Chen Qu, Susan W. S. Leung, Paul M. Vanhoutte, and Ricky Y. K. Man

Department of Pharmacology and Pharmacy, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong

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ABSTRACT

Acute inhibition of nitric-oxide synthase (NOS) unmasks the release of endothelium-derived contracting factors (EDCFs). The present study investigated whether chronic inhibition of NOS modulates endothelium-dependent contractions. Eighteen-week-old male Sprague-Dawley rats were treated by daily gavage for 6 weeks with the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (60 mg/kg) or vehicle (distilled water; 1 ml/kg). Chronic treatment with L-NAME increased arterial blood pressure. Isometric tension was measured in aortic rings with or without endothelium. Endothelium-dependent relaxations to acetylcholine and the calcium ionophore 5-(methylamino)-2-{[2R,3R,6S,8S,9R,11R]-3,9,11-trimethyl-8-[(1S)-1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl]-1,7-dioxaaspiro[5,5]undec-2-yl]methyl-4-benzoxazolocarboxylic acid (A23187) were reduced in preparations from L-NAME-treated rats. The reduction in relaxation to A23187 was partially reversed by L-arginine (1 mM). In quiescent aortic rings, A23187 caused contractions in the presence of L-NAME and intact endothelium. The A23187-induced contractions were greater in rings from the L-NAME-treated rats than in those from the control group. These contractions were abolished by the cyclooxygenase (COX)-2 inhibitor N-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide (NS-398) and the thromboxane-prostanoid (TP) receptor antagonist 3-[[6F]-6-[[4-chlorophenyl]sulfonyl]amido]-2-methyl-5,6,7,8-tetrahydrodiazepan-1-ylpropanoate (S18886), but not by the COX-1 inhibitor 5-[(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (SC-560). Chronic L-NAME treatment reduced the level of nitric oxide in the plasma but increased COX activity in the aortic rings. Western blotting and immunohistochemical staining showed that endothelial NOS expression was reduced in the aortae of the chronic L-NAME-treated group, COX-1 expression was augmented slightly, whereas COX-2 expression was up-regulated markedly. The TP receptor expression was comparable with control. These experiments demonstrate that chronic NOS inhibition increases endothelium-dependent contractions of the rat aorta by inducing COX-2 expression and augmenting the production of EDCF.

The endothelium plays an important role in the regulation of vasomotor tone through a balanced release of various vasodilators and vasoconstrictors (Furchgott and Vanhoutte, 1989; Vanhoutte et al., 2009). In hypertension, endothelial dysfunction with inappropriate release of vasoactive mediators is observed (Rubanyi 1993; Vanhoutte et al., 2009). Reduced production of endothelium-derived vasodilators, in particular nitric oxide (NO), and increased production of endothelium-derived vasoconstrictor prostanoids have been reported in many studies (Lüscher, 1990; Vanhoutte et al., 2005). Endothelium-dependent contractions in the rat aorta are augmented in the presence of Nω-nitro-l-arginine methyl ester (L-NAME), an analog of L-arginine that inhibits nitric-oxide synthases (NOSs) (Auch-Schwelk et al., 1992; Tang et al., 2005a,b). Indeed, inhibition of NOS by L-NAME results in increased production of endothelium-derived contracting factors (EDCFs), which are vasoconstrictor prostanoids pro-

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ABBREVIATIONS: NOS, nitric-oxide synthase; A23187, 5-(methylamino)-2-{[2R,3R,6S,8S,9R,11R]-3,9,11-trimethyl-8-[(1S)-1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl]-1,7-dioxaaspiro[5,5]undec-2-yl]methyl-4-benzoxazolocarboxylic acid; COX, cyclooxygenase; EDCF, endothelium-derived contracting factor; eNOS, endothelial nitric-oxide synthase; L-NAME, Nω-nitro-l-arginine methyl ester; NO, nitric oxide; NS-398, N-[2-cyclohexyloxy-4-nitrophenyl]methanesulfonamide; S18886, 3-[[6F]-6-[[4-chlorophenyl]sulfonyl]amido]-2-methyl-5,6,7,8-tetrahydrodiazepan-1-ylpropanoate; SC-560, 5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole; SHR, spontaneously hypertensive rat; TP, thromboxane-prostanoid; U46619, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F2α, PGF, prostaglandin F; TBS-T, Tris-HCl-buffered saline with 0.1% Tween 20.
duced from arachidonic acid by the enzyme cyclooxygenase (COX). EDCF's in turn activate thromboxane-prostanoid (TP) receptors on vascular smooth muscle, resulting in vasoconstriction (Auch-Schwelk et al., 1990, 1992; Yang et al., 2004a; Tang et al., 2005a).

EDCF-mediated contractions are augmented in arteries of spontaneously hypertensive rats (SHRs) (Lüscher and Vanhoutte, 1986; Yang et al., 2004a; Tang et al., 2005a). Acute and chronic inhibition of NOS causes hypertension in animals (Rees et al., 1989). The present study was designed to investigate whether hypertension resulting from the chronic inhibition of NOS by L-NAME modulates EDCF-mediated contractions of the rat aorta. When this seemed to be the case, the molecular mechanism involved was determined.

Materials and Methods

The experimental procedures used in this study followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Committee on the Use of Live Animals in Teaching and Research at the University of Hong Kong.

Animals and Experimental Design. Experiments were performed on aortae of 18-week-old male Sprague-Dawley rats obtained from the Laboratory Animal Unit of the University of Hong Kong. The animals were housed in a temperature-controlled room (21 ± 1°C) with a 12:12-h light/dark cycle and randomly divided into two groups. The treated group was given the NOS inhibitor L-NAME (50 mg/kg/day by gavage), and the control group was given vehicle (distilled water; 1 ml/kg/day, by gavage). The rats were fed a normal chow diet and had free access to tap water. After 6 weeks of treatment, rats were anesthetized with pentobarbitone sodium (70 mg/ml/kg i.p.), and their arterial blood pressure was measured through a cannula inserted into one of the carotid arteries. Arterial blood samples were collected for measuring the plasma concentration of nitrate (the metabolite of NO) detected as the amount of nitrite in the plasma.

Thoracic aortae were dissected free and placed into a modified Krebs-Ringer solution (pH 7.4) containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 25 mM NaHCO₃, and 11.1 mM glucose (control solution). The aortae were freed of connective tissue and fat, and then cut into rings of approximately 3-mm length. Care was taken during the dissecting procedure to prevent the endothelium from inadvertent damage. In some preparations, the endothelium was removed by perfusion with 1 ml of Triton X-100 solution (0.1%) for 30 s before cutting the rings. The presence or absence of relaxation to 2-(acetyloxy)-N,N,N',N'-tetramethyl-ethanaminium chloride (acetylcholine; 3 μM) was used to verify endothelial integrity or functional removal, respectively.

Isometric Tension Recording. Aortic rings were suspended by means of two l-shape stainless-steel wires inserted into the lumen in a organ chamber containing control solution kept at 37°C and continuously bubbled with a gas mixture of 95% O₂-5% CO₂. They were allowed to equilibrate for 1 1/2 h. The baseline tension was 2.5 g. Changes in isometric tension were recorded with a force transducer (MLT0201; ADInstruments Pty Ltd., Castle Hill, Australia) coupled to an octal bridge amplifier and a data acquisition system (ML119 and Powerlab model ML785, respectively; ADInstruments Pty Ltd.).

Relaxations were obtained during sustained contraction to phenylephrine (1 μM) and were expressed as percentage of that contraction. Constrictions in quiescent preparations are expressed as a percentage of a reference contraction to KCl (60 mM) obtained before the actual experiment. To elicit endothelium-dependent contractions, aortic rings with endothelium were exposed for 30 min to L-NAME (100 μM) before the application of the calcium ionophore 5-(methyloxiranyl)-2-[2R,3R,5S,7S,9R,11R]-3,9,11-trimethyl-8-[15]-1-methyl-2-oxo-2-(1H-pyrrol-2-yl)-ethyl-1,7-dioxaspiro[5.5]undec-2-yl)methyl-4-benzoazolcarboxylic acid (A23187; 0.1–10 μM). COX-1 and COX-2 inhibitors and TP receptor antagonist were administered 30 min before A23187-induced endothelium-dependent contractions. Contractions to the TP receptor agonist 9,11-dideoxy-9a,11α-methaneproxy prostaglandin F₂α (U46619; 0.1 nM to 1 μM) in the absence of endothelium were compared in rings with and without L-NAME treatment.

Measurement of Plasma Nitrate and COX Activity. The amount of nitrate (NO metabolite) in the plasma was determined by using a commercial kit (NO Assay Kit; Assay Designs, Ann Arbor, MI) according to the manufacturer's instructions. The amount of nitrate was determined by converting it to nitrite, followed by the colorimetric determination of the total concentration of nitrite as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm by using a microplate reader (Dynex MXR; Dynex Technologies, Chantilly, VA). COX activities were determined by assaying the peroxidase activity of COX colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-p-phenylenediamine at 590 nm with an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). To distinguish between COX-1 and COX-2 activities, isoenzyme-specific inhibitors [(5-(4-chlorophenyl)-1-(4-methoxyphenyl)-3-( trifluoromethyl)-1H-pyrazole (SC-560) for COX-1 and N-[2-cyclohexyloxy-4-nitrophenyl]methanesulphonamide (NS-398) for COX-2) were used in the assay (Futaki et al., 1994; Smith et al., 1998).

Prostanoids Immunoassay. The levels of 6-keto prostaglandin F₁α (6-keto PGF₁α; stable metabolite of prostacyclin), PGF₂α, and thromboxane B₂ (stable metabolite of thromboxane A₂) released into the buffer in the organ chamber by A23187-stimulated aortic rings were measured with enzyme immunoassay kits (Cayman Chemical). In brief, the buffer in the organ chamber was collected at the maximal level of aortic contraction induced by A23187 (10 μM) as described above and stored at −80°C until use (Gluisi et al., 2005). The buffer was assayed after different dilution (100× to 6-keto PGF₁α, 10× to PGF₂α, and 1× for thromboxane B₂) in triplicate and performed according to the manufacturer's instructions.

Western Blotting. The protein expressions of endothelial NOS (eNOS), COX-1, COX-2, and TP receptors were analyzed by Western blotting. Aortic rings with and without endothelium from L-NAME-treated and control rats were homogenized in a lysis buffer composed of 1 μg/ml leupeptin, 5 μg/ml aprotinin, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 mM EDTA, 1 mM NaF, and 2 mg/ml β-glycerophosphate. The lysates were centrifuged at 5000 rpm for 3 min at 4°C, and the supernatants were collected. The protein concentration was determined spectrophotometrically by using the Bradford protein assay. Homogenates containing 30 μg of protein (for COX-1 and COX-2) or 50 μg of protein (for eNOS and TP receptor) were separated electrophoretically by 7.5% SDS-polyacrylamide gel electrophoresis. Proteins were transferred onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA), which were blocked in nonfat milk (5%) in Tris-HCl-buffered saline with 0.1% Tween 20 (TBS-T). The membranes were then washed several times with TBS-T and incubated overnight at 4°C with primary antibodies against eNOS (1:2500 dilution; Transduction Laboratories, Lexington, KY), COX-1 (1:400; Cayman Chemical), COX-2 (1:200; Cayman Chemical), and TP receptors (1:100 Cayman Chemical). β-Actin was also determined and used as the reference. The primary antibodies were washed away by using TBS-T, and the membranes were incubated with the corresponding mouse and rabbit secondary antibodies (1:2000; Sigma-Aldrich, St. Louis, MO) in blocking buffer for 2 h. Immunoreactive proteins were detected by an enhanced chemiluminescence detection system (ECL reagents; GE Healthcare, Little Chalfont, Buckinghamshire, UK) and evaluated by densitometry (Bio-Rad Laboratories).
**Immunohistochemical Staining.** The localization and expression of eNOS, COX-1, and COX-2 were analyzed by immunohistochemical staining. Aorta samples were cut and prepared as paraffin-embedded sections. The sections were then dehydrated in xylene for 5 min and dehydrated in graded alcohol. The slides were immersed in H_2O (3%) for 15 min at room temperature. After blockade of nonspecific staining with phosphate-buffered saline (20 min, pH 7.3) containing bovine serum albumin (1%) and Tween 20 (1%), the slides were incubated overnight at 4°C with monoclonal goat anti-rat COX-1, COX-2, and eNOS antibodies (Sigma-Aldrich; diluted 1:400, 1:200, and 1:2500 with phosphate-buffered saline (0.01 M), respectively). The slides were then washed five times with phosphate-buffered saline and incubated at 37°C in rabbit anti-goat IgG conjugated with fluorescein isothiocyanate (1:50) for 60 min. Subsequently, antibody was washed free, and then the locations and expression of the target proteins were evaluated with a confocal microscope (LSM510; Carl Zeiss Inc., Thornwood, NY).

**Drugs.** Calcium ionophore A23187, L-NAME, and NS-398 were purchased from Sigma-Aldrich. Prostaglandin E_2 (PGE_2) and SC-560 were purchased from Cayman Chemical. The organic solvent dimethyl sulfoxide was purchased from Merck (Darmstadt, Germany). 3-((6R)-6-[(4-chlorophenyl)sulfonyl]amido)-2-methyl-5,6,7,8-tetrahydropyridazin-1-yl)propanoate (S18886; Terutroban), a selective TP receptor antagonist (Simonet et al., 1997), was a kind gift from the Institut de Recherches Servier (Suresnes, France). A23187 and NS-398 were dissolved with absolute dimethyl sulfoxide, and indomethacin was prepared in a sodium bicarbonate (1 mM) solution. All other compounds were prepared in deionized water. Concentrations are expressed as final molar concentrations in the buffer. All stock solutions were kept at −20°C.

**Data and Statistical Analysis.** Results are expressed as means ± S.E.M. for the number (n) of rats indicated. Results of eNOS, COX-1, COX-2, and TP receptor protein expression are expressed as the ratio of the optical density of these proteins to that of β-actin. Statistical analysis compared the curves obtained in the presence of the different substances with the control curves by repeated-measure two-way analysis of variance. Student’s t test for paired observation was used where appropriate. P values of less than 0.05 were considered to indicate statistically significant differences.

**Results**

**Body Weight and Arterial Blood Pressure.** The body weight of the animals subjected to L-NAME treatment (60 mg/kg/day) was not significantly different from that of the controls until day 35; after that time it decreased progressively (Supplemental Fig. 1). At the end of the treatment, both the diastolic and systolic arterial pressures were significantly higher in the chronic L-NAME-treated rats than controls until day 35; after that time it decreased progressively (Supplemental Fig. 1). At the end of the treatment, both the diastolic and systolic arterial pressures were significantly higher in the chronic L-NAME-treated rats than controls until day 35; after that time it decreased progressively (Supplemental Fig. 1).

**Endothelium-Dependent Relaxations.** After 6 weeks of L-NAME treatment, endothelium-dependent relaxations produced by the calcium ionophore A23187 in aortic ring were impaired significantly compared with those from control rats (Fig. 1A). The presence of L-arginine (1 mM, 30 min preincubation) improved the impaired relaxation of rings from L-NAME-treated rats, but not to the control level (Fig. 1A). Endothelium-independent relaxations to the NO⁺ donor sodium nitroprusside were comparable in the aortae of L-NAME-treated and control rats (Fig. 1B).

Incubation of aortic rings with SC-560 (1 μM), NS-398 (10 μM), or S18886 (100 nM) did not significantly modify A23187-induced relaxation in control aortae (Fig. 2A). In rings from chronic L-NAME-treated rats, the presence of NS-398 and S18886 significantly improved A23187-induced relaxation, whereas SC-560 failed to affect the response (Fig. 2B).

**Endothelium-Dependent Contractions.** In the presence of L-NAME (100 μM), A23187 produced endothelium-
Plasma Nitrate, COX Activity, and Prostanoids Release. After 6 weeks of L-NAME treatment, the plasma nitrate level (an indication of NO level), which was determined as the amount of nitrite in Griess assay, was significantly lower than that in the controls (Fig. 5A). There was a small, but significant, elevation in COX-1 activity after chronic L-NAME treatment. COX-2 activity was negligible in the aorta of control rats but was increased significantly in L-NAME-treated rats (Fig. 5B). The releases of 6-keto PGF$_{1α}$, PGF$_{2α}$, and thromboxane B$_2$ induced by A23187 were significantly increased after chronic administration of L-NAME compared with that in arteries of the vehicle-treated animals. The increase in production of the metabolites of arachidonic acid was abrogated by the COX-2 inhibitor NS-398 but not by the COX-1 inhibitor SC-560 (Fig. 5C).

Protein Expression. eNOS expression was reduced significantly in aortae from rats after chronic L-NAME treatment compared with the controls (Fig. 6A). COX-1 and COX-2 expression was significantly higher in aortae from L-NAME-treated rats than in control preparation. The protein expression of COX-1 and COX-2 was reduced after the removal of the endothelium (Fig. 6, B and C, respectively). The expression of TP receptors was comparable in aortae from treated and control rats (Fig. 6D).

Immunohistochemical analysis demonstrated that COX-1 and COX-2 were located mainly in the endothelium (Fig. 7, A and B). The presence of COX-1 and COX-2 was higher in the aortae after the rats were chronically treated with L-NAME (Fig. 7, A and B). Consistent with the results of Western immunoblotting, eNOS expression in the aorta from rats chronically treated with L-NAME was significantly reduced compared with the control group (Fig. 7C).

Discussion

In hypertension, the regulation of vascular tone is disturbed, resulting in increased endothelium-dependent vasoconstrictions and reduced endothelium-dependent vasodilatations (Vanhoutte et al., 2005, 2009). Endothelial dysfunction is one of the major factors leading to this impairment in vasomotor regulation during the development of hypertension. It is associated with impaired production of NO and increased release of EDCF (Mombouli and Vanhoutte, 1999; Tang et al., 2005b). NO and EDCF behave as functional antagonists, exerting opposite effects on the vascular smooth muscle. Thus, inhibitors of NOS acutely hinder the production of NO and augment endothelium-dependent contractions (Auch-Schwelk et al., 1992; Yang et al., 2004b). However, NO also negatively modulates endothelium-dependent contractions in a more prolonged manner. Indeed, rings of SHR aortae that are previously exposed to the NO$^+$ donor sodium nitroprusside or an endothelium-dependent vasodilator acetylcholine exhibit a reduced ability to produce endothelium-dependent contractions (Tang et al., 2005a). These observations led to the hypothesis that chronic deficiency in NO induced by long-term inhibition of eNOS may facilitate the release of EDCF and the occurrence of endothelium-dependent contractions and thus favor endothelial dysfunction. The main finding of the present study is that indeed chronic in vivo inhibition of NOS increases endothelium-dependent contractions in the rat aorta by augmenting the expression and activity of COX-2.

We observed that long-term inhibition of eNOS resulted in...
Fig. 5. Effect of chronic administration of L-NAME on plasma NO metabolite level (A), COX activity (B), and prostanoid or their metabolite levels (C) in aortae. Data are expressed as means ± S.E.M. of eight experiments. *, P < 0.05 compared with control group.

Fig. 6. Western blot analysis of protein expression of eNOS (A), COX-1 (B), COX-2 (C), and TP receptor (D) in aortae of control and L-NAME-treated rats. Protein expressions are expressed as the ratio to β-actin. Data are expressed as means ± S.E.M. of five experiments. *, P < 0.05, compared with control group or between +Endo and −Endo. #, P < 0.05, comparison between control and L-NAME group with endothelium. The blots are representative of five repeated experiments.
impairment of vascular relaxation. The inhibitory effect of L-NAME on NO production and vascular relaxation can be reversed by L-arginine, a substrate of NOS that can compete with L-NAME (Knowles and Moncada, 1994). In the present study, L-arginine failed to fully restore relaxation to the control level, indicating that the decrease in relaxation is not solely caused by L-NAME remaining in the aorta after chronic treatment. We also observed that the plasma NO levels as determined by measuring the metabolite of NO and aortic eNOS expression are reduced in rats chronically treated with L-NAME. These results are consistent with a chronic depression of the ability of the endothelial cells to release NO (Lüscher and Vanhoutte, 1990). When the production of NO from the endothelial cell is decreased, endothelium-dependent contractions are augmented, and endothelial dysfunction becomes established (Auch-Schwelk et al., 1992; Vanhoutte, 2003; Yang et al., 2004b).

Endothelium-mediated contractions depend on the activity of endothelial cyclooxygenase. Two isoforms of COX have been identified, and both of these heme proteins have an equal chance to oxidize arachidonic acid into prostaglandins (Garavito and DeWitt, 1999). In the SHR and Wistar Kyoto rat aorta, COX-2 is a highly inducible enzyme, whereas COX-1 is believed to be constitutively expressed in most tissues. Whereas preferential inhibitors of COX-1 abolish endothelium-dependent contractions in the aorta of the SHR (Ge et al., 1995; Yang et al., 2003, 2004a; Tang et al., 2005a), the present data suggest that COX-2 is involved in the production of EDCF in rats chronically treated with L-NAME. The difference in the subtype of COX involved may be related to the strain or species of the animal used or to underlying pathophysiology that leads to the development of endothelium-dependent contractions. Indeed, aging-induced endothelium-dependent contractions in Wistar rats, Sprague-Dawley rats, and hamsters are related to increased expression and activity of COX-2 (Heymes et al., 2000; Shi et al., 2008; Wong et al., 2009).

The present results confirm that A23187 produces concentration- and endothelium-dependent contractions in the rat aorta (Auch-Schwelk et al., 1992; Yang et al., 2004a; Gluais et al., 2006). These contractions are potentiated in the aortae of rats treated chronically with L-NAME, as they are in the aorta of the SHR (Lüscher and Vanhoutte, 1986; Yang et al., 2004a; Gluais et al., 2005).

In the present studies, Western blot analysis showed that COX-1 and COX-2 expression was increased in rings with endothelium after chronic L-NAME treatment. However, the overexpression of COX-2 in rats treated with L-NAME was to a much greater extent (approximately 5-fold compared with the control group) compared with that of COX-1. The comparison of aortae with and without endothelium allows the conclusion that COX-2 protein expression is localized mainly in the endothelium. This endothelial localization of the upregulated COX-2 was demonstrated further by immunohistochemical immunostaining. Thus endothelial COX-2 seems to represent the major isoform of the enzyme for the augmented generation of EDCF in the aortae of rats subjected to the chronic administration of L-NAME. The comparison of total and individual COX activities in the aortae before and after L-NAME administration confirmed the predominant role of COX-2, as there is a more obvious increase in COX-2 and total COX activity than in COX-1. The importance of the augmented COX-2 expression and activity in impaired relaxation and enhanced contraction was further established by the ability of a selective COX-2 inhibitor, but not of a COX-1 inhibitor, to reverse the changes in vascular responses observed in arteries from L-NAME-treated rats.

Although COX-2 expression increases under a number of pathophysiological conditions (Heymes et al., 2000; LaPointe et al., 2004; Shi et al., 2008), increased COX-1 expression may compensate for the adverse effects caused by COX-2 (Moncada 1982; Deeb et al., 2008). COX-2-mediated vasoconstriction is augmented in hypertension (Alvarez et al., 2005). In the present study, COX-1 blockade did not affect the A23187-induced EDCF-mediated contractions and did not improve the blunted A23187-induced relaxation after chronic NOS inhibition. By contrast, the presence of a COX-2 inhibitor in the organ chamber blocked the EDCF-mediated responses and attenuated the impaired relaxation in aortae from rats with chronic L-NAME treatment, suggesting that COX-2 is the isofrom responsible for the EDCF production in this experimental model, as it is in the aged hamster aorta (Wong et al., 2009). This conclusion is also validated by the changes in the production of the three major vasoconstrictors, namely prostacyclin, PGF$_{2\alpha}$, and thromboxane A$_2$. Indeed, the augmented levels of prostaglandin end-products (6-keto PGF$_{1\alpha}$, for prostacyclin and thromboxane B$_2$, for
thromboxane $A_2$ and PGF$_{2\alpha}$ in the presence of A23187 after chronic inhibition of eNOS were decreased by acute incubation with a selective COX-2 inhibitor but not by a selective COX-1 inhibitor. Although prostacyclin is generally regarded as a powerful vasodilator (Moncada 1982; Deeb et al., 2008), it has been established for many years that prostacyclin causes no relaxation in arteries of hypertensive or aged rats (e.g., Levy, 1980; Gluais et al., 2005). As such, all of the prostaglandin end-products produced by COX-2 may contribute to the endothelium-dependent contractions in the aorta of chronic l-NAME-induced hypertensive rats.

In hypertensive rat aorta, endothelium-dependent contractions ultimately are mediated by activating receptors on the vascular smooth muscle cells recognized as TP receptors, in view of the ability of TP receptor antagonist to abolish these responses (Auch-Schwelk et al., 1990; Yang et al., 2003). The results of the present experiments indicate that the expression of TP receptors at the protein level is not altered by chronic l-NAME administration, indicating that changes in their density are not a contributing mechanism in augmented endothelium-dependent contractions. A similar conclusion has been reached in the aorta of the SHR (Tang and Vanhoutte, 2008). Despite the unaltered density of TP receptors, the concentration-response curve to the TP agonist U46619 in rings without endothelium was shifted to the left compared with the control preparations. This suggests an increased sensitivity of the TP receptors of the smooth muscle cells of chronically l-NAME-treated rats, consistent with the augmented contractions to endothoperoxides observed in the SHR aorta study (Ge et al., 1995).

In addition to the conduit artery studied here, resistance blood vessels, such as small coronary arteries and mesenteric arteries, release cyclooxygenase-derived, endothelium-dependent contracting factor in experimental hypertensive animals (Lüscher et al., 1990; Kähönen et al., 1998; García-Redondo et al., 2009). The contribution of EDCF to endothelial dysfunction is also demonstrated in human studies, because endothelium-dependent vasodilatations in human hypertensive patients, but not in normotensive subjects, are improved by the inhibitor of COX, indomethacin (Taddei et al., 1993, 1997). The present findings, therefore, suggest that impairment of NO production (induced by chronic l-NAME treatment), as observed in hypertension (Vanhoutte et al., 2005, 2009), may lead to the occurrence of EDCF and hence exacerbate the initiation of vascular complications in hypertension.

In conclusion, the present study indicates that chronic NOS inhibition results in impairment of vascular relaxation and increases endothelium-dependent contractions by inducing COX-2 expression and augmenting the production of EDCF in the rat thoracic aorta.

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References


Address correspondence to: Professor Ricky Y. K. Man, Department of Pharmacology and Pharmacy, University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong. E-mail: rykman@hku.hk