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Contractile responses elicited by hydrogen peroxide in aorta from normotensive and hypertensive rats. Endothelial modulation and mechanism involved

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1 The present study analyses the influence of hypertension and endothelium on the effect induced by hydrogen peroxide (H_2O_2) on basal tone in aortic segments from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) of 6-month-old, as well as the possible mechanisms involved.

2 Single (1 mM) or cumulative (100 nM – 10 mM) concentrations of H_2O_2 produced a transient contraction or a concentration-dependent increase of basal tone, respectively, in segments from WKY and SHR. In both cases, the contractions were higher in intact segments from hypertensive than from normotensive rats, and increased by endothelium removal in both strains. Catalase (1000 u ml^{-1}, a H_2O_2 scavenger) abolished the contraction elicited by 1 mM H_2O_2 in both strains.

3 Superoxide dismutase (SOD, 150 u ml^{-1}) and dimethylsulphoxide (DMSO, 7 mM), scavengers of superoxide anions and hydroxyl radicals, respectively, did not alter H_2O_2-induced contractions in intact segments from both strains. However, L-N^G-nitroarginine methyl ester (L-NAME, 100 μM, a nitric oxide synthase inhibitor) increased the response to H_2O_2 in normotensive rats, although the increase was less than that produced by endothelium removal.

4 Incubation of segments with 1 mM H_2O_2 for 15 min and subsequent washout reduced the contractile responses induced by 75 mM KCl in intact segments from SHR and in endothelium-denuded segments from both strains; this effect being prevented by catalase (1000 u ml^{-1}).

5 Indomethacin (10 μM, a cyclo-oxygenase inhibitor) and SQ 29,548 (10 μM, a prostaglandin H_2/thromboxane A_2 receptor antagonist) practically abolished the contractions elicited by H_2O_2 in normotensive and hypertensive rats.

6 We conclude that: (1) the oxidant stress induced by H_2O_2 produces contractions mediated by generation of a product of the cyclo-oxygenase pathway, prostaglandin H_2 or more probably thromboxane A_2, in normotensive and hypertensive rats; (2) oxygen-derived free radicals are not involved in the effect of H_2O_2; (3) in normotensive rats, endothelium protects against H_2O_2-mediated injury to contractile machinery, determined by the impairment of KCl-induced contractions; and (4) endothelial nitric oxide has a protective role on the contractile effect induced by H_2O_2, that is lost in hypertension.

Keywords: Hydrogen peroxide; rat aorta; hypertension; endothelium; nitric oxide; free radicals; prostanoids

Introduction

Oxidative stress has been involved in vascular injury associated with a variety of conditions, such as inflammation, diabetes, atherosclerosis, hypertension and ischaemia-reperfusion (Rubanyi, 1988; Ward, 1991; Ross, 1993; Tesfamariam, 1994; Marín & Rodríguez-Martínez, 1995). Hydrogen peroxide (H_2O_2), the two-electron reduction product of oxygen, has been used as a model of oxidative stress. It can diffuse from its site of formation, crossing easily cell membranes and producing cellular oxidative damage (Rubanyi, 1988; Marín & Rodríquez-Martínez, 1995). At a vascular level, endothelial cells are a target for, and a source of, H_2O_2. The principal sources of H_2O_2 in endothelial cells are oxidative metabolic pathways, such as lipoxygenase, cytochrome P450 mono-oxygenase, xanthine/xanthine oxidase systems, mitochondrial respiration and superoxide dismutase (SOD)- catalyzed superoxide anion dismutation (Panus *et al*., 1993; Marín & Rodríguez-Martínez, 1995). Spontaneous dismutation of superoxide anion also occurs at a rate constant of $\sim 2 \times 10^5$ M^{-1} s^{-1}, this rate being $\sim 10^4$ times slower than the SOD-catalyzed dismutation (Yu, 1994; Marín & Rodríguez-Martínez, 1995). Moreover, endothelial cells are the target for H_2O_2 produced by inflammatory cells (Weiss *et al*., 1981; Warren & Ward, 1986). Thus, H_2O_2 concentration near activated neutrophils can reach several hundred of μmols (Selvaraj *et al*., 1974).

H_2O_2 is involved in two important functions in the vascular wall. The first function comes from its ability to produce endothelial barrier dysfunction by either modifying the membrane permeability to macromolecules (McQuaid *et al*., 1996) and the sodium-potassium pump activity (Meharg *et al*., 1993), or altering endothelial metabolic function (Harlan & Callahan, 1984; Whorton *et al*., 1985; Chen *et al*., 1996; Natarajan *et al*., 1996). The second function is derived from its capacity to modify vascular tone inducing either contraction (Sheenan *et al*., 1993; Jin & Rhoades, 1997) or relaxation (Burke-Wolin *et al*., 1991; Iesaki *et al*., 1994, 1996; Wei *et al*., 1996), depending on the vascular bed and experimental conditions. Different mechanisms have been proposed to

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explain the contractile and relaxant effects mediated by H_2O_2 either in vascular or nonvascular tissues. Thus, the relaxation induced by H_2O_2 is mediated by activating ATP-sensitive potassium channels in cat cerebral arterioles (Wei *et al*., 1996), and guanylate cyclase in rabbit intrapulmonary arteries (Burke-Wolin *et al*., 1991). However, smooth muscle contraction is mediated by activating tyrosine kinases in rat pulmonary arteries (Jin & Rhoades, 1997), serine esterases and/or phospholipase C in rabbit pulmonary arteries (Sheehan *et al*., 1993), whereas it is mainly dependent of products of the cyclo-oxygenase pathway in guinea-pig trachea (Gao & Vanhoutte, 1993).

Endothelial cells can modulate the vascular responses to H_2O_2 by generating vasoactive agents, such as nitric oxide (Monaco & Burke-Wolin, 1995; Mohazzab-H *et al*., 1996) and prostacyclin (Whorton *et al*., 1985; Wessels & Hempel, 1996), and by protecting smooth muscle cells from the oxidative injury mediated by H_2O_2 (Linas & Repine, 1997). Therefore, in de-endothelized arteries, and in hypertension, diabetes and atherosclerosis, in which endothelial cells are altered (Marín & Rodríquez-Martínez, 1997), higher sensitivity to oxidative stress induced by H_2O_2 could be expected.

The ability of H_2O_2 to modify vascular tone has been studied in different vascular preparations, however, to our knowledge, there are no studies that analyse the effect of H_2O_2 and its endothelial modulation in hypertension. For this, the objective of the present study was to assess the influence of hypertension and endothelium on the changes induced by H_2O_2 on vasomotor tone in aortic segments from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR), as well as the possible mechanisms involved. In the present study, we analysed: (1) the ability of H_2O_2 to alter basal tone; (2) the influence of either hypertension or free radicals on vascular changes induced by H_2O_2; (3) the role of endothelium as protecting smooth muscle cells from H_2O_2-mediated injury; and (4) the possible mechanisms involved in the vascular response to H_2O_2.

Methods

Animals

The present study was performed in 20 WKY and 20 SHR male rats of 6 months, which were born and fed with regular chow at the facilities of the Facultad de Medicina of the Universidad Autónoma of Madrid. A randomly group of 6 animals of each strain was weighed and mean arterial pressure (MAP) measured in right carotid artery. Weigh values were 508 ± 15 g for WKY and 364 ± 8 g ($P < 0.001$) for SHR. MAP values were 130 ± 8 mmHg for WKY and 170 ± 7 mmHg ($P < 0.001$) for SHR.

Reactivity experiments

Aortas from WKY and SHR were carefully dissected out, cleaned of connective tissue and divided into segments of 4 mm in length. For isometric tension recording, each segment was set up in an organ bath that contained 5 ml of Krebs Heinseleit solution (KHS) at 37°C continuously bubbled with a 95% O_2-5% CO_2 mixture, which gave a pH of 7.4. Two horizontally arranged stainless steel pins, 150 μm in diameter, were passed through the lumen of the vascular cylinder. One pin was fixed to the organ bath wall, while the other one was vertically connected to a strain gauge for isometric tension recording. The isometric contraction was recorded from a force-displacement transducer (Grass FTO3C; Quincy, MA, U.S.A.) connected to a polygraph (Grass, model 7D). Segments were subjected to a tension of 1.5 g (optimal resting tension), which was readjusted every 15 min during a 60 min equilibration period before drug administration. Vessels were previously 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 (ACh) to relax segments precontracted with 0.01 μM noradrenaline (NA). To remove vascular endothelium, some segments were incubated for 20 min with saponin (0.3 mg ml^{-1} KHS); the success of this procedure was confirmed by the inability of ACh to induce relaxations. The responses to 75 mM KCl were unaltered by the removal of endothelium.

Experimental protocol

To study the influence of oxidative stress caused by H_2O_2 on basal tone, segments with and without endothelium from WKY and SHR were exposed to 1 mM H_2O_2 for 15 min, and subsequently washed out with KHS. Some of these segments were preincubated with catalase (1000 u ml^{-1}, a H_2O_2 scavenger) for 10 min before the addition of H_2O_2. In another set of experiments, the mechanisms involved in the responses induced by cumulative concentrations of H_2O_2 (100 nM–10 mM) were analysed.

The modulation of the responses to H_2O_2 by free radicals, such as superoxide anion, hydroxyl radical and nitric oxide, was assessed in intact segments from both strains. For this, segments were incubated for 20 min with superoxide dismutase (SOD, 150 u ml^{-1}, a superoxide anion scavenger), dimethylsulphoxide (DMSO, 7 mM, a hydroxyl radical scavenger) or L-N^G-nitroarginine methyl ester (L-NAME, 100 μM, a nitric oxide synthase inhibitor) before the achievement of the corresponding concentration-response curve to H_2O_2.

To study the ability of H_2O_2 to mediate toxicity in smooth muscle cells, as well as the modulator role of endothelium, the contractile response induced by 75 mM KCl was considered as an index of the integrity of contractile machinery. Thus, after a first response to 75 mM KCl was achieved in segments with and without endothelium from both strains, some segments were incubated with H_2O_2 (0.1 or 1 mM) for 15 min, afterwards the segments were washed with KHS and 30 min later a second response to 75 mM KCl was achieved. In some experiments, a similar protocol was performed but the segments were preincubated with catalase (1000 u ml^{-1}) for 10 min before the addition of 1 mM H_2O_2.

To analyse whether prostanoids could be implicated in the mechanism of action of H_2O_2, segments with and without endothelium from WKY and SHR were incubated for 30 min with either 10 μM indomethacin (a cyclo-oxygenase inhibitor) or 10 μM SQ, 29,548 (a prostaglandin H_2/thromboxane A_2 receptor blocker) before a concentration-response curve to H_2O_2 was done.

Solutions and drugs

The composition of KHS (mM) was: NaCl 115, $CaCl_2$ 2.5, KCl 4.6, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, glucose 11.1 and Na_2EDTA 0.01. (−)-NA hydrochloride, ACh chloride, indomethacin, saponin, L-NAME, DMSO, bovine liver catalase (EC.1.11.1.6) and bovine erythrocyte SOD (EC.1.15.1.1) were purchased from Sigma Chemical Co (St. Louis, MO, U.S.A.); H_2O_2 and SQ 29,548 from Probus

(Barcelona, Spain) and ICN Ibérica, S.A. (Barcelona, Spain), respectively.

Stock solutions (10 mM) of NA, indomethacin and SQ 29,548 were prepared in saline (0.9% NaCl w/v)-ascorbic acid (0.01% w/v) solution, $HNaCO_3$ (0.5% w/v) and absolute ethanol, respectively. Daily solutions of saponin, DMSO, catalase and SOD were prepared in oxygenated KHS, whereas those of H_2O_2 were made in distilled water and protected from daylight. Experiments with SQ 29,548 were carried out under sodium vapour light. The concentrations of ethanol present in the bath (0.001%), when experiments with SQ 29,548 were done, did not modify the contractile responses elicited by H_2O_2.

Statistical analysis

Results are expressed as mean ± s.e.mean. Student's *t*-test was used to determine significant differences between means. In the reactivity experiments, a vertical pairwise contrast was used to determine the H_2O_2 concentrations at which a difference between groups of treatment was first apparent; *P* values were adjusted by the Dunn-Sidàk procedure (Ludbrook, 1994). A *P* value less than 0.05 was considered significant. More than three rats were used in each set of vascular reactivity experiments.

When concentration-response curves to H_2O_2 were done, the responses were expressed as a percentage of the previous contraction induced by 75 mM KCl.

Results

Effect of H_2O_2 on basal tone

A typical model showing the effect of 1 mM H_2O_2 on basal tone and its inhibition by catalase (1000 u ml^{-1}) in aortic segments with and without endothelium from WKY and SHR is shown in Figure 1. H_2O_2 produced a transient contraction, that appeared immediately after its addition. This contraction was higher in segments with endothelium from SHR than from WKY, and in segments without endothelium from both strains. The values of maximal response elicited by 1 mM H_2O_2 were of 243 ± 44 mg and 401 ± 56 mg ($P<0.05$) for segments with, and of 775 ± 73 mg and 700 ± 124 mg for segments without endothelium from WKY and SHR, respectively. Catalase (1000 u ml^{-1}) completely abolished the H_2O_2 effect (Figure 1). The relaxation that followed to the contraction induced by H_2O_2 was of such a magnitude that 90% of segments recover resting tone in less than 15 min,

As shown in Figure 2, H_2O_2 (100 nM – 10 mM) caused a concentration-dependent increase of basal tone in segments with and without endothelium from WKY and SHR, this increase being higher in segments without endothelium from both strains. Moreover, in intact segments, the contractions elicited by high H_2O_2 concentrations (1 and 10 mM) were greater in SHR than in WKY, whereas the responses in endothelium-denuded segments were similar in normotensive and hypertensive rats (Figure 2).

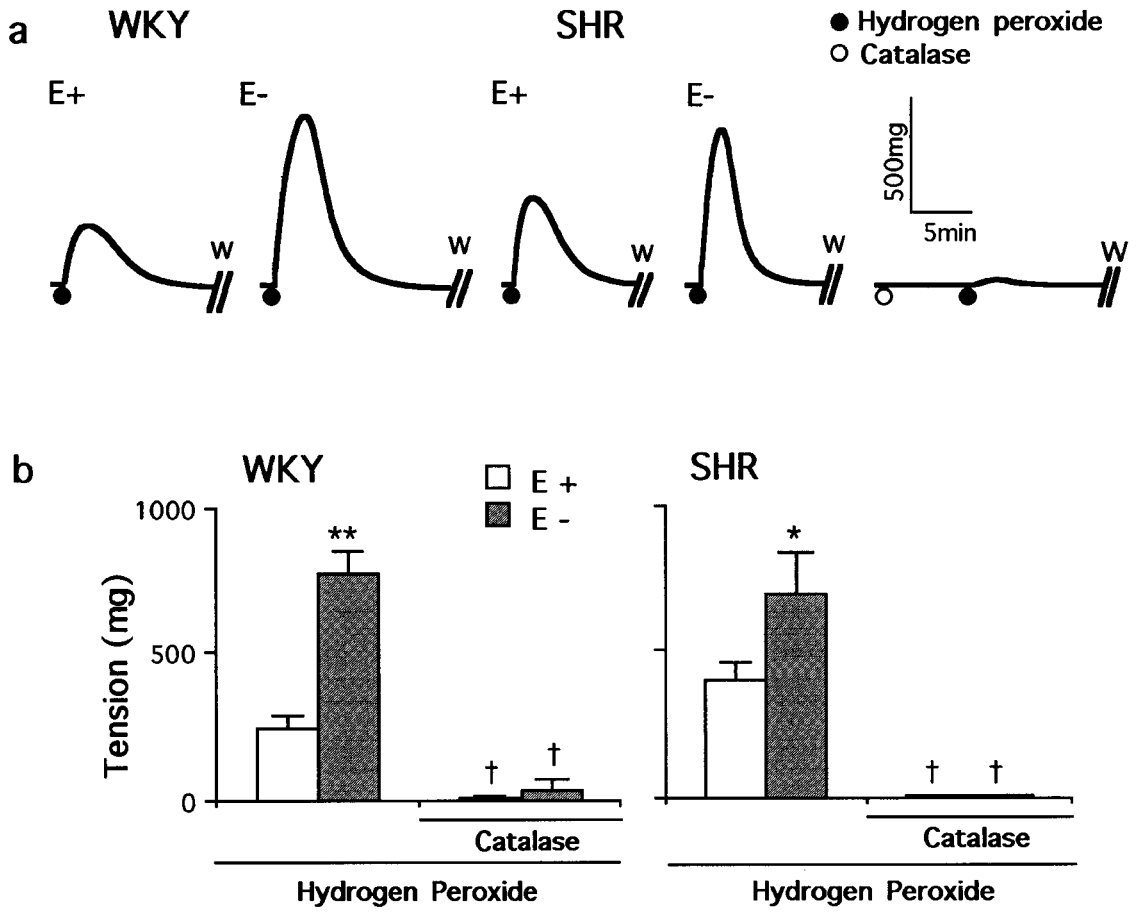

Figure 1 (a) Typical model showing the contractile effect of 1 mM hydrogen peroxide and its blockade by catalase (1000 u ml^{-1}) in aortic segments with (E+) and without (E−) endothelium from WKY and SHR. (b) The same protocol showing the results (mean ± s.e.mean) from six to ten arterial segments studied in each set of experiments and expressed in mg of tension developed. *$P<0.01$ and **$P<0.001$ E− vs E+; †$P<0.001$ catalase vs its respective control. W = washout.

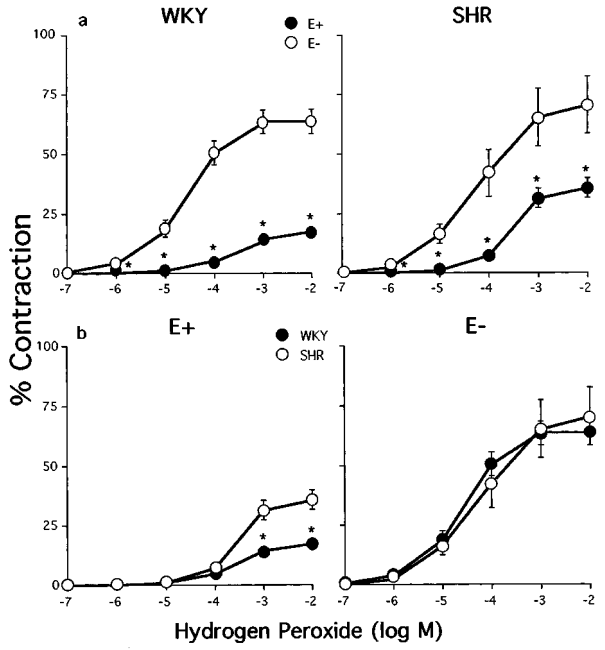

Figure 2 Effect of cumulative concentrations of hydrogen peroxide on basal tone. (a) Comparison of the results between aortic segments with (E+) and without (E−) endothelium in each strain. (b) Comparison of the results obtained in segments E+ and E− between WKY and SHR. Results (mean±s.e.mean) from 15–20 arterial segments used in each set of experiments are expressed as a percentage of the previous contraction with 75 mM KCl. Vertical pairwise contrast: $*P<0.001$.

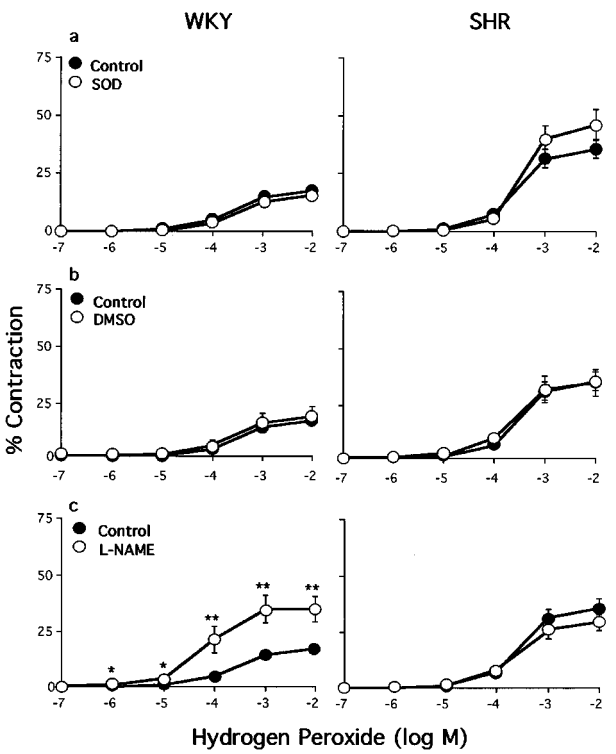

Figure 3 Effect of the blockade of free radicals on the contractile responses induced by hydrogen peroxide in intact aortic segments from WKY and SHR. Effect of scavenging superoxide anions (a), and hydroxyl radicals (b), and inhibition of nitric oxide synthase (c) with superoxide dismutase (SOD, 150 u ml^{-1}), dimethylsulphoxide (DMSO, 7 mM) and L-N^G-nitroarginine methyl ester (L-NAME, 100 μM), respectively, on responses to hydrogen peroxide. Results (mean±s.e.mean) from 10–20 arterial segments used in each set of experiments are expressed as a percentage of the previous contraction with 75 mM KCl. Vertical pairwise contrast: $*P<0.05$ and $**P<0.001$.

Influence of oxygen-derived free radicals and nitric oxide on the contractions elicited by H_2O_2

The contractile response induced by H_2O_2 were not modified by either 150 u ml^{-1} SOD or 7 mM DMSO in intact segments from WKY and SHR (Figure 3). However, 100 μM L-NAME increased these responses in normotensive rats, although to a lesser extent than that observed by endothelium removal (Figure 3). Only L-NAME modified basal tone in normotensive rats, producing an increase on basal tone of 220±25 mg ($n=8$).

Role of endothelium as protecting smooth muscle cells from H_2O_2-mediated injury

We observed that whereas 0.1 mM H_2O_2 did not cause any effect on the contractile responses elicited by 75 mM KCl (data not shown), 1 mM H_2O_2 produced a reduction of these responses in segments with endothelium from SHR and in segments without endothelium from WKY and SHR (Figure 4). The deleterious effect induced by H_2O_2 in smooth muscle cells was prevented by 1000 u ml^{-1} catalase (Figure 4). Additionally, there were not significant differences between two successive responses to 75 mM KCl in control situation, and catalase did not modify either basal tone or the responses elicited by 75 mM KCl (data not shown).

Role of prostanoids in the vascular effect of H_2O_2

Indomethacin (10 μM, Figure 5) or SQ 29,548 (10 μM, Figure 6) practically abolished the contractile responses induced by H_2O_2 in intact and endothelium-denuded segments from WKY and SHR. Both agents did not modify basal tone.

Discussion

The present study shows that, under resting conditions, 1 mM H_2O_2 was able to induce a transient contraction in rat aortic segments with and without endothelium from WKY and SHR. The contractions were blocked by catalase, an enzymatic antioxidant that scavenges H_2O_2, suggesting that these responses were caused by H_2O_2 rather than by a nonspecific stimulation. The ability of H_2O_2 to induce transient contractions has been described in vascular beds from different species. Thus, a transient contraction followed by a strong sustained contraction has been described in rat pulmonary arteries (Jin & Rhoades, 1997), as well as a transient contraction followed by prolonged 20 min dilation in pial arterioles of newborn pigs after topical application (Leffler *et al.*, 1990). However, this does not appear to be a generalized phenomenon, since H_2O_2 did not elicit measurable responses in quiescent rat mesenteric arteries (Hubel *et al.*, 1993).

We have found that the contractions induced by single or cumulative concentrations of H_2O_2 were greater in endothelium-denuded segments than in intact segments from both strains. Moreover, intact segments from hypertensive rats showed higher contractile responses to H_2O_2 that those from normotensive rats. These results indicate that the contractions elicited by H_2O_2 are negatively modulated by endothelium. This modulator/protector role of endothelium is altered in SHR, suggesting an enhanced sensitivity to oxidative stress in

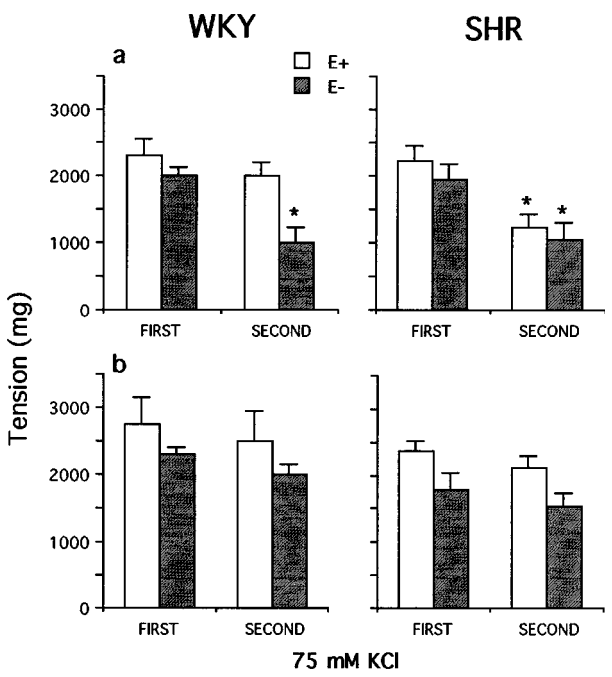

Figure 4 (a) Effect of first and second exposure to 75 mM KCl in aortic segments with (E+) and without (E−) from WKY and SHR. Between both responses the segments were incubated with 1 mM hydrogen peroxide for 15 min and subsequently washed out. (b) Similar protocol was followed, but in this case, 1000 u ml^{-1} catalase were applied for 10 min before H_2O_2 addition. Results (mean± s.e.mean) from six arterial segments used in each set of experiments are expressed as mg of tension developed. $^*P<0.05$ second vs first response to 74 mM KCl.

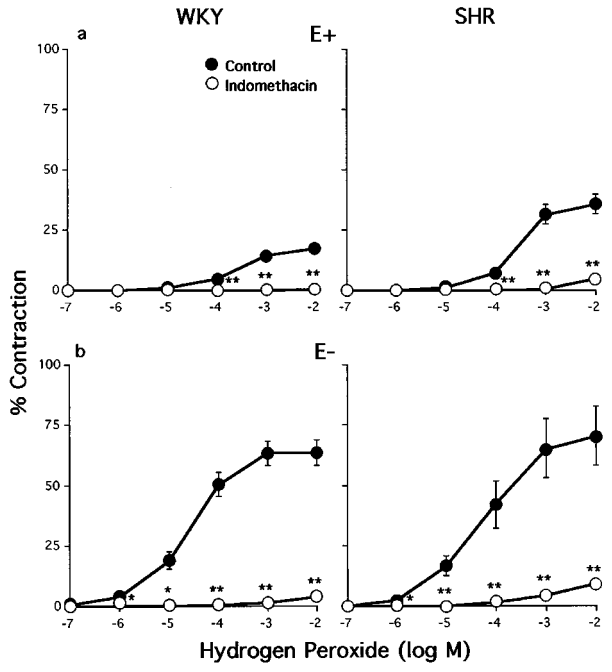

Figure 5 Effect of preincubation with 10 μM indomethacin on the contractile response elicited by hydrogen peroxide in intact (E+) and endothelium-denuded (E−) aortic segments from WKY and SHR. Results (mean±s.e.mean) from 10–20 arterial segments used in each set of experiments are expressed as a percentage of the previous contraction to 75 mM KCl. Vertical pairwise contrast: $^*P<0.05$ and $^{**}P<0.001$.

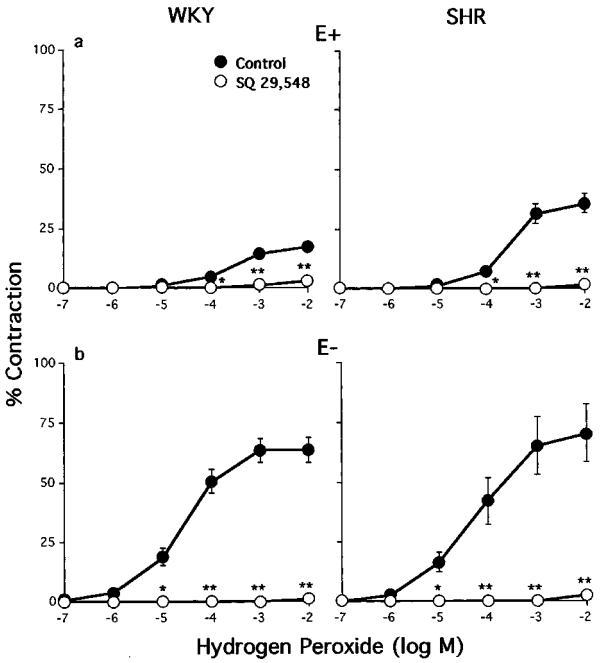

Figure 6 Effect of preincubation with 10 μM SQ 29,548 on the contractile response elicited by hydrogen peroxide in aortic segments with (E+) and without (E−) endothelium from WKY and SHR. Results (mean±s.e.mean) from 10–20 arterial segments used in each set of experiments are expressed as a percentage of the previous contraction to 75 mM KCl. Vertical pairwise contrast: $^*P<0.05$ and $^{**}P<0.001$.

hypertension. These findings agree with the fact that endothelium, among other vascular cells, is damaged in hypertension (Marín & Rodríguez-Martínez, 1997). The abnormalities of endothelial cells ranging from changes in the size and shape (Haudenschild *et al*., 1979) or in replication (Huttner & Gabbiani, 1983), to changes in their functionality, manifested by a predominant formation of endothelium-derived contracting factors (Auch-Schwelk *et al*., 1990; Lüscher, 1990; Bolger *et al*., 1991).

The involvement of oxyradicals in the contractile responses induced by H_2O_2 was assessed. We observed that SOD did not modify the contractile responses to H_2O_2 in intact segments from WKY and SHR. In addition, it is known that extracellular SOD is bound to heparin sulphate proteoglycan on endothelial cell surfaces (Karlsson *et al*., 1993). The presence of SOD on endothelial membranes could be essential to avoid the reaction of superoxide anion with hydrogen peroxide through Fenton's reaction, and therefore, the formation of hydroxyl radical. In this regard, the lack of effect of SOD suggest that neither superoxide anions nor probably hydroxyl radicals are involved in the vascular effect of H_2O_2. This assumption is supported by the fact that the contractions induced by H_2O_2 were not modified by the hydroxyl radical scavenger DMSO. Similar results were obtained in pial arterioles of newborn pigs, in which the initial contraction induced by H_2O_2 was unaltered by the hydroxyl radical scavenger deferoxamine (Leffler *et al*., 1990).

Concerning the role of nitric oxide in the responses to H_2O_2, we observed that L-NAME increased both basal tone and the contractile responses to H_2O_2 in intact segments from normotensive rats. These results suggest the existence of an important basal release of nitric oxide that modulates the resting tone and that plays a negative modulator role in the

H_2O_2-induced contractions. Thus, endothelial nitric oxide protects against the oxidant attack induced by H_2O_2 in normotensive rats. In this sense, other authors have also suggested that nitric oxide plays a critical role in the cytoprotection against endothelial oxidative stress mediated by H_2O_2. Nitric oxide, released from nitric oxide donors, protects against the H_2O_2-induced increase in endothelial monolayer permeability (McQuaid *et al*., 1996) and against H_2O_2-induced reduction of endothelial cells viability (Motterlini *et al*., 1996). In addition, we have found that L-NAME did not modify either the basal tone or the contractile responses elicited by H_2O_2 in hypertensive rats. These results, together with the fact that the contractile responses to H_2O_2 were higher in intact segments from hypertensive than from normotensive rats, indicate that the synthesis, release or action of nitric oxide, as well as its protective role is altered in hypertension, as previously described (Marín, 1993; Marín & Rodríquez-Martínez, 1997). Furthermore, our observations that endothelium removal increased the responses to H_2O_2 in both strains and that induced an increase in the responses to H_2O_2 higher than that produced by inhibition of nitric oxide synthesis in normotensive rats, suggest that other endothelial factors are involved in the endothelial protection against H_2O_2 attack. A possible candidate could be prostacyclin, since this potent vasodilator is released by cultured endothelial cells after exposure to either activated neutrophils or exogenous H_2O_2 (Harlan & Callahan, 1984).

The possible injury-mediated by H_2O_2 in smooth muscle cells was examined. We found that after exposure of segments to 1 mM H_2O_2 the responses to 75 mM KCl were reduced in segments with endothelium from SHR and in segments without endothelium from both strains. The impairment of contractile machinery was prevented by catalase, suggesting that H_2O_2 specifically, rather than its nonenzymatic reduction to the highly reactive hydroxyl radical, is responsible of this deleterious effect. These results also indicate that: (1) H_2O_2 produces a persistent impairment of contractile machinery, since this effect was observed after H_2O_2 removal; and (2) endothelium plays a protective role against the oxidant attack induced by H_2O_2 only in normotensive rats. The ability of endothelial cells to protect vascular smooth muscle cells from H_2O_2 attack has been reported in experiments of coincubation of endothelial and vascular smooth muscle cells (Linas & Repine, 1997). In these experiments, the endothelial protection was partially lost in the presence of nitric oxide synthase inhibitors (Linas & Repine, 1997).

To analyse whether prostanoids could be involved in the contractions induced by H_2O_2, we performed experiments in the presence of either the potent inhibitor of cyclo-oxygenase, indomethacin, or the blocker of prostaglandin H_2/thromboxane A_2 receptor, SQ 29,548. Both agents practically abolished the contractile responses elicited by H_2O_2 in segments with and without endothelium from both strains, suggesting that prostaglandin H_2, or more probably thromboxane A_2, is responsible of these responses. In addition, the almost complete inhibition of the vasoconstriction induced by H_2O_2 by indomethacin suggests that the cyclo-oxygenase-independent peroxidation of arachidonic acid to generate vasoconstrictor isoprostanes, as prostaglandin F_2-like compounds, does not mediate the effects of H_2O_2, at least in a significant extent. Since H_2O_2 is able to diffuse easily across hydrophobic membranes, it could activate the phospholipase A_2 in the cytosol of smooth muscle cells (Rao *et al*., 1995) and in the membrane of endothelial cells (Harlan & Callahan, 1984), thus, releasing arachidonic acid as a substrate for cyclo-oxygenase. The ability of H_2O_2 to induce indomethacin-sensitive contractions has been reported in pial arterioles from newborn pigs (Leffler *et al*., 1990), and in strips of guinea-pig trachea (Gao & Vanhoutte, 1993). Additionally, that indomethacin enhances the relaxant responses to H_2O_2 in precontracted rabbit intrapulmonary arteries (Burke-Wolin *et al*., 1991) supports the prostaglandin-mediated constrictor mechanism of H_2O_2).

In summary, the present results indicate that: (1) H_2O_2 produces contractions in rat aorta from normotensive and hypertensive rats that are mediated by generation of product of the cyclo-oxygenase pathway, prostaglandin H_2 or more probably thromboxane A_2; (2) oxygen-derived free radicals are not involved in the contractile effects of H_2O_2; (3) endothelium protects against oxidative injury caused by H_2O_2 in smooth muscle cells from normotensive but not from hypertensive rats; (4) endothelial nitric oxide has a protective role on the contractile effect induced by H_2O_2 in normotensive rats; and (5) this protective role of endothelial nitric oxide is lost in hypertension. This study supplies unknown information concerning the sensitivity to, and the mechanisms involved in, the oxidant attack induced by H_2O_2 in arteries from hypertensive animals.

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