Orchidectomy increases the formation of prostanoids and modulates their role in the acetylcholine-induced relaxation in the rat aorta

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Aims This study examines the effect of endogenous male sex hormones on thromboxane A2 (TXA2), prostaglandin (PG) I2, PGF2α, and PGE2 release, as well as their role in acetylcholine (ACH)-mediated relaxation in the aorta.

Methods and results Aortic segments from orchidectomized and control male Sprague-Dawley rats were used to measure COX-2 protein expression. ACh-induced relaxation of these segments was also determined in the absence and presence of the COX-2 inhibitor NS-398, the TXA2 synthesis inhibitor furegrelate, the PGI2 synthesis inhibitor tranylcypromine (TCP), or the thromboxane-prostaglandin (TP) receptor antagonist SQ-29 548. Furthermore, TXA2, PGI2, PGF2α, and PGE2 release as well as the vasomotor effect of exogenous TXA2, PGI2, PGF2α, and PGE2 were measured. COX-2 expression was increased in aortas from orchidectomized rats. NS-398 did not modify the ACh-induced relaxation in arteries from both control or orchidectomized rats. Furegrelate did not modify the ACh-induced relaxation in aortas from control animals but, in aortas from orchidectomized rats, it increased that response. TCP decreased the ACh-induced relaxation in both groups. The TP receptor antagonist, SQ29 548 failed to modify ACh-induced relaxation in aortas from either rat group. Pre-incubating arteries from orchidectomized rats with TCP plus furegrelate did not modify the decrease in the ACh response induced by TCP alone, but this response was restored by co-incubation of TCP plus SQ29 548. ACh-induced TXA2, PGI2, PGF2α, and PGE2 release were increased by orchidectomy. The presence of furegrelate plus TCP increased the ACh-induced PGE2 release more in arteries from orchidectomized than in those from control rats. The contractile responses induced by the TXA2 mimetic U-46619 or by exogenous PGF2α were similar in arteries from control and orchidectomized rats, while those induced by exogenous PGE2 were increased in arteries from orchidectomized rats; the vasodilator response induced by exogenous PGI2 was decreased in arteries from orchidectomized rats.

Conclusion These data show that endogenous male sex hormone deprivation increases COX-2 expression, the release of TXA2, PGI2, PGF2α, and PGE2 and the contractile response induced by exogenous PGE2 and TXA2, while it decreases the relaxation induced by exogenous PGI2. Despite the predominance of vasoconstrictor prostanoids derived from COX-2 in aortas from orchidectomized rats, the ACh-induced relaxation remains increased.

1. Introduction

Women develop cardiovascular diseases later in life than men. Although this gender difference involves more than sex hormones per se,1 it has traditionally been attributed to the loss of female sex steroid hormones at the time of menopause. However, the fact that recent clinical trials have indicated doubts on the cardioprotective effects of estrogens,2 coupled with studies demonstrating that low testosterone levels are associated with the development of cardiovascular diseases,3 has refocused interest on the role of androgens in cardiovascular function. In fact, some emerging data suggest that androgens are cardioprotective in males.4–6 Indeed, testosterone has been reported to have antiatherogenic actions7–10 and to improve myocardial
ischaemia in men with coronary artery disease. Proposed beneficial factors are the antioxidant properties of androgens and the endothelial nitric oxide (NO) system. Related to these issues, we previously reported that orchidectomy increased superoxide anion production in rat aorta, but did not affect either endothelial NO synthase (eNOS) expression or NO release.

Endothelial cells also release vasoconstrictor and vasodilator prostanoids, originated from the arachidonic acid metabolism through the cyclooxygenase (COX) pathway, to regulate vascular tone. One of the most frequently studied prostanoids is thromboxane A2 (TXA2), which has been implicated as a mediator in diseases such as myocardial infarction, hypertension, stroke, and bronchial asthma. However, little information is available on the role of androgens in the vascular effects of endogenous TXA2. Orchidectomy has been reported to either decrease or NO release. However, to the best of our knowledge studies analysing the effect of endogenous male sex hormones on the involvement of these prostanoids in vascular function are lacking. Taking all these observations together, the aim of this study was to assess whether endogenous male sex hormones regulate the involvement of TXA2, PGI2, PGF2α, and PGE2 in the acetylcholine (ACH)-induced response. Therefore, the expression of COX-2, the production and the vasomotor effect of these prostanoids derived from COX-2 were also analysed.

2. Methods

2.1 Animal housing and protocols

Male Sprague–Dawley rats (6 months old) were used. They were divided into two groups: control and orchidectomized males. All animals were housed in the Animal Facility of the Universidad Autónoma de Madrid (Registration number EX-021U) according to directives 609/86 CEE and RD. 233/88 of the Ministerio de Agricultura, Pesca y Alimentación of Spain. Male sex hormone deprivation was induced by gonadectomy at 7 weeks of age, and 4 months later the animals were sacrificed. Rats were sacrificed by the tail-cuff method, as previously reported (Letica, Digital Pressure Meter, LES5000, Barcelona, Spain).

2.2 Systolic blood pressure

Systolic blood pressure was indirectly measured in awake animals by the tail-cuff method, as previously reported (Leticia, Digital Pressure Meter, LES5000, Barcelona, Spain).

2.3 Serum levels of testosterone

Serum was obtained at the time of decapitation by collecting trunk blood, followed by centrifugation, and testosterone levels were determined using the monoclonal enzyme immunoassay kit (Cayman Chemical). The assay was performed according to the manufacturer’s instructions.

2.4 Western blot analysis of COX-2

For western blot analysis of COX-2 protein expression, aortic segments were homogenized in a boiling buffer composed of 1 mM sodium vanadate (a protease inhibitor), 1% SDS, and 0.01 M pH 7.4 Tris-HCl. Homogenates containing 15 μg protein were electrophoretically separated on a 10% SDS-polyacrylamide gel (SDS-PAGE) and then transferred to polyvinylidene difluoride membranes (Bio Rad Immun-Blot®) overnight at 4°C, 230 mA, using a Bio-Rad Mini Protean III system (Bio-Rad Laboratories, Hercules, CA, USA) containing 25 mM Tris, 190 mM glycine, 20% methanol, and 0.05% SDS. Prestained SDS-PAGE broad range standards (Bio-Rad Laboratories) were used as molecular mass markers. The membrane was blocked for 1 h at room temperature in Tris-buffered saline solution (100 mM, 0.9% w/v NaCl, 0.1% SDS) with 5% powdered fat-free milk before being incubated overnight at 4°C with rabbit polyclonal antibody for COX-2 (1:200 dilution, Cayman Chemical). After washing, the membrane was incubated with a 1:1000 dilution of anti-rabbit immunoglobulin G antibody conjugated to horseradish peroxidase (Amersham International Plc). The membrane was thoroughly washed and the immunocomplexes were detected using an enhanced horseradish peroxidase/luminol chemiluminescence system (ECL Plus, Amersham International Plc, Little Chalfont, UK) and subjected to autoradiography (Hyperfilm ECL, Amersham International Plc). Signals on the immunoblot were quantified using a computer program (NIH Image V1.56, National Institute of Health, Bethesda, MD, USA). The same membrane was used to determine α-actin expression, and the content of the latter was used to correct COX-2 expression in each sample by means of a monoclonal antibody anti α-actin (1:2000 dilution, Sigma).

2.5 Vascular reactivity

The method used for isometric tension recording has been described in full elsewhere. Briefly, two parallel stainless steel pins were introduced through the lumen of the vascular segment: one was fixed to the bath wall, and the other connected to a force transducer (Grass FT03C, Grass Instruments Co., Quincy, MA, USA); this in turn was connected to a model 7D Grass polygraph. Segments were suspended in an organ bath containing 5 mL of KHS at 37°C, continuously bubbled with a 95% O2–5% CO2 mixture (pH 7.4). The segments were subjected to a tension of 1 g which was re-adjusted every 15 min during a 90 min equilibration period before drug administration. After this, the vessels were exposed to KCl (75 mM/L) to check the functional integrity. After a washout period, the presence of vascular endothelium was confirmed by the ability of 10 μmol/L ACh to relax segments precontracted with 1 μmol/L 5-hydroxytryptamine (5-HT). The segments were rinsed several times with KHS for 1 h, and then cumulative ACh concentration–response curves (0.1 mM/L–10 μmol/L) were obtained in 5-HT precontracted segments. Only one cumulative ACh concentration–response curve was performed in each aortic segment to avoid desensitization and misinterpretation of the results. To investigate the possible participation of products derived from COX-2, some aortic segments were incubated for 30 min with the COX-2 inhibitor N-(2-cyclohexoxyloxy-4-nitrophenyl) methansulphonamide (NS-398; 10 μmol/L) before generating the ACh concentration–response curves.

In another set of experiments, to analyse the possible involvement of TXA2 in the ACh-induced relaxation, some segments were incubated with either the TXA2 synthase inhibitor, furegrelate
dilutions were made in KHS on the day of the experiment.

To investigate possible interactions between TXA2 and PGI2, concentration-response curves to ACh were performed in the presence of the PGI2 synthase inhibitor, tranylcypromine (TCP, 10 μmol/L), TCP plus furegrelate, or TCP plus SQ29 548.

To assess possible differences in the responses induced by TXA2, PGI2, PGF2α, or PGE2 in arteries from both groups, concentration-response curves for the TXA2 mimetic 15-hydroxy-11α,9 α-(epoxymethano)prosta-5,13-dienoic acid (U-46619, 1 nmol/L–10 μmol/L), exogenous PGI2 (0.1 nmol/L–1 μmol/L), PGF2α (1 nmol/L–1 μmol/L), or PGE2 (1 nmol/L–10 μmol/L) were performed in arteries from control and orchidectomized rats.

### 2.6 Prostanoid production

The production of TXA2, PGI2, PGF2α, and PGE2 in vivo is typically monitored by measuring the stable metabolite TXB2, 6-keto-PGF1α, 13,14-dihydro-15-keto PGF2α, and PGE2, respectively, using the respective enzyme immunoassay kit (Cayman Chemical). Segments of thoracic aorta were pre-incubated for 30 min in 5 mL of KHS at 37 °C, continuously gassed with a 95% O2–5% CO2 mixture (stabilization period). This was followed by two washout periods of 10 min in a bath of 0.2 mL of KHS, after which arteries were subjected to 1 μmol/L 5-HT for 2 min and then ACh concentration curve (0.1 nmol/L–10 μmol/L) was applied at 1 min interval. The different assays were carried out according to the manufacturer’s instructions. Results were expressed as pg prostanoid/mL mg tissue.

### 2.7 Drugs

5-HT, ACh chloride, furegrelate, TCP, PGI2, and PGF2α, (Sigma-Aldrich; Spain); PGE2 (Cayman Chemical), U-46619 and NS-398 (Calbiochem), and SQ29 548 (Biolink, SL). Stock solutions (10 mmol/L) of drugs were prepared in distilled water, except for SQ29 548, U-46619, and PGE2, which were dissolved in ethanol and administered from a prepared stock in such a way that the maximal ethanol concentration in the medium was <0.001% (vol/vol). All these solutions were stored at −20°C and appropriate dilutions were made in KHS on the day of the experiment.

### 2.8 Statistical analysis

Results are given as mean ± SEM. The responses elicited by KCl were expressed in milligrams and those elicited by U-46619 and PGE2 were expressed as percentage of the tone induced by 75 mmol/L KCl. The relaxation induced by ACh and PGI2 was expressed as a percentage of the initial contraction elicited by 5-HT. Statistical analysis was done by comparing the curve obtained in the presence of the PGI2 synthase inhibitor, tranylcypromine (TCP, 10 μmol/L), TCP plus furegrelate, or TCP plus SQ29 548.

### 3. Results

#### 3.1 Blood pressure

To study if orchidectomy induced haemodynamic changes, systolic blood pressure was measured in control and orchidectomized rats. We observed that orchidectomy did not modify systolic blood pressure levels (control: 137 ± 5.8 mmHg, n = 10; orchidectomized: 145 ± 6.2 mmHg, n = 12; P > 0.05), indicating that the alterations in vascular function to be shown later appears to be independent of blood pressure levels.

#### 3.2 Serum testosterone

The effectiveness of orchidectomy was analysed by measuring the concentration of testosterone in the serum from control and orchidectomized rats. We found that orchidectomy decreased the level of serum testosterone (control: 2404 ± 323 pg/mL; orchidectomized: 220 ± 49 pg/mL; n = 6; P < 0.001).

#### 3.3 COX-2 expression

The effect of orchidectomy on the expression of COX-2 protein was analysed by using western blot analysis. Orchidectomy increased the expression of COX-2 protein detected in homogenates from aortic segments (Figure 1).

#### 3.4 Vascular reactivity

The vasodilator response induced by ACh was greater in aortic segments from orchidectomized rats than those of controls (ANOVA, P < 0.01; Table 1), as previously described. Incubation with the specific COX-2 inhibitor NS-398 (10 μmol/L, 30 min) did not alter the ACh-induced response in either group of rats (Figure 2). However, despite the absence of differences in the ACh-induced response, it is possible to hypothesize that the two groups differed in terms of the products derived from COX-2. Therefore, the arteries were incubated with specific prostanoid synthase inhibitors or receptor blockers.

Pre-incubation with the TXA2 synthase inhibitor, furegrelate (1 μmol/L, 30 min) did not modify the relaxation induced by ACh in segments from control rats, although it increased the relaxation induced by ACh in arteries from orchidectomized rats (Figure 2A and B, Table 1). Pre-incubation with the TP receptor antagonist, SQ29 548 (1 μmol/L, 30 min) did not modify the response to ACh in arteries from either group of rats (Figure 2A and B, Table 1). These results indicate the participation of other prostanoids in addition to TXA2 in the ACh-induced response.

Pre-incubation with the PGI2 synthase inhibitor, TCP (10 μmol/L, 30 min), decreased ACh-induced relaxation in arteries from both control and orchidectomized rats (Figure 3, Table 1). Pre-incubation with TCP plus furegrelate or TCP plus SQ29 548 reversed the effect of TCP in arteries from control rats (Figure 3A). In arteries from orchidectomized rats, furegrelate did not reverse the effect of TCP, while SQ29 548 did (Figure 3B, Table 1). The effects of the different drugs used on the maximum response (Emax) to ACh and E50 are summarized in Table 1.

These results indicate the balanced action of PGI2 and TXA2 in arteries from control rats, and also suggest the existence of an additional prostanoid in arteries from orchidectomized rats that exerts vasoconstrictor action through TP receptors. It was for this reason that the formation of
PGI₂, TXA₂, PGF₂α, and PGE₂ was analysed, as well as their vasomotor effect. The TXA₂ mimetic U-46619 (1 nmol/L–10 μmol/L) induced a contractile response, which was similar in arteries from control and orchidectomized rats (Figure 4A). The vasodilator response induced by exogenous PGI₂ (1 nmol/L–1 μmol/L) was decreased by 36% in arteries from orchidectomized rats with regard to those of control rats (Figure 4B). The contractile response induced by exogenous PGF₂α was similar in arteries from control and orchidectomized rats (Figure 4C). The contractile response elicited by exogenous PGE₂ (1 nmol/L–10 μmol/L) was increased in arteries from orchidectomized rats (Figure 4D). Specifically, the contractile response induced by the highest concentration of exogenous PGE₂ in arteries from orchidectomized rats exceeded that induced in arteries from control rats by 206%.

The effect of orchidectomy on the Eₘₐₓ to the exogenous prostanoïds used and EC₅₀ are summarized in Table 2.

### 3.5 Prostanoid production

Orchidectomy increased the ACh-stimulated aortic production of TXB₂, 6-keto-PGF₁α, 13,14-dihydro-15-keto PGF₂α, and PGE₂ (Figure 5A–D). Pre-incubation with TCP plus furegrelate produced a greater increase in PGE₂ production in arteries from orchidectomized rats than in those of control rats (Figure 5D).

### 4. Discussion

Recent studies have reported several mechanisms behind the beneficial effects of androgens on cardiovascular function in males.²⁶,²⁷ One of the proposed mechanisms is the interaction between androgens and endothelial cells. It is known that endothelial cells possess androgen receptors where activation could modify intracellular signalling pathways, among them the NO pathway. Endothelial NO plays a crucial role in cardiovascular protection through its regulatory effects on platelet aggregation, oxidative stress, leukocyte adherence, and vascular smooth muscle cell proliferation, all of which ultimately modulate vascular tone.³⁶ In this respect, we have previously reported that orchidectomy did not alter either eNOS expression or endothelial NO release in rat aorta or mesenteric arteries; however, the ACH-induced relaxation in aortas from orchidectomized rats was greater than in those of control male rats due to superoxide-induced vasodilatory action through calcium-dependent potassium channels (KCa) activation.³² In the present study, we provide evidence that orchidectomy also regulates the release and function of prostanoids derived from COX-2, indicating the complexity of physiological systems in which multiple signalling pathways are simultaneously working.

The effects described earlier seem to be independent of previous blood pressure levels, since orchidectomy did not modify blood pressure; moreover, since endogenous hormone deprivation is the only variable used in our

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Changes in the maximum response (Eₘₐₓ, expressed as a percentage of relaxation) and log EC₅₀ to acetylcholine in aorta from control and orchidectomized rats</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Eₘₐₓ (%)</td>
</tr>
<tr>
<td>Control condition</td>
<td>88.15 ± 2.5</td>
</tr>
<tr>
<td>NS-398</td>
<td>89.53 ± 2.4</td>
</tr>
<tr>
<td>Furegrelate</td>
<td>92.95 ± 5.7</td>
</tr>
<tr>
<td>SQ29 548</td>
<td>92.89 ± 8.5</td>
</tr>
<tr>
<td>TCP</td>
<td>71.02 ± 3.5*</td>
</tr>
<tr>
<td>TCP+Furegrelate</td>
<td>80.76 ± 4.9</td>
</tr>
<tr>
<td>TCP+SQ29 548</td>
<td>83.31 ± 3.4</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. control rats.
**P < 0.01 vs. control rats.
³P < 0.01 vs. control condition in control rats.
⁴P < 0.01 vs. control condition in orchidectomized rats.
studies, the results obtained would have to be androgen-related, as confirmed by the decreased testosterone levels.

In addition to NO, endothelial cells also release vasoconstrictor and vasodilator prostanoids that are involved in the modulation of vascular tone. Therefore, it is possible to speculate that androgens could also modulate the release and/or function of prostanoids. Since prostanoids are derived from COX-2, we analysed the possible differences in COX-2 expression in arteries from control and orchidectomized rats. We found that COX-2 expression, in contrast to observations in mesenteric artery, was increased in aortas from orchidectomized rats indicating that endogenous male sex hormones act differently depending on the specific vessel. Our results also show that, in aorta from orchidectomized rats, COX-2 derivatives could also be increased and play a role in the regulation of vascular function. To test this hypothesis, we analysed the effect of the COX-2 inhibitor NS-398 on the ACh-induced response. In contrast to our assumptions, we found that NS-398 did not modify the ACh-induced relaxation in either group of rats, apparently indicating the lack of participation of COX-2-derived products in the ACh response. However, it has been recently reported that COX-2 selective inhibitors amplify NO/cGMP signalling by phosphodiesterase inhibition, this allows us to speculate that the contribution of different prostanoids to the vasodilator response mediated by ACh could be regulated by endogenous male sex hormones.

It is known that TXA2 is one of the most important vasoconstrictor prostanoids produced by the vascular wall to
participate in the endothelial dysfunction associated with different cardiovascular risk factors. Most of the studies analysing the influence of androgens on the vascular effects of TXA2 have been focused on describing its action on TP receptors, as well as on the contractile response elicited by TXA2 analogues. Thus, testosterone was shown to increase the density of TP receptors in platelets and vascular smooth muscle cells cultured from the rat aorta. Regarding the influence of androgens on constrictor response to the TXA2 mimetic, U-46619, both increases and a lack of change have been reported, but in different vessels. As we have previously reported that orchidectomy increased TXA2 production and its vascular involvement in the clonidine-induced contraction, in rat mesenteric artery, and since there is a lack of studies analysing the effect of endogenous male sex hormones on the whole TXA2 pathway under the same experimental conditions, we began by analysing, in aortic

Table 2  Effect of orchidectomy in the maximum response (E_max) and log EC_{50} to the vasomotor response elicited by the analogue of TXA2, U-46619, and the exogenous PGI2, PGF2α, or PGE2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Orchidectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-46619</td>
<td>E_max (%)</td>
<td>log EC_{50}</td>
</tr>
<tr>
<td>Control</td>
<td>213.1 ± 6.4</td>
<td>−7.74 ± 0.05</td>
</tr>
<tr>
<td>Orchidectomized</td>
<td>226.6 ± 13</td>
<td>−7.57 ± 0.1</td>
</tr>
<tr>
<td>PGI2</td>
<td>39.07 ± 2.4</td>
<td>−7.4 ± 0.2</td>
</tr>
<tr>
<td>PGF2α</td>
<td>12.82 ± 2.15</td>
<td>−7.25 ± 0.3</td>
</tr>
<tr>
<td>PGE2</td>
<td>30.12 ± 1.3</td>
<td>−5.58 ± 0.2</td>
</tr>
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*P < 0.05, **P < 0.001 vs. control rats.

Figure 4  Concentration–response curves to the TXA2 mimetic U-46619 (A), exogenous PGI2 (B), exogenous PGF2α (C), and PGE2 (D) in aortic segments from control and orchidectomized rats. Results (means ± SEM) are expressed as percentage of the previous tone elicited by 75 mmol/L KCl (A–D) or as percentage of inhibition of contraction induced by 1 μmol/L 5-HT (B). Number of animals is indicated in parenthesis. *P < 0.05, **P < 0.001 compared with control rats.
segments, the possible modulation of ACh-induced TXA2 production by endogenous male sex hormones and the involvement of TXA2 in ACh-induced relaxation. The results showed that the formation of TXA2 induced by ACh was increased in aortas from orchidectomized rats, a finding that is similar to those in mesenteric arteries from comparable animals stimulated with ACh37 or with the $\alpha_2$-receptor agonist clonidine.26

Once we had established that orchidectomy increased TXA2 release, we analysed the possible role of this prostanooid in the response to ACh by analysing the effect of the TXA2 synthase inhibitor, furegrelate, and the TP receptor antagonist, SQ29 548, on the vasodilator response to ACh. We observed that neither furegrelate nor SQ29 548 had any effect on the ACh-induced response in arteries from control animals, indicating that TXA2 did not participate in that response, in agreement with reports in other rat strains.28,29 However, in arteries from orchidectomized rats, furegrelate enhanced the vasodilator response to ACh, showing a functional involvement of TXA2. The fact that the contractile response to the TXA2 mimetic U-46619 was similar in arteries from control and orchidectomized rats demonstrated that sensitivity to TXA2 is not modified by orchidectomy, which agrees with reports in cerebral25 and mesenteric arteries; additionally, it also shows that differences in the TXA2 involvement in the ACh-response are due to increased synthesis rather than increased sensitivity to TXA2. However, the incubation with SQ29 548 did not affect the ACh-induced relaxation. This observation seems to contradict the results obtained with furegrelate. However, since interactions among different prostanoids have been reported,50,51 it is possible to hypothesize that when TXA2 synthesis is inhibited, the production of other prostanoids, which counterbalance the TXA2 effect, could be increased. Therefore, we investigated the effect of inhibiting PGI2 synthesis on the ACh-induced response. We found that the presence of the PGI2 synthesis inhibitor TCP decreased the vasodilator response to ACh to a greater extent in arteries from orchidectomized than in those of control rats, which would indicate a greater involvement of this vasodilator prostanooid in the former arteries, a circumstance that could be due to alterations in PGI2 synthesis and/or the vasomotor effect. We observed that the ACh-induced PGI2 release was increased in arteries from orchidectomized rats, probably due to the superoxide anion overproduction observed in aortas from orchidectomized rats,18 supporting the concept of redox regulation of vascular prostanoid synthesis proposed by Bachschmid et al.51 Moreover, the increased production of PGI2 is in line with that reported in human syndromes involving platelet activation in which PGI2 biosynthesis is elevated along with TXA2.52,53 It is known that PGI2 can induce both vasodilation, through activation of prostacyclin receptors (IP) and thereby increasing cyclic-AMP, and vasoconstriction through activation of TP receptors.21 In the present study, we found that exogenous PGI2 induced relaxation in rat aorta, and that it was decreased in arteries from orchidectomized rats, which could be due to differences in the expression of IP receptors rather than differences in cell signalling operating after receptor activation; we have observed that
the relaxation induced by the activator of adenylate cyclase, forskolin, was similar in arteries from control and orchidectomized rats (unpublished data).

Since considerable evidence exists for cross-talk between the TXA2 and PGI2 systems,50 we analysed the functional effect of inhibiting the synthesis of both prostanoids. We observed that co-incubation of arteries with TCP plus furegrelate, or TCP plus SQ29 548, reversed the decreased response to ACh caused by TCP in arteries from control rats, showing the existence of a balance between TXA2 and PGI2 in these arteries. However, in arteries from orchidectomized rats, the co-incubation with TCP plus furegrelate did not modify the decreased ACh response caused by TCP, indicating the participation of prostanoids other than PGI2 and TXA2 that could induce contraction. Moreover, these other prostanoids would activate TP receptors since co-incubation with TCP and SQ29 548 completely reversed the decrease in the ACh response induced by TCP.

Among COX-2 derivatives, other than TXA2 and PGI2, that can activate TP receptors, PGE2 is the most plausible candidate,29,54 since the ACh-induced PGE2 production and its vasoconstrictor effect were both very limited. Therefore, we investigated the ACh-induced PGE2 release, as well as its vasoconstrictor effect. We found that both ACh-induced PGE2 production and PGE2-induced vasoconstrictor response were greater in arteries from orchidectomized than in those of control rats. Consequently, we analysed the effect of TXA2 and PGI2 synthesis inhibition on the ACh-induced PGE2 release. We found that, under this experimental condition, the ACh-induced PGE2 production further increased, probably as a consequence of increased PGH2 production and subsequent transformation into PGE2;54,55 and, what is more important, the PGE2 increase was more pronounced in arteries from orchidectomized than in those of control rats. This finding confirms our hypothesis that when the synthesis of PGI2 and TXA2 was inhibited, the release of PGE2 was increased in arteries from orchidectomized rats, but raises the question as to why the PGE2 produced in the presence of TCP plus furegrelate did not affect the ACh-induced relaxation in arteries from control animals. The possible explanation could be that the PGE2 release was not sufficient to induce a vasomotor effect and/or the PGE2-induced contraction in arteries from control rats was diminished as a consequence of different expression of EP receptor subtypes. By itself, this finding is of physiological relevance, since PGE2 release and the vasoconstrictor effect are both increased in orchidectomized animals.

In summary, this study demonstrates that orchidectomy enhances COX-2 expression, and, that when ACh activates the muscarinic receptor (M-R) the release of prostaglandin (PG) I2, PGE2, and thromboxane (TX) A2 is induced; orchidectomy increases the ACh-induced release of PGI2, PGE2, and TXA2. Additionally, when the synthesis of PGI2 and TXA2 is inhibited with tranylcypromine (TCP) and furereglate (Fure.), respectively, the release of PGE2 is increased, and orchidectomy increases that effect. These prostanoids through their respective specific receptors (IP-R, specific receptor for PGI2; EP-R, specific receptors for PGE2; FP-R, specific receptor for PGF2a; TP-R, receptor for TXA2 and prostanoids) exert a net vasoconstrictor effect in orchidectomized rats. Despite of these modifications, the response induced by ACh remains increased in aortas from orchidectomized rats. This result probably is a consequence of compensatory mechanisms such as the dual effect of superoxide anion (O2-), whose formation is increased in orchidectomized rats, by decreasing endothelial nitric oxide (NO) bioavailability18 and by activating calcium-dependent potassium channels (Kca),32 NS-398, specific COX-2 inhibitor; SQ29 548, TP-receptor antagonist.
from orchidectomized rats, probably as a consequence of compensatory mechanisms, such as the activation of BKCa channels by superoxide anion, the formation of which is increased in orchidectomized rats[12] (see Figure 6).

This intriguing information makes it essential to perform studies in vascular function taking into account different cell signalling pathways that are working simultaneously.

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