Orchidectomy increases the formation of non-endothelial thromboxane A₂ and modulates its role in the electrical field stimulation-induced response in rat mesenteric artery

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Abstract

The aim of this study was to analyze whether endogenous male sex hormones influence the release of thromboxane A₂ (TXA₂) and its role in the electrical field stimulation (EFS)-induced response, as well as the mechanism involved. For this purpose, endothelium-denuded mesenteric arteries from control and orchidectomized male Sprague–Dawley rats were used to measure TXA₂ release; EFS-induced response, nitric oxide (NO), norepinephrine (NA), and prostaglandin (PG) I₂ release were also measured in the presence of the TXA₂ synthesis inhibitor furegrelate. Orchidectomy increased basal and EFS-induced TXA₂ release. Furegrelate decreased the EFS-induced contraction in arteries from control rats, but did not modify it in arteries from orchidectomized rats. The EFS-induced neuronal NO release and vasodilator response were increased by furegrelate in arteries from control rats, but were not modified in arteries

from orchidectomized rats. Furegrelate did not modify the EFS-induced NA release or vasoconstrictor response in arteries from either control or orchidectomized rats. The EFS-induced PGI₂ release was not modified by furegrelate in arteries from control rats, but was increased in arteries from orchidectomized rats. The results of the present study show that endogenous male sex hormone deprivation i) increases non-endothelial TXA₂ release and ii) regulates the effect of endogenous TXA₂ on the EFS-induced response through different mechanisms that, at the least, involve the NO and PGI₂ systems. In arteries from control rats, inhibition of TXA₂ formation decreases the EFS-induced response by increasing neuronal NO release. In arteries from orchidectomized rats, the EFS-induced response is unaltered after the inhibition of TXA₂ formation, by increasing PGI₂ release.

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Introduction

Clinical and epidemiological studies indicate the existence of gender differences in the incidence of cardiovascular disease and have established the existence of vascular protective effects of endogenous estrogens (Teede 2007). On their part, androgens have generally been associated with impaired vascular reactivity; nevertheless, recent studies have reported beneficial properties of androgens in male vascular function (Blanco-Rivero *et al.* 2006a,b) similar to the effects of estrogens in women (Alexandersen *et al.* 1999, Ng 2007). In addition, low levels of testosterone have been reported in patients with hypertension (Phillips *et al.* 1993), atherosclerosis (Alexandersen *et al.* 1996), and coronary disease (Wranicz *et al.* 2005).

Vascular tone is regulated by several mechanisms in which, depending on the type of the vessel, innervation plays a more or less important role. This regulation involves the adrenergic, cholinergic, nitrergic, peptidergic, and/or sensory innervations (Vanhoutte *et al.* 1981, Marco *et al.* 1985, Kawasaki *et al.* 1988) that are specific to the vascular bed under

consideration. Nitric oxide (NO) is an important neurotransmitter in both the central (Bredt *et al.* 1992) and peripheral (Marín & Balfagón 1998) nervous systems. Electrical field stimulation (EFS) has been shown to induce NO release from nitrergic nerves in rat mesenteric arteries (Marín & Balfagón 1998, Ferrer *et al.* 2000, Ferrer & Balfagón 2001, del Carmen Martín *et al.* 2005), thus producing relaxation by stimulating soluble guanylate cyclase and increasing the intracellular levels of cGMP in the smooth muscle cells of the arterial wall (Holzmann 1982, Ignarro & Kadowitz 1985). Sex hormones have been described to modulate the release and/or function of the neuronal NO in male (del Carmen Martín *et al.* 2005) and female (Minoves *et al.* 2002) rat mesenteric arteries.

Vascular tone is also regulated by prostanoids originated by arachidonic acid metabolism through the cyclooxygenase pathway (Henrion *et al.* 1997, Blanco-Rivero *et al.* 2005, Félétou & Vanhoutte 2006). One of the most studied prostanoids is thromboxane A₂ (TXA₂) that has been implicated as a mediator in diseases such as myocardial infarction, hypertension and stroke (FitzGerald *et al.* 1987,

Narumiya *et al.* 1999). We previously reported that, in mesenteric artery from comparable rats, endogenous male sex hormones modulate endothelial TXA₂ production, whether in basal conditions or after stimulation with either clonidine (Blanco-Rivero *et al.* 2006a) or acetylcholine (Blanco-Rivero *et al.* 2007), without modifying the TXA₂ vasoconstrictor effect. Additionally, we have also shown that endogenous male sex hormones regulate the functional involvement of endogenous TXA₂ in vascular responses of aorta (Martorell *et al.* 2008) and mesenteric artery (Blanco-Rivero *et al.* 2006a, 2007).

On the other hand, while NO has been reported to modulate the prostanoid system (Salvemini *et al.* 1996, Laemmel *et al.* 2003), there have been few reports on the action of prostanoids on the NO system (Ferrer *et al.* 2004, Mollace *et al.* 2005). Moreover, regarding the specific action of TXA₂ on the NO system, a decrease in inducible (Yamada *et al.* 2003) as well as endothelial (Miyamoto *et al.* 2007) NO release has been reported. However, details about the action of TXA₂ on neuronal NO release in vascular tissue remain unknown.

In the light of these considerations, the present study was designed to investigate whether endogenous male sex hormones influence the release of TXA₂ and the role of the latter in the EFS-induced response, as well as the mechanism involved in this response.

Materials and Methods

Animal housing and protocols

Male Sprague–Dawley rats (6 months old) were used. Animals were housed in the Animal Facility of the Universidad Autónoma de Madrid (registration number EX-021U) in accordance with directives 609/86 CEE and RD 233/88 of the Ministerio de Agricultura, Pesca y Alimentación of Spain. Deprivation of male sex hormones was induced by gonadectomy at 7 weeks of age, and 4 months later the animals were killed. The observation of seminal vesicles atrophy confirmed successful surgery. The rats were weighed and killed by CO₂ inhalation; the first branch of the mesenteric artery was carefully dissected out, cleaned of connective tissue, and placed in Krebs–Henseleit solution (KHS; in mM: NaCl, 115; CaCl₂, 2.5; KCl, 4.6; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; NaHCO₃, 25; glucose, 11.1; Na₂EDTA, 0.03) at 4 °C. The endothelium was removed to eliminate the main source of vasoactive substances, including NO. This avoided possible actions on endothelial cells by different drugs that could lead to misinterpretation of results. The endothelium was removed by gently rubbing the luminal surface of the segments with a thin wooden stick. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the USA National Institutes of Health (publication no. 85-23 revised 1985).

TXA₂, norepinephrine (NA), and prostaglandin I₂ (PGI₂) release

The production of TXA₂ and PGI₂ *in vivo* was typically monitored by measuring their stable metabolites TXB₂ and 6-keto-PGF_{1α} respectively, using a TXB₂ or a 6-keto-PGF_{1α} EIA kit (Cayman Chemical, Ann Arbor, MI, USA). NA was measured using Noradrenaline Research EIA (Labor Diagnostika Nord, Nordhorn, Germany).

The endothelium-denuded rat mesenteric segments were preincubated for 30 min in 5 ml KHS at 37 °C, continuously gassed with a 95% O₂–5% CO₂ mixture (stabilization period). After several 10-min washout periods in a bath containing 400 µl KHS, the medium was collected to measure basal release. Once the chamber was refilled, cumulative EFS periods of 30 s at 1, 2, 4, 8, and 16 Hz at 1-min intervals were applied, and the medium was collected to measure the EFS-induced release. Each assay was performed following the manufacturer's instructions. Results were expressed as pg/ml × mg tissue for TXA₂ and PGI₂ release and as ng/ml × mg tissue for NA release.

Vascular reactivity

The method used for isometric tension recording has been described in full elsewhere (Nielsen & Owman 1971). 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 FTO3C; Quincy, MA, USA); this was connected in turn to a model 7D Grass polygraph. For the EFS experiments, the segments were mounted between two platinum electrodes 0.5 cm apart and connected to a stimulator (Grass, model S44) modified to supply appropriate current strength. The segments were suspended in an organ bath containing 5 ml KHS at 37 °C continuously bubbled with a 95% O₂–5% CO₂ mixture (pH 7.4). The segments were subjected to a tension of 0.5 g, which was readjusted every 15 min during a 90-min equilibration period before drug administration. After this, the vessels were exposed to 75 mM KCl to check their functional integrity. The endothelium removal did not alter the contractions elicited by 75 mM KCl. After a washout period, the absence of vascular endothelium was tested by the inability of 10 µM acetylcholine (ACh) to relax segments precontracted with 1 µM NA.

Frequency–response curves for EFS (1, 2, 4, 8, and 16 Hz) and concentration–response curves for NA (10 nM–10 µM) were obtained. The parameters used for EFS were 200 mA, 0.3 ms, and 1–16 Hz, for 30 s with an interval of 1 min between each stimulus, the time required to recover basal tone. A washout period of at least 1 h was necessary to avoid desensitization between consecutive curves. Three successive frequency–response curves separated by 1-h intervals produced similar contractile responses.

To determine the effect of endogenous TXA₂ on the response induced by EFS, the TXA₂ synthase inhibitor,

furegrelate (1 μ M), was added to the bath 30 min before the second frequency–response curve.

To determine the possible effect of endogenous TXA₂ on the NA-induced vasoconstrictor response, furegrelate was added to the bath 30 min before performing the NA concentration–response curve. The possible effect of furegrelate on the vasodilator effect of NO was also analyzed by obtaining concentration–response curves for the NO donor sodium nitroprusside (SNP) in 30-min furegrelate preincubated arteries.

NO release

Endothelium–denuded mesenteric arteries from control and orchidectomized rats were subjected to a resting tension of 0.5 g as indicated for the reactivity experiments. After an equilibration period of 60 min, arteries were incubated with the fluorescent probe 4,5-diaminofluorescein (DAF-2, 0.5 μ M) for 45 min. Then the medium was collected to measure basal NO release. Once the organ bath was refilled, cumulative EFS periods of 30 s at 1, 2, 4, 8, and 16 Hz at 1-min intervals were applied. The fluorescence of the medium was measured at room temperature using a spectrofluorimeter (LS50 Perkin–Elmer instruments; FL WinLab Software) with excitation wavelength set at 495 nm and emission wavelength at 515 nm. This method has been validated by comparing the results obtained with DAF and with those obtained by nitrites measurement (del Carmen Martín *et al.* 2005).

The interference of endogenous TXA₂ on NO release was studied by incubating the arteries with the TXA₂ synthase inhibitor furegrelate (1 μ M) 30 min before collecting medium.

Each data was calculated by subtracting the blank measures from the corresponding NO release obtained. Blank measures were collected in the same way from segment-free medium in order to subtract background emission. The specificity of the method has already been demonstrated (del Carmen Martín *et al.* 2005, Blanco-Rivero *et al.* 2006c). The amount of NO released was expressed as arbitrary units/mg tissue.

Drugs

L-NA hydrochloride, ACh chloride, L-NAME hydrochloride, SNP and DAF-2 were obtained from Sigma–Aldrich and furegrelate from Cayman chemical (Europe). Stock solutions (10 mM) of drugs were made in distilled water, except for NA, which was dissolved in an NaCl (0.9%)–ascorbic acid (0.01% w/v) solution. These solutions were kept at -20°C and appropriate dilutions were made in KHS on the day of the experiment.

Data analysis

The responses elicited by EFS or NA were expressed as a percentage of the contraction induced by 75 mM KCl. The relaxation induced by SNP was expressed as a percentage of the initial contraction elicited by 1 μ M NA. Results are given as

mean $\pm$ S.E.M. Statistical analysis was done by comparing the curve obtained in the presence of the different substances with the previous or control curve by means of repeated-measures ANOVA. For the experiments on TXA₂, NO, NA, and PGI₂ release, the statistical analysis was done using Student's *t*-test for unpaired experiments. $P < 0.05$ was considered significant.

Results

Body weight

Orchidectomy slightly decreased rat body weight (control, 469.7 ± 8.4 g; orchidectomized, 428 ± 5.4 g; $n = 10$; $P < 0.05$), but this did not affect the size of mesenteric artery.

TXA₂ release

Orchidectomy increased basal TXA₂ release. The EFS-induced TXA₂ release was greater in arteries from orchidectomized than control male rats (Fig. 1).

Vascular reactivity

Preincubation with the TXA₂ synthase inhibitor furegrelate (1 μ M) did not modify basal tone in arteries from either rat group; furegrelate decreased the EFS-induced contraction in arteries from control male rats (Fig. 2a), but did not modify it in arteries from orchidectomized rats (Fig. 2b).

Preincubation with furegrelate did not modify the NA-induced contraction in arteries from control (Fig. 3a) or orchidectomized rats (Fig. 3b).

Preincubation with furegrelate increased the SNP-induced relaxation in arteries from control male rats (Fig. 4a), but did not affect that induced in arteries from orchidectomized rats (Fig. 4b).

Figure 1 Basal and EFS-induced thromboxane A₂ (TXA₂) release in mesenteric denuded segments from (a) control and (b) orchidectomized rats. Results (mean $\pm$ S.E.M.) are expressed as pg/ml $\times$ mg tissue. Number of animals is indicated in parenthesis. * $P < 0.05$ compared with basal TXA₂ release. + $P < 0.05$ compared with the respective control.

Figure 2 Effect of furegrelate on the frequency–response curve in mesenteric artery segments from (a) control and (b) orchidectomized rats. Results (mean $\pm$ S.E.M.) are expressed as percentage of the tone induced by 75 mM KCl. *n*, number of animals.

NO release

Basal and EFS-induced NO release was similar in mesenteric arteries from both control and orchidectomized male rats (ANOVA, $P > 0.05$). In segments from control male rats, preincubation with the TXA₂ synthase inhibitor, furegrelate, did not modify the basal NO release, but did increase the EFS-induced NO release (Fig. 5a). By contrast, in segments from orchidectomized rats, preincubation with furegrelate did not affect the basal or EFS-induced NO release (Fig. 5b); similar results were obtained when the furegrelate concentration was increased to 10 μM (Fig. 5b).

NA release

Basal and EFS-induced NA release was similar in mesenteric arteries from both control and orchidectomized male rats (ANOVA, $P > 0.05$), as already reported using the tritium release method for measuring NA release (Blanco-Rivero *et al.* 2006c). The presence of furegrelate did not modify either the basal or the EFS-induced NA release in arteries from control (Fig. 6a) or orchidectomized (Fig. 6b) rats.

Figure 3 Effect of furegrelate on the concentration–response curves for norepinephrine in mesenteric artery segments from (a) control and (b) orchidectomized rats. Results (mean $\pm$ S.E.M.) are as percentage of the tone induced by 75 mM KCl. Number of animals is indicated in parenthesis.

PGI₂ release

Basal and EFS-induced PGI₂ release was similar in mesenteric arteries from both control and orchidectomized male rats (ANOVA, $P > 0.05$). Furegrelate did not modify this release in arteries from control rats (Fig. 7a), but increased it in arteries from orchidectomized rats (Fig. 7b).

Discussion

Recent studies have reported beneficial effects of androgens in cardiovascular function in males through different mechanisms that involve NO and prostanoids (Jones *et al.* 2004, Martorell *et al.* 2008). The activation of androgen receptors, expressed in both the endothelial (Yu *et al.* 2007) and smooth muscle (Ma *et al.* 2005) cells, regulates cell signaling pathways. However, the vascular effects of male sex hormones have also been reported to be independent of androgen receptor activation (Liu *et al.* 2008) and to interact

Figure 4 Effect of furegrelate on the concentration-dependent curves for sodium nitroprusside in mesenteric artery segments from (a) control and (b) orchidectomized rats. Results (mean $\pm$ S.E.M.) are expressed as percentage of inhibition of contraction induced by 1 μ M NA. Number of animals is indicated in parenthesis.

with intracellular calcium regulatory mechanisms, as reported for other sex steroids hormones (Zhang *et al.* 2006).

The role of NO and prostanoids in regulating vascular tone is well established (Henrion *et al.* 1997, Ferrer & Osol 1998, Busse & Fleming 2003), and interaction between these two systems has been described, particularly that of NO acting on prostanoids release (Laemmel *et al.* 2003, Mollace *et al.* 2005); however, reports describing the action of prostanoids on the NO system are scarce (Ferrer *et al.* 2004). TXA₂ is one of the most important vasoconstrictor prostanoids with stimulatory action on proliferation or hypertrophy of vascular smooth muscle cells (Hanasaki *et al.* 1990), and is implicated as a mediator in diseases such as myocardial infarction, hypertension, and stroke (FitzGerald *et al.* 1987, Narumiya *et al.* 1999). We previously reported that endothelial TXA₂ release in mesenteric artery (Blanco-Rivero *et al.* 2006a, 2007) and aorta (Martorell *et al.* 2008) was increased in arteries from orchidectomized rats. The fact that the levels of testosterone

Figure 5 Effect of furegrelate (Fure.) on the basal and EFS-induced NO release in mesenteric denuded segments from (a) control and (b) orchidectomized rats. Results (means $\pm$ S.E.M.) are expressed as arbitrary units/mg tissue. Number of animals is indicated in parenthesis. * P < 0.05 compared with basal TXA₂ release. + P < 0.05 compared with the respective control.

dramatically decreased in orchidectomized rats (Martorell *et al.* 2008) seems to indicate that the vascular effects observed are testosterone dependent. However, the involvement of hormones and/or gonadal factors other than testosterone cannot be ruled out. Thus, vascular endothelial growth factor, basic fibroblast growth factor, transforming growth factor- β , or hyaluronidase with gonadal origin (Lissbrant *et al.* 2003) could all play an important role in vascular function (Rahmanian & Heldin 2002).

Therefore, we studied the possible modification of EFS-induced TXA₂ release by endogenous male sex hormones. We found that orchidectomy increased the basal TXA₂ release, as previously reported in endothelium intact mesenteric arteries from comparable animals (Blanco-Rivero *et al.* 2006a, 2007); it is important to mention that basal release in arteries without endothelium was lower than that in arteries with intact endothelium, confirming endothelial and smooth muscle cells as sources of TXA₂ production.

We have previously demonstrated that EFS induced similar contractile responses in mesenteric arteries from control and orchidectomized rats, responses that appear to be mediated by NA release from adrenergic nerve terminals and the

Figure 6 Effect of furegrelate on the basal and EFS-induced norepinephrine (NA) release in mesenteric denuded segments from (a) control and (b) orchidectomized rats. Results (means $\pm$ S.E.M.) are expressed as ng/ml $\times$ mg tissue. Number of animals is indicated in parenthesis. * $P < 0.05$ compared with basal NA release. + $P < 0.05$ compared with the respective control.

subsequent activation of α -adrenoceptors (del Carmen Martín *et al.* 2005); in addition, we found that the contractile response to exogenous NA was decreased by orchidectomy (del Carmen Martín *et al.* 2005), suggesting that EFS could increase NA release in arteries from orchidectomized rats; however, we later demonstrated that the EFS-induced NA release was not modified by orchidectomy (Blanco-Rivero *et al.* 2006c), which indicates that other vasoconstrictor factors could be released when the artery was electrically stimulated. Since the EFS-induced release of TXA₂ has been demonstrated in hypertensive rats (Aras-López *et al.* 2007), we analyzed the EFS-induced TXA₂ release in normotensive rats, as well as the possible role of endogenous male sex hormones in that release. We observed that EFS induced a greater TXA₂ formation in arteries from orchidectomized than control rats, which is in line with reports showing increased TXA₂ release after activation of different receptors (Blanco-Rivero *et al.* 2006a, 2007). This result also indicates that TXA₂ could be the contractile factor that is released when the artery is electrically stimulated, as suggested previously (Blanco-Rivero *et al.*

Figure 7 Effect of furegrelate on the basal and EFS-induced PGI₂ release in mesenteric denuded segments from (a) control and (b) orchidectomized rats. Results (means $\pm$ S.E.M.) are expressed as pg/ml $\times$ mg tissue. Number of animals is indicated in parenthesis. * $P < 0.05$ compared with basal PGI₂ release. + $P < 0.05$ compared with the respective control.

2006c). Increased TXA₂ release would explain the non-modification of the EFS-induced response in arteries from control and orchidectomized rats, in spite of the fact that the NA response was diminished in arteries from the latter animals.

The next step was to analyze the function of endogenous TXA₂ in the EFS-induced response, as well as the dependence on male sex hormones. Preincubation with furegrelate did not modify the basal tone in arteries from control or orchidectomized rats, indicating that endogenous TXA₂ does not have a direct effect on vascular tone regulation in basal conditions. We showed that furegrelate decreased the EFS-induced response in arteries from control rats, but did not modify it in arteries from orchidectomized rats, indicating that the effect of endogenous TXA₂ on the EFS response is under male sex hormone regulation.

It is widely reported that mesenteric arteries possess nitrergic (Marín & Balfagón 1998, del Carmen Martín *et al.* 2005), sympathetic (Li & Duckles 1992), and sensory (Kawasaki *et al.* 1988) innervations that modulate vasomotor tone; therefore, the EFS-induced contraction is the result of a balance between opposing vasoconstrictor and vasodilator factors (Vanhoutte

1996, Ferrer & Balfagón 2001). Since, in our experimental conditions, we have demonstrated that sensory innervation did not modulate the vasomotor response to EFS (del Carmen Martín *et al.* 2005); we studied whether the differences in the EFS-induced contractions observed in the presence of furegrelate were due to alterations in nitrergic and adrenergic innervations.

Since there is a lack of studies analyzing the effect of TXA₂ on neuronal NO release, and since we previously reported that endogenous prostanoids different from TXA₂, i.e. PGI₂ increased neuronal NO release (Ferrer *et al.* 2004), it is possible to speculate that endogenous TXA₂ could regulate neuronal NO and vasomotor function. Therefore, we studied the effect of the TXA₂ synthesis inhibitor furegrelate, on the release and function of neuronal NO, as well as the dependence on endogenous male sex hormones. We found that in arteries from control rats, furegrelate increased the neuronal NO release, which is in line with reports describing an inhibitory effect of TXA₂ on inducible (Yamada *et al.* 2003) and endothelial (Miyamoto *et al.* 2007) NO release. The vasodilator response induced by the NO donor, SNP, was also increased by furegrelate, showing that endogenous TXA₂ negatively modulates both the release and the vasodilator effect of neuronal NO. These results could also explain the decreased the EFS-induced contraction in the presence of furegrelate, but alterations in the release and function of neurotransmitters other than NO cannot be ruled out.

Different modulating effects of TXA₂ on NA release have been reported, including inhibition (Nishihara *et al.* 2000) and non-modification (Rump & Schollmeyer 1989). The fact that furegrelate did not modify either NA release or its vasomotor response, as reported previously (Molderings *et al.* 1998, Hoang *et al.* 2003), indicates that endogenous TXA₂ does not alter the function of sympathetic innervation in arteries from control rats.

By contrast, in arteries from orchidectomized rats, furegrelate did not modify the basal and EFS-induced NO release; since TXA₂ formation was greater in arteries from orchidectomized than control rats, we used a higher concentration of furegrelate, and still obtained similar results. In addition, the vasodilator response induced by SNP was not modified by furegrelate. These results show that endogenous TXA₂ does not regulate the release or function of neuronal NO in arteries from orchidectomized rats, in contrast to what occurs in arteries from control rats.

Regarding noradrenergic neurotransmission, we found that furegrelate modified neither the NA release nor the vasoconstrictor response induced by exogenous NA, indicating that the function of the sympathetic innervation is not regulated by endogenous TXA₂ in arteries from orchidectomized rats, as was also observed in arteries from control rats.

Since TXA₂ was higher in arteries from orchidectomized rats, and since endogenous TXA₂ did not modify either the release or function of NO or NA, the unaltered EFS-induced response observed in the presence of furegrelate could be explained through the release of vasodilator factors that would counterbalance the vasoconstrictor effect of TXA₂.

One of the more plausible candidates would be PGI₂, since crosstalk between TXA₂ and PGI₂ systems has been reported (Cheng *et al.* 2002, Martorell *et al.* 2008) and joint increases in PGI₂ and TXA₂ synthesis have been shown in pathological conditions (FitzGerald 1991, Caughey *et al.* 2001). Therefore, we measured the production of PGI₂ in the presence of furegrelate in mesenteric arteries from both control and orchidectomized rats. First, we found that orchidectomy did not modify either the basal or EFS-induced PGI₂ release, in contrast to the increased PGI₂ formation observed in aorta from comparable animals (Martorell *et al.* 2008); these results are in line with reports showing smooth muscle (Wang *et al.* 1993, Ferrer *et al.* 2004) and/or neuronal (Snitsarev *et al.* 2005) cells as cellular sources of PGI₂. Concerning the effect of endogenous TXA₂ on PGI₂ production, we observed that the inhibition of the endogenous TXA₂ synthesis did not modify the release of PGI₂ in arteries from control rats, although it did increase PGI₂ release in arteries from orchidectomized rats. This result reinforced the suggestion that the possible increase in the release of vasodilator substances that counterbalance the vasoconstrictor effect of TXA₂, although the involvement of substances other than PGI₂ cannot be ruled out.

In summary, this study demonstrates that non-endothelial TXA₂ release is increased in mesenteric arteries from orchidectomized rats. The effect of endogenous TXA₂ on the EFS-induced response is also regulated in a gonad-dependent way, suggesting that male sex hormones modulate different simultaneous cell signaling pathways. In arteries from control rats, inhibition of TXA₂ formation decreases the EFS-induced response by increasing neuronal NO release, but in arteries from orchidectomized rats, the EFS-induced response is unaltered after the inhibition of TXA₂ formation, by increasing PGI₂ release.

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References

- Alexandersen P, Haarbo J & Christiansen C 1996 The relationship of natural androgens to coronary heart disease in males: a review. *Atherosclerosis* **125** 1–13.
- Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H & Christiansen C 1999 Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circulation Research* **84** 813–819.
- Aras-López R, Blanco-Rivero J, Xavier FE, Salices M, Ferrer M & Balfagón G 2007 Dexamethasone decreases contraction to electrical field stimulation in

- mesenteric arteries from spontaneously hypertensive rats through decreases in thromboxane A₂ release. *Journal of Pharmacological and Experimental Therapeutics* **322** 1129–1136.
- Blanco-Rivero J, Cachofeiro V, Lahera V, Aras-Lopez R, Márquez-Rodas I, Salaices M, Xavier FE, Ferrer M & Balfagón G 2005 Participation of prostacyclin in endothelial dysfunction induced by aldosterone in normotensive and hypertensive rats. *Hypertension* **46** 107–112.
- Blanco-Rivero J, Balfagón G & Ferrer M 2006a Orchidectomy modulates α 2-adrenoceptor reactivity in rat mesenteric artery through increased Tromboxane A₂ formation. *Journal of Vascular Research* **43** 101–108.
- Blanco-Rivero J, Sagredo A, Balfagón G & Ferrer M 2006b Orchidectomy increases expression and activity of Cu/Zn-superoxide dismutase, while decreasing endothelial nitric oxide bioavailability. *Journal of Endocrinology* **190** 771–778.
- Blanco-Rivero J, Aras-López R, Del Campo L, Sagredo A, Balfagón G & Ferrer M 2006c Orchidectomy increases beta-adrenoceptor activation-mediated neuronal nitric oxide and noradrenaline release in rat mesenteric artery. *Neuroendocrinology* **84** 378–385.
- Blanco-Rivero J, Sagredo A, Balfagón G & Ferrer M 2007 Protein kinase C activation increases endothelial nitric oxide release in mesenteric arteries from orchidectomized rats. *Journal of Endocrinology* **192** 189–197.
- Bredt DS, Ferris CD & Snyder SH 1992 Nitric oxide synthase regulatory sites. Phosphorylation by cyclic AMP-dependent protein kinase, protein kinase C, and calcium/calmodulin protein kinase; identification of flavin and calmodulin binding sites. *Journal of Biological Chemistry* **267** 10976–10981.
- Busse R & Fleming I 2003 Regulation of endothelium-derived vasoactive autacoid production by hemodynamic forces. *Trends in Pharmacological Sciences* **24** 24–29.
- del Carmen Martín M, Balfagón G, Minoves N, Blanco-Rivero J & Ferrer M 2005 Androgens deprivation increases neuronal nitric oxide metabolism and its asodilator effect in rat mesenteric arteries. *Nitric Oxide* **12** 163–176.
- Caughey GE, Cleland LG, Gamble JR & James MJ 2001 Up-regulation of endothelial cyclooxygenase-2 and prostanoid synthesis by platelets. Role of thromboxane A₂. *Journal of Biological Chemistry* **276** 37839–37845.
- Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA & FitzGerald GA 2002 Role of prostacyclin in the cardiovascular response to thromboxane A₂. *Science* **296** 539–541.
- Féltout M & Vanhoutte PM 2006 Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *American Journal of Physiology. Heart and Circulatory Physiology* **291** 985–1002.
- Ferrer M & Balfagón G 2001 Aging alters neuronal nitric oxide release from eat mesenteric arteries: role of presynaptic β -adrenoreceptors. *Clinical Science* **101** 321–328.
- Ferrer M & Osol G 1998 Estrogen replacement modulates resistance artery smooth muscle and endothelial α 2-adrenoceptor reactivity. *Endothelium* **6** 133–141.
- Ferrer M, Marín J & Balfagón G 2000 Diabetes alters neuronal nitric oxide release from rat mesenteric arteries: role of protein kinase C. *Life Sciences* **66** 337–345.
- Ferrer M, Salaices M & Balfagón G 2004 Endogenous prostacyclin increases neuronal nitric oxide release in mesenteric artery from spontaneously hypertensive rats. *European Journal of Pharmacology* **506** 151–156.
- FitzGerald GA 1991 Mechanisms of platelet activation: thromboxane A₂ as an amplifying signal for other agonists. *American Journal of Cardiology* **68** 11–15.
- FitzGerald GA, Healy C & Daugherty J 1987 Thromboxane A₂ biosynthesis in human disease. *Federation Proceedings* **46** 154–158.
- Hanasaki K, Nakano T & Arita H 1990 Receptor-mediated mitogenic effect of thromboxane A₂ in vascular smooth muscle cells. *Biochemical Pharmacology* **40** 2535–2542.
- Henrion D, Dechaux E, Dowell FJ, MacIour J, Samuel JL, Lévy BI & Michel JB 1997 Alteration of flow-induced dilatation in mesenteric resistance arteries of L-NAME treated rats and its partial association with induction of cyclooxygenase-2. *British Journal of Pharmacology* **121** 83–90.
- Hoang D, Macarthur H, Gardner A, Yang CL & Westfall TC 2003 Prostanoid-induced modulation of neuropeptide Y and noradrenaline release from the rat mesenteric bed. *Autonomic and Autacoid Pharmacology* **23** 141–147.
- Holzmann S 1982 Endothelium-induced relaxation by acetylcholine associated with larger rises in cyclic GMP in coronary arterial strips. *Journal of Cyclic Nucleotide Research* **8** 409–419.
- Ignarro LJ & Kadowitz PJ 1985 The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. *Annual Review of Pharmacology and Toxicology* **25** 171–191.
- Jones RD, Hugh Jones T & Channer KS 2004 The influence of testosterone upon vascular reactivity. *European Journal of Endocrinology* **151** 29–37.
- Kawasaki H, Takasaki K, Saito A & Goto H 1988 Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat. *Nature* **335** 164–167.
- Laemmel E, Bonnardel-Phu E, Hou X, Seror J & Vicaut E 2003 Interaction between nitric oxide and prostanoids in arterioles of rat cremaster muscle *in vivo*. *American Journal of Physiology. Heart and Circulatory Physiology* **285** 1254–1260.
- Li YJ & Duckles SP 1992 Effect of endothelium on the actions of sympathetic and sensory nerves in the perfused rat mesentery. *European Journal of Pharmacology* **210** 23–40.
- Lissbrant IF, Lissbrant E, Persson A, Damber JE & Bergh A 2003 Endothelial cell proliferation in male reproductive organs of adult rat is high and regulated by testicular factors. *Biology of Reproduction* **68** 1107–1111.
- Liu D, Iruthayanathan M, Homan LL, Wang Y, Yang L, Wang Y & Dillon JS 2008 Dehydroepiandrosterone stimulates endothelial proliferation and angiogenesis through ERK 1/2-mediated mechanisms. *Endocrinology* **149** 889–898.
- Ma R, Wu S & Lin Q 2005 Homologous up-regulation of androgen receptor expression by androgen in vascular smooth muscle cells. *Hormone Research* **63** 6–14.
- Marco EJ, Balfagón G, Salaices M, Sanchez-Ferrer CF & Marín J 1985 Serotonergic innervation of cat cerebral arteries. *Brain Research* **338** 137–139.
- Marín J & Balfagón G 1998 Effect of clenbuterol on non-endothelial nitric oxide release in rat mesenteric arteries and the involvement of beta-adrenoceptors. *British Journal of Pharmacology* **124** 473–478.
- Martorell A, Blanco-Rivero J, Aras-López R, Sagredo A, Balfagón G & Ferrer M 2008 Orchidectomy increases the formation of prostanoids and modulates their role in the acetylcholine-induced relaxation in the rat aorta. *Cardiovascular Research* **77** 590–599.
- Minoves N, Balfagón G & Ferrer M 2002 Role of female sex hormones in neuronal nitric oxide release and metabolism in rat mesenteric arteries. *Clinical Science* **103** 239–247.
- Miyamoto A, Hashiguchi Y, Obi T, Ishiguro S & Nishio A 2007 Ibuprofen or ozagrel increases NO release and L-nitro arginine induces TXA₂ release from cultured porcine basilar arterial endothelial cells. *Vascular Pharmacology* **46** 85–90.
- Molderings GJ, Likungu J & Göthert M 1998 Modulation of noradrenaline release from the sympathetic nerves of human right atrial appendages by presynaptic EP3- and DP-receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* **358** 440–444.
- Mollace V, Muscoli C, Masini E, Cuzzocrea S & Salvemini D 2005 Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors. *Pharmacological Reviews* **57** 217–252.
- Narumiya S, Sugimoto Y & Ushikubi F 1999 Prostanoid receptors: structures, properties, and functions. *Physiological Reviews* **79** 1193–1226.
- Ng MK 2007 New perspectives on Mars and Venus: unravelling the role of androgens in gender differences in cardiovascular biology and disease. *Heart, Lung and Circulation* **16** 185–192.
- Nielsen KC & Owman C 1971 Contractile response and amine receptor mechanism in isolated middle cerebral artery of the cat. *Brain Research* **27** 25–32.
- Nishihara M, Yokotani K, Inoue S & Osumi Y 2000 U-46619, a selective thromboxane A₂ mimetic, inhibits the release of endogenous noradrenaline from the rat hippocampus *in vitro*. *Japanese Journal of Pharmacology* **82** 226–231.
- Phillips GB, Jing TY, Resnick LM, Barbagallo M, Laragh JH & Sealey JE 1993 Sex hormones and hemostatic risk factors for coronary heart disease in men with hypertension. *Journal of Hypertension* **11** 699–702.
- Rahmanian M & Heldin P 2002 Testicular hyaluronidase induces tubular structures of endothelial cells grown in three-dimensional collagen gel through a CD44-mediated mechanism. *International Journal of Cancer* **97** 601–607.
- Rump LC & Schollmeyer P 1989 Effects of endogenous and synthetic prostanoids, the thromboxane A₂ receptor agonist U-46619 and arachidonic acid on [3H]-noradrenaline release and vascular tone in rat isolated kidney. *British Journal of Pharmacology* **97** 819–828.

- Salvemini D, Currie MG & Mollace V 1996 Nitric oxide-mediated cyclooxygenase activation. A key event in the antiplatelet effects of nitrovasodilators. *Journal of Clinical Investigation* **97** 2562–2568.
- Snitsarev V, Whiteis CA, Chapleau MW & Abboud FM 2005 Neuronal prostacyclin is an autocrine regulator of arterial baroreceptor activity. *Hypertension* **46** 540–546.
- Teede HJ 2007 Sex hormones and the cardiovascular system: effects on arterial function in women. *Clinical and Experimental Pharmacology and Physiology* **34** 672–676.
- Vanhoutte PM 1996 Endothelium-dependent responses in congestive heart failure. *Journal of Molecular and Cellular Cardiology* **28** 2233–2240.
- Vanhoutte PM, Verbeuren JT & Clinton R 1981 Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. *Physiological Reviews* **61** 151–247.
- Wang W, Brändle M & Zucker IH 1993 Indomethacin reduces acute baroreceptor resetting in the dog. *Journal of Physiology* **469** 139–151.
- Wranicz JK, Cygankiewicz I, Rosiak M, Kula P, Kula K & Zareba W 2005 The relationship between sex hormones and lipid profile in men with coronary artery disease. *International Journal of Cardiology* **101** 105–110.
- Yamada T, Fujino T, Yuhki K, Hara A, Karibe H, Takahata O, Okada Y, Xiao CY, Takayama K, Kuriyama S *et al.* 2003 Thromboxane A₂ regulates vascular tone via its inhibitory effect on the expression of inducible nitric oxide synthase. *Circulation* **108** 2381–2386.
- Yu J, Eto M, Akishita M, Kaneko A, Ouchi Y & Okabe T 2007 Signaling pathway of nitric oxide production induced by ginsenoside Rb1 in human aortic endothelial cells: a possible involvement of androgen receptor. *Biochemical and Biophysical Research Communications* **353** 764–769.
- Zhang H, Xiao D, Longo LD & Zhang L 2006 Regulation of alpha1-adrenoceptor-mediated contractions of uterine arteries by PKC: effect of pregnancy. *American Journal of Physiology. Heart and Circulatory Physiology* **291** 2282–2289.

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