Testosterone treatment increases thromboxane function in rat cerebral arteries

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Gonzales, Rayna J., Amir A. Ghaffari, Sue P. Duckles, and Diana N. Krause. Testosterone treatment increases thromboxane function in rat cerebral arteries. Am J Physiol Heart Circ Physiol 289: H578–H585, 2005. First published March 11, 2005; doi:10.1152/ajpheart.00958.2004.—We previously showed that testosterone, administered in vivo, increases the tone of cerebral arteries. A possible underlying mechanism is increased vasoconstriction through the thromboxane A2 (TxA2) pathway. Therefore, we investigated the effect of chronic testosterone treatment (4 wk) on TxA2 synthase levels and the contribution of TxA2 to vascular tone in rat middle cerebral arteries (MCAs). Using immunofluorescence and confocal microscopy, we demonstrated that TxA2 synthase is present in MCA segments in both smooth muscle and endothelial layers. Using Western blot analysis, we found that TxA2 synthase protein levels are higher in cerebral vessel homogenates from testosterone-treated orchiectomized (ORX) rats compared with orchiectomized (ORX) control animals. Functional consequences of changes in cerebrovascular TxA2 synthase were determined using cannulated, pressurized MCA segments in vitro. Constrictor responses to the TxA2 mimetic U-46619 were not different between the ORX+T and ORX groups. However, dilator responses to either the selective TxA2 synthase inhibitor furegrelate or the TxA2-endoperoxide receptor (TP) antagonist SQ-29548 were greater in the ORX+T compared with ORX group. In endothelium-denuded arteries, the dilation to furegrelate was attenuated in both the ORX and ORX+T groups, and the difference between the groups was abolished. These data suggest that chronic testosterone treatment enhances TxA2-mediated tone in rat cerebral arteries by increasing endothelial TxA2 synthase synthesis without altering the TP receptors mediating constriction. The effect of in vivo testosterone on cerebrovascular TxA2 synthase, observed here after chronic hormone administration, may contribute to the risk of vasospasm and thrombosis related to cerebrovascular disease.

Although the risk for cardiovascular disease and stroke is higher in males, the influence of testosterone on vascular function is not well understood (25). This lack of information also fuels concerns about the vascular impact of testosterone therapy for elderly men (33) and postmenopausal women (28) as well as the use of anabolic androgens in young athletes, both male and female (30). Androgens appear to influence a variety of cardiovascular risk factors including lipid profile, platelet aggregation, blood pressure, and vascular reactivity (1, 25, 31, 44). Data on vascular reactivity, however, are limited and often conflicting. For example, acute exposure of arteries to testosterone has been found to cause either vasodilation or vasoconstriction (6, 7, 25, 40, 43), whereas chronic testosterone exposure in vivo results in increased vascular tone (13–15, 31). A better understanding of how testosterone modulates vascular function is needed to explain these observations.

In the cerebral circulation, the critical vascular bed in stroke, arterial tone is elevated after in vivo treatment with testosterone (14, 15, 31). Using rat middle cerebral arteries (MCAs), we demonstrated that this effect is mediated, in part, by suppression of the vasodilation normally produced by EDHFs (15). There was no apparent effect of chronic testosterone treatment on the endothelium dilator nitric oxide (NO), nor was there a change in the level of the potent constrictor endothelin (15). Testosterone treatment also had no effect on cerebrovascular levels of cyclooxygenase-1 (COX-1), the rate-limiting enzyme for prostaglandin production. COX-1 converts arachidonic acid to the intermediate endoperoxide prostaglandin H2 (PGH2), which is then converted to one of a number of prostanooids, depending on the terminal enzyme present. Under normal conditions, the synthase that produces the vasodilator prostacyclin is predominant in blood vessels. Testosterone had no effect on prostacyclin production in cerebral vessels (15).

Several studies, however, suggest testosterone affects another prostanoid, thromboxane A2 (TxA2), which has potent vasoconstrictor and platelet-aggregating actions. Testosterone was shown to increase the density of TxA2-endoperoxide receptors (TP) in platelets (1, 27) and vascular smooth muscle cells cultured from the rat aorta or canine coronary artery (18, 26). Testosterone also increased constrictor responses to the TxA2 mimetic U-46619 in the isolated aorta and coronary and renal arteries (17, 21, 27, 34). Interestingly, TP receptors in vascular tissue from males were more susceptible to testosterone modulation than those in female tissue (18, 21, 26). These data suggest that the TxA2 pathway might contribute to the elevated cerebral artery tone observed after chronic testosterone treatment of orchiectomized (ORX) male rats (14, 15). To test this hypothesis, we investigated whether testosterone treatment affected cerebrovascular TxA2 synthase and/or TP-mediated constrictor responses to endogenous TxA2 and the agonist U-46619.

MATERIALS AND METHODS

Animal Procedures

Animal handling and experimental protocols were approved by the Animal Care and Use Committee of the University of California-Irvine. Male Fischer-344 rats (3 mo old; Harlan) were anesthetized with ketamine (91 mg/kg im) and xylazine (9 mg/kg im). Animals were orchietomized under aseptic surgical conditions, and rats were divided into two groups. The first group received Silastic testosterone propionate-filled pellets (1.57 mm inner diameter, 15 mm length) implanted subcutaneously at the base of the neck (ORX+T). The second group of rats did not receive an implant (ORX; testosterone-deficient controls). Postsurgery, all animals were treated with an injection (im) of penicillin (penicillin G benzathine-penicillin G procain, 30,000 units) and, after recovery from anesthesia, were

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returned to housing (12:12-h light-dark cycle) with fresh water, food, and bedding.

Four weeks after the surgery/implant, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip), and the thoracic cavity was exposed. A direct cardiac puncture was used to collect blood samples for serum testosterone measurements using a radioimmunoassay (DiSorin; Stillwater, MN). After blood samples were collected, animals were heparinized and exsanguinated. This was achieved by exposing the right and left carotid arteries via a 3-cm midline neck incision. Catheters were placed in the right and left carotid arteries using polyethylene tubing (PE-50) and secured with 3-0 surgical silk ligatures. Caudal vessels were connected to a small pressure transducer and pressure-servo control unit (Living Systems) to monitor infusion pressure; this did not exceed 60 Torr. Next, the right and left jugular/subclavian veins were cut, and saline containing heparin (100 U/0.1 ml) was infused until the cerebrovasculature was completely rinsed of blood and platelets. After euthanasia and exsanguination, brains were removed and placed in a Sylgard-coated dissection dish containing ice-cold PBS or rapidly frozen and stored at −80°C for future use.

**Confocal Laser Scanning Microscopy**

Confocal imaging was used to determine the localization of TxA2 synthase in MCA segments. The MCA was microscopically dissected from the brains of ORX + T rats, cut into small segments, fixed in 3% formaldehyde (30 min), and permeabilized with Triton X-100 (0.1%) for 5 min. After the addition of 1% BSA, arterial segments were incubated with primary antibodies specific for TxA2 synthase or endothelial NO synthase (eNOS), each at 1:50 dilution (TxA2 synthase and eNOS, Transduction Labs) for 3 h at room temperature. Vessels were washed (30 min, buffer change every 5 min) and incubated with a fluorescent secondary antibody at 10 μg/ml (Oregon green or Texas red, Molecular Probes) overnight at 4°C, washed again, and then arranged on microscope slides coverslipped with VectaShield mounting medium containing 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Vector Laboratories) to counterstain cellular nuclei. Images were obtained using a Bio-Rad model 1024 laser scanning confocal microscope equipped with standard and UV lasers. In control staining conditions, vessels were incubated with the secondary antibody alone but showed no fluorescence.

**Western Blot Analysis**

Western blot analysis was used to determine whether chronic testosterone treatment alters the protein levels of TxA2 synthase in cerebral blood vessels. Before Western blot analysis, perfused brains of ORX and ORX + T rats were thawed on ice, and cerebral vessels were isolated using a previously published procedure (29). In brief, the whole brain was gently homogenized (glass Dounce) in cold PBS (pH 7.4) and centrifuged (720 g for 10 min at 4°C). The supernatant was discarded, and pellets were resuspended in cold PBS. The mixture was layered over a 15% dextran solution (35–45 kDa) and separated by centrifugation (1,300 g for 30–40 min at 4°C), and the pellet containing blood vessels was collected and washed with ice-cold PBS over a nylon mesh (50 μm). Next, vessels were collected from the mesh using fine tip forceps and transferred to a small glass homogenizer containing lysis buffer (4°C). Light microscopy verified that the vessel fraction consisted of a mixture of arteries, arterioles, venules, veins, and capillaries.

After incubation in lysis buffer on ice (20 min), homogenates were centrifuged (4,500 g for 10 min at 4°C), the supernatant was drawn off, and an aliquot was analyzed for protein concentration using a bicinchoninic acid protein assay (Pierce; Rockford, IL). Samples were then dissolved in Tris-glycine SDS sample buffer and boiled for 6 min. Equal amounts of sample protein (40 μg/lane) were then loaded and separated in 8% polyacrylamide gels using SDS-PAGE. In addition to ORX and ORX + T samples, gels were loaded with a standard molecular weight marker (Bio-Rad), and isolated human platelet homogenate was used as a positive control. Separated proteins were transferred to polyvinylidenefluoride membranes and blocked overnight at 4°C in PBS (0.1% Tween and 6.5% nonfat dry milk). Membranes were probed with antibodies specific for TxA2 synthase (1:200, Cayman Chemical; Ann Arbor, MI) or smooth muscle α-actin (1:20,000, Sigma Chemical). Enhanced chemiluminescence development in conjunction with a horseradish peroxidase-labeled secondary antibody (1:15,000) was used to visualize the proteins of interest. Densitometry of bands was analyzed with UN-SCAN-it software (Silk Scientific; Orem, UT).

**Isolated, Pressurized Cerebral Artery Preparations**

Functional studies were used to assess the ability of chronic testosterone to modulate the role of the TxA2 pathway in the maintenance of vascular tone. Brains isolated from ORX and ORX + T rats were placed in ice-cold physiological saline solution (PSS) containing Ca2+ and pinned in a Sylgard-coated dissection dish aerated with an oxygen and carbon dioxide gas mixture. Small segments of second-order MCAs (200–250 μm diameter) were carefully dissected and placed in a small vessel chamber (Living Systems; Burlington, VT) with PSS. The proximal end of the vessel was mounted on a glass micropipette and secured with a nylon ligature, and the lumen was gently rinsed with PSS to ensure removal of any remaining blood elements. The proximal cannula was connected to a pressure transducer and reservoir containing PSS equilibrated with 21% O2–6% CO2–balance N2. Next, the distal end was mounted on a second cannula and tied in place. A stopcock located distal to the vessel was closed, and the vessel was gradually pressurized to 60 Torr and then maintained at constant pressure with a pressure-servo control unit (Living Systems). A constant-flow peristaltic pump continuously superfused (25 ml/min) the tissue with warmed PSS (37°C, pH 7.4) aerated with 21% O2–6% CO2–balance N2. The vessel preparation was viewed using an inverted microscope (Nikon) equipped with a charge-coupled device camera/computer interface system (IonOptix; Milton, MA). An electronic dimension analyzer (Living Systems) was used to measure intraluminal diameter. Changes in intraluminal diameter were recorded using a computer-based data-acquisition edge-detection system (IonOptix).

**TxA2 receptor agonist (U-46619).** After segments were equilibrated for 1 h at 60 Torr and showed the development of spontaneous tone, a concentration-response curve (10−12–10−6 mol/l) to the TxA2 mimetic (15)-hydroxy-11α,9α-(epoxymethano)prosta-5,13-dienoic acid (U-46619) was generated in MCA segments isolated from ORX and ORX + T rats. At the end of the experiment, the passive intraluminal vessel diameter was measured at 60 Torr in Ca2+-free PSS containing EDTA (3 mM). TxA2 synthase inhibition and TP receptor blockade. Arterial segments were equilibrated for 1 h at 60 Torr. After segments developed spontaneous tone, vessel diameter was recorded during increasing step pressure changes to 60, 80, and 100 Torr in the presence of PSS. Next, arteries were incubated in PSS containing the NO synthase inhibitor L-NAME (100 μM) for an additional 30 min. At the end of each experiment, passive responses to step pressure changes were determined in Ca2+-free PSS containing EDTA (3 mM). Tissues were incubated in Ca2+-free PSS for 20 min, and intraluminal step pressure changes (60, 80, and 100 Torr) were then applied to obtain passive blood vessel diameters. On the basis of passive diameters, artery...
Table 1. Intraluminal diameters of MCA segments isolated from ORX and ORX + T rats in the presence of L-NAME, after endothelium removal in the presence of L-NAME, or in the presence of Ca²⁺-free PSS (passive diameter)

<table>
<thead>
<tr>
<th>Pressure, Torr</th>
<th>60</th>
<th>80</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺-free PSS + EDTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORX (n = 5)</td>
<td>211±10</td>
<td>214±11</td>
<td>215±11</td>
</tr>
<tr>
<td>ORX + T (n = 5)</td>
<td>200±8</td>
<td>205±9</td>
<td>209±8</td>
</tr>
<tr>
<td>+ Endo with L-NAME</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORX (n = 5)</td>
<td>82±4</td>
<td>84±5</td>
<td>83±5</td>
</tr>
<tr>
<td>ORX + T (n = 5)</td>
<td>77±5</td>
<td>78±5</td>
<td>77±7</td>
</tr>
<tr>
<td>Ca²⁺-free PSS + EDTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORX (n = 3)</td>
<td>227±2</td>
<td>236±1</td>
<td>241±3</td>
</tr>
<tr>
<td>ORX + T (n = 3)</td>
<td>228±2</td>
<td>245±5</td>
<td>247±7</td>
</tr>
<tr>
<td>− Endo with L-NAME</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ORX (n = 3)</td>
<td>135±6</td>
<td>141±14</td>
<td>138±10</td>
</tr>
<tr>
<td>ORX + T (n = 3)</td>
<td>143±35</td>
<td>148±31</td>
<td>151±28</td>
</tr>
</tbody>
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Values are means ± SE of vessel diameter (in μm); n, no. of animals, MCA, middle cerebral artery; ORX, orchietomized rats; ORX + T, ORX rats treated with testosterone; L-NAME, Nω-nitro-L-arginine methyl ester (100 μM); PSS, physiological saline solution. There were no significant differences between groups.

Segments used in the isolated vessel experiments were similar in size for the ORX and ORX + T groups (Table 1).

Solutions and Chemicals

PSS bicarbonate-phosphate buffer containing (in mM) 122 NaCl, 5.1 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 25.5 NaHCO₃, and 0.03 EDTA was prepared weekly as a stock solution. Before each experiment, superfusate buffer was prepared from the PSS stock solution by adding 11.5 mM glucose and 1.6 mM CaCl₂. PBS was prepared from premade packets. Stock solutions for drugs in the tissue bath experiments were prepared by dissolving L-NAME and furegrelate (Cayman Chemical) in double-distilled H₂O and SQ-29548 (Cayman Chemical) and U-46619 (Cayman Chemical) in ethanol. Final concentrations for all drugs were prepared in PSS. Lysis buffer for Western blots, containing β-glycerophosphate (50 mmol/l), sodium orthovanadate (100 μmol/l), magnesium chloride (2 mmol/l), EGTA (1 mmol/l), and Triton X-100 (0.5%), was prepared fresh; dithiothreitol (1 mmol/l), phenylmethylsulfonyl fluoride (1 mmol/l), pepstatin (20 μmol/l), leupeptin (20 μmol/l), and aprotonin (0.1 U/ml) were then added from stock solutions (dissolved in solvent, double-distilled H₂O or DMSO, and stored at −20°C). Unless noted otherwise, all drugs and chemicals were purchased from Sigma Chemical.

Data Analysis

Data are reported as means ± SE. Data were compared between ORX and ORX + T groups using Student’s paired t-test or ANOVA for repeated measures as appropriate. Multiple comparisons were made with the Student-Newman-Keuls test when ANOVA indicated that differences existed. P ≤ 0.05 was considered statistically significant for all comparisons.

RESULTS

Serum Testosterone Levels and Body Weight

We verified our hormone replacement animal model by measuring serum levels of total circulating testosterone after 4 wk of in vivo testosterone treatment. Serum testosterone concentrations for the ORX + T group (1.29 ± 0.13 ng/ml) were in the range of those previously measured in intact male rats (14, 15). In ORX rats not receiving hormone-filled implants, serum testosterone levels were below the radioimmunoassay level of detection (0.03 ng/ml). In addition to serum levels, body weight also was used as an indicator of testosterone levels. Body weights of ORX + T (290 ± 9 g) rats were significantly greater compared with ORX controls (273 ± 4 g), as expected after chronic testosterone treatment (14, 15).

TxA₂ Synthase Is Expressed in MCAs

In the present study, immunofluorescence with confocal microscopy was used to verify the presence and localization of TxA₂ synthase in the MCA within the cerebral vasculature. Containing with the nuclear specific stain DAPI was used as a focal plane marker to distinguish the endothelial and smooth muscle layers within the MCA segments. Characteristically, endothelial cell nuclei are oriented in the direction of blood flow (Fig. 1A), whereas smooth muscle nuclei are perpendicular to the direction of flow (Fig. 1B). This identification is confirmed by the presence of eNOS in the endothelial layer (Fig. 1E) and the absence of eNOS staining in the smooth muscle layer (Fig. 1F). Colabeling with an antibody selective for TxA₂ synthase resulted in fluorescent staining in both the intimal (endothelial) (Fig. 1C) and medial (smooth muscle) (Fig. 1D) layers, indicating the presence of the enzyme TxA₂ synthase in both cell types within the MCA.

Testosterone Increases TxA₂ Synthase Levels in Cerebral Blood Vessels

Because confocal imaging verified the presence of TxA₂ synthase in the cerebrovasculature, we next determined if chronic testosterone treatment affects TxA₂ synthase levels in cerebral blood vessels. Using blood-free vessel homogenates, we measured the levels of TxA₂ synthase with Western blot techniques. Human platelet whole cell lysate was used as a positive marker (band at 60 kDa), and levels of α-actin (40 kDa) were used to verify equal protein loading of ORX and ORX + T samples within each experiment (Fig. 2A). Interestingly, the level of TxA₂ synthase was significantly greater in cerebral blood vessel homogenates from ORX + T compared with ORX rats (Fig. 2).

Chronic Testosterone Treatment Does Not Alter Constrictor Responses to the TP Agonist U-46619 in MCAs

Possible effects of testosterone treatment on cerebrovascular TxA₂ receptors (TP receptors) were assessed using a functional assay. Constrictor responses to the specific TP agonist U-46619 were measured in pressurized MCAs isolated from ORX and ORX + T rats. As shown in Fig. 3, arteries from both ORX and ORX + T rats developed significant, and similar, constriction when exposed to increasing concentrations of U-46619. The concentration-response curves of the two groups were not different, suggesting that testosterone treatment does not alter the affinity, number, or signaling of TP receptors in cerebral arteries.

Testosterone Treatment Augments Dilator Responses Caused by TxA₂ Synthase Inhibition or TP Receptor Blockade

Because chronic treatment with testosterone resulted in an increase in TxA₂ synthase protein levels (Fig. 2), experiments...
were designed to investigate whether the contribution of basal TxA2 production to cerebrovascular tone is altered after chronic in vivo testosterone treatment. Two approaches were used: inhibition of TxA2 synthase with furegrelate or blockade of the TP receptor with SQ-29548. In initial experiments with pressurized MCA segments, dilatory responses to furegrelate or SQ-29548 were very weak (data not shown). However, the addition of the NO synthase inhibitor L-NAME was found to enhance tone and facilitate observation of dilation in response to TxA2 pathway inhibitors, similar to what was reported by Benyo et al. (3). Previous studies in our laboratory demonstrated that chronic testosterone treatment does not alter vasoconstrictor responses to L-NAME (15). Therefore, all inhibitor studies were conducted in the presence of L-NAME. Under this condition, diameters of both intact and endothelium-denuded vessels were not different between the ORX and ORX + T groups (Table 1). Inhibition of TxA2 synthase with furegrelate caused an increase in the diameter of arteries isolated from both ORX and ORX + T animals. As shown in Fig. 4A, this dilation was significantly greater in MCAs from ORX + T compared with ORX animals at all intraluminal pressures tested (60–100 Torr). However, in vessels denuded of endothelium, furegrelate responses were relatively small, and there were no significant differences between the ORX and ORX + T groups (Fig. 4B).

Inhibition of the TP receptor using the selective antagonist SQ-29548 also caused dilation of pressurized MCA segments treated with L-NAME (Fig. 5). This effect was significantly greater in MCA segments from ORX + T rats compared with the ORX rats, regardless of the intraluminal pressure.

DISCUSSION

The present study demonstrates functional increases in TxA2, a potent vasoconstrictor and prothrombotic factor, in cerebral blood vessels after chronic testosterone administration in vivo. Protein levels of TxA2 synthase were higher in cerebral vessels taken from ORX + T rats compared with ORX controls. Vascular TxA2 production, functionally determined in MCA segments using inhibitors of the synthase (furegrelate) and receptor (SQ-29548) for TxA2, was also increased in ORX + T arteries. TxA2 synthase was visualized by confocal
microscopy to both endothelial and smooth muscle layers of the MCA, but functional experiments indicated that endothelium-derived TxA2 contributed to resting tone and was increased after chronic testosterone treatment. In contrast, no differences were found in constrictor responses to the TxA2 receptor agonist U-46619 between the ORX and ORX/H11001T groups, implying that cerebral artery TP receptors are unaffected by in vivo testosterone treatment. Overall, these findings suggest that cerebrovascular TxA2 production may contribute to in vivo effects of testosterone treatment with possible implications for hormone therapy, androgen abuse, and gender differences in stroke.

TxA2, a prostanoid metabolite of arachidonic acid, is synthesized by a cascade of reactions terminating in the conversion of the prostaglandin endoperoxide intermediate PGH2 via the enzyme TxA2 synthase (35). Pfister et al. (32) proposed that the source of TxA2 for blood vessels is derived from an endothelium-derived precursor of the arachidonic acid pathway that is transferred to platelets for further metabolism to TxA2. In rabbit pulmonary arteries, TxA2 synthase was found in cell types, e.g., macrophages and platelets, that, when activated,
We demonstrated functional consequences of vascular TxA2 production using isolated, pressurized segments of MCAs that were pretreated with l-NAME (100 μM). Diameter changes in response to SQ-29548 (1 μM) were measured in vessels constricted with l-NAME (100 μM). Data are calculated as the percent change: [diameter in l-NAME − diameter in l-NAME with SQ-29548]/diameter in l-NAME] × 100. *P ≤ 0.05 vs. ORX (n = 5).

Our data agree with earlier reports of TxA2 activity in cerebral arteries (3, 22, 23, 36, 41). We demonstrated functional consequences of vascular TxA2 production using isolated, pressurized segments of MCAs that were pretreated with the NO synthase inhibitor l-NAME. Under these conditions, we measured small, but significant, vasodilation after inhibition of either TxA2 synthase or TP receptors, consistent with a selective TxA2 synthase inhibitor furegrelate and SQ-29548 in pressurized middle cerebral arteries isolated from ORX and ORX+T males, consistent with enhanced TxA2 synthesis. All studies were conducted in the presence of l-NAME, but this inhibitor produced similar levels of constriction in ORX and ORX+T arteries, in agreement with our previous finding that testosterone treatment does not affect the TxA2 pathway observed in the present study.

We are not aware of any previous reports showing an effect of testosterone on TxA2 synthase. A number of transcription factors have been identified that regulate TxA2 synthase expression (46), but possible interactions with androgen receptors remain to be determined. Alternatively, because testosterone exposure occurred in vivo, we cannot rule out indirect effects that could modulate the levels of TxA2 synthase. For example, changes in shear stress were recently shown in human umbilical veins to affect TxA2 expression; however, unlike testosterone exposure, prostacyclin synthase was also upregulated by shear (11). While the mechanisms remain to be elucidated, an effect on TxA2 synthase is consistent with an earlier observation that monkeys treated with testosterone exhibit elevated plasma levels of TxA2 (45).

Both the selective TxA2 synthase inhibitor furegrelate and the TP receptor antagonist SQ-29548 had greater effects in arteries from ORX+T males, consistent with enhanced TxA2 synthesis. All studies were conducted in the presence of l-NAME, but this inhibitor produced similar levels of constriction in ORX and ORX+T arteries, in agreement with our previous finding that testosterone treatment does not affect the NO pathway in rat cerebral arteries (15). We also considered the possibility that in vivo testosterone treatment increased the responsiveness of TP receptors in cerebral arteries. However, concentration-response curves to the TP agonist U-46619 were identical in pressurized MCA segments from ORX and ORX+T rats. This finding implies there was no change in cerebral artery TP receptors, in contrast with studies on other vascular beds, cultured vascular smooth muscle, and platelets in which testosterone increased TP receptor density and U-46619 responses (1, 17, 21, 27, 34). Because the mechanisms underlying testosterone effects on TP receptors are unknown, it is difficult at this point to explain why cerebral arteries differ. Isoforms of the TP receptor exist, and they may be regulated differently among vascular beds (35). Alternatively, the effect of testosterone on TP receptors may require...
other stimuli not active in cerebral vessels under resting physiological conditions. Clearly, more investigation is needed to understand these discrepancies.

With no apparent change in TP function, our results with furogrelate and SQ-29548 point to an increase in the synthesis of \( \text{TXA}_2 \) as the primary effect of testosterone on cerebrovascular prostanooid pathways. Higher production of \( \text{TXA}_2 \) increases the vasoconstrictor contribution to net resting tone. This is consistent with previous findings that chronic exposure to testosterone augments the vascular tone of cerebral arteries (14, 15, 31). Interestingly, the latter effect was endothelium dependent (15). In the present study, endothelial removal also greatly reduced dilation to furogrelate, and the differences between ORX and ORX+T were abolished. Thus MCA resting tone appears to be modulated by endothelium-derived \( \text{TXA}_2 \), and this pathway is influenced by chronic testosterone administration. While there was no apparent contribution of smooth muscle-derived \( \text{TXA}_2 \) to smooth muscle tone under our conditions, the presence of \( \text{TXA}_2 \) synthase in the medial layer no doubt has important roles, and future studies should address possible modulation by testosterone treatment.

In our earlier work, we found that testosterone treatment suppresses an endothelial vasodilator with the characteristics of EDHF (15). Therefore, the enhanced vascular tone in cerebral arteries induced by testosterone treatment involves both increases in vasoconstrictor and decreases in vasodilator factors. Interestingly, a recent study (8) in the rat mesenteric artery suggests that \( \text{TXA}_2 \) suppresses EDHF-mediated hyperpolarization and dilation by reducing the activity of smooth muscle small-conductance \( \text{Ca}^{2+} \)-activated \( \text{K}^+ \) channels. This suggests a possible relationship between the increased level of \( \text{TXA}_2 \) and decreased EDHF dilation observed after testosterone treatment.

In the present study, we focused on cerebral arteries under normal, physiological conditions. Under these circumstances, the contribution of \( \text{TXA}_2 \) to resting tone is relatively small and is balanced by the endothelium dilators NO and prostacyclin. However, \( \text{TXA}_2 \) production in cerebral arteries is stimulated by a number of vasoconstrictors including endothelin (37), serotonin (10), UTP (23), and isoprostanes (4, 19), and even relatively low levels of \( \text{TXA}_2 \) can facilitate constrictor responses in cerebral resistance vessels (2, 36). Thus, by increasing levels of \( \text{TXA}_2 \) synthase, testosterone may enhance cerebrovascular constriction by a number of agents. Increased levels of \( \text{TXA}_2 \) in cerebral arteries also would increase the risk of thrombosis, vasospasm (39), and ischemic injury (20). The impact of cerebrovascular \( \text{TXA}_2 \) is highly relevant during pathophysiological conditions such as endothelial dysfunction (3, 4, 22, 41), cerebral vasomotion (22, 39), oxidative stress (4, 19), hypoxia (38), and stroke (20). Effects of testosterone on \( \text{TXA}_2 \) synthase may contribute to the detrimental influence of this hormone on ischemic brain injury (16). Future studies are needed, however, to elucidate the mechanisms by which testosterone affects cerebrovascular \( \text{TXA}_2 \) synthase and directly assess the impact of this effect in pathological states.

GRANTS

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