British Journal of Pharmacology (2001) 134, 1639–1646

www.nature.com/bjp

Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A_2 production

[1]Y.J. Gao & *,[1]R.M.K.W. Lee

[1]Department of Anaesthesia, McMaster University, Hamilton, Ontario, Canada. L8N 3Z5

1 Hydrogen peroxide (H_2O_2) caused a transient contraction in endothelium-intact (E+) and -denuded (E−) mesenteric arteries (MA) from 8–10-month-old spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) in a concentration-dependent manner (10^{-5} M to 10^{-3} M).

2 The contraction to H_2O_2 in MA (E+ or E−) was greater in SHR than in WKY. Removal of endothelium potentiated the contraction to H_2O_2 in WKY but not in SHR. Tachyphylaxis to H_2O_2 was less prominent in SHR than in WKY.

3 The contraction of aorta to H_2O_2 (5×10^{-4} M), expressed as a percentage of 80 mM KCl-induced contraction, was approximately half of that found in the MA. A greater contraction was found in E+ but not E− SHR aortic rings.

4 The contraction of MA to H_2O_2 (5×10^{-4} M) was greatly inhibited by SQ 29548 and ICI 192605 (thromboxane A_2 (TXA_2)/prostaglandin H_2 receptor antagonists), quinacrine (a phospholipase A_2 (PLA_2) inhibitor), indomethacin and diclofenac (cyclooxygenase (COX) inhibitors), and furegrelate (a TXA_2 synthase inhibitor).

5 Production of thromboxane B_2 induced by H_2O_2 (5×10^{-4} M) was greater in SHR MA than in WKY, and was inhibited by quinacrine, indomethacin and diclofenac, and furegrelate, but not by SQ 29584 and ICI 192605.

6 These results suggested (1) that SHR MA exhibits a higher contraction involving an increased smooth muscle reactivity and less tachyphylaxis to H_2O_2 than WKY; (2) that a greater production of TXA_2 through activation of PLA_2-COX-TXA_2 synthase pathway appeared to be responsible for the enhanced contraction in SHR MA. The enhanced vascular response to H_2O_2 may be related to hypertension in SHR.

British Journal of Pharmacology (2001) **134,** 1639–1646

Keywords: Hydrogen peroxide; hypertension; mesenteric artery; reactive oxygen species; spontaneously hypertensive rats; thromboxane A_2; Wistar-Kyoto rats

Abbreviations: COX, cyclooxygenase; DMSO, dimethyl sulphoxide; E+, endothelium-intact; E−, endothelium-denuded; EC_{50}, concentration for 50% maximal response; H_2O_2, hydrogen peroxide; MA, mesenteric artery; $O_2^{-\cdot}$, superoxide; $OH^{\cdot}$, hydroxyl radical; PLA_2, phospholipase A_2; ROS, reactive oxygen species; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; TP, thromboxane A_2/prostaglandin H_2; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; WKY, Wistar- Kyoto rats

Introduction

Although hydrogen peroxide (H_2O_2) is a non-radical form of reactive oxygen species (ROS) and only possesses moderate oxidant reactivity, it is probably more harmful than superoxide anion ($O_2^{-\cdot}$), because H_2O_2 can easily diffuse across plasma membrane and enter the inner compartments of a cell (Bergendi *et al*., 1999). $O_2^{-\cdot}$ can be produced by NAD(P)H oxidase (Griendling *et al*., 2000) and serves as the substrate for superoxide dismutase (SOD) to generate H_2O_2. Previous studies on ROS were mainly focused on their deleterious effects on the cells or organisms including oxidation of membrane lipids, inactivation and oxidization of certain enzymes and proteins, and damage to DNA by ROS (Machlin & Bendich, 1987; Oliver, 1987). Recent finding that some forms of ROS (e.g. H_2O_2) may serve as an important second messenger molecule in certain vital processes such as cell growth (Bass & Berk, 1995; Sundaresan *et al*., 1995) has turned our attention to other physiological roles that ROS may play.

Vascular tissues including endothelial cells, smooth muscle cells and fibroblasts are a rich source of ROS (Griendling & Alexander, 1997). The vasoactive effects of ROS have been shown in various arteries from normal animals including rat aorta (Rodriguez-Martinez *et al*., 1998; Hibino *et al*., 1999), canine basilar artery (Katusic *et al*., 1993), bovine pulmonary artery (Wolin *et al*., 1985), and human placental arteries (Omar *et al*., 1992). The mitogenic or apoptotic effects of ROS on vascular smooth muscle cells have also been reported (Bass & Berk, 1995; Li *et al*., 1997). Taken together, these results suggest that ROS may participate in physiological or pathological regulation of vascular function and structure.

*Author for correspondence; E-mail: rmkwlee@mcmaster.ca

In hypertension, the role of ROS is gaining increasing attention. Patients with uncontrolled hypertension showed a higher level of H_2O_2 and $O_2^{-\cdot}$, and these high levels had reverted to normal levels after the control of high blood pressure (Prabha *et al*., 1990). In animal models of hypertension, deoxycorticosterone acetate-salt hypertensive rats and Dahl hypertensive rats have been shown to exhibit enhanced vascular $O_2^{-\cdot}$ production (Swei *et al*., 1997; Somers *et al*., 2000). Hypertension produced by exogenously applied angiotensin II or by renovascular ligation also showed similar increase of ROS level (Rajagopalan *et al*., 1996; Harrison *et al*., 1999). In the spontaneously hypertensive rats (SHR), a higher level of ROS and lipid peroxidation in mesenteric arterioles and myocardium has been reported (Ito *et al*., 1992; Suzuki *et al*., 1995). In vascular reactivity studies with isolated vessels from SHR, higher contractile responses to exogenously applied H_2O_2 or $O_2^{-\cdot}$ or lipid peroxides have been shown (Auch-Schwelk *et al*., 1989; Rodriguez-Martinez *et al*., 1998; Hibino *et al*., 1999; Garcia-Cohen *et al*., 2000). However, these results were all derived from aorta, which is a large elastic conduit artery. It is not known if similar changes also occur in smaller arteries such as the mesenteric arteries (MA).

Although H_2O_2 is widely used in *in vitro* vascular reactivity studies to induce oxidative stress, the detailed pathway and the mediator responsible for H_2O_2-induced contraction are not fully understood. Activation of several key enzymes such as phospholipase A_2 (PLA_2) (Chakraborti *et al*., 1989), phospholipase C (Sheehan *et al*., 1993), tyrosine kinase (Jin & Rhoades, 1997), protein kinase C (Yang *et al*., 1998), cyclooxygenase (COX) (Rodriguez-Martinez *et al*., 1998) has been suggested in H_2O_2-induced vascular contraction. For the mediator of H_2O_2-induced contraction of SHR artery, as far as we know, there is only one study which suggested that thromboxane A_2 (TXA_2) / prostaglandin H_2 (TP) may be the candidate in the aorta but the measurement of tissue level of TXA_2 metabolite was not carried out (Rodriguez-Martinez *et al*., 1998). Therefore, information on the mediator(s) of H_2O_2-induced vascular contraction in hypertensive animals is still lacking.

In this study, we used a combined functional and biochemical approach to examine the reactivity of isolated SHR MA to H_2O_2 stimulation and measured the vascular production of TXA_2. The possible pathway and the mediator involved in the contraction were studied and the responses to H_2O_2 in MA and aorta were compared.

Methods

Animals

Male SHR and WKY at the age of 8–10 months were obtained from the rat colonies maintained at the McMaster University Central Animal Facilities. These colonies originated from the Charles River strains, and we have maintained these inbred colonies at our institute for over 20 years. The care and the use of these animals were in accordance with the guidelines of the Canadian Council on Animal Care.

Reactivity experiments

The rats were anaesthetized with sodium pentobarbitone (50 mg kg^{-1} i.p.), and then exanguinated by bleeding from the abdominal aorta. The mesentery and the thoracic aorta were isolated by dissection and placed in oxygenated Krebs solution at 4°C. The superior mesenteric artery and the aorta were carefully dissected out, cleaned of connective tissues and cut into 4 mm segments under a dissecting microscope. Each mesenteric arterial ring was mounted in a 3 ml organ bath with a resting tension of 1.5 g, and each aortic ring in 5 ml organ bath with 4 g resting tension, which we had determined to be the optimal resting tensions, and isometric contractile response was recorded. The composition of Krebs solution was (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.08; $NaHCO_3$, 25; Glucose, 11; HEPES, 8. The organ chamber was continuously bubbled with 95% O_2 and 5% CO_2, and maintained at 37°C. The solution in the organ bath was changed every 15–20 min. After 1 h of equilibration, the rings were challenged with 80 mM KCl until a stable contraction was achieved. In some segments, the endothelium was removed by rubbing the internal surface of the ring with a fine wooden stick. Successful removal of endothelium was indicated by the absence of relaxation response to carbamycholine chloride (3×10^{-6} M) in rings precontracted with phenylephrine (10^{-6} M).

Experimental protocol

To assess the contractile response to H_2O_2, arterial rings with and without endothelium was exposed to different concentrations of H_2O_2 for 5 min, followed by a thorough wash with Krebs solution. In experiments to assess the effects of catalase, SOD, and dimethylsulphoxide (DMSO), the rings with endothelium were incubated with one of these agents for 5–10 min before exposure to H_2O_2. In most cases, one ring was exposed to only one concentration of H_2O_2 because of the tachyphylaxis to H_2O_2 we had noticed in our preliminary experiments. In experiments to quantify the tachyphylaxis phenomenon, the same concentration of H_2O_2 was added at an interval of 30 min after the tension had returned to the basal level. The concentration-response curves for H_2O_2 were constructed using different rings. In assessing the effects of various enzyme inhibitors and receptor antagonists, endothelium-denuded rings were used and 25 min of incubation was allowed before exposure to H_2O_2. Generally, 5–6 rings were obtained from the middle part of the superior mesenteric arcade from each rat. We randomly took one ring as control, and the other rings to test various agents. The contraction to H_2O_2 was normalized with the contraction to 80 mM KCl. The contraction of the aorta and the mesenteric arteries to 80 mM KCl was similar between SHR and WKY (in grams, aorta: 3.2 ± 0.22 for SHR, 2.9 ± 0.17 for WKY; mesenteric arteries: 0.6 ± 0.12 for SHR, 0.63 ± 0.11 for WKY), and removal of the endothelium also did not affect the response of these vessels to 80 mM KCl (in grams, aorta 3.17 ± 0.18 for SHR, 3.03 ± 0.27 for WKY; mesenteric arteries: 0.62 ± 0.14 for SHR, and 0.66 ± 0.12 for WKY) ($n=6-9$). The inhibitory effects of enzyme inhibitor or receptor antagonist were expressed as per cent contraction of the control. All these inhibitors and receptor antagonists at the concentrations used did not affect the basal tension or the contractions to 80 mM KCl. The only exception was that quinacrine (10^{-4} M) slightly slowed down the onset of KCl-induced contraction. The concentration-response curve for U46619 was made in endothelium-denuded mesenteric arterial rings, and was

expressed as per cent of 80 mM KCl-induced contraction. Concentration for 50% maximal response (EC_{50}) was estimated by fitting each concentration-response curve. At the end of each experiment, the viability of the artery reactivity was tested by exposing the arteries to 80 mM KCl, and the wet weight of the artery was measured.

Measurement of thromboxane B_2 (TXB_2)

Samples of the bathing solution in the organ bath were taken before and 5 min after adding H_2O_2, and stored at $-80°C$. The incubation time was set to 5 min because the contraction to H_2O_2 was transient and had returned to the baseline within 5 min in most cases. The concentration of TXB_2 was measured using a TXB_2 enzyme-linked immunosorbent assay kit (Neogen, U.S.A.). The content of TXB_2 was expressed as pg mg^{-1} wet weight of the arterial rings.

Chemicals

The following chemicals were used: H_2O_2 and DMSO (BDH Inc. Toronto, Canada); carbamycholine chloride, catalase, furegrelate sodium salt, indomethacin, quinacrine dihydrochloride, SOD, U46619 (Sigma, U.S.A.); diclofenac sodium, SQ 29548 (RBI, Sigma, U.S.A.); ICI 192605 (Tocris, U.S.A.). Indomethacin was dissolved in 0.5% sodium carbonate, diluted in deionized water, and freshly prepared before use; SQ 29548 and U46619 were dissolved in absolute ethanol and diluted in 50% ethanol; ICI 192605 was dissolved in DMSO and diluted in deionized water. All other agents were dissolved in deionized water and prepared fresh daily.

Statistical analysis

Contractile response to H_2O_2 was assessed by the tension developed. The results were expressed as mean ± s.e.mean, where n represents the number of rats. Statistical analysis was performed by one-way ANOVA or unpaired Student's t-test. The differences were considered significant when $P<0.05$.

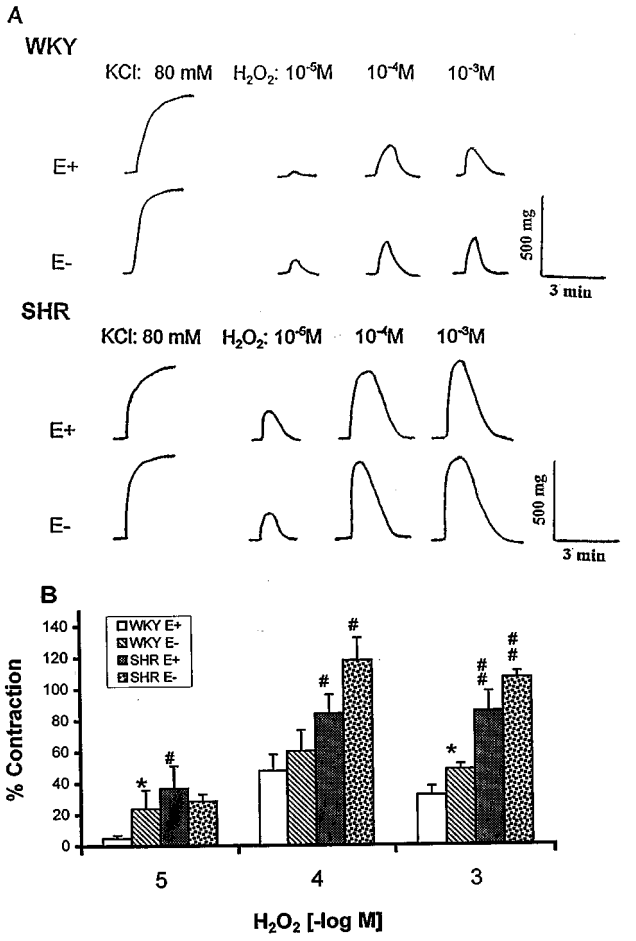

Figure 1 (A) Typical tracings showing the contractile effects of hydrogen peroxide (H_2O_2, 10^{-5} M, 10^{-4} M, and 10^{-3} M) in WKY and SHR mesenteric arteries with intact (E+) or denuded (E−) endothelium. (B) Concentration-response relation of H_2O_2. Results (mean ± s.e.mean) were from 4–6 rats and expressed as the percentage of the contraction elicited by 80 mM KCl. $*P<0.05$, compared with respective E+ arteries; $\#P<0.05$, $\#\#P<0.01$ between SHR and respective WKY arteries with or without endothelium.

Results

At 8–10 months of age, body weight of SHR (387 ± 20 g, $n=18$) was lighter than WKY (436 ± 18 g, $n=15$, $P<0.05$). Systolic blood pressure measured using the tail-cuff compression method was significantly higher in the SHR (196 ± 9 mmHg) than WKY (133 ± 6 mmHg, $P<0.01$).

The contractile response of MA to a single concentration of H_2O_2 was composed of a quick onset and a transient component, and in most cases the tension developed had returned to the baseline within 5 min (Figure 1A). H_2O_2 at concentrations of 10^{-5} M, 10^{-4} M, and 10^{-3} M produced concentration-dependent contractile responses in MA. Removal of endothelium increased the contraction to H_2O_2 at 10^{-5} M, and 10^{-3} M in WKY but not in SHR. A higher level of contraction was found in endothelium-intact (E+) MA of SHR at the three concentrations of H_2O_2 tested and in endothelium-denuded (E−) MA of SHR at the two higher concentrations (10^{-4} M, and 10^{-3} M) as compared with WKY (Figure 1B).

Based on the concentration-response results, we had selected 5×10^{-4} M of H_2O_2 for further investigation on contraction mechanisms, because the tension developed at this concentration was higher in both E+ and E− MA from SHR than WKY, and endothelium removal only increased the contraction to H_2O_2 in WKY (Figure 2A). Contractile response to H_2O_2 (5×10^{-4} M) in MA was nearly abolished by catalase (1000 u/ml) but not affected by SOD (150 u/ml) or DMSO (5 mM) in E+ MA (Figure 2B).

The contraction of MA to H_2O_2 (5×10^{-4} M) was compared with that of the aorta. H_2O_2 also caused a transient contractile response in aorta with or without endothelium, but the magnitude of contractions, expressed as per cent of contraction induced by 80 mM KCl, was only approximately half of that found in the MA (Figure 3A). Removal of endothelium increased the contraction in WKY but not in SHR. A higher level of contraction was found in SHR as compared with WKY in aortic rings with intact endothelium, but not in the rings where endothelium was removed (Figure 3B).

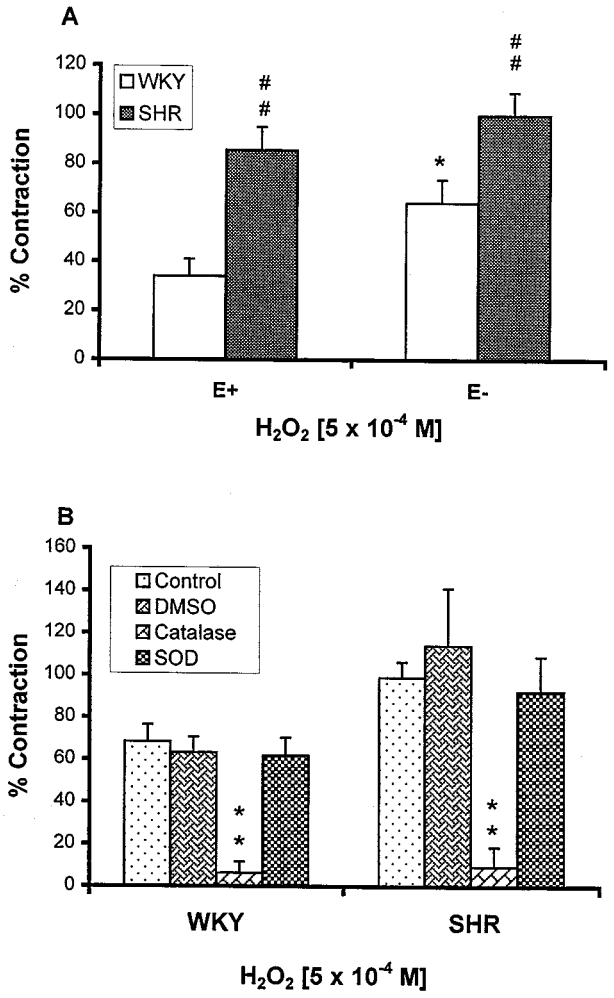

Figure 2 (A) Contractile effects of H_2O_2 (5×10^{-4} M) on the mesenteric artery with (E+) or without (E−) endothelium from SHR and WKY. $*P<0.05$ compared with E+ arteries; $\#P<0.05$, $\#\#P<0.01$, compared with WKY arteries. (B) Effects of dimethylsulphoxide (DMSO, 5 mM), catalase (1000 u/ml), and superoxide dismutase (SOD, 150 u/ml) on the contraction induced by H_2O_2 (5×10^{-4} M) in the E+ mesenteric artery of 4–6 rats each from WKY and SHR. $**P<0.01$ compared with their respective control.

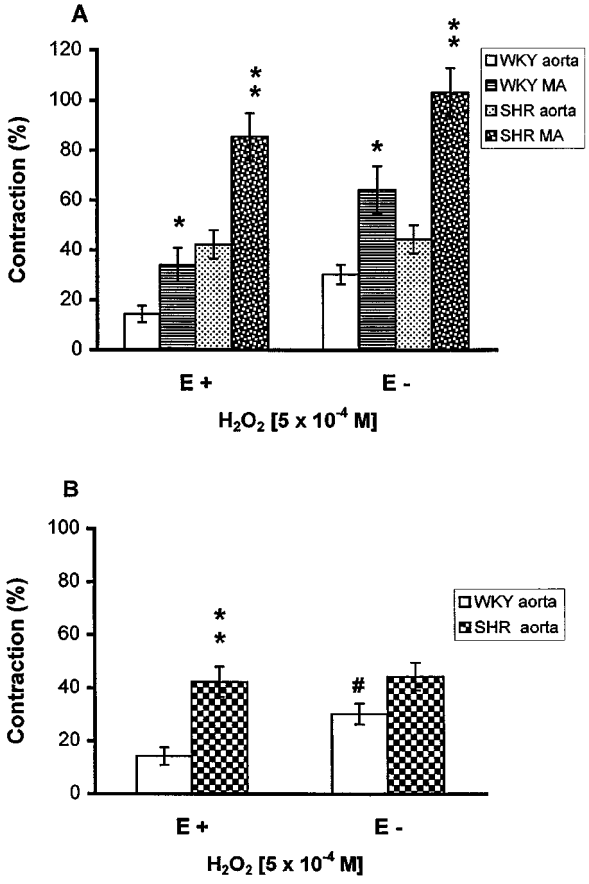

Figure 3 (A) Comparison of the contractile effects of H_2O_2 (5×10^{-4} M) on the mesenteric artery (MA) and the aorta, with (E+) or without (E−) endothelium, from SHR and WKY. $*P<0.05$, $**P<0.01$ compared with aorta; (B) Contraction to H_2O_2 (5×10^{-4} M) in E+ and E− aorta from SHR and WKY. $**P<0.01$, compared with WKY aorta, $\#P<0.05$ compared with E+ rings. Results (mean±s.e.mean) were from 6–11 rats and expressed as the percentage of the contraction elicited by 80 mM KCl.

Upon repeated exposure to the same concentration of H_2O_2 (5×10^{-4} M), the contraction of the MA became smaller after the first challenge. In E− MA, the reduction in the amplitude of response was less in SHR than in WKY: the contraction in SHR arteries remained around 40% of KCl-induced contraction after the 4th challenge but only around 10% in WKY (Figure 4).

The mechanisms responsible for the contractile response to H_2O_2 were investigated in E− MA. SQ 29548 and ICI 192605, both are thromboxane A_2/prostaglandin H_2 (TP) receptor antagonists, significantly inhibited the contractile response of arteries to H_2O_2 in both SHR and WKY at concentrations of 1 and 3×10^{-8} M (Figure 5A), and nearly abolished the contraction at the concentration of 10^{-6} M (data not shown). The contractile response to U46619, a TP receptor agonist, in E− MA was similar between SHR and WKY as shown in Figure 5B (EC_{50}: 17.9±0.3 nM for WKY, $n=5$; 17.5±0.7 nM for SHR, $n=4$).

Incubation with quinacrine (a PLA_2 inhibitor, 10^{-4} M), indomethacin and diclofenac (both are non-selective COX inhibitors, 1 and 3×10^{-6} M), and furegrelate (a TXA_2 synthase inhibitor, 10^{-4} M) significantly inhibited the contractile response to H_2O_2 in both SHR and WKY MA (Figure 6).

The concentration of TXB_2, a stable metabolite of TXA_2, was measured in the bathing solutions from the contractility studies with E− MA. The basal levels of TXB_2 were not different between SHR and WKY, but H_2O_2 (5×10^{-4} M) induced a greater increase of TXB_2 in SHR than WKY. Incubation with quinacrine (10^{-4} M), indomethacin and diclofenac (10^{-6} M), and furegrelate (10^{-4} M) significantly reduced the production of TXB_2 in response to H_2O_2, while SQ 29548 (10^{-8} M) and ICI 192605 (10^{-8} M) did not affect TXB_2 production (Figure 7).

Discussion

The purpose of the present study was to compare the contractile response to exogenously applied H_2O_2 in the isolated MA from SHR and WKY, and to investigate the mechanisms underlying H_2O_2-induced contraction. The contractile responses to H_2O_2 in MA and in the aorta were

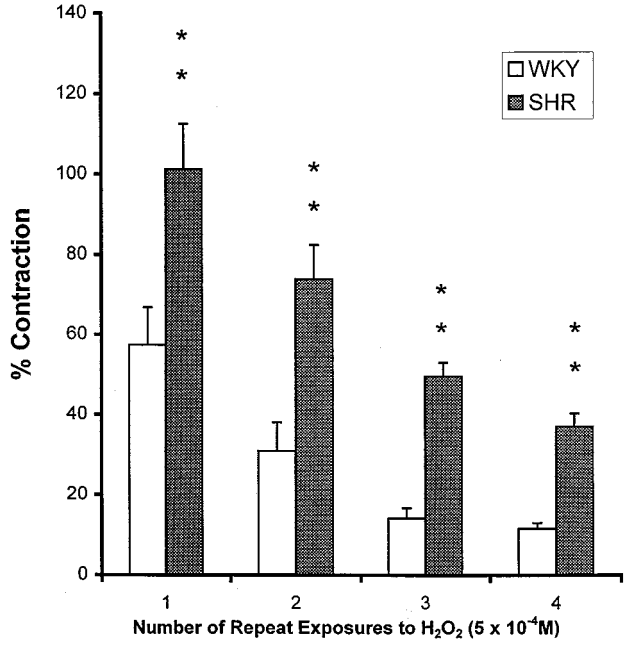

Figure 4 Tachyphylaxis of the contraction by H_2O_2 (5×10^{-4} M) in endothelium-denuded mesenteric artery from WKY and SHR. Results (mean ± s.e.mean) were from four each of SHR or WKY and expressed as the percentage of the contraction elicited by 80 mM KCl. $^{**}P<0.01$ compared with respective WKY arteries.

also compared. The main findings of this study were (1) that MA from SHR exhibited a higher contraction and less tachyphylaxis to H_2O_2 as compared with MA from WKY; (2) that an increase in smooth muscle reactivity was involved in the enhanced contraction to H_2O_2 in SHR MA; (3) that a greater production of TXA_2 through activation of PLA_2-COX-TXA_2 synthase pathway appeared to be responsible for the enhanced contraction in SHR. We also found that MA contracted more to H_2O_2 than the aorta and the higher contraction presented in SHR aorta was an event associated with reduced negative modulation of endothelium on the response to H_2O_2.

Among ROS, hydroxyl radical ($OH^{\cdot}$) is regarded as the most potent oxidant. The $OH^{\cdot}$ can be generated when H_2O_2 comes into contact with copper ion (Cu^{2+}) or iron ion (Fe^{2+}) by Fenton reaction (Fenton, 1984). In bovine pulmonary artery smooth muscle cells, H_2O_2 markedly stimulated $OH^{\cdot}$ formation (Roychoudhury *et al*., 1996). It has been shown that $OH^{\cdot}$ can contract rat aorta (Shen *et al*., 2000a). $O_2^{-\cdot}$ can interact with H_2O_2 by Haber-Weiss reaction to form $OH^{\cdot}$ (Cheeseman & Slater, 1993) in addition to its own vasoactive action (Somers *et al*., 2000). Therefore it is necessary to clarify if the contraction to H_2O_2 is caused by H_2O_2 itself, or by other related ROS. Our results showed that the contraction induced by H_2O_2 in rat MA was not through $OH^{\cdot}$ or O_2^-, because catalase, the decomposing enzyme of H_2O_2, nearly abolished H_2O_2-induced contraction, while SOD, a O_2^- scavenger, or DMSO, a $OH^{\cdot}$ scavenger, did not affect H_2O_2-induced contraction.

H_2O_2 can cause either vasoconstriction or relaxation depending on the contractile state of the vessels. Vasoconstriction was reported in vessels without pre-contraction. In arteries precontracted with phenylephrine (Zembowicz *et al*., 1993), norepinephrine (Fujimoto *et al*., 2001), or prostaglandin $F_{2\alpha}$ (Beny & Von der Weid, 1991), H_2O_2 produced a dose-dependent relaxation response. The relaxation involves endothelium-dependent or -independent mechanisms, which could be mediated by nitric oxide (Zembowicz, *et al*., 1993), cyclic GMP (Iesaki *et al*., 1996), cytochrome P450 products (Yang *et al*., 1999), or membrane hyperpolarization (Beny & Von der Weid, 1991). A recent report by Matoba *et al*. (2000) suggested that H_2O_2 is an endothelium-derived hyperpolarizing factor in acetylcholine-induced relaxation of mouse small MA, while another report by Hamilton *et al*. (2001) did not support this notion in the study on the relaxation response of human radial arteries to carbachol. The difference in animal species and vessel types may contribute to the difference in findings in these two reports.

Previous studies on the contractile response of MA to H_2O_2 include those by Hubel *et al*. (1993) who reported that there was no observable response to H_2O_2 in quiescent mesenteric arteries, and by Pelaez *et al*. (2000) who found that H_2O_2 induced contraction in MA from Sprague-Dawley rats through a calcium-independent mechanism. Our study is probably the first to show that H_2O_2 can induce prominent contraction of MA from SHR and WKY in a concentration-dependent manner. In order to find out whether MA is more reactive to H_2O_2 than aorta, we compared the contractile response to H_2O_2 in these vessels. Aorta is probably the most commonly used rodent vessel in *in vitro* vascular functional studies, but it is a large elastic conduit vessel and contributes little if any to the regulation of blood flow or peripheral resistance. Results generated using the aorta may not be applicable to smaller artery because their responses may differ qualitatively or quantitatively. For example, norbormide relaxes rat aorta by calcium entry blocker activity but contracts MA by stimulating phospholipase C-protein kinase C pathway (Bova *et al*., 2000). Another study showed that serotonin contracted rat aorta and MA with different EC_{50}s (Adegunloye & Sofola, 1997). In this study, we found that MA from both SHR and WKY generated twice as much tension as those generated by the aorta. This showed that the MA was more sensitive to H_2O_2 than the aorta. It is possible that H_2O_2 may affect local blood flow by its vasomotor action on reactive vessels such as MA. Future studies on small resistance vessels will be helpful in understanding the role of H_2O_2 in blood pressure regulation.

Existing reports suggested that endothelium may participate in the vascular contractile response to H_2O_2 in certain arteries. In quiescent rat aorta, contraction to H_2O_2 was augmented by endothelium removal (Rodriguez-Martinez *et al*., 1998), but in human placental artery, removal of endothelium did not affect H_2O_2-induced contraction (Omar *et al*., 1992). It is well known that endothelium is an important regulator of vascular tone, and endothelial dysfunction may exist in SHR (Bauersachs *et al*., 1998). In the current study, we found that removal of endothelium potentiated H_2O_2-induced contractions of MA and aorta in normotensive rats WKY but not in SHR. This indicated that the negative modulatory role of endothelium in H_2O_2-induced contraction was impaired in SHR vessels.

Comparing to WKY, an enhanced contractile response to higher concentrations of H_2O_2 (10^{-4} M, and 10^{-3} M) was found in MA from SHR without intact endothelium, suggesting that the enhanced response to these concentra-

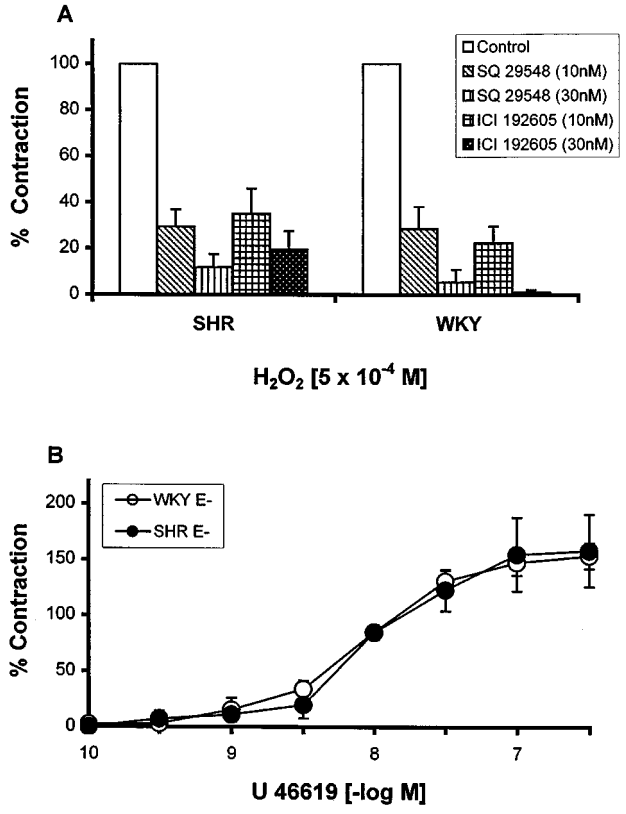

Figure 5 (A) Inhibitory effects of SQ 29548 and ICI 192605 on H_2O_2 (5×10^{-4} M) induced contraction in the endothelium-denuded (E−) mesenteric artery from WKY and SHR. Results (mean ± s.e.mean) were from four each of SHR or WKY and expressed as the percentage of the contraction of the control arteries. (B) Contraction by U46619 in the E− mesenteric artery from WKY and SHR. Results (mean ± s.e.mean) were from four each of SHR or WKY and expressed as the percentage of the contraction elicited by 80 mM KCl.

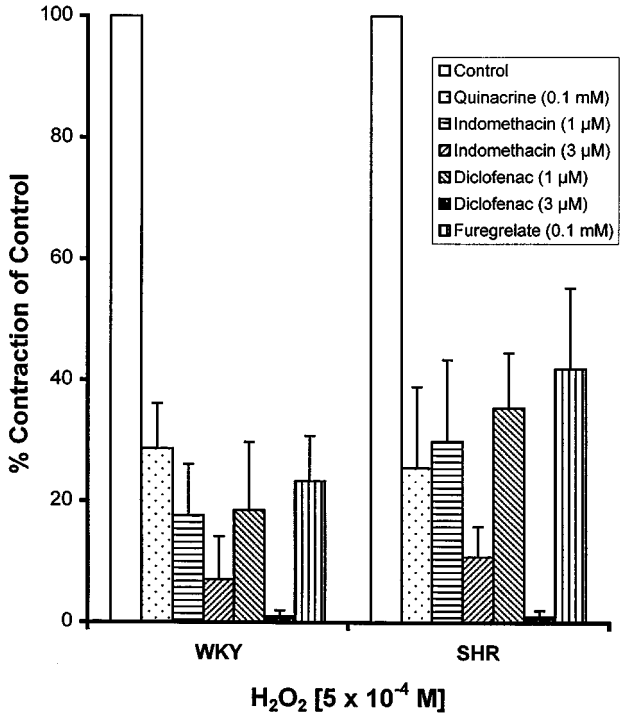

Figure 6 Inhibitory effects of quinacirne, indomethacin, diclofenac, and furegrelate on the contraction induced by H_2O_2 (5×10^{-4} M) in E− mesenteric artery from WKY and SHR. Results (mean ± s.e.mean) were from four each of SHR or WKY and expressed as the percentage of the contraction in the control arteries.

tions of H_2O_2 was mediated by an increased reactivity of smooth muscles to H_2O_2. This is different from what was found in the aorta. In the aorta from SHR, the enhanced contraction to H_2O_2 was found in E+ aortic rings but removal of the endothelium eliminated the difference between SHR and WKY, indicating that endothelial dysfunction which resulted in a reduced modulating effect on smooth muscle contraction was responsible for the enhanced response to H_2O_2 in SHR aorta. However, in the MA endothelium removal only blunted the difference in the response to H_2O_2 found in E+ MA of SHR and WKY at low concentration (10^{-5} M). Taken together, in contrast to the results found in SHR and WKY aorta where difference in H_2O_2 response was related to endothelial function, in SHR MA an increased smooth muscle reactivity was involved in the enhanced response to exogenously applied H_2O_2. Furthermore, upon repeated exposure to the same concentration of H_2O_2, MA from SHR showed less tachyphylaxis than WKY vessels, suggesting that the hyperreactivity to H_2O_2 was well maintained in SHR even in the case of repeated stimulation by H_2O_2. This may be another mechanism through which an enhanced contractile response to H_2O_2 is maintained in the arteries from SHR as compared with those from WKY, which will affect the regulation of local blood flow and resistance.

Experiments were done in E− MA to explore the possible mechanisms responsible for the contractile response to H_2O_2. It has been reported that H_2O_2 can activate PLA_2 in bovine pulmonary endothelial cells (Chakraborti *et al*., 1989) and rabbit pulmonary smooth muscle cells (Heinle, 1982; Chakraborti & Michael, 1993). In this study, we found that incubation with quinacrine, a PLA_2 inhibitor, significantly reduced the contractile response, suggesting the involvement of PLA_2 in H_2O_2-induced contraction of MA. It is still not known how H_2O_2 activates PLA_2. Involvement of protein kinase C (Chakraborti & Michael, 1993) or mediation by P_2-purinoceptors in H_2O_2-induced contraction (Shen *et al*., 2000b) has been suggested as possible mechanisms.

A number of reports have shown that COX is involved in ROS-induced contraction (Rodriguez-Martinez *et al*., 1998; Hibino *et al*., 1999; Garcia-Cohen *et al*., 2000). In this study, we found that indomethacin and diclofenac, two non-selective COX inhibitors, significantly inhibited the contraction to H_2O_2, confirming the participation of COX metabolite-related signal pathway in H_2O_2-induced vessel contraction. Furthermore, TP receptor antagonists (SQ 29548 and ICI 192605) significantly inhibited the contractile response to H_2O_2 at lower concentrations (1 and 3×10^{-8} M), and nearly abolished the response at a higher concentration (10^{-6} M). We also found that furegrelate (10^{-4} M), a TXA_2 synthase inhibitor, significantly reduced the contraction to H_2O_2. These results strongly suggest that TXA_2 generated from PLA_2-COX-TXA_2 synthase pathway may serve as the mediator of H_2O_2-induced contraction. There has been a report showing that TXA_2 may act as the mediator in $O_2^{-\cdot}$-stimulated contraction of SHR aorta

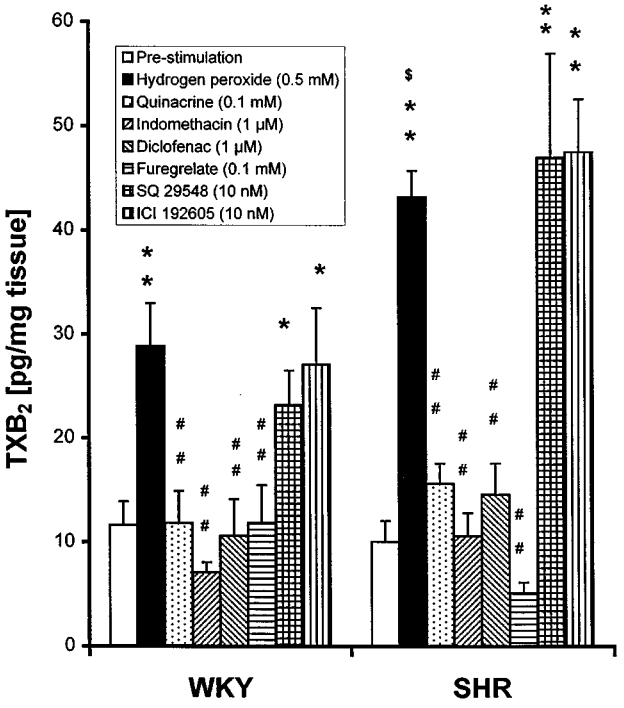

Figure 7 Thromboxane B_2 (TXB_2) production (pg/mg wet tissue), stimulated by H_2O_2 (5×10^{-4} M), and the effects of enzyme inhibitors (quinacirne, indomethacin, diclofenac, and furegrelate) and of thromboxane A_2 and/or prostaglandin H_2 receptor antagonists (SQ 29548 and ICI 192605) in E− mesenteric artery from WKY and SHR. Results (mean ± s.e.mean) were from 4–6 rats each from SHR and WKY. $*P<0.05$, $**P<0.01$ compared with respective pre-stimulation level; $\#\#P<0.01$, compared with H_2O_2; $\$P<0.05$ compared with respective WKY.

(Hibino *et al*., 1999), but for H_2O_2-induced contraction of SHR artery, as far as we know, there is only one functional study with aorta suggesting that COX metabolites may be the mediator(s) but the measurement of tissue production of TXA_2 was not carried out (Rodriguez-Martinez *et al*., 1998). Our results on the measurement of TXB_2 (a stable TXA_2 metabolite) clearly showed that H_2O_2 stimulated the production of TXB_2 in the MA, and this production was greater in the arteries from SHR than from WKY. Incubation with enzyme inhibitors including quinacrine, indomethacin, diclofenac, and furegrelate inhibited H_2O_2-stimulated TXB_2 production along with the inhibition of contraction. Treatment with TP receptor antagonists SQ 29584 or ICI 192605 did not inhibit H_2O_2-stimulated TXB_2 production, but markedly inhibited H_2O_2-induced contraction because of the blockade of TP receptors. These are in agreement with our findings in the functional studies, and indicate that TXA_2 is involved in H_2O_2-induced contraction.

The reactivity to U46619, a TP receptor agonist, was compared in the MA of SHR and WKY, because the greater contractile responses induced by H_2O_2 could be due to an enhanced reactivity of the SHR arteries to TXA_2 as compared with WKY arteries. However, our results which showed that the contractile response of MA to U46619 was similar between SHR and WKY (either in maximal response or EC_{50}), indicated that the enhanced contraction response to H_2O_2 in SHR was not due to the altered vascular reactivity to TXA_2.

In summary, SHR MA exhibits a higher contractile response to H_2O_2 than MA from WKY, which involves an increased smooth muscle reactivity and less tachyphylaxis to H_2O_2. A greater production of TXA_2 through activation of PLA_2-COX-TXA_2 synthase pathway appeared to be responsible for the enhanced contraction in SHR MA.

We thank Dr E.E. Daniel for the use of his equipment, Dr C.Y. Kwan and Mr Z.J. Shen for their discussion, and Mr Mathew Butler, Miss Angela Demeter and Nermin Attia for their technical assistance. Dr Y.J. Gao is a Research Fellow supported by the Joint Canadian Hypertension Society/Medical Research Council of Canada Special Initiative Program. This study was supported by the Heart and Stroke Foundation of Ontario.

References

ADEGUNLOYE, B.I. & SOFOLA, O.A. (1997). Differential responses of rat aorta and mesenteric artery to norepinephrine and serotonin in vitro. *Pharmacology,* **55,** 25–31.

AUCH-SCHWELK, W., KATUSIC, Z.S. & VANHOUTTE, P.M. (1989). Contractions to oxygen-derived free radicals are augmented in aorta of the spontaneously hypertensive rat. *Hypertension,* **13,** 859–864.

BASS, A.S. & BERK, B.C. (1995). Differential activation of mitogen-activated protein kinases by H_2O_2 and O_2^- in vascular smooth muscle cells. *Circ. Res.,* **77,** 29–36.

BAUERSACHS, J., BOULOUMIÉ, A., MÜLSCH, A., WIEMER, G., FLEMING, I. & BUSSE, R. (1998). Vasodilator dysfunction in aged spontaneously hypertensive rats: changes in NO synthase III and soluble guanylyl cyclase expression, and in superoxide anion production. *Cardiovasc. Res.,* **37,** 772–779.

BENY, J.L. & VON DER WEID, P.Y. (1991). Hydrogen peroxide: an endogenous smooth muscle cell hyperpolarizing factor. *Biochem. Biophys. Res. Commun.,* **176,** 378–384.

BERGENDI, L., BENES, L., DURACKOVA, Z. & FERENCIK, M. (1999). Chemistry, physiology and pathology of free radicals. *Life Sci.,* **65,** 1865–1874.

BOVA, S., TREVISI, L., CIMA, L., LUCIANI, S., GOLOVINA, V. & CARGNELLI, G. (2000). Signaling mechanisms for the selective vasoconstrictior effect of norbormide on the rat small arteries. *J. Pharmacol. Exp. Ther.,* **296,** 458–463.

CHAKRABORTI, S., GURTNER, G.H. & MICHAEL, J.R. (1989). Oxidant-mediated activation of phospholipase A2 in pulmonary endothelium. *Am. J. Physiol.,* **257,** L430–L437.

CHAKRABORTI, S. & MICHAEL, J.R. (1993). Role of protein kinase C in oxidant-mediated activation of phospholipase A2 in rabbit pulmonary arterial smooth muscle cells. *Mol. Cell. Biochem.,* **122,** 9–15.

CHEESEMAN, K.H. & SLATER, T.F. (1993). An introduction to free radical biochemistry. *Br. Med. Bull.,* **49,** 481–493.

FENTON, H.J.H. (1984). Oxidation of tartaric acid in the presence of iron. *J. Chem. Soc.,* **65,** 899–910.

FUJIMOTO, S., ASANO, T., SAKAI, M., SAKURAI, K., TAKAGI, D., YOSHIMOTO, N. & ITOH, T. (2001). Mechanisms of hydrogen peroxide-induced relaxation in rabbit mesenteric small artery. *Eur. J. Pharmacol.,* **412,** 291–300.

GARCIA-COHEN, E.C., MARIN, J., DIEZ-PICAZO, L.D., BAENA, A.B., SALAICES, M. & RODRIGUEZ-MARTINEZ, M.A. (2000). Oxidative stress induced by tert-butyl hydroperoxide causes vasoconstriction in the aorta from hypertensive and aged rats: role of cyclooxygenase-2 isoform. *J. Pharmacol. Exp. Ther.,* **293,** 75 – 81.

GRIENDLING, K.K. & ALEXANDER, R.W. (1997). Oxidative stress and cardiovascular disease. *Circulation,* **96,** 3264 – 3265.

GRIENDLING, K.K., SORESCU, D. & USHIO-FUKAI, M. (2000). NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ. Res.,* **86,** 494 – 501.

HAMILTON, C.A., McPHADEN, A.R., BERG, G., PATHI, V. & DOMINICZAK, A.F. (2001). Is hydrogen peroxide an EDHF in human radial arteries? *Am. J. Physiol.,* **280,** H2451 – H2455.

HARRISON, D., SCHIAVI, P. & FALGUI, B. (1999). Renovascular hypertension, aortic superoxide production: effect of perindopril and indapamide. *Am. J. Hypertens.,* **12,** 50A.

HEINLE, H. (1982). Peroxide induced activation of glycogen phosphorylase A activity in vascular smooth muscle. *Biochem. Biophys. Res. Commun.,* **107,** 597 – 601.

HIBINO, M., OKUMURA, K., IWAMA, Y., MOKUNO, S., OSANAI, H., MATSUI, H., TOKI, Y. & ITO, T. (1999). Oxygen-derived free radical-induced vasoconstriction by thromboxane A_2 in aorta of the spontaneously hypertensive rat. *J. Cardiovasc. Pharmacol.,* **33,** 605 – 610.

HUBEL, C.A., DAVIDGE, S.T. & McLAUGHLIN, M.K. (1993). Lipid hydroperoxides potentiate mesenteric artery vasoconstrictor responses. *Free Radic. Biol. Med.,* **14,** 397 – 407.

IESAKI, T., OKADA, T., SHIMADA, I., YAMAGUCHI, H. & OCHI, R. (1996). Decrease in CA^{2+} sensitivity as a mechanism of hydrogen peroxide-induced relaxation of rabbit aorta. *Cardiovasc. Res.,* **31,** 820 – 825.

ITO, H., TORII, M. & SUZUKI, T. (1992). A comparative study on defense systems for lipid peroxidation by free radicals in spontaneously hypertensive and normotensive rat myocardium. *Comp. Biochem. Physiol. [B],* **103,** 37 – 40.

JIN, N. & RHOADES, R.A. (1997). Activation of tyrosine kinases in H_2O_2-induced contraction in pulmonary artery. *Am. J. Physiol.,* **272,** H2686 – H2692.

KATUSIC, Z.S., SCHUGEL, J., COSENTINO, F. & VANHOUTTE, P.M. (1993). Endothelium-dependent contractions to oxygen-derived free radicals in the canine basilar artery. *Am. J. Physiol.,* **264,** H859 – H864.

LI, P.F., DIETZ, R. & VON HARSDORF, R. (1997). Reactive oxygen species induce apoptosis of vascular smooth muscle cell. *FEBS Lett.,* **404,** 249 – 252.

MACHLIN, L.J. & BENDICH, A. (1987). Free radical tissue damage: protective role of antioxidant nutrients. *FASEB,* **1,** 441 – 445.

MATOBA, T., SHIMOKAWA, H., NAKASHIMA, M., HIRAKAWA, Y., MUKAI, Y., HIRANO, K., KANAIDE, H. & TAKESHITA, A. (2000). Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J. Clin. Invest.,* **106,** 1521 – 1530.

OLIVER, C.N. (1987). Inactivation of enzymes and oxidative modification of proteins by stimulated neutrophils. *Arch. Biochem. Biophys.,* **253,** 62 – 72.

OMAR, H.A., FIGUEROA, R., OMAR, R.A., TEJANI, N. & WOLIN, M.S. (1992). Hydrogen peroxide and reoxygenation cause prostaglandin-mediated contraction of human placental arteries and veins. *Am. J. Obstet. Gynecol.,* **167,** 201 – 207.

PELAEZ, N.J., BRAUN, T.R., PAUL, R.J., MEISS, R.A. & PACKER, C.S. (2000). H_2O_2 mediates CA^{2+} - and MLC_{20} phosphorylation-independent contraction in intact and permeabilized vascular muscle. *Am. J. Physiol.,* **279,** H1185 – H1193.

PRABHA, P.S., DAS, U.N., KORATKAR, R., SAGAR, P.S. & RAMESH, G. (1990). Free radical generation, lipid peroxidation and essential fatty acids in uncontrolled essential hypertension. *Prostaglandins Leukot. Essent. Fatty Acids,* **41,** 27 – 33.

RAJAGOPALAN, S., KURZ, S., MUNZEL, T., TARPEY, M., FREEMAN, B.A., GRIENDLING, K.K. & HARRISON, D.G. (1996). Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J. Clin.Invest.,* **97,** 1916 – 1923.

RODRIGUEZ-MARTINEZ, M.A., GARCIA-COHEN, E.C., BAENA, A.B., GONZALEZ, R., SALAICES, M. & MARIN, J. (1998). Contractile responses elicited by hydrogen peroxide in aorta from normotensive and hypertensive rats. Endothelial modulation and mechanism involved. *Br. J. Pharmacol.,* **125,** 1329 – 1335.

ROYCHOUDHURY, S., GHOSH, S.K., CHAKRABORTI, T. & CHAKRABORTI, S. (1996). Role of hydroxyl radical in the oxidant H_2O_2-mediated CA^{2+} release from pulmonary smooth muscle mitochondria. *Mol. Cell. Biochem.,* **159,** 95 – 103.

SHEEHAN, D.W., GIESE, E.C., GUGINO, S.F. & RUSSELL, J.A. (1993). Characterization and mechanisms of H_2O_2-induced contractions of pulmonary arteries. *Am. J. Physiol.,* **264,** H1542 – H1547.

SHEN, J.Z., ZHENG, X.F. & KWAN, C.Y. (2000a). Differential contractile actions of reactive oxygen species on rat aorta: selective activation of ATP receptor by H_2O_2. *Life Sci.,* **66,** PL 291 – PL 296.

SHEN, J.Z., ZHENG, X.F. & KWAN, C.Y. (2000b). Evidence for P(2)-purinoceptors contribution in H_2O_2-induced contraction of rat aorta in the absence of endothelium. *Cardiovas. Res.,* **47,** 574 – 585.

SOMERS, M.J., MAVROMATIS, K., GALIS, Z.S. & HARRISON, D.G. (2000). Vascular superoxide production and vasomotor function in hypertension induced by deoxycorticosterone acetate-salt. *Circulation,* **101,** 1722 – 1728.

SUNDARESAN, M., YU, Z.X., FERRANS, V.J., IRANI, K. & FINKEL, T. (1995). Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science,* **270,** 296 – 299.

SUZUKI, H., SWEI, A., ZWEIFACH, B.W. & SCHMID-SCHONBEIN, G.W. (1995). In vivo evidence for microvascular oxidative stress in spontaneously hypertensive rats. Hydroethidine microfluorography. *Hypertension,* **25,** 1083 – 1089.

SWEI, A., LACY, F., DELANO, F.A. & SCHMID-SCHONBEIN, G.W. (1997). Oxidative stress in the Dahl hypertensive rat. *Hypertension,* **30,** 1628 – 1633.

WOLIN, M.S., RODRIGUES, A.M. & YU, J.M. (1985). Peroxides cause dose-dependent relaxant and contractor responses in isolated bovine intrapulmonary arterial and venous rings. *Fed. Prol. Am. Soc. Exp. Biol.,* **44,** 821 – 826.

YANG, Z., ZHANG, A., ALTURA, B.T. & ALTURA, B.M. (1999). Hydrogen peroxide-induced endothelium-dependent relaxation of rat aorta. Involvement of CA^{2+} and other cellular metabolites. *Gen. Pharmacol.,* **33,** 325 – 336.

YANG, Z.W., ZHENG, T., ZHANG, A., ALTURA, B.T. & ALTURA, B.M. (1998). Mechanisms of hydrogen peroxide-induced contraction of rat aorta. *Eur. J. Pharmacol.,* **344,** 169 – 181.

ZEMBOWICZ, A., HATCHETT R.J., JAKUBOWSKI, A.M. & GRYGLEWSKI, R.J. (1993). Involvement of nitric oxide in the endothelium-dependent relaxation induced by hydrogen peroxide in the rabbit aorta. *Br. J. Pharmacol.,* **110,** 151 – 158.

(Received June 18, 2001
Revised September 21, 2001
Accepted September 28, 2001)