertensive rats, a decrease in these contractions was observed in segments with endothelium from all ages and in segments without endothelium from 24-month-old rats (Table 1). Due to this different response to KCl between strains and because the contractions induced by t-BOOH were expressed in a percentage of those elicited by KCl, we analyzed whether the increased responses to t-BOOH observed in SHR were maintained when the results were expressed in milligrams of contraction elicited by 75 mM KCl.

**Discussion**

The results described in this study show the ability of the hydroperoxide t-BOOH, which induces oxidative stress, to...
produce concentration-dependent contractions in aortic segments with and without endothelium from normotensive and hypertensive rats. The contractile responses induced by t-BOOH were greater in segments from hypertensive rats of 6-, 18-, and 24-month-old rats than in age-matched normotensive rats. It has been reported that high t-BOOH concentrations (≥1 mM) produce vasoconstriction in quiescent rat mesenteric arteries (Hubel et al., 1993) and that the same effect can be observed by perfusing isolated rabbit lung with this agent (Gurtner and Burke-Wolin, 1991).

Previous results from our laboratory indicate that H₂O₂, another membrane-permeant oxidant, also induces vasoconstrictor responses in the aorta from WKY and SHR of 6 months (Rodrı́guez-Martı́nez et al., 1998b). In this vascular bed, the contractions induced by 10 mM t-BOOH were similar to those elicited by 10 mM H₂O₂, except in intact segments from SHR, where t-BOOH-induced responses were greater. In addition, the contractions elicited by either H₂O₂ or t-BOOH were larger in intact segments from SHR than from WKY, as well as in endothelium-denuded segments from both strains. These results suggest (1) an increased sensitivity of vascular wall to inducers of oxidative stress in hypertension and (2) the existence of common vascular pathways of response against oxidant agents. It is known that endothelial changes after t-BOOH perfusion, such as cauliflower-like blebs and crater-like holes, are frequently observed in aorta from SHR but rarely seen in aorta from WKY rats (Ito et al., 1995), supporting the previous assumption of a greater sensitivity to oxidative stress in hypertension. It is interesting that the contractions induced by these oxidants were similar to those elicited by low concentrations of the receptor-mediated vasoconstrictor norepinephrine (30 nM) and lower than those elicited by the receptor-independent vasoconstrictor KCl (75 mM). Furthermore, both oxidants induced greater contractile responses in endothelium-denuded segments than in intact segments from both strains, with this effect not observed in response to both KCl and norepinephrine. These results support the idea that the ox-
dants use a different pathway to induce vasoconstriction than the classic vasoconstrictor agents and that endothelium plays an important role in modulating the vasoconstriction induced by oxidants.

We have found that the contractions induced by cumulative concentrations of t-BOOH were greater in endothelium-denuded segments than in intact segments from both strains, suggesting a negative modulator role of endothelium on the vasoconstriction elicited by t-BOOH. However, this negative modulator role of endothelium seems to be partially lost in hypertensive rats. This assumption is supported by the fact that the contractions induced by t-BOOH were greater in intact segments from SHR than in those from WKY rats and similar in intact segments from SHR than in endothelium-denuded segments from WKY. We analyzed whether vasodilator compounds, such as NO or prostacyclin, could be responsible for the negative modulator role played by endothelium on the vasoconstriction elicited by t-BOOH. We observed that L-NAME and tranylcypromine, inhibitors of NO synthase and prostacyclin synthase, respectively, increased basal tone and potentiated the contractile responses elicited by t-BOOH in intact segments from normotensive rats, with these effects not observed in hypertensive rats. These results indicate the existence of a basal release of NO and prostacyclin that negatively modulates the t-BOOH-induced vasoconstriction in normotensive rats. In addition, these results suggest an alteration in the synthesis, release, or action of NO in hypertensive rats, as previously described (Marín, 1993; Küng and Lüscher, 1995; Marín and Rodríguez-Martínez, 1997). Related to prostacyclin, our results agree with the recent evidence that prostacyclin receptor mRNA levels in aorta from SHR are consistently lower than those in WKY rats, despite prostacyclin synthase mRNA and protein levels in SHR being significantly higher than those in WKY rats (Numaguchi et al., 1999). In addition, t-BOOH-induced contractions were greater in endothelium-denuded segments from WKY rats than in intact segments incubated with L-NAME or tranylcypromine. This suggests that another endothelium-derived relaxing factor could be involved in its negative modulator role or that contracting factors are counteracting the effect of the relaxing ones. In this regard, preliminary results using superoxide dismutase, a superoxide anion scavenger, suggest a t-BOOH-mediated generation of superoxide anion that could react with NO, counteracting its vasodilator action (data not shown).

Aging scarcely modified the vasocontractile responses induced by t-BOOH in WKY and SHR. Only a slight decrease in these responses in segments without endothelium from 18-month-old WKY rats was observed. This small decrease in the response is unlikely to be due to a dysfunction in the contractile machinery because the responses to 75 mM KCl were unaltered in 18-month-old WKY rats compared with 6-month-old rats (see Table 1).

In analyzing the involvement of prostanoids in the vasoconstrictor responses induced by t-BOOH, we have found that indomethacin, a nonspecific COX inhibitor, and SQ 29,548, a PGH₂/TXA₂ receptor blocker, practically abolished the response elicited by t-BOOH in segments with and without endothelium from 6-, 18-, and 24-month-old WKY and SHR. These results indicate that prostanoids acting on PGH₂/TXA₂ receptor are involved in the vascular effect of t-BOOH and that the mechanism of action of t-BOOH is independent of age and hypertension. In addition, the greater contractile responses induced by t-BOOH in SHR could suggest an enhanced production of COX metabolites in response to oxidative stress or an increased number of PGH₂/TXA₂ receptors in SHR. Different PGH₂/TXA₂ receptor antagonists play an important role in the control of blood pressure (Lin et al., 1991; Wilcox et al., 1996), as well as in attenuating the increased contractile responses induced by the calcium ionophore A23187, endothelin, angiotensin II, or oxygen-derived free radicals (Lin and Nasjletti, 1991, 1992; Hibino et al., 1999) in rat aorta in different models of hypertension. However, whether these effects are due to an increased number of receptors or to their overstimulation by the elevated generation of vasoconstrictor PGs or isoprostanes in the hypertensive rats is still unclear.

To further investigate the potential involvement of COX products in the mechanism of action of t-BOOH, we used aortic segments from 6-month-old rats. We found that NS-398, a specific COX-2 inhibitor, abolished the t-BOOH-induced contractions in the same manner as indomethacin in segments with endothelium from WKY and SHR. However, in segments without endothelium from both strains, NS-398 reduced the t-BOOH-induced contractions, whereas indomethacin abolished them. These results indicate that the t-BOOH-induced vasoconstriction is essentially mediated through COX-2 activation, with scarce participation of COX-1 at the smooth muscle cell level. In this regard, it has been described that activation of the gene for inducible COX-2 is an early response (45–90 min) to injury mediated by different mitogenic stimuli in vascular smooth muscle cells (Rimarachin et al., 1994). However, t-BOOH responses showed a quick development, and gene expression is unlikely in such a short time. Therefore, the responses to t-BOOH appear to be the result of an activation of COX-2 constitutively expressed in vascular endothelial and smooth muscle cells. To confirm this hypothesis, the expression of COX-2 isoform was evaluated by Western blotting. We found that COX-2 protein is expressed in aorta from WKY and SHR in either basal situation or after stimulation with t-BOOH. In addition, our results appear to suggest an enhancement of COX-2 expression in the hypertensive strain. The fact that the mRNA for COX-2 has been detected in freshly prepared rat aorta with either intact or disrupted endothelium (Bishop-Bailey et al., 1997) supports the idea that COX-2 is constitutively expressed in this vascular bed.

In addition, imidazole, a TX synthase inhibitor, did not alter the contractile responses induced by t-BOOH in WKY and SHR, indicating that TXA₂ is not involved in the vasoconstriction induced by t-BOOH. The results obtained in both strains indicate that the oxidant t-BOOH mediates its vasoconstrictor effect by generating vasoactive products distinct from TXA₂, through COX-2 activation. Two different hypotheses appear to be the most feasible. The first one suggests that the vasoconstrictor agent responsible for the contractions induced by t-BOOH is PGH₂ or a PG synthesized from this substrate, such as PGF₂α. Such a suggestion is supported by the fact that the contractions induced by t-BOOH were abolished by blocking the receptors for PGH₂/TXA₂, with no participation of TXA₂ in these contractions. Moreover, it has been reported that PGH₂ produces contractions in rabbit aortic rings that are greater in endothelium-denuded segments and blocked by SQ 29,548 (Tesfamariam and Cohen, 1992). The second hypothesis suggests that in the
Role of Oxidative Stress in Hypertension and Aging

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Oxidative stress plays a significant role in the pathogenesis of various diseases, including hypertension and aging. This review focuses on the role of oxidative stress in these conditions, highlighting recent findings and potential therapeutic strategies.

1. Introduction

Oxidative stress is a condition characterized by an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) over the body's antioxidant defenses. This imbalance can lead to cellular damage and dysfunction, contributing to various health conditions, including hypertension and aging.

2. Oxidative Stress and Hypertension

A. Pathophysiology

Increased oxidative stress is associated with the development and progression of hypertension. ROS and RNS can damage vascular endothelial cells, leading to impaired endothelial function and increased vascular tone.

B. Mechanisms

1. Endothelial dysfunction: Oxidative stress can impair endothelial cell function, reducing nitric oxide (NO) bioavailability, a key regulator of vascular tone.

C. Clinical Implications

1. Oxidative stress is a potential target for therapeutic intervention in hypertension.

3. Oxidative Stress and Aging

A. Cellular Changes

Oxidative stress plays a significant role in the aging process, contributing to cellular damage and dysfunction. ROS can induce oxidative modifications of proteins, DNA, and lipids, leading to cellular senescence and organ dysfunction.

B. Clinical Relevance

1. Oxidative stress is associated with various age-related diseases, including cardiovascular disease.

4. Conclusions

Oxidative stress is a critical factor in both hypertension and aging. Understanding the mechanisms involved can provide new insights into the pathogenesis of these conditions and the development of targeted therapeutic strategies.

References


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