British Journal of Pharmacology (2002) 136, 104–110

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Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats

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1 Experiments were designed to investigate whether or not oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in the aorta of spontaneously hypertensive rat (SHR).

2 Isometric tension was measured in aortic rings taken from adult male SHR and Wistar-Kyoto rat (WKY) in the presence of N^G-nitro-L-arginine.

3 Endothelium-dependent contractions to acetylcholine were significantly greater in rings from SHR compared to WKY. Oxygen-derived free radicals, generated from xanthine plus xanthine oxidase, induced contractions that were larger in aortas from SHR than from WKY. Contractions to acetylcholine and free radicals were abolished by a selective TP-receptor antagonist, S 18886, and a preferential inhibitor of cyclo-oxygenase-1, valeryl salicylate, but not by a preferential inhibitor of cyclo-oxygenase-2, NS-398.

4 Allopurinol, deferoxamine and the combination of superoxide dismutase plus catalase inhibited the contractions to oxygen-derived free radicals but did not significantly affect those to acetylcholine. In contrast, diethyldithiocarbamic acid, an inhibitor of superoxide dismutase, or Tiron, a scavenger of superoxide anion, reduced endothelium-dependent contractions to acetylcholine in aortas from SHR. The effect of these two drugs was additive.

5 In SHR chronically treated with dimethylthiourea endothelium-dependent contractions to acetylcholine were decreased, and reduced further by acute *in vitro* exposure to deferoxamine or the combination of superoxide dismutase plus catalase.

6 These results suggest that in the SHR aorta acetylcholine-induced endothelium-dependent contractions involve endothelial superoxide anion production and the subsequent dismutation into hydroxyl radicals and/or hydrogen peroxide. The free radicals activate cyclo-oxygenase-1, most likely to produce endoperoxides. Activation of TP-receptors is required to observe endothelium-dependent contractions to acetylcholine or endothelium-independent contractions in response to free radical generation.

British Journal of Pharmacology (2002) **136,** 104–110

Keywords: Cyclo-oxygenase; endothelium-dependent contractions; endothelium-derived contracting factor(s); oxygen-derived free radicals; TP receptor; spontaneously hypertensive rat

Abbreviations: DETCA, diethyldithiocarbamic acid; DMTU, dimethylthiourea; EDCF, endothelium-derived contracting factor; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; WKY, Wistar-Kyoto rat

Introduction

The endothelium plays a crucial role in the regulation of vascular tone by releasing relaxing and contracting substances in response to different stimuli. Under several pathological conditions including hypertension, diabetes, and atherosclerosis, the endothelial cells are dysfunctional. This dysfunction is characterized by impaired relaxation in response to endothelium-dependent dilators such as acetylcholine (De Vriese *et al*., 2000; Taddei *et al*., 1993; Wolin, 2000). In human essential hypertension and several animal models of the disease, the impairment of endothelium-dependent vasodilatation is due either to the generation of endothelium-derived contracting factor(s) (EDCF) or to the reduced bioavailability of nitric oxide (Vanhoutte & Boulanger, 1995). In the aorta of the spontaneously hypertensive rat (SHR), an EDCF is generated in response to acetylcholine which can be scavenged by nitric oxide (Auch-Schwelk *et al*., 1992; Lüscher & Vanhoutte, 1986). The response to this EDCF involves activation of cyclo-oxygenase-1 and TP-receptors (Vanhoutte & Boulanger, 1995).

In the basilar artery of the dog, superoxide anions mediate endothelium-dependent contractions, which are prevented by inhibitors of cyclo-oxygenase (Katusic & Vanhoutte, 1989).

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Oxygen-derived free radicals, in particular superoxide anions, are also likely candidates as EDCF in the SHR (Vanhoutte & Boulanger, 1995). Indeed, superoxide anions can be released by cultured endothelial cells (Matsubara & Ziff, 1986). Their generation is greater in blood vessels from rats with genetic or experimental hypertension and in essential hypertensive patients than in normotensive blood vessels (Maytin *et al*., 1999; Wu *et al*., 2001). In the aortas of stroke-prone SHR, an excessive production of superoxide anions occurs in endothelium (Kerr *et al*., 1999). The artificial generation of superoxide anions causes contractions that are larger in aortas from SHR than normotensive rats (Auch-Schwelk *et al*., 1989). These contractions can also be inhibited by TP-receptor antagonists and inhibitors of cyclo-oxygenase (Auch-Schwelk *et al*., 1989; Hibino *et al*., 1999). Altogether, those findings are consistent with the hypothesis that oxygen-derived free radicals are EDCF in the aorta of the SHR. However, the endothelium-dependent contractions to acetylcholine in contrast to the contraction produced by the generation of oxygen-derived radicals, are not inhibited either by the combination of superoxide dismutase plus catalase or by deferoxamine (Auch-Schwelk *et al*., 1989).

The present study was designed to examine further whether or not oxygen-derived free radicals are indeed involved in the endothelium-dependent contractions to acetylcholine in the aorta of the SHR.

Methods

All experiments were performed on the thoracic aortas from male SHR aged 30–36 weeks and age-matched male normotensive Wistar-Kyoto rats (WKY). Some of the SHR were treated with dimethylthiourea for 10 days by injection (500 mg kg^{-1} body weight intraperitoneally for the first injection, followed by two daily injections of 125 mg kg^{-1} body weight). On the day of the experiment, all rats were anaesthetized with pentobarbitone sodium (50 mg kg, i.p.) and exsanguinated. The thoracic aorta was dissected free, excised and placed into cold modified Krebs-Ringer bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2SO_4 1.2, $NaHCO_3$ 25.0, and edetate calcium di-sodium 0.026; glucose 11.1 (control solution). The blood vessels were cut into rings (4–5 mm in length). In some preparations the endothelium was removed mechanically. In the other rings the presence of endothelium was confirmed by relaxations to thrombin (1 u ml^{-1}) during contractions to phenylephrine (0.1 μM). The rings were suspended in organ chambers which contained control solution (37°C) aerated with 95% O_2 and 5% CO_2, and were connected to a force transducer. Changes in isometric tension were recorded. All experiments were performed in the presence of N^G-nitro-L-arginine (100 μM) to amplify the endothelium-dependent contraction induced by acetylcholine (Auch-Schwelk *et al*., 1992).

Acetylcholine hydrochloride, allopurinol, catalase, deferoxamine, diethyldithiocarbamic acid, dimethylthiourea, N^G-nitro-L-arginine, phenylephrine, superoxide dismutase, Tiron (4,5-dihydroxy-1,3-benzene-disulphonic acid), xanthine, xanthine oxidase were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). S 18886 (3-[(6-amino-(4-chlorobenzensulphonyl)-2-methyl-5,6,7,8-tetrahydronapht]-1-yl)propionic acid) was synthesized at the Institut de Recherches Servier (Suresnes, France). NS-398 (N[-2-(cyclohexyloxy)-4-nitrophenyl]-methanesulphonamide) and valeryl salicylate (2-[(1-oxopenytyl)oxy]-benzoic acid) were purchased from Cayman Chemical Company (Ann Arbor, MI, U.S.A.). Drug concentrations are expressed as final molar concentrations in the bath solution. Most drugs were dissolved in distilled water. Allopurinol and xanthine were alkalized with NaOH (1N). N^G-nitro-L-arginine was sonicated for 15 min. NS-398 was dissolved in ethanol. Tiron and valeryl salicylate were dissolved directly in control solution. The incubation time was 5 min for superoxide dismutase, catalase and deferoxamine and 40 min for other drugs. Xanthine was added 10 min before xanthine oxidase.

Changes in tension were expressed as the percentage (%) of the maximal response to KCl (60 mM) obtained at the beginning of the experiment. The results were given as means ± s.e.mean; *n* refers to the number of rats from which the thoracic aorta was taken. Statistical analysis was done by two-tailed Student's *t*-test for paired (rings with and without treatment from the same animal) or unpaired (rings from different animals) observations. Differences were considered to be statistically significant when P was less than 0.05.

Results

Control SHR and WKY

Endothelium-dependent contractions In the presence of N^G-nitro-L-arginine (100 μM), acetylcholine (0.3–100 μM) evoked concentration-dependent contractions, which were significantly larger in rings with endothelium from SHR compared to WKY (Figure 1). Acetylcholine did not contract the preparations without endothelium from both strains (data not shown). The contractions were abolished by valeryl salicylate (3 mM) and S 18886 (0.1 μM), and reduced significantly by NS-398 (1 μM) (Figure 1). The combination of superoxide dismutase (120 u ml^{-1}) plus catalase (1200 u ml^{-1}), or deferoxamine (100 μM) did not significantly affect the endothelium-dependent contractions induced by acetylcholine in aortas with endothelium from SHR (Table 1). Similarly, dimethylthiourea (100 μM) had no significant acute effect on endothelium-dependent contractions (Table 1). In contrast, both diethyldithiocarbamic acid (100 μM) or Tiron (10 mM) depressed significantly the endothelium-dependent contractions evoked by acetylcholine in SHR. The combination of diethyldithiocarbamic acid plus Tiron had an additive effect (Figure 2). The two drugs did not significantly affect contractions evoked by phenylephrine (1 nM–100 μM) (data not shown).

Contractions to oxygen-derived free radicals Xanthine oxidase (0.001–0.03 u ml^{-1}) in the presence of xanthine (100 μM) caused concentration-dependent contractions in rings with or without endothelium from both SHR and WKY. The contractions were significantly larger in rings from SHR than from WKY (Figure 3). Allopurinol (100 μM), the combination of superoxide dismutase (120 u ml^{-1}) plus catalase (1200 u ml^{-1}), or deferoxamine (100 μM) significantly decreased the contractions in SHR (Figure 4). In aortas from SHR, S 18886 (0.1 μM), valeryl salicylate (3 mM)

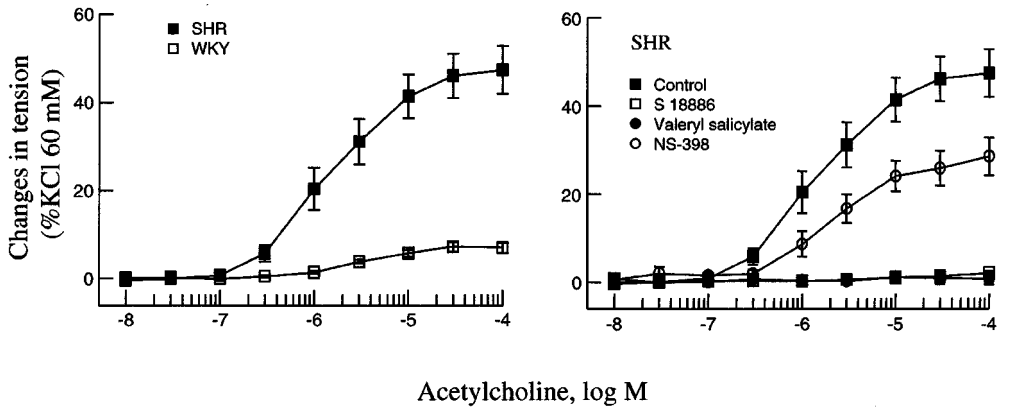

Figure 1 Endothelium-dependent contractions to acetylcholine in aortic rings (presence of N^G-nitro-L-arginine: 100 μM). Left panel: comparison between SHR and WKY. The response in aortic rings taken from SHR is significantly different than that of WKY ($P<0.05$). Right panel: effect of S 18886 (0.1 μM), valeryl salicylate (3 mM) and NS-398 (1 μM) in aortas from SHR. Each treatment produced statistically significant inhibition when compared to control. Additionally, a statistically significant difference was observed between NS-398 treatment and other treatments ($P<0.05$). Data are shown as means$\pm$s.e.mean, ($n=8$).

Table 1 Superoxide dismutase (SOD) plus catalase, deferoxamine, or dimethylthiourea and endothelium-dependent contractions to acetylcholine (30 μM) in SHR

	SOD (120 u ml^{-1})+ *catalase* (1200 u ml^{-1})	*Deferoxamine* (100 μM)	*Dimethylthiourea* (100 μM)
Control	50.8$\pm$6.0 ($n=10$)	50.8$\pm$6.0 ($n=10$)	59.1$\pm$5.2 ($n=4$)
Treated	42.6$\pm$5.0 ($n=10$)	41.8$\pm$5.9 ($n=10$)	51.1$\pm$7.4 ($n=4$)

Aortas with endothelium were taken from spontaneously hypertensive rats (SHR). Data are shown as means$\pm$s.e. mean; n indicates the number of animals studied.

and to a lesser extent NS-398 (1 μM) significantly reduced the contractions produced by xanthine plus xanthine oxidase (Figure 4).

Chronic treatment with dimethylthiourea

Endothelium-dependent contractions In aortas from SHR treated chronically with dimethylthiourea, the endothelium-dependent contractions to acetylcholine were reduced significantly compared with those in untreated SHR (Figure 5). Superoxide dismutase (120 u ml^{-1}) plus catalase (1200 u ml^{-1}), or deferoxamine (100 μM) significantly decreased the amplitude of the endothelium-dependent contractions in aortas taken from dimethylthiourea-treated SHR (Figure 5).

Contractions to oxygen-derived free radicals

The contractions induced by xanthine plus xanthine oxidase were reduced significantly in rings without endothelium from SHR treated chronically with dimethylthiourea when compared to untreated SHR (Figure 6).

Discussion

The present study demonstrates that oxygen-derived free radicals are involved in endothelium-dependent contractions evoked by acetylcholine in aortas from SHR. Endothelium-dependent contractions of the SHR aorta are enhanced by analogues of L-arginine, inhibitors of nitric oxide synthase, by oxyhaemoglobin, a scavenger of nitric oxide, but not by inhibitors of guanylyl cyclase. This indicates that the EDCF released from the SHR interacts chemically with nitric oxide (Auch-Schwelk *et al*., 1992). In the present study, experiments were performed in the presence of N^G-nitro-L-arginine, an inhibitor of nitric oxide synthase, in order to optimize endothelium-dependent contractions (Auch-Schwelk *et al*., 1992).

The acetylcholine evoked endothelium-dependent contractions are abolished by S 18886, a selective TP-receptor antagonist (Simonet *et al*., 1998), and valeryl salicylate, but only partially inhibited by NS-398. Valeryl salicylate and NS-398 are poorly selective inhibitors of cyclo-oxygenase-1 and cyclo-oxygenase-2, respectively (Johnson *et al*., 1995). However, the concentrations of valeryl salicylate and NS-398 used in the present study were within the range of their respective selectivity (Cyclo-oxygenase-1: 0.8 mM and 0.15 μM, cyclo-oxygenase-2: 15 mM and 220 μM for valeryl salicylate and NS-398, respectively). The partial inhibitory effect of NS-398 should be attributed to its non-specific inhibition of cyclo-oxygenase-1 and it could be concluded that cyclo-oxygenase-1 is involved in the response, confirming previous results (Ge *et al*., 1995). Thereafter, these arachidonic acid metabolites must activate TP-receptors, since the response to acetylcholine is also abolished by TP-receptor antagonists (Auch-Schwelk *et al*., 1990; Ge *et al*., 1995). The contractions are not prevented by inhibitors of thromboxane synthase (Auch-Schwelk *et al*., 1990; Lüscher & Vanhoutte, 1986). This suggests that the metabolite of arachidonic acid involved is an endoperoxide(s) (Vanhoutte & Boulanger, 1995), possibly prostaglandin H_2 (Auch-Schwelk *et al*., 1990), the immediate product of the cyclo-oxygenase pathway. This interpretation is supported by the demonstration of an augmented expression of cyclo-oxygenase-1 in the SHR aorta, a greater release of endoperoxides and a hypersensitivity of the SHR aorta to prostaglandin H_2 (Ge *et al*., 1995). The precise location of cyclo-oxygenase-1 and TP-receptors is still uncertain as a satisfactory bioassay of EDCF has not yet been performed.

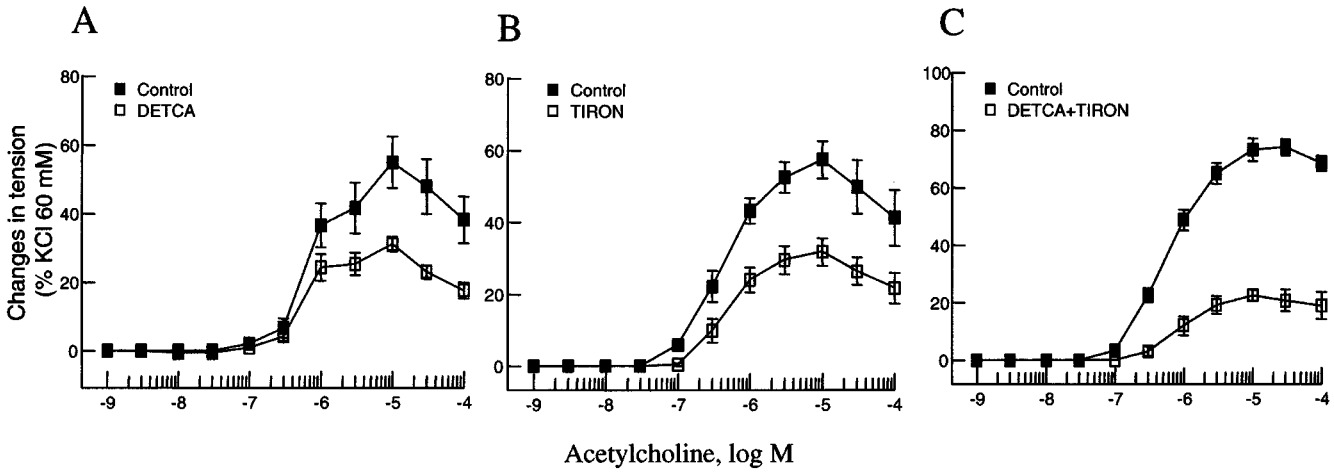

Figure 2 Superoxide anion and endothelium-dependent contraction in SHR (presence of N^G-nitro-L-arginine: 100 μM). (A) Effect of diethyldithiocarbamic acid (DETCA, 100 μM, $n=7$). (B) Effect of Tiron (10 mM, $n=8$). (C) Effect of the combination of DETCA plus Tiron ($n=5$). Data are shown as means$\pm$s.e.mean. Each treatment produced a statistically significant inhibition when compared to control ($P<0.05$).

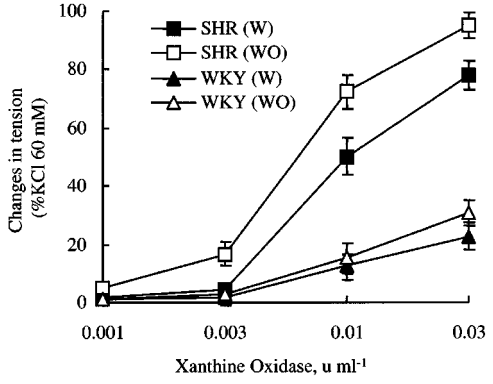

Figure 3 Contractions to xanthine oxidase in the presence of xanthine (100 μM) in aortic rings with endothelium (W) and without endothelium (WO) taken from SHR ($n=7$) and WKY ($n=8$; presence of N^G-nitro-L-arginine: 100 μM). Data are shown as means$\pm$s.e.mean. The responses in aortic rings with and without endothelium taken from SHR were significantly different than those of WKY ($P<0.05$).

An increased production of oxygen-derived free radicals is observed in hypertensive vascular walls (Kerr *et al*., 1999; Wu *et al*., 2001). Endothelial cells contain several enzymes, which can be responsible for the generation of oxygen-derived free radicals and for instance, in the aorta of the Stroke-Prone SHR, endothelial cells contribute to this excessive production (Kerr *et al*., 1999). Reactive oxygen species may act directly on vascular smooth muscle or interact with endogenous nitric oxide in the endothelial cells (Auch-Schwelk *et al*., 1989; Huie & Padmaja, 1993). Hence, these radicals could play a role in endothelium-dependent contractions in hypertension, as they do in the canine basilar artery (Katusic & Vanhoutte, 1989).

Xanthine oxidase in the presence of xanthine caused concentration-dependent, endothelium-independent contractions in the aortas from both SHR and WKY. The contractions were inhibited by allopurinol, an inhibitor of xanthine oxidase, by superoxide dismutase plus catalase or by deferoxamine, indicating that the generation of oxygen-derived free radicals is responsible for the contractions. Hydroxyl radicals are the likely mediator of these contractions since deferoxamine abolished the response, in confirmation of earlier experiments (Auch-Schwelk *et al*., 1989). The inhibitory effect of superoxide dismutase plus catalase is explained best by the scavenging of superoxide anions and hydrogen peroxide, which reduces the formation of hydroxyl radicals, although a contribution of hydrogen peroxide itself in response to xanthine oxidase cannot be excluded. Indeed, exogenous hydrogen peroxide can cause contractions in aortas from SHR and normotensive rats (Rodriguez-Martinez *et al*., 1998). The contractions evoked by oxygen-derived free radicals were augmented in aortas without endothelium of the SHR when compared to that of WKY and were inhibited by S 18886 and valeryl salicylate, but not by NS-398. The present results are in line with earlier observations (Auch-Schwelk *et al*., 1989; Hibino *et al*., 1999) indicating that the activation of the smooth muscle cyclo-oxygenase-1 is required during contractions to oxygen-derived free radicals. The production of a metabolite of arachidonic acid, possibly prostaglandin H_2 and (or) thromboxane A_2 (Auch-Schwelk *et al*., 1990; Taddei & Vanhoutte, 1993), activates TP-receptors on the vascular smooth muscle. The augmented expression of cyclo-oxygenase-1 and the hypersensitivity to prostaglandin H_2 in the vascular smooth muscle layer of SHR (Ge *et al*., 1995) then explains the larger contractions to xanthine plus xanthine oxidase. Likewise, in aortic segments from SHR and WKY, contractions to exogenous hydrogen peroxide are also mediated by a product of the cyclo-oxygenase pathway (Rodriguez-Martinez *et al*., 1998). Oxygen-derived free radicals probably activate phospholipase A_2 in the vascular smooth muscle cells, as they do in cultured endothelial cells (Harlan & Callahan, 1984; Rao *et al*., 1995), producing the substrate for cyclo-oxygenase.

Comparing the endothelium-dependent contractions to acetylcholine with the endothelium-independent contractions to oxygen-derived free radicals in the SHR aorta, it seems plausible to assume a role of oxygen-derived free radicals in the endothelium-dependent response. Indeed, these two

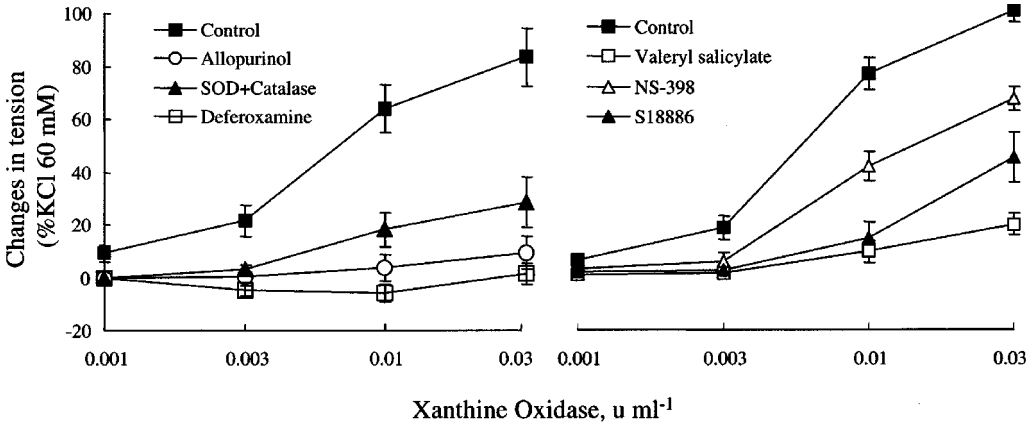

Figure 4 Contractions to xanthine oxidase in the presence of xanthine (100 μM) in aortic rings without endothelium taken from SHR (presence of N^G-nitro-L-arginine: 100 μM). Left panel: effect of allopurinol (100 μM), the combination of superoxide dismutase (SOD, 120 u ml^{-1}) plus catalase (1200 u ml^{-1}), and deferoxamine (100 μM; $n=10$). Right panel: effect of S 18886 (0.1 μM), valeryl salicylate (3 mM), and NS-398 (1 μM; $n=7$). Data are shown as means$\pm$s.e.mean. Each treatment produced a statistically significant inhibition when compared to control ($P<0.05$).

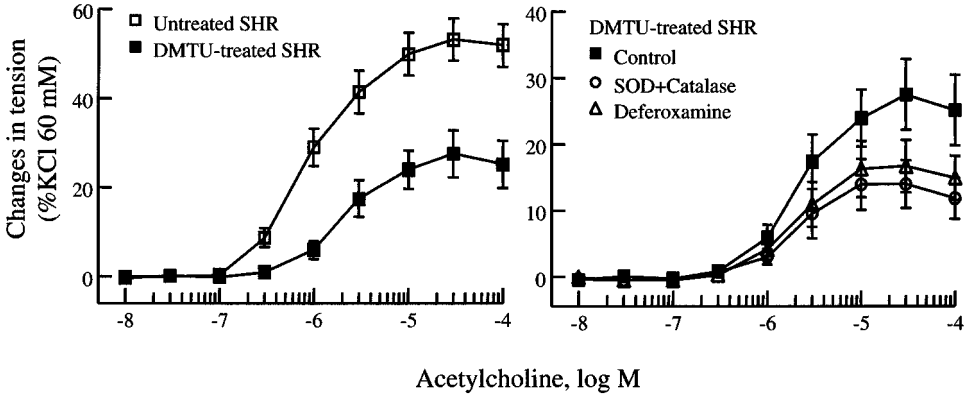

Figure 5 Chronic *in vivo* treatment with dimethylthiourea (DMTU) and endothelium-dependent contractions to acetylcholine in aortas with endothelium from SHR (in the presence of N^G-nitro-L-arginine: 100 μM). Left panel: SHR control and chronically treated *in vivo* with DMTU ($n=8$). Data are means$\pm$s.e.mean. The contraction in aortic rings taken from chronically treated SHR was significantly inhibited when compared to non-treated SHR ($P<0.05$). Right panel: SHR chronically treated *in vivo* with DMTU ($n=8$). Effects of *in vitro* treatment with superoxide dismutase (SOD, 120 u ml^{-1}) plus catalase (1200 u ml^{-1}), or deferoxamine (100 μM). Data are means$\pm$s.e.mean. Each treatment produced a statistically significant inhibition when compared to control ($P<0.05$).

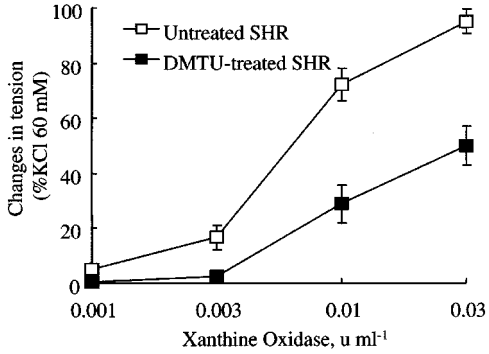

Figure 6 Contractions to oxygen-derived free radicals generated from xanthine (100 μM) plus xanthine oxidase in aortas without endothelium from SHR treated ($n=8$) or not treated ($n=7$) chronically *in vivo* with dimethylthiourea (DMTU; presence of N^G-nitro-L-arginine: 100 μM). Data are shown as means$\pm$s.e.mean. The contraction in aortic rings taken from chronically treated SHR was significantly inhibited when compared to non-treated SHR ($P<0.05$).

contractions are inhibited by the inhibition of cyclo-oxygenase-1 and the blockade of TP-receptor. However, the present study also confirms that the endothelium-dependent contractions to acetylcholine in aortas from SHR are not sensitive to the combination of superoxide dismutase plus catalase or to deferoxamine (Auch-Schwelk *et al.*, 1989). Superoxide dismutase and catalase are large molecules and may not have access to the site of production of the oxygen-derived free radicals while the ability of deferoxamine to enter cells is very poor (Paller & Hedlund, 1994). Alternatively or additionally, these drugs could be unable to sufficiently scavenge the radicals generated in the hypertensive vascular wall. Diethyldithiocarbamic acid at the concentration used is an inhibitor of endogenous Cu/Zn superoxide dismutase (Wu *et al.*, 2001), while Tiron is a cell-permeable non-enzymatic scavenger of superoxide anions (Greenstock & Miller, 1975). The specific inhibition of the endothelium-dependent contractions by diethyldithiocarbamic acid or Tiron strongly supports the hypothesis that superoxide dismutase, catalase

or deferoxamine could not gain access to the site of production of oxygen-derived free radicals. Superoxide anion is a primary oxygen-derived free radical, it can be dismutated to form hydrogen peroxide by superoxide dismutase. Metal-catalyzed interaction between hydrogen peroxide and superoxide anion produces hydroxyl radical (Fridovich, 1986). The vascular level of superoxide anion is determined by the superoxide dismutase activity and the amount of nitric oxide released by endothelial cells (Huie & Padmaja, 1993; Oury *et al*., 1996). Since the present experiments were performed in the presence of an inhibitor of nitric oxide synthase, inactivation of superoxide anion by nitric oxide can be ruled out. An increased production of superoxide anions should result in an increase in hydrogen peroxide and hydroxyl radicals and favour the occurrence of endothelium-dependent contractions as observed in present study and earlier work (Katusic & Vanhoutte, 1989). Inhibition of the endogenous Cu/Zn superoxide dismutase activity with diethyldithiocarbamic acid should reduce the formation of hydrogen peroxides and hydroxyl radicals. Tiron should effectively scavenge intracellular superoxide anions resulting in decreased generation of hydrogen peroxides and hydroxyl radicals in the vascular wall. In the presence of diethyldithiocarbamic acid, the increased level of intracellular superoxide anion, resulting from the inhibition of endogenous Cu/Zn superoxide dismutase activity (Heikkila & Cohen, 1977), was not associated with an increase in endothelium-dependent contractions. This indicates that superoxide anions *per se* are not sufficient to evoke contractions, but that they must be dismutated first into hydroxyl radicals. This then indirectly confirms the role of hydroxyl radicals in endothelium-dependent contractions. The lack of full inhibition of endothelium-dependent contractions with either diethyldithiocarbamic acid or Tiron is explained best by their incomplete intracellular action. Indeed, for example, in canine basilar arteries, even higher concentration of diethyldithiocarbamic acid only inhibits half of the total vascular superoxide dismutase activity (Wambi-Kiesse & Katusic, 1999).

To curtail the presence of oxygen-derived free radicals in the aorta from SHR, the animals were treated chronically with dimethylthiourea at a dose known to scavenge hydroxyl radicals *in vivo* and to limit their production (Mayhan & Patel, 1998; Pieper *et al*., 1996). The endothelium-dependent contractions to acetylcholine in the treated SHR were decreased significantly compared with untreated animals. Furthermore, the chronic treatment with dimethylthiourea unmasked an inhibitory effect of superoxide dismutase plus catalase or deferoxamine on the reduced endothelium-dependent contractions to acetylcholine. These findings strongly support the interpretation that oxygen-derived free radicals contribute to endothelium-dependent contractions in the SHR aorta and that hydroxyl radicals are involved. Since the *in vitro* administration of dimethylthiourea does not decrease endothelium-dependent contractions to acetylcholine in aortas from untreated control SHR, the attenuation of the response in blood vessels from treated animals can be attributed to a decreased generation of hydroxyl radicals *in vivo*. Likewise, the unmasking of an inhibitory effect of superoxide dismutase plus catalase or deferoxamine on endothelium-dependent contractions in dimethylthiourea-treated SHR indicates that the chronic limitation of the production of hydroxyl radicals allows an effective scavenging by superoxide dismutase plus catalase or deferoxamine.

Like that to acetylcholine, the responses to oxygen-derived free radicals generated by xanthine plus xanthine oxidase in aortas without endothelium from dimethylthiourea-treated SHR were reduced by the chronic treatment. This probably reflects a diminished hypersensitivity of the hypertensive smooth muscle exposed to exaggerated oxidative stress. This decrease in sensitivity of the smooth muscle to oxygen-derived free radicals following the chronic treatment with dimethylthiourea, parallels a similar effect on the response to acetylcholine, and this supports the role of oxygen-derived free radicals in endothelium-dependent contractions in the hypertensive aorta. The chronic inhibition of the production of oxygen-derived free radicals *in vivo* could result in inhibition of endothelium-dependent contractions because of either a decreased synthesis of EDCF and/or a reduced sensitivity to endothelial mediator.

In summary, the present study suggests that reactive oxygen species, and in particular hydroxyl radicals and/or hydrogen peroxide, play a key role in endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat.

This study was supported by an Educational grant from the Institut de Recherches Servier, France and by a grant from the Education Administration of Jiangsu Province, China.

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(Received November 13, 2001
Revised December 20, 2001
Accepted February 11, 2002)