Nitric oxide synthase-inhibition hypertension is associated with altered endothelial cyclooxygenase function

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Submitted 24 June 2004; accepted in final form 9 August 2004

Nitric oxide synthase-inhibition hypertension is associated with altered endothelial cyclooxygenase function. Am J Physiol Heart Circ Physiol 287: H2394–H2401, 2004.—We reported previously that endothelium-intact superior mesenteric arteries (SMA) from N-nitro-l-arginine (l-NNA)-treated hypertensive rats (LHR) contract more to norepinephrine (NE) than SMA from control rats. Others have shown that nitric oxide (NO) synthase (NOS) inhibition increases cyclooxygenase (COX) function and expression. We hypothesized that augmented vascular sensitivity to NE in LHR arteries is caused by decreased NOS-induced dilation and increased COX product-induced constriction. We observed that the EC50 for NE is lower in LHR SMA compared with control SMA (control −6.37 ± 0.04, LHR −7.89 ± 0.09 log mol/l; P < 0.05). Endothelium removal lowered the EC50 (control −7.95 ± 0.11, LHR −8.44 ± 0.13 log mol/l; P < 0.05) and increased maximum tension in control (control 1.036 ± 38 vs. 893 ± 21 mg; P < 0.05) but not LHR (928 ± 30 vs. 1,066 ± 31 mg) SMA. Thus augmented NE sensitivity in LHR SMA depends largely on decreased endothelial dilation. NOS inhibition (l-NNA, 10−4 mol/l) increased maximum tension and EC50 in control arteries but not in LHR arteries. In contrast, COX inhibition decreased maximum tension in control arteries, suggesting that COX products augment contraction. Indomethacin did not affect NE-induced contraction in l-NNA-treated or denuded arteries. In control SMA loaded with the fluorescent NO indicator 4-amino-5-methylamino-2′,7′-difuorofluorescein diacetate, indomethacin increased and L-NNA decreased NO release. Therefore, COX products appear to inhibit NO production to augment NE-induced contraction. With chronic NOS inhibition, this modulating influence is greatly diminished. Thus, in NOS-inhibition hypertension, decreased activity of both COX and NOS pathways profoundly disrupts endothelial modulation of contraction.

nepinephrine; acetylcholine; endothelial cells; vascular smooth muscle cells

Hypertension is characterized by increased vascular smooth muscle tone, which leads to increased peripheral resistance and elevated blood pressure. In disease states such as hypertension, isolated arteries respond abnormally to many stimuli and the vascular endothelium is especially prone to dysfunction. Endothelial dysfunction, the imbalanced synthesis and/or release of constrictors and dilators, is common in both animal and clinical studies of hypertension.

Impaired release of the endothelial vasodilator nitric oxide (NO) has been demonstrated repeatedly in hypertension. This signaling molecule maintains blood pressure by regulating vascular tone under normal and pathological conditions.

Indeed, NO appears requisite to prevent hypertension given that loss of endothelial NO synthase (NOS) activity genetically (41) or pharmacologically (14) causes hypertension.

In the face of NO deficiency, other endothelial dysfunctions may result that contribute to the development of hypertension. Excess production of constricting cyclooxygenase (COX) products such as PGH2 (24) and thromboxane A2 (1) have been implicated. Furthermore, COX-1 (37) and COX-2 (19) expression and activity (36) are increased in some forms of hypertension, and COX-2 inhibitors improve endothelial function and lower blood pressure in hypertensive patients (44, 46). In consequence, it has been suggested that constricting COX products augment vascular reactivity to contribute to the development of hypertension, including NOS-inhibition hypertension (44).

Alternatively, COX products may contribute to endothelial dysfunction by inhibiting NO production. In cultured endothelial cells, COX inhibition with indomethacin increases NO synthesis without affecting NOS expression (33, 45). Under certain conditions, COX can synthesize reactive oxygen species (ROS) that bind to and quench NO (43). In stroke-prone spontaneously hypertensive rats (SHRSP) (21) and in arteries from diabetic rats (47), COX inhibition augments endothelial dilation and lowers blood pressure. Therefore, both the enzymes and the products of the COX pathway can be altered in hypertension, but it is unclear whether vascular COX activity is increased or decreased in this condition. Several studies also suggest that NO directly inhibits COX activity (18, 28) or that COX products inhibit NOS activity (33). Therefore, it is also unclear how these two systems interact to modulate endothelial function and how chronic loss of NO synthesis affects COX regulation of vascular tone.

We previously reported (23) that endothelium-intact arteries from rats made hypertensive with NOS inhibition have elevated vascular smooth muscle sensitivity to both adrenergic and depolarization-induced contraction. However, it is not known whether these changes are dependent on decreased NO production, increased prostanoid synthesis, or both. In the current study, we tested the hypothesis that, in the face of inhibited NO production, endothelial COX products contribute more to adrenergic vasoconstriction in endothelium-intact arteries from N-nitro-l-arginine (l-NNA)-treated hypertensive rats (LHR) than in arteries from control rats. We compared the effect of NOS inhibition and COX inhibition on norepinephrine (NE)-induced contraction and NO production in arteries from NOS-inhibited (LHR) and control rats. We also compared the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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endothelium removal with NOS inhibition, COX inhibition, and combined inhibition. If loss of NOS activity leads to increased COX activity that augments NE-induced contraction in NOS-inhibited arteries, then inhibition of COX ex vivo should cause control and NOS-inhibited arteries to respond similarly. Furthermore, combining NOS and COX inhibition should be equivalent to removing the endothelium if these factors mediate all endothelial modulation of NE-induced contraction.

METHODS

Animals. Male Sprague-Dawley rats (200–300 g) were divided into two groups. Control rats drank tap water, whereas treated rats were made hypertensive with water containing L-NNA (0.5 g/l) as described previously (4). Systolic blood pressure was measured with the tail-cuff method (plethysmographic detection; IITC, Woodland Hills, CA). After 2-wk treatment, when blood pressure was elevated, animals were anesthetized with pentobarbital sodium (50 mg/kg) and exsanguinated. The superior mesenteric artery (SMA) above the second branch artery was rapidly removed and placed in cold physiological saline solution (PSS) containing (mmol/l) 130 NaCl, 4.7 KCl, 1.18 KH2PO4, 1.17 MgSO4, 1.17 MgSO4, 7H2O, 14.9 NaHCO3, 5.5 dextrose, 0.026 CaNa2EDTA, and 1.6 CaCl2, pH 7.4. All procedures were approved by the animal use committee at the University of New Mexico and conformed to National Institutes of Health guidelines for animal use.

Contractile studies. SMA from LHR and control rats were cleaned and cut into 3-mm segments. In some segments, endothelial cells were removed by gently rubbing the lumens with forceps tips. Artery rings were attached to force transducers and placed in tissue baths containing PSS warmed to 37°C and bubbled with 95% O2-5% CO2. Rings were stretched with 800-mg tension and equilibrated for 60 min as previously described (4). After equilibration, tissue viability was confirmed by contraction to NE (10−7 mol/l). Rings that did not contract >800 mg were excluded. Endothelial integrity was assessed by relaxation to ACh (10−6 mol/l) in NE-contracted rings. For control arteries, segments with intact endothelium were only included if ACh relaxed NE contraction >80%, whereas tissues with endothelium removed were only included if ACh relaxation was <5% of NE contraction. All LHR SMA exhibited relaxations similar to those in denuded arteries. Tissues were washed for an additional 60 min with PSS to remove NE and ACh and then exposed to cumulative concentrations of NE (10−11–10−5 mol/l) to generate baseline curves. The tissues were washed with PSS for 1 h. During the final 30 min, a COX inhibitor or its vehicle was added [indomethacin (10−6 mol/l), COX-1 inhibitor valeryl salicylate (5 × 10−5 mol/l), COX-2 inhibitor NS-398 (10−6 mol/l)]. Inhibitors were used at concentrations previously shown to be effective at selectively inhibiting COX-1 [valeryl salicylate (9), COX-2 [NS-398 (7), or both isoforms [indomethacin (48)]. A second cumulative NE curve was obtained in the continued presence of the COX inhibitor. Tissues were washed with PSS for 1 h. During the final 30 min, both COX inhibitors or vehicles were added. A third NE curve was generated in the continued presence of both COX inhibitors. The order of addition of COX inhibitors was randomized, and vehicle treatments confirmed the ability to generate three equal concentration-response curves. Sets of NE curves were generated in endothelium-intact, endothelium-denuded, and endothelium-intact L-NNA (10−4 mol/l)-treated rings.

Western blots for COX-1 and COX-2. Endothelium-intact SMA were homogenized in ice-cold homogenization buffer [50 mmol/l Tris·HCl, 10 mmol/l EDTA, 50 μg/ml PMSF, 1.6 mg/ml benzamidine, 30 μl/ml Complete protease inhibitor (Roche, Mannheim, Germany)]. Homogenates were spun for 3 min at 13,000 g at 4°C. Protein concentration of the supernatant was assayed by the Lowry method (Pierce, Rockford, IL). Proteins were separated in 7.5% polyacrylamide gels by electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were incubated overnight with a monoclonal COX-1 or COX-2 antibody (1:1,000; Cayman Chemicals, Ann Arbor, MI), followed by horseradish peroxidase-linked secondary antibody (1:5,000; Sigma, St. Louis, MO). Enhanced chemiluminescence (Amersham, Piscataway, NJ) was used to visualize labeled proteins. Coomassie blue staining was used to evaluate protein loading, and optical density of immuno-reactive bands was divided by the optical density of stained bands for each lane. Density of digitized images was calculated with Sigma Gel (Jandel Scientific).

NO measurements. NO production in endothelium-intact SMA was measured with the fluorescent indicator 4-aminophenylmethylnitrobenzofurazan-2,7'-difluorofluorescein diacetate (DAF-FM; Molecular Probes, Eugene, OR). DAF-FM is a nonfluorescent, cell-permeant molecule that becomes weakly fluorescent and cell-impermeant when cleaved by intracellular esterases. On NO binding, a benzotriazole derivative is formed and fluorescence increases proportionally to NO concentration and reversibly. Thus steady NO production produces a linear increase in fluorescence with a slope that is proportional to the rate of NO production. Immediately before loading, DAF-FM was mixed in DMEM and diluted with HEPES-buffered PSS to a final concentration of 5 × 10−6 mol/l. Segments of SMA were incubated with DAF-FM for 45 min at 37°C in the dark and then washed with PSS for 15 min to allow complete deesterification of the dye. The vessel chamber was transferred to the stage of a Nikon Diaphot 300 microscope equipped with a ×10 Nikon fluorescence objective (numerical aperture 0.30). Fluorescent images were obtained with a standard FITC filter before the vessel was loaded with dye to allow background subtraction and after loading with DAF-FM to measure basal NO release. All studies were conducted in the dark. Images were generated with a cooled digital charge-coupled device camera (SenSys 1400) and processed with MetaFluor 4.5 software (Universal Imaging). Images were taken every 3 min for 15 min, and the slope of fluorescence over time was used to measure NO release. Fluorescence was recorded in the presence of vehicle, L-NNA (10−4 mol/l, 30 min), or indomethacin (10−6 mol/l, 30 min). Normalized fluorescence intensity is defined as average color scale values for all pixels in the field above background and is expressed as the slope of fluorescence over time.

Data analysis and statistics. Data are reported as means ± SE and were analyzed by two-way or three-way ANOVA with Student-Newman-Keuls post hoc test for significance. NO production and EC50 values were compared with one-way ANOVA (for more than 2 groups) or t-tests. EC50 was calculated with SigmaPlot (Jandel Scientific). Differences were considered significant at P < 0.05, and n represents number of tissues from individual rats.

RESULTS

Adrenergic contraction is augmented in mesenteric arteries from LHR. Our previous studies (23) demonstrated that arteries from LHR contract more to adrenergic agonists than control arteries. We report here that endothelium-intact mesenteric arteries from LHR are also more sensitive to NE than arteries from control rats (EC50: control = 6.37 ± 0.04, LHR = 7.89 ± 0.09 log mol/l; P < 0.05; Fig. 1A). Removing the endothelium shifted the NE response leftward only in control arteries (Fig. 1B), similar to previous observations in aorta (23). These data suggest that endothelial factors oppose NE contraction only in control SMA. Removing the endothelium did not increase maximum tone and, if anything, decreased it in NOS-inhibited arteries. These results suggest that endothelial factors counter NE-induced contraction in control arteries but may augment it in NOS-inhibited arteries.

NO production. To determine whether COX inhibition indeed regulates NO production in SMA, the NO-sensitive indicator dye DAF-FM was used to evaluate NO release from
decreased NO production compared with vehicle-treated arteries, whereas indomethacin (10^{-6} mol/l) attenuated NE-induced contraction (540 \pm 61 vs. 1,102 \pm 56 mg; P < 0.05; Fig. 3A). In the presence of L-NNA, however, indomethacin increased NE-induced contraction above that in vehicle-treated arteries and above that in L-NNA-treated arteries. Thus COX inhibition reduces NE-induced contraction when NOS is active but augments contractions when NOS is inactive, suggesting that COX products both inhibit NOS and act as direct vasoconstrictors. Because combining L-NNA and indomethacin increased maximum tension above that in either denuded control arteries or LHR arteries, other endothelial factors may augment NE-induced contraction in control SMA. L-NNA and indomethacin did not affect NE responses in denuded arteries (maximum tension data).

In LHR arteries, L-NNA did not affect NE-induced contraction (Fig. 3B). In addition, even in the presence of L-NNA, LHR arteries were more sensitive to NE than control arteries (EC50 control \(-7.64 \pm 0.10\), LHR \(-8.20 \pm 0.20\) log mol/l; \(P < 0.05\)). Indomethacin inhibited NE-induced constriction in LHR arteries but less so than in control arteries (Fig. 3B) compared with Fig. 3A: 878 \pm 92 vs. 1,096 \pm 40 mg; \(P < 0.05\).

Effects of NOS and COX inhibition on NE-induced contraction in endothelium-intact SMA. L-NNA (10^{-4} mol/l) was used to inhibit NOS and evaluate endogenous NO modulation of contraction, whereas indomethacin (10^{-6} mol/l) was used to evaluate COX product modulation of contraction. In control arteries, L-NNA potentiated NE-induced contraction, whereas indomethacin (10^{-6} mol/l) attenuated NE-induced contraction (540 \pm 61 vs. 1,102 \pm 56 mg; P < 0.05; Fig. 3A). In the presence of L-NNA, however, indomethacin increased NE-induced contraction above that in vehicle-treated arteries and above that in L-NNA-treated arteries. Thus COX inhibition reduces NE-induced contraction when NOS is active but augments contractions when NOS is inactive, suggesting that COX products both inhibit NOS and act as direct vasoconstrictors. Because combining L-NNA and indomethacin increased maximum tension above that in either denuded control arteries or LHR arteries, other endothelial factors may augment NE-induced contraction in control SMA. L-NNA and indomethacin did not affect NE responses in denuded arteries (maximum tension data).

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In the presence of L-NNA, indomethacin augmented contraction and shifted the response curves to the right (EC50: LHR \(-7.75 \pm 0.10\) vs. L-NNA alone \(-8.20 \pm 0.20\) log mol/l; Fig. 3), suggesting again that NOS inhibition unmasks the presence of a dilator COX product. As in control SMA, indomethacin and L-NNA did not affect NE responses in denuded arteries (data not shown). These data suggest that endothelial COX product(s) augment NE-induced contraction by inhibiting NOS and inhibit contraction via direct relaxation.

Selective inhibition of COX-1. COX-1- or COX-2-selective inhibitors were next used to evaluate the isoform mediating these responses. In control arteries, COX-1 inhibition with valeryl salicylate \((5 \times 10^{-5}\) mol/l) had no affect on NE-induced contraction in the absence of L-NNA and caused an increase in contraction in the presence of L-NNA (Fig. 4A). These data suggest that COX-1 products do not contribute to the apparent inhibition of NOS in SMA but do cause relaxation in control arteries.

In LHR SMA, valeryl salicylate produced effects similar to indomethacin. Thus NE-induced contraction was decreased in vehicle-treated intact arteries. However, in the presence of L-NNA, valeryl salicylate did not affect NE-induced contraction in LHR SMA (Fig. 4B).

Fig. 4. COX-1 inhibition on NE-induced contraction. A: effects of valeryl salicylate (Val, \(5 \times 10^{-5}\) mol/l) on NE-induced contraction in control SMA. Val had no effect on NE-induced contraction in control SMA \((n = 5)\). As expected, NE-induced contraction was augmented in the presence of L-NNA \((10^{-4}\) mol/l, \(n = 9\)) and further augmented when valeryl salicylate was added in the presence of L-NNA \((n = 5)\). These data suggest that COX-1 products do not participate in NE-induced contraction in control SMA but cause dilation when NO synthesis is eliminated. B: effects of valeryl salicylate on NE-induced contraction in LHR SMA. Valeryl salicylate \((5 \times 10^{-5}\) mol/l) attenuated NE-induced contraction in LHR SMA \((n = 5)\). L-NNA \((10^{-4}\) mol/l) or L-NNA + valeryl salicylate combined with Val did not affect contraction \((n = 5)\). Because NE-induced contraction of LHR arteries in the presence of valeryl salicylate or L-NNA + valeryl salicylate resembled that in untreated or valeryl salicylate-treated controls, COX-1 products may contribute to increased NE sensitivity in LHR SMA. *Difference from vehicle treated at \(P < 0.05\).

Valeryl salicylate did not affect contraction in denuded arteries in either group (data not shown). Therefore, COX-1 products appear to inhibit residual NOS activity in LHR arteries and to act as vasodilators in control arteries.

Selective inhibition of COX-2. COX-2 inhibition with NS-398 \((10^{-6}\) mol/l) decreased NE-induced contraction in endothelium-intact arteries from both groups but much more in control than LHR (Fig. 5). In the presence of L-NNA, NS-398 did not affect NE-induced contraction (Fig. 5). NS-398 attenuated NE-induced contraction less in LHR SMA than in control SMA. Therefore, COX-2 products may augment contraction by inhibiting NOS.

Effects of combined COX-1 and COX-2 inhibition. In control arteries, inhibiting both COX-1 and COX-2 in the absence of L-NNA inhibited contraction similar to that seen with indomethacin or NS-398 alone, supporting the conclusion that COX-2 metabolites inhibit NO production. In the presence of L-NNA, the combined inhibition was similar to that seen with either indomethacin or valeryl salicylate, suggesting that COX-1 products cause dilation (Fig. 6A).

In LHR SMA, the antagonists additively inhibited contraction (Fig. 6B). These data suggest that COX-2 products either directly or indirectly augment NE-induced contraction in LHR
arteries whereas they do not in control SMA. Neither inhibitor affected contraction in L-NNA-treated LHR arteries (Fig. 6B). Therefore, endothelium-dependent relaxation is absent in LHR arteries. There was no effect in any of the denuded arteries (data not shown).

COX Western blots. To determine whether the apparent increased affect of COX-2 inhibition and decreased effect of COX-1 in LHR arteries were caused by altered expression, Western blot analysis was performed for COX-1 and COX-2. A single 70-kDa immunoreactive band was present in both LHR and control SMA homogenates probed with an antibody against either COX-1 or COX-2 (Fig. 7). Expression of the COX-1 protein was not different between groups (normalized densitometric values: LHR 2.60 ± 0.51, control 2.7 ± 0.64; n = 5; Fig. 7A). However, expression of immunoreactive COX-2 was significantly decreased in LHR arteries (normalized densitometric values: LHR 2.90 ± 0.32, control 4.81 ± 0.72; n = 5; Fig. 7B). These data suggest that chronic NOS inhibition hypertension decreases COX-2 expression so that diminished effects of NS-398 on NE-induced contraction in LHR arteries may be due to both decreased COX-2 activation and decreased NOS activity (Fig. 8). In contrast, differences in response to COX-1 inhibition in control and LHR arteries are not due to changes in expression.

Fig. 6. Combined COX-1 and COX-2 inhibition on NE-induced contraction. A: effects of the combination of valeryl salicylate and NS-398 on NE-induced contraction in control SMA. Treating tissues with both COX antagonists attenuated NE-induced contraction in control SMA (n = 5), similar to treating with NS-398 alone. These data suggest that only COX-2 metabolites contribute to NE-induced contraction. As expected, NE-induced contraction was augmented in the presence of L-NNA (n = 9), whereas adding both COX antagonists in the presence of L-NNA augmented contraction similar to that seen with valeryl salicylate alone (n = 5). These data suggest that COX-1 products act as dilators in SMA, but this effect is masked by NO production. Furthermore, COX-1 and COX-2 products modulate NE-induced contraction very differently. B: effects of valeryl salicylate and NS-398 on NE-induced contraction in LHR SMA. Addition of valeryl salicylate (5 × 10⁻⁷ mol/l) and NS-398 (10⁻⁶ mol/l) attenuated NE-induced contraction in LHR SMA (n = 5) more than either antagonist alone, suggesting that products of both isoforms contribute to NE-induced contraction in LHR SMA. The addition of L-NNA or L-NNA combined with COX inhibitors did not affect contraction (n = 5). *Difference from vehicle at P < 0.05.

Fig. 7. COX-1 and COX-2 expression. A: COX-1 immunoreactive protein detected by Western blotting (~70 kDa) in homogenized control and LHR SMA. COX-1 expression was not different between groups (n = 4). B: COX-2 immunoreactive protein as detected by Western blotting (~73 kDa) in homogenates of control and LHR SMA was significantly decreased in LHR SMA (n = 5). *Difference from control at P < 0.05.

Fig. 8. Proposed interactions between COX and NOS in vascular endothelial cells. Our data and previous observations suggest that endothelial cell COX-1 products are primarily vasodilatory (30), whereas COX-2 products are constricting (7, 20). Our results, together with previous studies, further suggest that NO upregulates COX-2 expression (8) whereas COX-2 products decrease both expression and activity of NOS (6). Thus NOS inhibition modulates vascular smooth muscle contraction both by removing the dilatory influence of NO, decreasing COX-2 expression, and by limiting COX-2 efficacy by eliminating a primary target, active NOS. These two pathways appear to interact in a complex way to maintain vascular tone under normal conditions so that perturbations in the system have multiple results in endothelial function.
DISCUSSION

This study addresses the cellular mechanism of endothelial dysfunction in arteries from NOS-inhibited rats and evaluates potential interactions between NOS and COX products to modulate NE-induced contraction. The major findings are that COX-2 inhibition diminishes NE-induced contraction when NOS is active and increases NO release from endothelium-intact SMA. Thus a COX-2 product appears to inhibit NOS. Second, in control endothelium-intact arteries, COX-1 inhibition augments NE-induced contraction when NOS is inhibited but does not affect contraction when NOS is active, suggesting that COX-1 produces a vasodilator that is masked by basal NO production. In LHR arteries, COX-1 inhibition only inhibits contraction. Thus production of endothelial COX-1 vasodilator products is diminished in NOS-inhibition hypertension. Finally, removing the endothelium augments contraction more than combined inhibition of NOS and COX, suggesting that a non-COX, non-NOS endothelial factor augments NE-induced contraction. Given that all three of these effects are diminished in LHR arteries, endothelial modulation of NE-induced contraction is dramatically reduced when NOS activity is chronically reduced. Furthermore, COX products appear to both regulate and be regulated by NO, as illustrated in Fig. 8.

In addition to examining interactions between COX and NOS products, a primary goal of this study was to determine whether endothelial COX products contribute to the augmented vascular response to NE in LHR arteries. We observed that endothelium-intact LHR arteries are more sensitive to NE than control rings, whereas endothelium-denuded arteries only have a modest increase in sensitivity. These data suggest that endothelial factors play a major role in augmenting NE responses in LHR arteries. Importantly, our data also suggest that endothelium-derived COX products augment NE-induced contraction by decreasing NO-induced dilation in healthy arteries, but this response is diminished in arteries from hypertensive rats. Thus NOS inhibition evx vivo augments contraction more in control arteries than in LHR arteries. Other studies have similarly demonstrated compensatory changes when NOS is chronically inhibited. For example, endothelial NOS knockout animals express more neuronal NOS in pial artery vascular smooth muscle (34) and have augmented NOS-independent flow-mediated dilation in coronary arterioles (16). N^G-nitro-L-arginine methyl ester (L-NAME)-treated rats have augmented dilator responses to adenosine and isoproterenol (31). Therefore, loss of NO appears to cause compensatory changes to offset the resultant augmented vasoconstriction.

To determine how much of the augmented NE-induced contraction in endothelium-intact LHR arteries was due solely to loss of NO, NOS was inhibited evx vivo in control and LHR arteries. This caused control arteries to contract more than both LHR arteries and denuded control arteries, suggesting that NO is not the only endothelial product affecting NE-induced contraction. In LHR arteries, treatment in the tissue bath with L-NAME (100 μM) did not affect NE-induced contraction, suggesting the in vivo treatment is still effectively inhibiting NOS in these arteries. However, even in L-NAME-treated arteries, there was a significantly lower threshold of contraction in LHR compared with control SMA. Thus, in LHR arteries, loss of NO is a major factor in the augmented contraction to NE but does not fully explain the altered endothelial function.

One of the apparent differences is the altered role of endothelial COX-2 products. Indeed, COX-2-selective inhibitor NS-398 inhibited NE-induced contraction in LHR arteries but augmented it in control arteries. Therefore, different COX-2 products are evidently produced in LHR arteries. This is similar to previous observations that endothelial production of constrictor COX constrictors is augmented in hypertension (13, 15). In previous studies of the effects of COX products in the vasculature, COX-2 inhibition improved endothelial dilation in diseased arteries (44, 46). This was attributed to blocked synthesis of contractor prostanooids. However, studies in cultured endothelial cells demonstrate that indomethacin augments NO synthesis (10, 45), suggesting that COX products inhibit NO production.

In the current studies, observations that indomethacin and NS-398 inhibit contraction only when NOS are active are consistent with this possibility. DAF-FM measurements of indomethacin-induced increases in NO synthesis in endothelium-intact SMA segments also suggest that COX products inhibit NO synthesis. Thus it appears that earlier observations of COX-2 inhibitors improving endothelial dilation in coronary arteries (7) and lowering blood pressure in hypertensive subjects (44, 46) might have been caused, at least in part, by inhibition of NO synthesis.

We also observed that augmented NE-induced contraction resulting from loss of NO synthesis is partially offset by diminished COX-2 expression and function. Western blot analysis of mesenteric artery homogenates revealed that COX-2 expression in LHR arteries was about half of that in control arteries. This is similar to a previous study in SHRSP aorta in which diminished vascular expression of COX-2 was reported (21) but is in contrast to a report that thoracic aorta from male Sprague-Dawley rats made hypertensive with 21 days of L-NAME treatment have increased COX-2 expression (44). In this previous study in L-NAME-treated Sprague-Dawley rats, NOS inhibition was of longer duration and the treated rats had significant weight loss and signs of renal failure. Therefore, the upregulation of renal and aortic COX-2 in those animals may have been a secondary response to the renal failure in the L-NAME group. Of interest, the COX-2 inhibitor NS-398 lowered blood pressure and prevented the development of renal failure in this study of L-NAME hypertension. This is in agreement with our observation that NS-398 inhibits NE-induced constriction in isolated mesenteric arteries and with other reports that COX-2 inhibition lowers blood pressure in hypertension (44, 46). Thus COX-2 products consistently appear to augment or cause vasoconstriction. Although this effect is not consistently altered in hypertension, blockade of COX-2 does consistently lower blood pressure and improve endothelial dilation.

In contrast, COX-1 products appear to function primarily as vasodilators in healthy arteries. However, in LHR arteries this vasodilator response is gone and COX-1 products appear to augment NE-induced constriction. Thus decreased NO-induced dilation and increased COX-1-induced constriction act together to augment NE-induced constriction in LHR arteries, whereas diminished COX-2 expression and activity counter these effects. These results suggest that NOS and COX products normally interact to modulate smooth muscle contraction. However, in hypertension and other disease states these pathways do not function normally, contributing to increased vas-
cular reactivity (11, 47). Therefore, the adaptive changes of diminished COX-2 expression observed in the current study may be common in cases of NO impairment.

Combining NOS inhibition with COX inhibition did not diminish NE-induced contraction compared with NOS inhibition alone in either control or LHR arteries. Rather, contraction was modestly augmented, suggesting that COX-1 products inhibit contraction. This would be consistent with the production of a vasodilator such as prostacyclin, which has been established as a COX-1 (2, 38) product.

One potential mechanism for COX inhibition of NO production is via synthesis of ROS. ROS can inactivate NO to reduce dilation and bioavailability (12, 22). ROS have previously been implicated in the vascular pathology of hypertension (32, 40), and multiple studies demonstrate that ROS production is increased in arteries from hypertensive rats (17, 29). Under certain conditions, both the NOS enzymes (29) and COX products (39, 49) can synthesize ROS and inactivate NO (25, 42). Therefore, inhibition of COX appears to directly or indirectly increase bioavailable NO, as suggested by the observation that indomethacin increases NO-induced dilation and NO generation in isolated artery segments (Fig. 8).

Perspectives

The current study shows that NE-induced contraction in mesenteric arteries from t-NAME-treated rats is augmented in part by altered endothelial cell function. This may contribute to augmented contractile sensitivity and elevated peripheral resistance in NOS-inhibition hypertension. In LHR arteries, endothelial dysfunction includes loss of NOS activity as expected, which may be exerting direct or indirect effects. Our results further suggest that COX-1 products relax vascular smooth muscle whereas COX-2 products augment contraction, in part through inhibiting NO-induced dilation. Chronic decrease in NO production diminishes these buffering effects so that the ability to regulate local tone is greatly diminished by the loss of both NO and these modulating COX influences.

ACKNOWLEDGMENTS

The authors give special thanks to Pam Allgood for expert technical assistance.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-03852, an American Heart Association (AHA) Desert Mountain Affiliate Grant-in-aid, and a Research Allocation Committee grant from the University of New Mexico. N. L. Kanagy is an established investigator of the AHA.

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