Dihydrotestosterone alters cyclooxygenase-2 levels in human coronary artery smooth muscle cells

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Submitted 18 November 2009; accepted in final form 20 January 2010

Osterlund KL, Handa RJ, Gonzales RJ. Dihydrotestosterone alters cyclooxygenase-2 levels in human coronary artery smooth muscle cells. Am J Physiol Endocrinol Metab 298: E838–E845, 2010. First published January 26, 2010; doi:10.1152/ajpendo.00693.2009.—Both protective and nonprotective effects of androgens on the cardiovascular system have been reported. Our previous studies show that the potent androgen receptor (AR) agonist dihydrotestosterone (DHT) increases levels of the vascular inflammatory mediator cyclooxygenase (COX)-2 in rodent cerebral arteries independent of an inflammatory stimulus. Little is known about the effects of androgens on inflammation in human vascular tissues. Therefore, we tested the hypothesis that DHT alters COX-2 levels in the absence and presence of induced inflammation in primary human coronary artery smooth muscle cells (HCASMC). Furthermore, we tested the ancillary hypothesis that DHT’s effects on COX-2 levels are AR-dependent. Cells were treated with DHT (10 nm) or vehicle for 6 h in the presence or absence of LPS or IL-1β. Similar to previous observations in rodent arteries, in HCASMC, DHT alone increased COX-2 levels compared with vehicle. This effect of DHT was attenuated in the presence of the AR antagonist bicalutamide. Conversely, in the presence of LPS or IL-1β, increases in COX-2 were attenuated by cotreatment with DHT. Bicalutamide did not affect this response, suggesting that DHT-induced decreases in COX-2 levels occur independent of AR stimulation. Thus we conclude that DHT differentially influences COX-2 levels under physiological and pathophysiological conditions in HCASMC. This effect of DHT on COX-2 involves AR-dependent and independent mechanisms, depending on the physiological state of the cell.

vacular smooth muscle; interleukin-1β; lipopolysaccharide; inflammation; androgen

PRECLINICAL AND CLINICAL RESEARCH suggests that vascular inflammation is a critical regulator in the etiology and progression of cardiovascular disease that, if not treated or managed, can eventually lead to fatal clinical end points, such as stroke and myocardial infarction. Clinical data consistently show that cardiovascular disease is more prevalent in men than in premenopausal women (39). Numerous studies have focused on the role of estrogens in contributing to the sex-related disparities in cardiovascular disease, but the cardiovascular actions of androgens are not well understood. Among the few documented studies, there is disagreement as to whether androgens exacerbate or beneficially contribute to the development and progression of cardiovascular disease. Because chronic vascular inflammation contributes significantly to the pathogenesis of cardiovascular diseases (13, 31, 37), it is important to investigate the role of androgens under normal and pathophysiological conditions.

During vascular inflammation, monocytes and macrophages penetrate the vessel wall and produce inflammatory cytokines (34), such as interleukin-1β (IL-1β), leading to activation of NF-κB, a transcription factor for inflammatory mediators such as cytokines, VCAM-1, cyclooxygenase (COX)-2, and inducible nitric oxide synthase (30). Vascular inflammation reduces the stability of atherosclerotic plaques (13, 31, 37), thus increasing the probability of a thrombotic event, such as myocardial infarction or stroke. In this study, we use COX-2 as a marker for vascular inflammation. Two major isoforms of COX have been described, COX-1 and COX-2. COX-1 is constitutively expressed in most cell types and is involved in normal physiological responses. COX-2 is the inducible isoform that is minimally expressed in vascular tissue under normal conditions but, on induction, can play an important role in mediating inflammation (23). COX-1 and COX-2 are responsible for converting arachidonic acid to PGH2, which can be converted to an array of prostanooids. Production of these prostanooids can lead to many biological effects, including vasodilation, vasoconstriction, platelet aggregation, anti-platelet aggregation, and apoptosis (19).

Our previous studies demonstrated that dihydrotestosterone (DHT) increases COX-2 levels in rodent cerebral arteries in the absence of induced inflammation (14). Although much of the work regarding androgens and vascular inflammation has been conducted in endothelial cells (8, 16, 24, 25, 27), there are few reports of the effects of androgens on inflammation in vascular smooth muscle (32). We have hypothesized that androgens can increase COX-2 expression in the absence and presence of induced inflammation in human coronary artery smooth muscle cells (HCASMC) grown in vitro. Furthermore, we hypothesized that DHT’s effects on COX-2 protein levels would be androgen receptor (AR)-dependent. This study focuses on the effects of DHT on the inflammatory mediator COX-2 because of the significant contribution of COX-2 to the inflammatory response in vascular tissue (35) and because COX-2 has been implicated in vascular diseases (33).

MATERIALS AND METHODS

Cell culture and hormone/drug treatment. Primary HCASMC (Cascade Biologies, Portland, OR) were grown in a 5% CO2-95% room air atmosphere at 37°C in RPM 231 medium (Cascade Biologies) supplemented with smooth muscle growth supplement (Cascade Biologies) containing 5% FBS. Primary human brain microvascular endothelial cells (HBMEC; Applied Cell Biology Research Institute, Kirkland, WA) were used in some of the studies for comparison. HBMEC were cultured in Cell Systems Complete medium containing 10% FBS.

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Hormone/drug treatments were performed on cells at 70–80% confluency and at passage 5 or 6. Cell treatments were carried out in hormone-free medium supplemented with charcoal-stripped FBS (Cocalico Biologicals, Reamstown, PA). A cytokine or endotoxin exposure time course was determined in HCASMC by treatment with IL-1β (5 ng/ml), LPS (100 μg/ml), or vehicle (100 μM PBS) for 3, 6, 9, or 12 h. Since LPS and IL-1β resulted in a significant increase in COX-2 levels at 3, 6, and 9 h and 4 h of DHT treatment has been shown to induce significant changes in COX-2 mRNA level in human endothelial cells (27), the 6-h time point was selected for subsequent studies of the effects of DHT treatment on COX-2 protein expression in the absence or presence of IL-1β- or LPS-induced inflammation. Furthermore, 6 h would also allow adequate time for DHT to exert its effects while remaining within the peak 3- to 9-h window for cytokine- or endotoxin-induced COX-2 expression. Depending on the experiment, cells were treated with DHT (1, 5, or 10 nM) or vehicle (0.001% ethanol + 100 μM PBS) for 6 h in the absence or presence of IL-1β (5 ng/ml) or LPS (100 μg/ml). A separate set of dose-response experiments was conducted to determine the optimal dose of the AR antagonist bicalutamide (100 nM, 1 μM, or 10 μM) for inhibition of DHT’s effect on COX-2 and AR expression. Increases in COX-2 and AR expression in response to 10 nM DHT were inhibited equally by 1 and 10 μM bicalutamide, whereas 100 nM bicalutamide was ineffective (data not shown). Therefore, the lowest effective concentration of bicalutamide of 1 μM was selected for subsequent studies. HCASMC were pretreated for 1 h with the AR-antagonist bicalutamide (1 μM, dissolved in DMSO) and then cotreated for 6 h with bicalutamide including vehicle (0.001% ethanol + 0.01% DMSO), DHT, or DHT + IL-1β.

Immunocytochemical labeling for AR. Immunocytochemistry was used to verify AR expression in HCASMC and HBMEC. Cells were plated on glass coverslips precoated with poly-L-lysine. Culture medium was replaced with medium containing 5% (HCASMC) or 10% (HBMEC) charcoal-stripped FBS containing DHT (10 nM) or vehicle (0.001% ethanol). Cells were grown for an additional 4 h and then fixed (4% formaldehyde), washed in PBS (pH 7.4), and permeabilized in 1% Triton X-100. Cells were incubated in 1% BSA in PBS to block nonspecific binding, incubated with anti-AR (catalog no. N20, Santa Cruz Biotechnology, Santa Cruz, CA; 1:5,000 dilution) for 1 h in PBS containing 1% BSA, PBS (5 times for 5 min each), incubated with Cy3 goat anti-mouse secondary antibody (Invitrogen, Carlsbad, CA; 1:5,000 dilution) for 1 h in PBS containing 1% BSA, and washed in PBS (5 times for 5 min each). Coverslips were mounted on glass slides, with mounting medium containing 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) used to label nuclei. Cells were visualized using a confocal microscope (model 710, Carl Zeiss International, Heidelberg, Germany). Omission of the primary antibody removed the fluorescence signal and was used to determine specificity of binding (data not shown).

Western blotting for COX-1 and COX-2. Levels of COX-1 and COX-2 protein were examined using standard immuno blotting methods. Cells were rinsed twice with ice-cold PBS containing 100 μM sodium orthovanadate, scraped from the flask over ice, and centrifuged (Sorval Legend RT+, Thermo Fisher Scientific, Waltham, MA) at 800 g for 10 min. The pellet was resuspended in ice-cold lysis buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 1 mM EGTA, 1 mM EDTA, and 1 mM DTT) containing protease inhibitors (20 μM PCMB, 20 μM leupeptin, 0.1 μM aprotinin, and 0.1 mM PMSF), homogenized, sonicated, and centrifuged (Accuspin Micro 17R, Thermo Fisher Scientific) at 4,500 g for 10 min at 4°C, and lysate was collected. Total protein content of whole cell lysate was determined using a bichoninic acid protein assay kit (Thermo Fisher Scientific) and measured on a MultiSkan Spectrum using SkanIt RE software (Thermo Fisher Scientific). Next, samples were diluted in Tris-glycine SDS sample buffer (Invitrogen) and boiled for 5 min. Two-color fluorescent standard (LI-CORE Biosciences, Lincoln, NE) and diluted samples were loaded into 7.5% Smart Gels (LI-CORE). Proteins were separated via SDS-PAGE in Smart Gel running buffer (LI-CORE) at 145 V using a Mini PROTEAN Tetra electrophoresis system (Bio-Rad Laboratories, Hercules, CA). Separated proteins were transferred to nitrocellulose membranes, and nonspecific binding was blocked by incubation at room temperature for 30 min in PBS containing 1% Tween (TPBS) and 3% dry milk. Membranes were incubated in COX-2 (1:1,000 dilution) or COX-1 (1:400 dilution) monoclonal antibodies (Cayman Chemical, Ann Arbor, MI) overnight at 4°C in TPBS with 3% dry milk. After TPBS washes (5 times for 5 min each), the membranes were incubated in goat anti-mouse IR 800 dye secondary antibody (LI-CORE) for 1 h at room temperature. COX-2 antibody specificity was verified with LPS-stimulated RAW 264.7 (mouse macrophage) cell lysate (Santa Cruz Biotechnology), which is a positive control for COX-2 protein (data not shown). After additional PBS washes (5 times for 5 min each), proteins were visualized using an Odyssey infrared imager, and data were analyzed using Odyssey version 3.0 software (LI-CORE). After they were imaged, all blots were stained with Coomassie Brilliant Blue (Bio-Rad) for verification of equal loading of protein.

PGE2 measurements. PGE2, a downstream metabolite of COX-2, was measured in HCASMC incubation medium after hormone/drug treatment using a PGE2 monoclonal enzyme immunoassay kit (Cayman Chemical). The enzyme immunoassay was performed according to the manufacturer’s instructions and measured on a MultiSkan Spectrum using SkanIt RE software (Thermo Fisher Scientific).

Reagents. All reagents were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise noted.

Statistical analysis. Samples from each treatment were run on the same Western blot or 96-well plate (PGE2 assay) for direct comparison, and treatments were repeated for statistical analysis (n = 4 – 24). Data from Western blots are expressed as an optical density ratio relative to vehicle. Values are means ± SE. Data were compared using ANOVA, and differences were compared using post hoc (Student-Newman-Keuls) tests (Prism Software, Irvine, CA). P < 0.05 was considered significant.

RESULTS

ARs are present in HCASMC and HBMEC. Using immunohistochemistry, we detected AR immunoreactivity in the cytoplasmic and nuclear compartments in HCASMC and HBMEC. A representative confocal image of HCASMC is shown in Fig. 1A. Since AR activation has previously been shown to increase AR expression (15), AR protein levels were measured by Western blotting after treatment with vehicle or increasing concentrations (1, 5, and 10 nM) of DHT. DHT increased AR protein expression in HCASMC and HBMEC at all concentrations tested (Fig. 1B).

DHT increases COX-2 levels in an AR-dependent fashion. To determine the effect of DHT treatment on COX-2 protein expression in the absence of induced inflammation, HCASMC were treated with various concentrations of DHT for 6 h: 10 nM DHT increased COX-2 levels compared with vehicle (P < 0.001); lower concentrations (1 and 5 nM) of DHT had no effect on COX-2 levels (Fig. 2A). The AR antagonist bicalutamide was used to determine whether increases in COX-2 expression induced by 10 nM DHT were AR dependent. Cotreatment of cells with vehicle and bicalutamide (1 μM) had no effect on COX-2 levels compared with vehicle. However, cotreatment with DHT (10 nM) and bicalutamide (1 μM) inhibited the DHT-induced increases in COX-2 protein (P < 0.001 vs. DHT; Fig. 2B).
Time course of COX-2 increases following endotoxin or cytokine treatment in HCASMC. In a time-course study of COX-2 induction by LPS or IL-1β administration, COX-2 levels were increased at 3 h ($P < 0.001$), 6 h ($P < 0.001$), and 9 h ($P < 0.01$) compared with vehicle, but COX-2 levels were not significantly different after 12 h of LPS treatment (Fig. 3A). IL-1β treatment increased COX-2 levels at 3, 6, 9, and 12 h compared with vehicle ($P < 0.001$; Fig. 3B). On the basis of these results, the 6-h time point for endotoxin or cytokine exposure was selected for further studies of the effects of DHT on COX-2 following an induced inflammatory stimulus.

Cytokine-induced COX-2 levels are more robust in HCASMC than in HBMEC. To compare the inflammatory response in smooth muscle and endothelial cells, we measured COX-2 protein following IL-1β or LPS stimulation in HCASMC and HBMEC. A representative blot is shown in Fig. 4A. COX-2 expression was not significantly different between vehicle-treated HCASMC and HBMEC (Fig. 4). After LPS or IL-1β stimulation, COX-2 expression was significantly elevated in HCASMC ($P < 0.001$) and HBMEC ($P < 0.001$) compared with vehicle. IL-1β and LPS increased COX-2 levels to a greater extent in HCASMC than in HBMEC ($P < 0.001$). Because we detected a significant and robust response in HCASMC compared with HBMEC, we pursued this finding in HCASMC in subsequent studies.

DHT attenuates COX-2 levels in the presence of LPS- or IL-1β-induced inflammation in HCASMC. To determine the effect of DHT on COX-2 expression following induced inflammation, we treated HCASMC cells with IL-1β or LPS in the absence or presence of DHT. IL-1β caused significant increases in COX-2 compared with vehicle ($P < 0.001$), whereas 10 nM DHT attenuated these increases ($P < 0.01$ vs. IL-1β; Fig. 5A). Lower doses (1 or 5 nM) of DHT had no effect on COX-2 induction by IL-1β (data not shown). LPS treatment also resulted in significant increases in COX-2 in HCASMC compared with vehicle-treated cells ($P < 0.001$). This effect was prevented by 10 nM DHT ($P < 0.001$ vs. LPS; Fig. 5B).

Attenuation of IL-1β-induced increases in COX-2 by DHT is AR-independent. To determine whether DHT (10 nM) attenuates IL-1β-induced increases in COX-2 via AR activation, we treated HCASMC with the AR antagonist bicalutamide and either vehicle or DHT. Consistent with our previous findings, the AR antagonist had no effect on COX-2 induction by IL-1β (data not shown). LPS treatment also resulted in significant increases in COX-2 in HCASMC compared with vehicle-treated cells ($P < 0.001$). This effect was prevented by 10 nM DHT ($P < 0.001$ vs. LPS; Fig. 5B).

Fig. 1. Androgen receptors (AR) are present in human coronary artery smooth muscle cells (HCASMC) and human brain microvascular endothelial cells (HBMEC). A: androgen receptor (AR) antibody and fluorescent secondary labeling detected the presence of AR in the cytoplasm and nucleus of HCASMC (green). 4′,6-Diamidino-2-phenylindole (DAPI) was used to verify nuclear boundaries (blue). B: representative Western blot of whole cell homogenates from AR antibody-labeled HCASMC (top) and HBMEC (bottom) treated with dihydrotestosterone (DHT) for 6 h at 1, 5, or 10 nM and vehicle (VEH). Mouse testis lysate served as positive control.
DHT does not alter PGE2 levels in absence or presence of IL-1β-induced inflammation. PGE2, an end product of the COX-2 enzymatic pathway, was measured in HCASMC culture medium by ELISA to serve as an indirect measurement of COX-2 enzymatic activity. IL-1β treatment increased PGE2 levels compared with vehicle (P < 0.001; Fig. 7). However, DHT (10 nM) did not alter PGE2 levels in the absence or presence of IL-1β-induced inflammation (Fig. 7).

Neither DHT nor IL-1β increases COX-1 levels in HCASMC. To rule out any compensatory PGE2 production due to changes in COX-1 levels, we measured COX-1 levels in HCASMC following DHT and/or IL-1β treatment. COX-1 was detected in all treatment groups, and levels remained unchanged compared with vehicle following DHT (10 nM) treatment. In the presence of IL-1β or DHT + IL-1β, COX-2 levels were decreased compared with vehicle (P < 0.01; Fig. 8).

DISCUSSION

The goal of this study was to determine the effects of androgens on inflammation in HCASMC. Using COX-2 protein levels as a marker for vascular inflammation, we examined the effects of the nonaromatizable androgen DHT on COX-2 levels in HCASMC in the absence or presence of endotoxin- or cytokine-induced inflammation. Our results demonstrate that DHT treatment increased COX-2 protein levels in the absence of induced inflammation in HCASMC, an effect that was AR dependent. In contrast, after endotoxin or cytokine-induced inflammation, DHT treatment caused an unexpected attenuation of LPS- and IL-1β-induced increases in COX-2 levels. The inability of bicalutamide to block this response suggested that this effect was AR-independent. Thus it appears that, in HCASMC, modulation of the inflammatory mediator COX-2 by DHT under physiological conditions may differ from that under pathophysiological conditions.

To our knowledge, this is the first report of DHT’s effects on COX-2 levels in the absence of induced inflammation in a human vascular cell model. We found a significant increase in COX-2 levels in cells treated with 10 nM DHT compared with vehicle-treated cells; however, at 1 or 5 nM DHT, COX-2 levels were not altered. Furthermore, the DHT-dependent increases in COX-2 could be blocked with the classical AR antagonist bicalutamide. The DHT concentration (10 nM) used in this study is well within the range of DHT concentrations (1–400 nM) used in previous studies of the effects of DHT on inflammation in human endothelial cells (8, 24, 27) and the range of DHT concentrations (3–300 nM) used to examine the effects of DHT on human vascular smooth muscle cell proliferation (32). Although a lower concentration of DHT (0.1 nM) has been shown to reduce vascular inflammation, as measured by VCAM and COX-2 levels in human vein endothelial cells (27), data for vascular smooth muscle cells had previously been lacking. In the present study, we show a greater increase in COX-2 after IL-1β or LPS stimulation in HCASMC than in...
HBMEC. Although our two vascular cell types were isolated from different vascular beds (i.e., cerebral circulation vs. heart circulation), it is possible that endothelium and smooth muscle have different thresholds for modulation of inflammation because of differences in the number of Toll-like 4 and IL-1 receptors, receptor affinity, or cytokine production. For example, a comparison of the intracellular calcium response to 1 ng/ml IL-1 in human aortic endothelial cells and human aortic smooth muscle cells revealed that only the smooth muscle cells responded with a significant increase in calcium, suggesting that the IL-1 receptor level or receptor affinity for IL-1 may be higher in smooth muscle cells than in endothelial cells (4).

Since COX-2 levels were increased after DHT treatment in the absence of inflammation, we predicted that DHT would also increase COX-2 levels in the presence of induced inflammation. Surprisingly, DHT actually attenuated COX-2 levels in the presence of cytokine or endotoxin stimulation. The AR antagonist bicalutamide was unable to block this effect of DHT. Thus it appears that, in the absence of an inflammatory stimulus, DHT acts via the classical AR to increase COX-2 levels, but in the presence of cytokine-induced inflammation, DHT acts via an AR-independent mechanism to reduce COX-2 levels.

Other reports have demonstrated AR-independent actions of androgens in the vasculature and in nonvascular tissues. Aside from the AR, DHT can activate the sex hormone-binding globulin receptor to increase cAMP and protein kinase A (12, 26). DHT can also increase intracellular calcium via an unidentified membrane-bound receptor (2, 3). Furthermore, several authors also hypothesized that a membrane-bound AR may exist that is not blocked by classical AR antagonists (2, 3, 18, 20).

Fig. 4. COX-2 protein levels in response to cytokine stimulation or endotoxin in HCASMC and HBMEC. A: representative Western blot of COX-2 protein levels in HCASMC (n = 4) and HBMEC (n = 4) treated for 6 h with vehicle or IL-1β (5 ng/ml). B: Western blot analysis of COX-2 following 6 h of treatment with IL-1β (5 ng/ml) or LPS (100 μg/ml) in HCASMC and HBMEC (n = 4 per group). *P < 0.001 vs. vehicle. #P < 0.05, HCASMC LPS vs. HBMEC LPS; P < 0.001, HCASMC IL-1β vs. HBMEC IL-1β.

Fig. 5. DHT attenuated cytokine- and endotoxin-induced increases in COX-2. A: Western blot analysis of COX-2 levels in response to 6 h of treatment with vehicle (n = 18), IL-1β (5 ng/ml, n = 14), or IL-1β + DHT (5 ng/ml and 10 nM, respectively, n = 17) in HCASMC. B: Western blot analysis of COX-2 levels in response to 6 h of treatment with vehicle (n = 18), LPS (100 μg/ml, n = 6), or LPS + DHT (100 μg/ml and 10 nM, respectively, n = 4) in HCASMC. *P < 0.01 vs. vehicle. #P < 0.01.

Fig. 6. DHT attenuated cytokine-induced increases in COX-2 via an AR-independent mechanism. Western blot analysis of COX-2 levels in response to 6 h of treatment with vehicle (n = 18), vehicle + bicalutamide (1 μM, n = 9), IL-1β (5 ng/ml, n = 14), IL-1β + DHT (5 ng/ml and 10 nM, respectively, n = 17), or IL-1β + DHT + bicalutamide (5 ng/ml, 10 nM, and 1 μM, respectively, n = 9) in HCASMC. *P < 0.01 vs. vehicle. #P < 0.01. NS, not significant.

Fig. 7. DHT did not alter PGE2 production. ELISA of PGE2 production following 6 h of treatment with vehicle (n = 3), DHT (10 nM, n = 6), IL-1β (5 ng/ml, n = 3), or IL-1β + DHT (5 ng/ml and 10 nM, respectively, n = 3) in HCASMC. *P < 0.001.
levels for androgens, cytokines, and endotoxins may also change in culture. To minimize the risk of changes in culture, all experiments were performed after a small number (5 or 6) of passages. Furthermore, at the time of experiments, the smooth muscle cells still expressed the smooth muscle cell markers smoothelin and α-actin, while the endothelial cells still expressed the endothelial cell marker von Willebrand factor (data not shown).

Androgens may be cardioprotective and anti-inflammatory. For example, low levels of testosterone (T) are associated with coronary artery disease (9), hypertension, adverse lipid profile, and procoagulable factors (10). Furthermore, androgens have been shown to suppress the activity of proinflammatory cytokines while enhancing the activity of anti-inflammatory factors in an induced inflammatory environment (22). For example, androgens have been shown to decrease IL-1 production in human monocytes (21), decrease LPS-induced TNF-α, IL-1, and IL-6 levels in mouse macrophages (28), decrease TNF-α-induced VCAM-1 and NF-κB expression in human aortic endothelium (16), and decrease LPS- and TNF-α-induced VCAM-1, IL-6, monocyte chemoattractant protein-1, Toll-like receptor-4, and COX-2 mRNA expression in human umbilical vein endothelial cells (27). Furthermore, T has been shown to enhance proliferation of human vascular smooth muscle cells (38), potentially helping maintain the fibrous cap of atherosclerotic plaques (22). Accordingly, castration of male rabbits has been shown to increase aortic atheroma by 100%, an effect that is inhibited by T replacement (1). T replacement has also been used in clinical settings to relieve symptoms of angina (11) and has been shown to decrease IL-1β and TNF-α levels in men (27). Although many of these studies used the aromatizable androgen T, in our experimental design, we used the more potent AR agonist DHT and noted similar findings. Thus these data, along with our present data, suggest that androgen therapy might protect against vascular inflammation in men already predisposed to cardiovascular events.

By contrast to the beneficial effects of androgens, androgenic effects on inflammation remain controversial, since some studies have shown proinflammatory effects of androgens (8, 14, 29), as well as detrimental effects of androgens on lesion size, following cerebral ischemia (5, 17). For example, the effects of androgens on outcome after cerebral ischemia are age- and dose-dependent. DHT was found to be protective at a low physiological dose or in aged male rodents but detrimental at a high dose or in young male rodents (6, 36). The present data suggest a similar story for vascular smooth muscle cells, where vascular inflammatory actions of androgens may also be condition-dependent, with different effects in the absence and presence of induced inflammation.

In summary, we have shown that DHT increases COX-2 protein levels in the absence of induced inflammation via an AR-dependent mechanism, but attenuates IL-1β-induced increases in COX-2 levels via an AR-independent mechanism, in primary HCASMC. The findings suggest that DHT may be proinflammatory, by augmenting COX-2 levels under physiological conditions, but anti-inflammatory, by attenuating COX-2 under pathophysiological conditions. Surprisingly, the effects of DHT on COX-2 levels did not translate to changes in PGE2 production, although other COX-2-derived end products (prostacyclin, PGF2α, and PGD2) may prove to be androgen targets. Finally, we found that HCASMC are more responsive...
(i.e., increases in COX-2) to IL-1β or LPS stimulation than vascular endothelial cells. Investigations of mechanisms associated with inflammatory effects on human vascular smooth muscle cells have been largely ignored in the literature in favor of the more commonly studied vascular endothelial cells. Thus we have identified human vascular smooth muscle cells as potentially important targets for androgens in the vascular response to inflammation. This is of particular importance, because investigation of the mechanisms by which androgens modulate vascular inflammation may lead to better therapeutic targets for cardiovascular diseases, such as atherosclerosis, myocardial infarction, and stroke.

ACKNOWLEDGMENTS
We thank Anthony Gutierrez for technical assistance.

GRANTS
This work was supported by an American Heart Association grant (to R. J. Gonzales), an American Heart Association predoctoral fellowship (to K. L. Losterlund), and National Institute of Neurological Disorders and Stroke Grant NS-039951 (to R. J. Handa).

DISCLOSURES
No conflicts of interest are declared by the author(s).

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