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RESEARCH PAPER

Altered arachidonic acid metabolism via COX-1 and COX-2 contributes to the endothelial dysfunction of penile arteries from obese Zucker rats

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Background and purpose: The aim of the current study was to investigate the role of arachidonic acid (AA) metabolism via cyclooxygenase (COX) in the endothelial dysfunction of penile arteries from pre-diabetic, obese Zucker rats (OZR).
Experimental approach: Penile arteries from OZR and from lean Zucker rats (LZR) were mounted in microvascular myographs to assess vascular function and COX expression was determined by immunohistochemistry.
Key results: Acetylcholine (ACh) and AA elicited relaxations that were impaired in arteries from OZR. Inhibition of both COX-1 and COX-2 reduced the relaxant effects of ACh and AA in LZR but not in OZR. Inhibitors of COX-1 and of the TXA_2/PGH_2 (TP) receptor enhanced the relaxations induced by AA in both LZR and OZR, whereas COX-2 inhibition enhanced these responses only in OZR. TP receptor blockade did not restore ACh relaxant responses in arteries from OZR. Inhibition of COX-1 increased basal tension in OZR and this contraction was blunted by TP receptor blockade. The vasoconstrictor responses to noradrenaline were augmented by indomethacin and by COX-2 inhibition in LZR but not in OZR. Immunohistochemical staining showed that both COX-1 and COX-2 are expressed in the endothelium of penile arteries from both LZR and OZR.
Conclusions and implications: Vasoactive prostanoids were formed via constitutively active COX-1 and COX-2 pathways in normal rat penile arteries. Under conditions of insulin resistance, the release and/or effects of vasodilator prostanoids were impaired, contributing to the blunted endothelium-dependent vasodilatation and to the enhanced vasoconstriction.
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Keywords: insulin resistance; penile arteries; endothelial dysfunction; arachidonic acid; COX-1; constitutive COX-2; obese Zucker rat

Abbreviations: AA, arachidonic acid; ED, erectile dysfunction; L-NNA, N^{ω}-nitro-L-arginine; LZR, lean Zucker rat; OZR, obese Zucker rat

Introduction

Erectile dysfunction (ED) is a highly prevalent condition in men with cardiovascular risk factors and is now considered to be an early sign of systemic endothelial dysfunction and subclinical vascular disease (Billups, 2005). The metabolic syndrome, a cluster of metabolic abnormalities associated with obesity and including insulin resistance, hyperglycemia and hyperlipidemia, has become a major clinical and public health problem and a large group of patients are at increased risk for developing diabetes and cardiovascular disease. Approximately 35% to 75% of men with diabetes mellitus suffer ED (Vickers and Wright, 2004). Some aspects of the metabolic syndrome have also been associated with ED and an increased prevalence has been demonstrated in men with metabolic syndrome (Espósito *et al.*, 2005; Fonseca and Jawa, 2005). On the other hand, the prevalence of obesity and associated risk factors in men reporting symptoms of ED is remarkably high and strong epidemiological evidence links the risk of ED to diabetes type 2 (Vickers and Wright, 2004; Espósito *et al.*, 2005; Fonseca and Jawa, 2005).

Penile erection is a complex neurovascular process primarily achieved by the relaxation of penile smooth muscle and further expansion and filling of the cavernous sinusoids, due to nerve- and endothelial-derived nitric oxide (NO), released

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upon sexual stimulation (Prieto, 2008). Studies in diabetic animal models and in human cavernosal tissue from diabetic men have pointed to the multifactorial mechanisms of the diabetes-associated ED and both endothelial and neural NO-mediated relaxant responses have been shown to be impaired (Saenz de Tejada *et al*., 1989; Keegan *et al*., 1999; Angulo *et al*., 2003). Metabolic disorders associated with diabetes include hyperglycemia, excess free fatty acids and insulin resistance, components of the metabolic syndrome that are risk factors for ED and also characterized by abnormal endothelial function (Espósito *et al*., 2005; Fonseca and Jawa, 2005). Although diabetic endothelial dysfunction has primarily been considered to reflect a deficiency of NO, the mechanisms responsible for the impaired endothelium-dependent vasodilatation in diabetes and metabolic syndrome still need to be fully elucidated (De Vriese *et al*., 2000; Kuboki *et al*., 2000; Okon *et al*., 2005; Vanhoutte *et al*., 2009).

An imbalance between the production of vasodilator and vasoconstrictor prostanoids has recently been shown to underlie endothelial dysfunction and abnormal vascular smooth muscle reactivity in arteries from rodent models of both type 1 and type 2 diabetes (Matsumoto *et al*., 2007; Shi and Vanhoutte, 2008) and metabolic syndrome (Goodwill *et al*., 2008; Xiang *et al*., 2008). In healthy blood vessels, most prostanoids are formed by the constitutive isoform of cyclooxygenase 1 (COX-1). However, these mediators may also be synthesized by the inducible COX isoform COX-2 that is usually expressed at undetectable levels in vascular cells but can be up-regulated by inflammatory, mitogenic and physical stimuli (Seibert *et al*., 1994; Herranz *et al*., 2004; Warner and Mitchell, 2004). Moreover, in the last years, it has become evident that prostanoid production from constitutively expressed COX-2 is also involved in the modulation of vascular responses (Henrion *et al*., 1997; Baber *et al*., 2003; Qui *et al*., 2006).

We have recently demonstrated profound alterations in vascular structure that correlate with endothelial dysfunction in penile arteries from the obese Zucker rat (OZR) (Villalba *et al*., 2009), a well-established genetic model of insulin resistance and metabolic syndrome caused by a dysfunctional gene of the leptin receptor (Guerre-Millo, 1997). The purpose of the present study was to further elucidate the mechanisms underlying penile endothelial dysfunction and to assess whether there is a specific role of altered vascular prostanoid metabolism in the impaired dilatation and abnormal vasoconstriction of penile arteries from OZR.

Methods

Animal model

All animal care and experimental protocols conformed to the European Union Guidelines for the Care and the Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Madrid Complutense University. Male OZR (*fa/fa, n* = 63) and their control strain, lean Zucker rats (LZR) (*fa/-, n* = 60) were purchased from Charles River Laboratories (Barcelona, Spain) at 8–10 weeks of age. Animals were housed at the Pharmacy School animal care facility and maintained on standard chow and water *ad libitum*, until they were used for study, at 17–18 weeks of age.

Dissection of microvessels, mounting and force measurement

Rats were killed by cervical dislocation and exanguination. The penile arteries, first- or second-order branches of the rat dorsal penile artery from LZR and OZR rats were carefully dissected by removing the connective and fat tissue, as described previously (Villalba *et al*., 2009). Segments of dorsal penile arteries were mounted in parallel in double microvascular myographs (Danish Myotechnology, Denmark) by inserting two 40 μm tungsten wires into the vessel lumen. After mounting, the arteries were equilibrated for 30 min in Krebs solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.17, $MgSO_4$ 1.18, $CaCl_2$ 1.5, EDTA 0.027 and glucose 11, mantained at 37°C and continuously gassed with a mixture of 5% CO_2/95% O_2 to maintain pH at 7.4. The relationship between passive wall tension and internal circumference was determined for each individual artery and from this, the internal diameter, l_1, that yielded a circumference equivalent to 90% of that given by an internal pressure of 100 mmHg was calculated.

Experimental procedures for the functional experiments

At the beginning of each experiment, arteries were challenged twice with 120 mM K^+ (KPSS) in order to test vessel viability. The vasoactive effects of acetylcholine (ACh) (Sigma Chemical Co., St Louis, MO, USA) and of the prostanoid precursor, arachidonic acid (AA) (Sigma Chemical), were evaluated by adding cumulative concentrations of these agents on arteries precontracted with 1 μM phenylephrine (Sigma Chemical). The effects of the NOS inhibitor N^{ω}-nitro-L-arginine (L-NNA, 100 μM) (Sigma Chemical) and of the non-selective COX inhibitor indomethacin (1 μM) (Sigma Chemical) were initially tested on the ACh-induced relaxations. The responses to exogenous ACh, AA and noradrenaline (Sigma Chemical) were further obtained in the absence and presence of specific inhibitors of COX-1 (SC-560, 1 μM) (Sigma Chemical), COX-2 (NS-398, 1 μM) (Tocris Cookson, Bristol, UK) and the antagonist of the TXA_2/PGH_2 (TP) receptor, (ICI-192, 1 μM) (Tocris). The effect of the TXA_2 agonist U46619 was also assessed in penile arteries of LZR and OZR, in the absence and presence of ICI-192. The drugs were added to the myograph chamber 30 min before the construction of a second concentration–response curve for the corresponding agonist. The role of the vascular endothelium was examined in arteries were the endothelium was mechanically removed by inserting a human hair in the vessel lumen and guiding it back and forwards several times. The absence of functional endothelium was confirmed by the lack of relaxation to ACh (10 μM).

Immunohistochemistry

Tissue samples from the penis containing the dorsal penile artery were inmersion-fixed in 4% paraformaldehyde in 0.1 M sodium phosphate-buffer (PB), cryoprotected in 30% sucrose in PB and snap-frozen in liquid nitrogen and stored at –80°C. Tissue sections were processed following the avidin-biotin-peroxidase complex (ABC) method (Hsu *et al*., 1981). Sections were first immersed in a mixture of 1% H_2O_2 and 90% methanol in distilled water for 30 min, washed in PB and the pre-incubated in 10% normal goat serum in PB containing 0.3%

Triton-X-100 for 2–3 h. Then, sections were incubated with either a mouse monoclonal anti-COX-1 (Cayman Chemical, Ann Arbor, MI, USA), diluted at 1:100 or a rabbit anti COX-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:200 for 48 h, washed and reacted with a biotinylated goat secondary serum (Chemicon International Inc) (anti-mouse for the COX-1 and anti-rabbit for the COX-2) diluted 1:400 for 2h at room temperature, followed by incubation with avidin-biotin-complex (ABC, Vector) 1:100 dilution for 90 min at room temperature. The immunocomplex was visualized with 0.05% 3.3 diaminobenzidine and 0.001% in H_2O_2 in PB. No immunoreactivity could be detected in sections incubated in the absence of the primary antisera. Preadsorption with COX-1 and COX-2 protein showed no cross-reactivity for the antibodies.

Data presentation and statistical analysis

Results are expressed as either tension (as $N{\cdot}m^{-1}$) or as a percent of the response to either phenylephrine or KPSS in each artery, as means ± SEM of six to eight arteries (one to two from each animal). The sensitivity of the arteries to the relaxant and vasoconstrictor agonists is expressed in terms of pEC_{50} values, where pEC_{50} is $-\log EC_{50}$. EC_{50} is the concentration of the agonist required to producing 50% of the response and was calculated by non-linear curve fitting of the concentration–response curves for the inhibitor to the classical Hill Equation by using a standart computer software (Prism 5.0, GraphPad, San Diego, CA, USA). The EC_{50} value for each individual curve was first obtained and thereafter the average value for a given set of experiments was calculated. The statistical differences between means were analysed by using one-way ANOVA, paired or unpaired Student's *t*-test when appropiate. Probability levels smaller than 5% were considered significant.

Drug and molecular target nomenclature follows Alexander *et al*. (2008).

Results

General parameters

At the time of the experiment (17–18 weeks of age), OZR were significantly heavier than LZR (483 ± 5 g vs. 375 ± 5 g, $P < 0.001$, $n = 51$). We have recently reported that animals from the OZR group exhibit mild hyperglycemia, hyperinsulinemia and dyslipidemia with elevated total cholesterol and triglyceride levels (Villalba *et al*., 2009). The normalized internal lumen diameters, $l_{1,}$ were significantly smaller in penile arteries from OZR (135 ± 3 μm) compared with LZR (150 ± 3 μm, $P < 0.01$, $n = 51$). The contractions to KPSS were reduced in the OZR group (1.8 ± 0.1 $N{\cdot}m^{-1}$) compared with LZR (2.4 ± 0.1 $N{\cdot}m^{-1}$, $P < 0.01$; $n = 51$), indicating reduced contractility of arterial smooth muscle. Although maximal contractile responses to noradrenaline were reduced, sensitivity was augmented in OZR (pEC_{50} 6.96 ± 0.11 vs. 6.48 ± 0.08, $P < 0.0001$, $n = 27$, in LZR and OZR respectively). The endothelium-dependent responses to ACh were markedly reduced in penile arteries from OZR compared with LZR (27 ± 5% vs. 60 ± 4%, $P < 0.0001$, $n = 24$), thus indicating endothelial dysfunction.

Effect of COX inhibitors on ACh relaxant responses

In order to assess whether changes in the AA metabolism via COX may be involved in the endothelial dysfunction observed in penile arteries from OZR, the effect of the non-selective COX inhibitor, indomethacin (1 μM) and of the selective COX-1 and COX-2 blockers SC-560 and NS-398, were examined on the relaxant responses to ACh. Indomethacin reduced the relaxations elicited by ACh in arteries from LZR and to a lesser degree in those from OZR (Figure 1, Table 1). Treatment with L-NNA (100 μM) markedly reduced the relaxations to ACh in both LZR and OZR, this inhibition being larger than that produced by indomethacin in LZR (Figure 1). Combined blockade of COX and NOS abolished

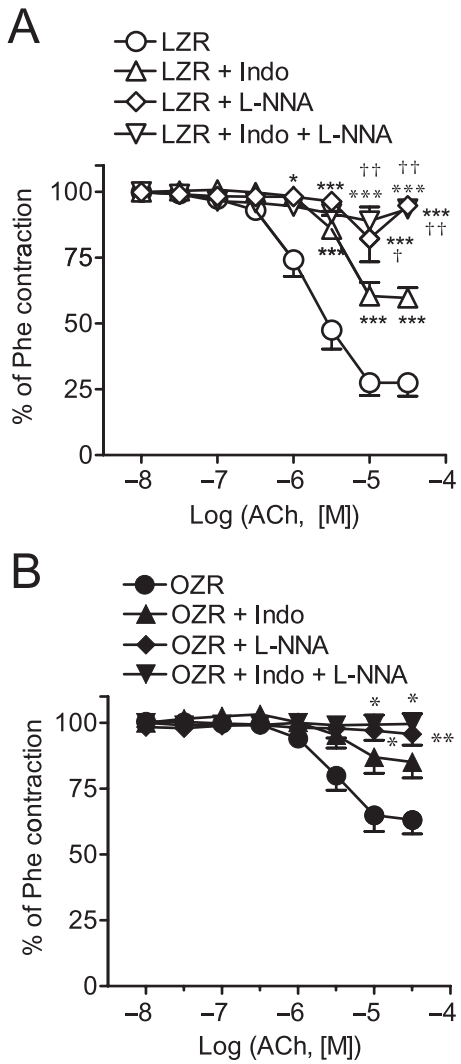

Figure 1 Effect of the inhibitor of COX, indomethacin (indo) (1 μM) and the inhibitor of the NO synthesis, L-NNA (100 μM) on the relaxant responses to acetylcholine (ACh) in penile arteries from LZR (A) and OZR (B). Results are expressed as percentage of the pre-contraction induced by phenylephrine (Phe). Data are shown as the means ± SEM of 6–13 arteries. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control before treatment. †$P < 0.01$; ††$P < 0.001$ versus indomethacin-treated. COX, cyclooxygenase; L-NNA, N^{ω}-nitro-L-arginine; LZR, lean Zucker rats; NO, nitric oxide; OZR, obese Zucker rats.

Table 1 Effects of indomethacin (indo) (1 μM), of the selective COX-1 inhibitor SC-560 (1 μM) and of the COX-2 inhibitor NS-398 (1 μM) on the vasodilator responses to acetylcholine (ACh) of penile arteries from LZR and OZR

	ACh					
	LZR			*OZR*		
	pEC_{50}	*Emax (%)*	n	pEC_{50}	*Emax (%)*	n
ACh	5.77 ± 0.06	73.1 ± 7.3	7	5.50 ± 0.18	37.6 ± 9.7	6
+Indo	5.57 ± 0.15	40.3 ± 3.8**	7	–	14.9 ± 6.0*	6
ACh	6.55 ± 0.05	60.0 ± 7.6	8	5.64 ± 0.43	24.6 ± 4.3	8
+SC-560	6.51 ± 0.27	38.4 ± 6.1*	8	–	15.6 ± 5.0*	8
ACh	6.10 ± 0.09	62.4 ± 7.2	8	6.02 ± 0.25	33.4 ± 9.9	10
+NS-398	5.89 ± 0.08*	52.1 ± 6.9	8	6.11 ± 0.17	26.6 ± 5.2	10

Values represent mean ± SE of the number *n* of individual arteries. pEC_{50} is $-\log EC_{50}$, being the concentration of agonist giving half maximal response (Emax). Significant differences from controls were analysed by a paired Student's *t*-test.
*$P < 0.05$; **$P < 0.01$.
COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

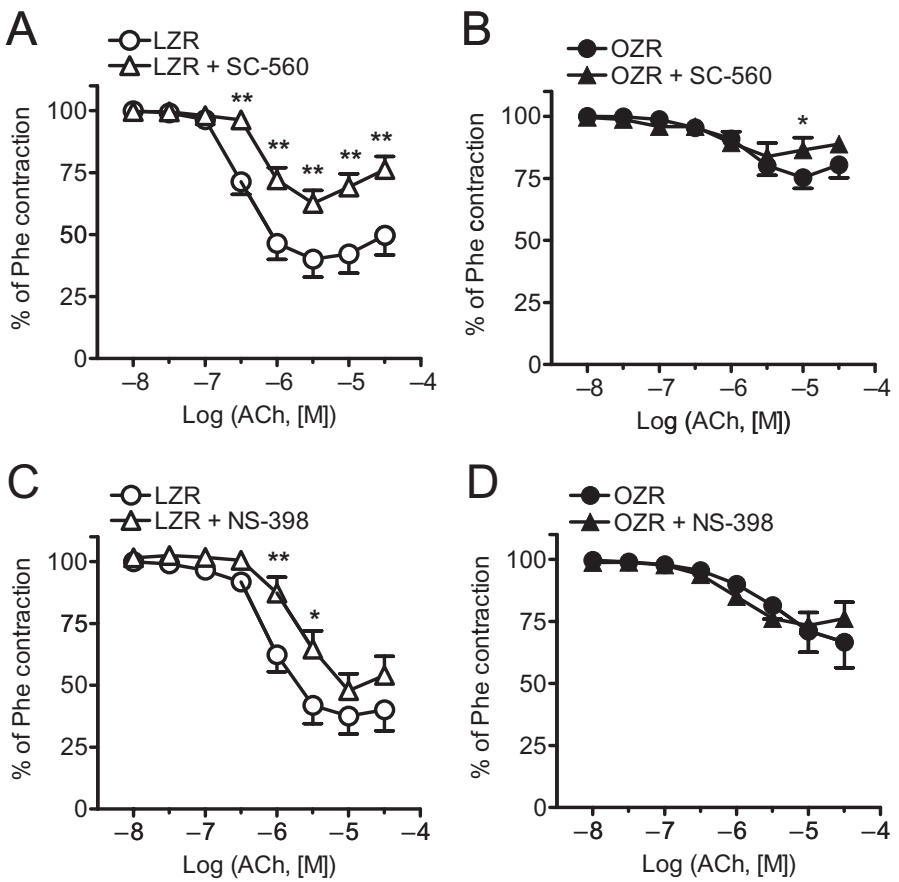

Figure 2 Effects of the selective COX-1 inhibitor SC-560 (1 μM) (A, B) and the selective COX-2 inhibitor NS-398 (1 μM) (C, D) on the average relaxant responses to acetylcholine (ACh) in penile arteries from LZR (A, C) and OZR (B, D). Results are expressed as percentage of the pre-contraction induced by phenylephrine (Phe). Data are shown as the means ± SEM of 8–10 arteries. *$P < 0.05$, **$P < 0.01$ versus control before treatment. COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

the relaxant responses to ACh in both LZR and OZR (Figure 1), suggesting the involvement of both NO and prostanoids. Treatment with 1 μM of the selective COX-1 antagonist SC-560 inhibited the relaxations induced by ACh in penile arteries from LZR (Figure 2A, Table 1) but not from OZR (Figure 2B, Table 1). Furthermore, selective inhibition of COX-2 with NS-398 (1 μM) also reduced the relaxations to ACh in LZR but did not affect those in OZR (Figure 2C,D, Table 1). These results suggest that the endothelium-dependent vasodilator responses to ACh involve both COX-1- and COX-2-derived vasodilator prostanoids in LZR which are impaired in OZR.

Effect of COX inhibitors and TP receptor antagonism on responses to AA

Exogenously administered AA (0.1–10 μM) induced concentration-dependent relaxations in penile arteries from LZR precontracted with phenylephrine, which were impaired in arteries from OZR (Figure 3).

In order to investigate the metabolism of AA by COX under basal conditions, the effects of the selective inhibitors of COX-1 and COX-2 and TP receptor were investigated. The COX-1 inhibitor SC-560 reduced the relaxations to the lower doses of AA (1 μM) in arteries from LZR and enhanced those at higher doses used (10 μM) in arteries from both LZR and OZR (Figure 4A,B). These results suggest that constrictor prostanoids are produced from AA via COX-1 in penile arteries from LZR and OZR. The selective COX-2 inhibitor NS-398 (1 μM) reduced the relaxations to AA in LZR (Figure 4C), but increased these relaxations in OZR (Figure 4D). These results indicate that there is a constitutive production of relaxant prostanoids via COX-2 in LZR that is changed to the formation of vasoconstrictor prostanoids in OZR.

The selective PGH_2/TXA_2 receptor antagonist IC-192 (1 μM) enhanced the relaxant effects of AA in penile arteries from both LZR and OZR, suggesting an endogenous basal production of PGH_2/TXA_2 that usually counterbalances the relaxant effects of AA (Figure 4E,F).

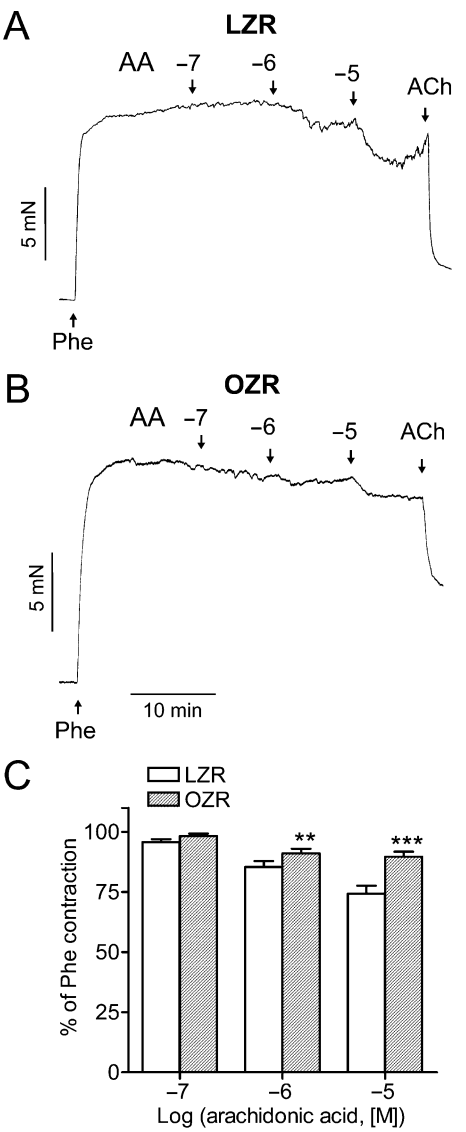

Figure 3 Relaxant responses to AA in penile arteries from LZR and OZR. (A, B) Representative traces showing the AA-induced relaxations in penile arteries from (A) LZR and (B) OZR. (C) Average concentration–response curves for the relaxation to AA. Data are shown as the means ± SEM of 37 and 39 arteries (one to two per animal). $^{**}P < 0.01$, $^{***}P < 0.001$ versus LZR. AA, arachidonic acid; LZR, lean Zucker rats; OZR, obese Zucker rats.

Effects of COX inhibitors and TP receptor antagonist on basal tone

Treatment with SC-560 (1 μM) significantly enhanced basal tone in arteries from OZR but not from LZR and this constriction was abolished by blockade of the TP receptor with ICI-192 (Figure 5A). However, no significant changes in baseline tension of penile arteries from either LZR or OZR were observed after blockade of COX-2 with NS-398 (Figure 5B). These results suggest that blockade of COX-1 unmasks an increased basal production of PGH_2/TXA_2 by the COX-2 pathway in penile arteries from OZR.

Effect of TP receptor antagonism on U46619 and ACh responses

The TXA_2 mimetic U46619 induced concentration-dependent contractions that were enhanced in penile arteries from OZR, pD_2 values for U46619 being 7.21 ± 0.09 ($n = 7$) in LZR, and 7.46 ± 0.05 ($P < 0.01$, $n = 10$) in OZR. The contractile effect of the TXA_2 analogue was inhibited by the TP receptor antagonist, ICI-192 (1 μM) (Figure 6A,B). In order to assess whether an increased TP receptor-mediated vasoconstriction could be involved in the blunted ACh relaxant responses of penile arteries from OZR, the effect of ICI-192 (1 μM) was tested. Treatment with ICI-192 did not alter the ACh-induced relaxation in arteries form either LZR or OZR (Figure 6C,D).

Effects of COX inhibitors on responses to noradrenaline

Treatment with indomethacin augmented noradrenaline-induced contractions in penile arteries from LZR and to a lesser extent those from OZR (Table 2). The contractile responses elicited by noradrenaline were unaltered by treatment with the COX-1 inhibitor SC-560 in penile arteries from either LZR or OZR (Figure 7A,B, Table 2). However, they were significantly enhanced by the COX-2 inhibitor NS-398 in penile arteries from LZR but not from OZR (Figure 7C,D, Table 2), indicating that there is a basal production of a vasodilator prostanoid via COX-2 in LZR rats which is absent in OZR.

Immunohistochemistry of COX-1 and COX-2

Staining of arterial sections with a monoclonal antibody against COX-1 revealed that this constitutive COX isoform was widely and uniformly distributed throughout the endothelial lining of penile arteries, being absent in the smooth muscle layer. No apparent differences in either the distribution or

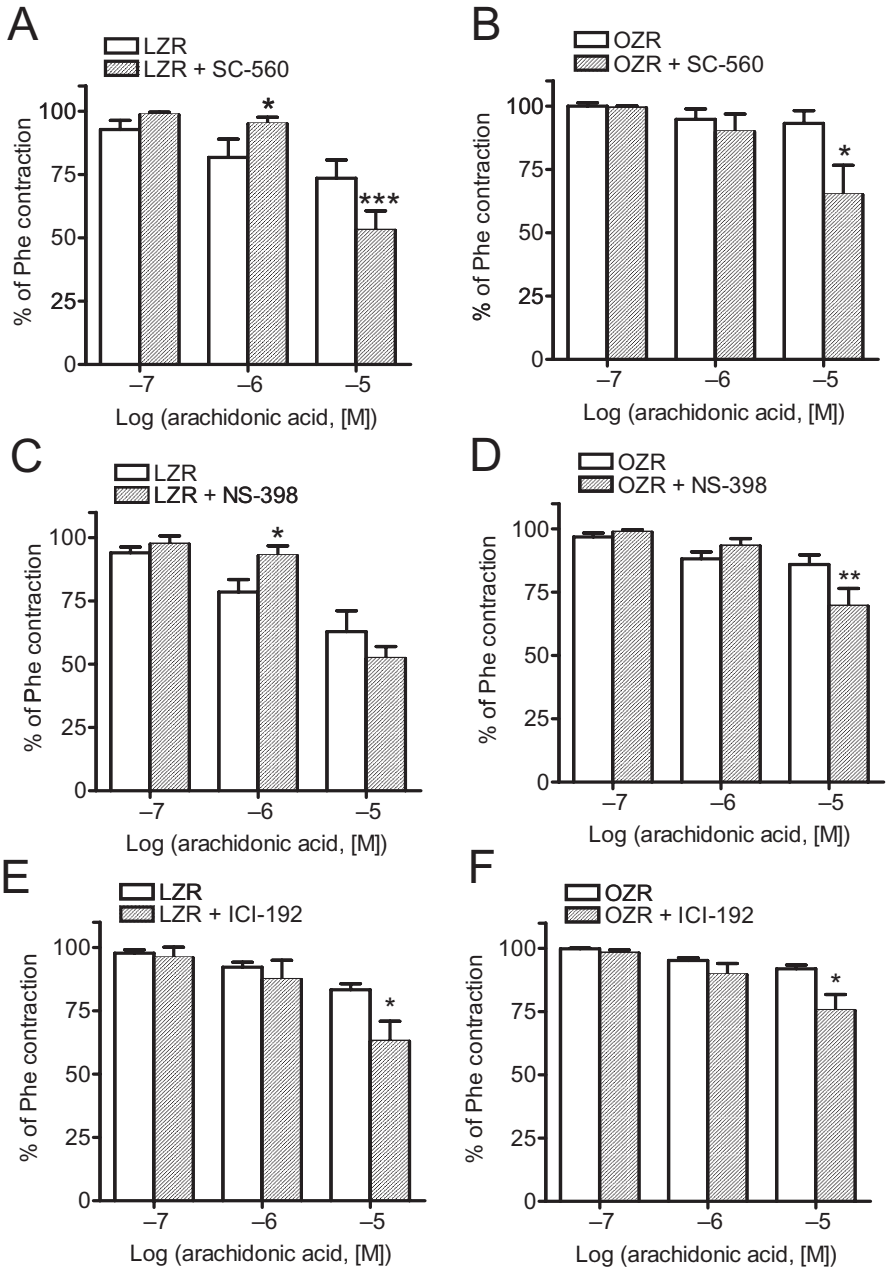

Figure 4 Effect of the COX-1 inhibitor SC-560 (1 μM) (A, B), the COX-2 inhibitor NS-398 (1 μM) (C, D) and the TXA_2/PGH_2 receptor antagonist ICI-192 (1 μM) (E, F) on the average concentration–response curves for the relaxation to AA. Results are expressed as percentage of the precontraction induced by phenylephrine (Phe). Data are shown as means ± SEM of 10 arteries (A, B) and 11 arteries (C, D) and 8 arteries (E, F). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus controls in the absence of treatment. COX, cyclooxygenase.

density of COX-1 immunolabeling were observed between LZR and OZR (Figure 8B). By using a polyclonal antibody against COX-2, this COX isoform was also found to be primarily expressed in the penile endothelium of both LZR and OZR, although a diffuse COX-2 immunostaining, more intense in penile arteries from OZR, was also observed in the smooth muscle layer (Figure 8C). The endothelial COX-2 immunolabeling was sparser and less uniform compared with that for COX-1 and it was restricted to small foci of endothelial cells (marked with arrows in Figure 8C). Furthermore, additional but less intense immunostaining for both COX-1 and COX-2 isoforms was also found in the adventitia and in the surrounding trabecular tissue in the case of COX-1, probably associated with macrophages and mast cell-like cells.

Discussion

In the present study we have investigated the role of AA metabolism through COX in the pathogenesis of the endothelial dysfunction of penile arteries in an animal model of the metabolic syndrome. Our results first demonstrated that both COX-1 and COX-2 were constitutively expressed in the endothelium of penile arteries and primarily produced

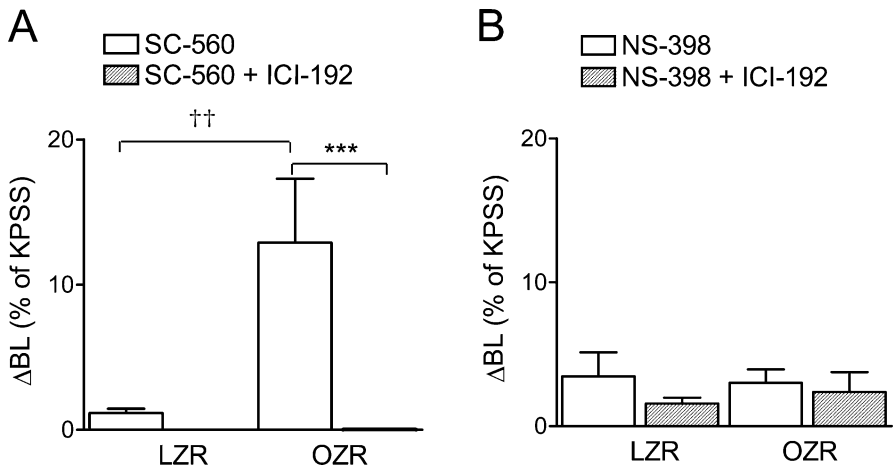

Figure 5 Effect of the COX-1 inhibitor SC-560 (1 μM) (A) and of the COX-2 inhibitor NS-398 (1 μM) (B) alone or in the presence of the TXA_2/PGH_2 receptor antagonist ICI-192 (1 μM) on the basal tone of penile arteries from LZR and OZR. Results are shown as the increase in baseline tension (ΔBL), expressed as % of the contraction to 120 mM K^+ (% of KPSS), after addition of the antagonists. Data are shown as the means ± SEM of 10 arteries (A, B). ††$P < 0.01$ versus OZR. ***$P < 0.001$ versus controls in the absence of treatment. COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

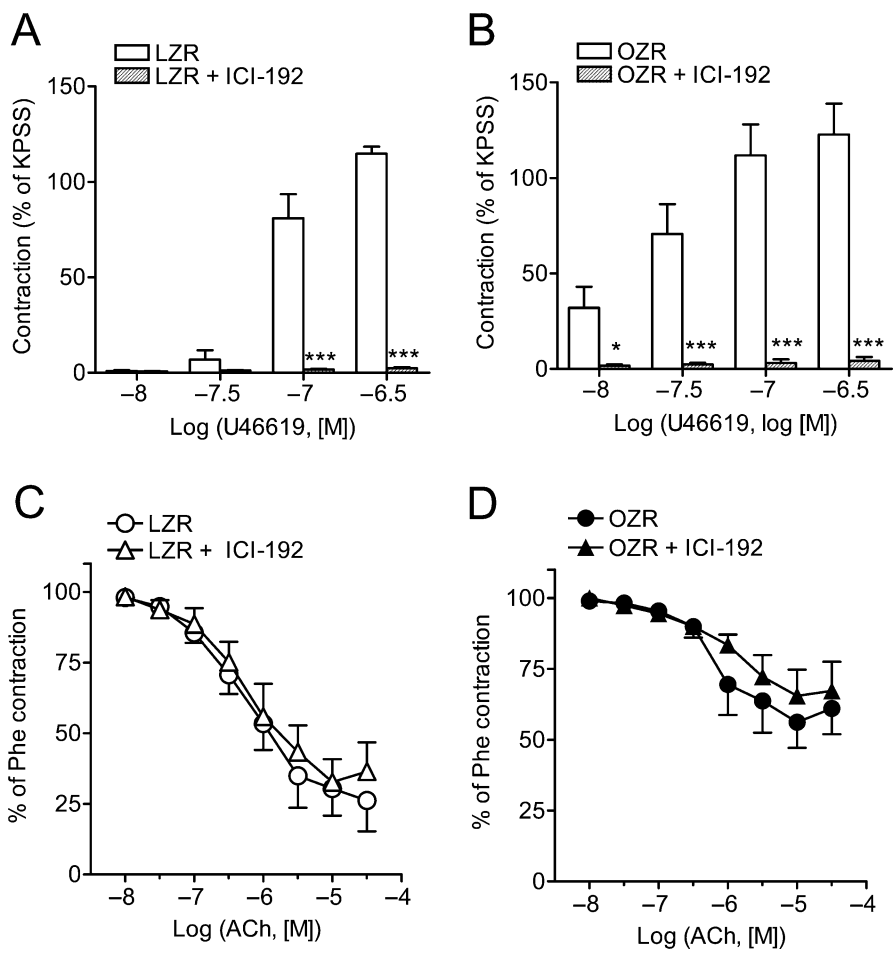

Figure 6 Effect of the TXA_2/PGH_2 receptor with ICI-192 (1 μM) on the contractile effects of the of TXA_2 analogue U-46619 [A, B; expressed as % of the contraction to 120 mM K^+ (% of KPSS)] and on the relaxant responses to acetylcholine (ACh) (C, D) in penile arteries from LZR (A, C) and OZR (B, D). Data are shown as the means ± SEM of 10 arteries (A, B), 8 arteries (C) and 6 arteries (D). *$P < 0.05$, ***$P < 0.001$ versus controls in the absence of treatment. LZR, lean Zucker rats; OZR, obese Zucker rats.

Table 2 Effects of indomethacin (indo) (1 μM), of the selective COX-1 inhibitor SC-560 (1 μM) and of the COX-2 inhibitor NS-398 (1 μM) on the vasoconstrictor responses to noradrenaline (NA) of penile arteries from LZR and OZR

	NA					
	LZR			*OZR*		
	pEC_{50}	*Emax* (Nm^{-1})	n	pEC_{50}	*Emax* (Nm^{-1})	n
NA	6.26 ± 0.12	3.0 ± 0.5	9	6.58 ± 0.01	2.1 ± 0.2	10
+Indo	6.57 ± 0.14**	2.9 ± 0.5	9	6.78 ± 0.15*	1.9 ± 0.2	10
NA	6.80 ± 0.10	2.8 ± 0.5	7	7.16 ± 0.18	2.2 ± 0.5	8
+SC-560	6.57 ± 0.11	2.9 ± 0.3	7	6.98 ± 0.40	2.1 ± 0.4	8
NA	6.51 ± 0.13	3.5 ± 0.3	12	7.00 ± 0.17	2.6 ± 0.3	9
+NS-398	6.69 ± 0.12**	3.5 ± 0.4	12	6.49 ± 0.17*	2.4 ± 0.3*	9

Values represent mean ± SE of the number *n* of individual arteries. pEC_{50} is $-\log EC_{50}$, being the concentration of agonist giving half maximal response (Emax). Significant differences from controls were analysed by a paired Student's *t*-test.
*$P < 0.05$; **$P < 0.01$.
COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

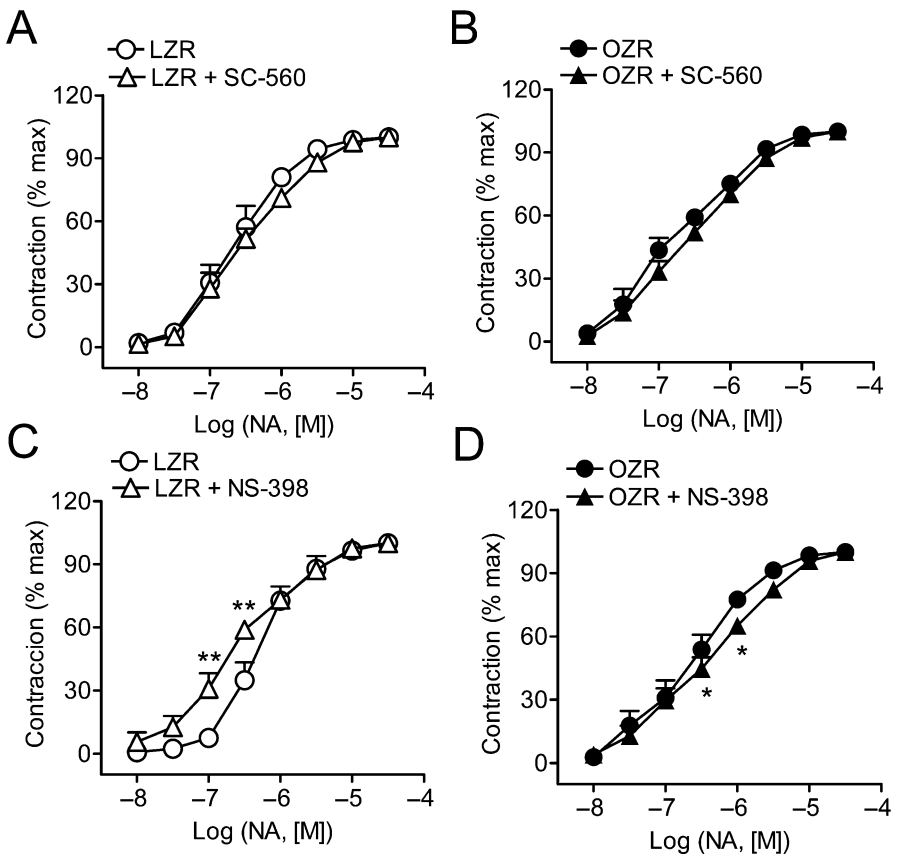

Figure 7 Effects of the selective COX-1 inhibitor SC-560 (1 μM) (A, B) and of the selective COX-2 inhibitor NS-398 (1 μM) (C, D) on the average contractile responses to noradrenaline (NA) in penile arteries from LZR (A, C) and OZR (B, D). Results are expressed as percentage of the maximum contraction induced by NA in each artery. Data are shown as the means ± SEM of seven to eight arteries (A, B) and 9–12 arteries (C, D). *$P < 0.05$, **$P < 0.01$ versus control before treatment. COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

relaxant prostanoids in healthy animals. Under conditions of insulin resistance or the metabolic syndrome, there is an impairment of the COX-1- and COX-2-mediated vasodilator effects that contributes to the blunted ACh endothelium-dependent relaxations and to the enhanced vasoconstriction induced by noradrenaline. An increased basal COX-2-mediated production of vasoconstrictor prostaglandins was also found in arteries from OZR. The present study therefore demonstrates that alterations in the AA metabolism through the COX pathway are involved in the vascular dysfunction of penile arteries from insulin-resistant OZR.

Penile erectile tissues synthesize and locally metabolize several relaxant and contractile prostanoids. In the corpus cavernosum and penile veins, there is a predominant

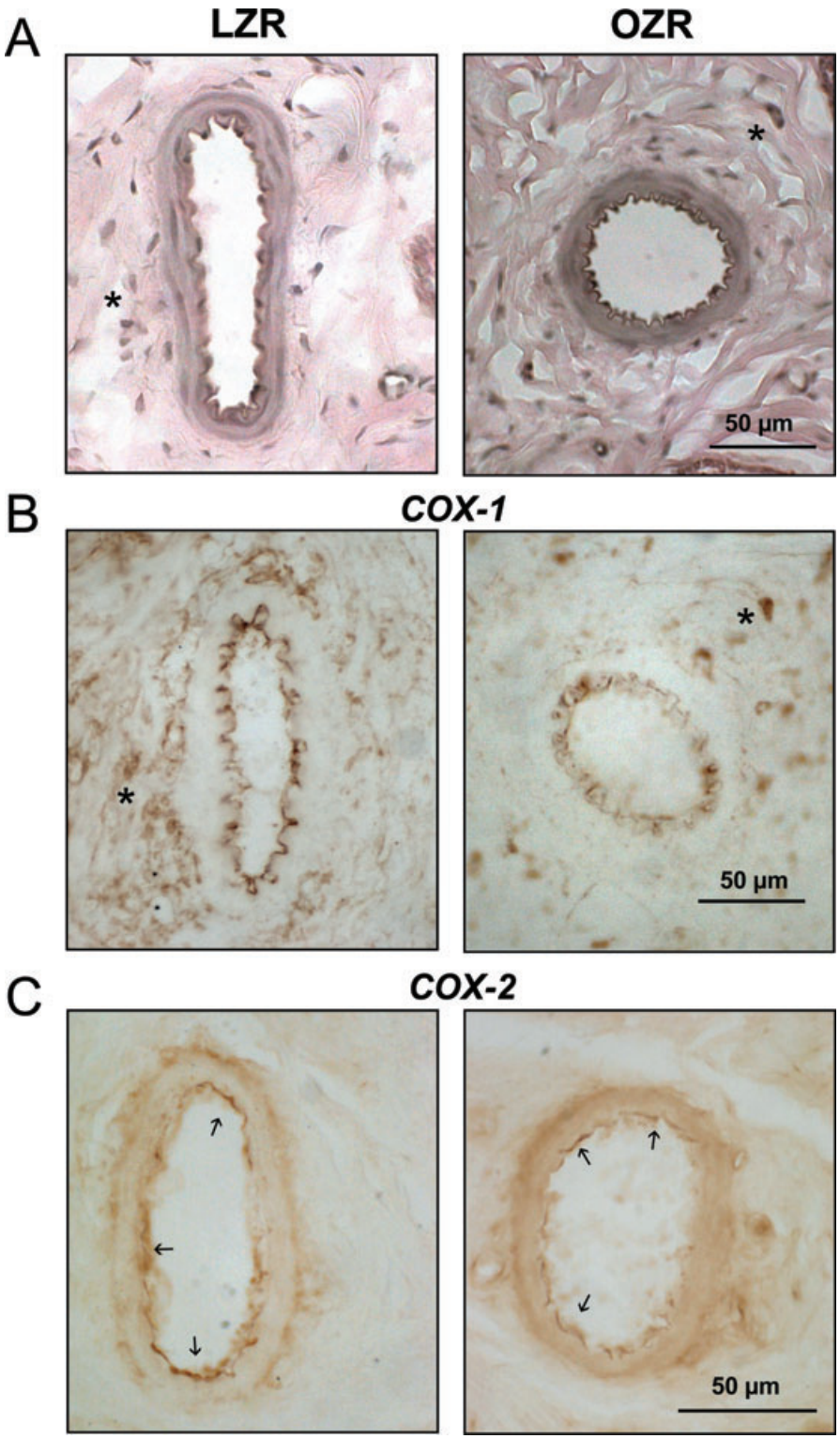

Figure 8 Immunohistochemical staining of COX-1 and COX-2 in the endothelium of penile arteries from LZR and OZR. (A) Haematoxylin and eosin-stained cross sections showing the vascular structure of penile arteries from LZR and OZR. Inmunohistochemical demonstration of COX-1 (B) and COX-2 (C) in LZR and OZR. (B) COX-1 isoform was widely distributed throughout the endothelial lining of penile arteries and absent in the smooth muscle layer. Additional COX-1 immunostaining was also found in the trabecular tissue, probably associated with macrophages and mast cell-like cells (asterisk). (C) COX-2 immunolabeling was sparse and restricted to small foci of endothelial cells marked with arrows. A diffuse COX-2 immunostaining was also observed in the smooth muscle layer. COX, cyclooxygenase; LZR, lean Zucker rats; OZR, obese Zucker rats.

production of contractile prostanoids such as $PGF_{2\alpha}$, TXA_2 and PGE_2 modulated by O_2 tension and reduced by hypoxia (Christ *et al.*, 1990; Azadzoi *et al.*, 1992; Daley *et al.*, 1996; Martínez *et al.*, 2005). In penile resistance arteries, relaxant prostanoids modulate arterial spontaneous tone and they can contribute to the ACh-induced endothelium-dependent responses in some species and humans (Prieto *et al.*, 1998; Simonsen *et al.*, 2001; Angulo *et al.*, 2002; Ruiz Rubio *et al.*, 2004). The present study demonstrated that the ACh-induced endothelium-dependent relaxations of penile arteries from control LZR involved both NO and vasodilator prostanoids. Although COX-1 is constitutively expressed in vascular tissues and platelets and COX-2 is regarded as an inducible isoform up-regulated by inflammatory stimuli, our results further demonstrate that the formation of vasoactive prostanoids occurs via constitutively active COX-1 and COX-2 pathways in normal rat penile arteries. Thus, the immunohistochemical study showed the expression of both COX isoforms primarily in the penile arterial endothelium and non-selective (indomethacin) and selective COX-1 and COX-2 inhibitors significantly reduced the relaxations elicited by both ACh and AA in arteries from LZR. The functional experiments of the present study are consistent with the predominant localization of both COX isoforms in the endothelium of normal penile arteries. Thus, the COX-1 inhibitor SC-560 and the COX-2 inhibitor NS-398 reduced the ACh-elicited relaxations in LZR, thus suggesting the release of both COX-1- and COX-2-derived endothelial vasodilator prostanoids upon agonist stimulation. COX-2 constitutive expression in the vascular system has earlier been reported in a few organs such as the kidney (Qui *et al.*, 2006) and the lung (Ermert *et al.*, 1998; Baber *et al.*, 2003; 2005). In our study, COX-2 was found to be mainly distributed in the penile arterial endothelium of LZR, although a diffuse distribution in the smooth muscle layer was also found, which is in agreement with that reported in the lung where COX-2 was localized in both the endothelium and smooth muscle of small pulmonary arteries (Ermert *et al.*, 1998; Baber *et al.*, 2003).

As regards the functional experiments, our results further demonstrate a basal release of COX-2-derived relaxant prostanoids involved in the spontaneous tone of rat penile arteries from LZR. Thus, AA elicited a net relaxant effect that was reduced by the COX-2 inhibitor NS-398. Furthermore, both non-selective (indomethacin) and selective COX-2 inhibition enhanced noradrenaline-induced contraction, suggesting a basal release of COX-2-derived relaxant prostanoids that counterbalance vasoconstriction. The predominant formation of vasodilator prostaglandins via COX-2 in arteries from healthy animals would be in agreement with previous studies in the rat showing that selective COX-2 inhibitors reduced systemic vasodilator responses to AA with no effect of TXA_2 receptor blockade (Baber *et al.*, 2003). COX-2 has been demonstrated to preferentially couple to the cellular synthesis of PGI_2 and PGE_2 (Brock *et al.*, 1999) and to be up-regulated by steady laminar shear stress, hence being proposed as a major isoform responsible for the production of PGI_2 under physiological conditions (Topper *et al.*, 1996; Henrion *et al.*, 1997; Okahara *et al.*, 1998). The results of the present study further extend the concept that constitutive COX-2 may functionally contribute to the formation of vasodilator prostanoids in the vascular system (Baber *et al.*, 2003), although its role in penile erection remains to be elucidated. Interestingly, recent studies have demonstrated that COX-2 is highly expressed in penile corpus cavernosum and COX-2-derived contractile prostanoids are involved in the generation of spontaneous contractions. However, in contrast to the primary location of COX-2 in the endothelium of penile arteries, COX-2 was found in a population of interstitial-like cells in the corpus cavernosum and proposed to contribute to the neural regulation of corporal smooth muscle tone (Hashitani *et al.*, 2005).

On the other hand, the present study also demonstrated the release of COX-1-derived vasoconstrictor prostaglandins that contributed to the spontaneous tone of penile arteries from healthy animals, as shown by the enhancing effect of both the COX-1 blocker SC-560 and of the selective TP receptor antagonist ICI-192, on the relaxations induced by high doses of AA. These results are consistent with studies showing an extensive co-distribution of COX-1 with both TXA_2 and PGI_2 synthases in the aortic endothelium (Kawka *et al.*, 2007), along with a marked reduction of PGI_2 and TXA_2 metabolites by COX-1 inhibitors (Qui *et al.*, 2006; Kawka *et al.*, 2007), thus indicating the major functional contribution of endothelial COX-1 to the basal production of both PGI_2 and TXA_2 in the normal arterial wall.

Cyclooxygenase 2 is up-regulated by inflammatory stimuli and is involved in the abnormal vascular reactivity of arteries from type 2 diabetic mice (Bagi *et al.*, 2005; Guo *et al.*, 2005), although recent studies have shown that both COX-1 and COX-2 may contribute to the endothelial dysfunction (Matsumoto *et al.*, 2007) and to the hypersensitivity of vascular smooth muscle (Shi and Vanhoutte, 2008) in arteries from type 2 and type 1 diabetic rats respectively. We have recently demonstrated that penile arteries from animals with the metabolic syndrome exhibit endothelial dysfunction, as shown by the reduced ACh-induced relaxations and the impaired NO basal activity (Villalba *et al.*, 2009). In the present study, we confirmed this dysfunction and further demonstrated that alterations in the AA metabolism via both COX-1 and COX-2 were involved in the loss of the predominant basal and endothelial agonist-induced release of vasodilator prostaglandins in arteries from OZR, thereby contributing to the endothelial dysfunction in these animals. Thus, COX-1 and COX-2 inhibitors failed to inhibit the relaxations induced by both ACh and AA in penile arteries from OZR, suggesting a blunted formation and/or impaired effects of relaxant prostanoids. Moreover, COX-2 inhibition no longer enhanced noradrenaline vasoconstriction.

The present results showing that the relaxant effects of AA are impaired in penile arteries from OZR suggest a shift in the AA metabolism towards an enhanced formation of vasoconstrictor prostanoids. The specific involvement of COX-2 is suggested by two findings. First, both the COX-2 inhibitor NS-398 and the TP receptor antagonist ICI-192 enhanced the relaxant effect of high concentrations of AA in OZR and restored them to levels similar to those in LZR. Second, COX-1 inhibitors unmasked a marked increase in basal tension in penile arteries from OZR, which was abolished by TP receptor blockade, indicating an enhanced COX-2-mediated basal production of vasoconstrictor prostanoids that seems to be balanced by an increased basal production of

COX-1-derived relaxant prostaglandins. The present results would be consistent with those reported for skeletal muscle arteries from OZR (Xiang *et al.*, 2008) and from type 2 diabetic mice (Bagi *et al.*, 2005), where the impaired AA relaxant responses were improved and the increased arteriolar basal tone was inhibited by TP receptor antagonism. However, measurements of TXA_2 metabolites in pooled skeletal muscle arteries from OZR showed no significant changes in either basal or AA-stimulated TXA_2 levels (Goodwill *et al.*, 2008; Hodnett *et al.*, 2009). In the present study, we have found that the responses to the exogenous TXA_2 analogue U46619 were augmented in penile arteries from OZR, suggesting that an enhanced TP receptor activity might be in part responsible for the augmented basal vasoconstrictor activity in these arteries.

Penile arteries from OZR exhibit an impaired basal release of NO despite unaltered eNOS expression (Villalba *et al.*, 2009). In arteries from healthy LZR, both NOS and COX contribute to the maintenance of penile arterial tone through the production of NO and predominantly vasodilator prostanoids. In several blood vessels, NO is responsible for a permanent feedback inhibition which restricts the release of constrictor prostanoids, and reduced NO bioavailability has been shown to increase this constrictor activity through the enhanced formation of reactive oxygen species (Yang *et al.*, 2002; Laemmel *et al.*, 2003; Vanhoutte *et al.*, 2009). Therefore, an impaired endothelial NOS activity, as occurs in penile arteries from OZR under conditions of insulin resistance (Villalba *et al.*, 2009), could favour the synthesis/release of vasoconstrictor prostanoids and thus contribute to the enhanced COX-2-mediated basal constrictor activity observed in the present study.

In human corpus cavernosum from diabetic patients, enhanced TP receptor-mediated contractile responses have been shown to underlie the impaired endothelium-dependent relaxations to ACh (Angulo *et al.*, 2006). However, TP receptor antagonism could not restore the blunted ACh-elicited relaxations in penile arteries from OZR, unlike its enhancing effect on the AA-induced relaxations, and in contrast to that reported for diabetic penile corporal tissue. Neither selective nor selective inhibition of COX could significantly enhance either ACh relaxant responses in penile arteries unlike that observed in mesenteric arteries from type 2 diabetic rats where an augmented production of TXA_2 and PGE_2 impairs ACh-induced relaxations and treatment with indomethacin improves these responses (Matsumoto *et al.*, 2007). Although an enhanced formation of vasoconstrictor prostaglandins other than TXA_2 cannot be ruled out (Vanhoutte *et al.*, 2009), the present data suggest that other factors might contribute to the blunted prostanoid-mediated endothelium-dependent relaxations of penile arteries in insulin-resistant OZR. Recent studies have demonstrated an attenuated production of PGI_2 associated to the increased nitration of tyrosine residues of the PGI_2 synthase in skeletal muscle arterioles from OZR (Goodwill *et al.*, 2008; Hodnett *et al.*, 2009). Increased mitochondrial fatty acid oxidation with the subsequently augmented superoxide production is responsible for enhanced tyrosine nitration and inactivation of PGI_2 synthase in the aortic endothelium of OZR (Du *et al.*, 2006). We have recently demonstrated that acute antioxidant treatment partially restored the enhanced vasoconstriction of penile and coronary resistance arteries from OZR (Villalba *et al.*, 2009). Therefore, enhanced oxidative stress is likely to contribute to the impaired relaxant prostanoid formation and thus to be involved in the endothelial/vascular dysfunction of penile arteries of insulin-resistant OZR, as reported for arteries from diabetic and hypertensive animals (Álvarez *et al.*, 2007; Matsumoto *et al.*, 2007; Shi and Vanhoutte, 2008; Vanhoutte *et al.*, 2009).

In summary, the present results demonstrate that constitutively active COX-1 and COX-2 pathways contribute to the regulation of vascular tone in normal rat penile arteries through the predominant formation of vasodilator prostanoids. Although endothelial dysfunction in penile vasculature from diabetic animals has primarily been ascribed to deficiencies in the NO pathway, here we demonstrate that impaired AA metabolism with reduced release/effects of vasodilator prostaglandins from both COX-1 and COX-2 pathways, plays a key role in the pathogenesis of both endothelial dysfunction and augmented vasoconstriction in penile arteries under conditions of insulin resistance and the metabolic syndrome.

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Conflicts of interest

None.

References

Alexander SPH, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edition (2008 revision). *Br J Pharmacol* **153** (Suppl.2): S1–S209.

Álvarez Y, Pérez-Giron JV, Hernanz R, Briones AM, García-Redondo A, Beltrán A *et al.* (2007). Losartan reduces the increased participation of cyclooxygenase-2-derived products in vascular responses of hypertensive rats. *J Pharmacol Exp Ther* **321** (1): 381–388.

Angulo J, Cuevas P, Fernández A, Allona A, Moncada I, Martín-Morales A *et al.* (2006). Enhanced thromboxane receptor-mediated responses and impaired endothelium-dependent relaxation in human corpus cavernosum from diabetic impotent men: role of protein kinase C activity. *J Pharmacol Exp Ther* **319** (2): 783–789.

Angulo J, Cuevas P, Fernández A, Gabancho S, Allona A, Martín-Morales A *et al.* (2003). Diabetes impairs endothelium-dependent relaxation of human penile vascular tissues mediated by NO and EDHF. *Biochem Biophys Res Commun* **312** (4): 1202–1208.

Angulo J, Cuevas P, La Fuente JM, Pomerol JM, Ruiz-Castane E, Puigvert A *et al.* (2002). Regulation of human penile smooth muscle tone by prostanoid receptors. *Br J Pharmacol* **136**: 23–30.

Azadzoi KM, Kim N, Brown ML, Goldstein I, Cohen RA, Saenz de Tejada I (1992). Endothelium-derived nitric oxide and cyclooxygenase products modulate corpus cavernosum smooth muscle tone. *J Urol* **147**: 220–225.

Baber RS, Hunter CC, Trinity JB, Hyman AL, Kadowitz PJ (2003). Role

of Cyclooxygenase-2 in the generation of vasoactive prostanoids in the rat pulmonary and systemic vascular beds. *Circulation* **108**: 896–901.

Baber SR, Deng W, Rodriguez J, Master RG, Bivalacqua TJ, Hyman AL *et al*. (2005). Vasoactive prostanoids are generated from arachidonic acid by COX-1 and COX-2 in the mouse. *Am J Physiol Heart Circ Physiol* **289** (4): H1476–H1487.

Bagi Z, Erdei N, Toth A, Li W, Hintze TH, Koller A *et al*. (2005). Type 2 diabetic mice have increased arteriolar tone and blood pressure: enhanced release of COX-2-derived constrictor prostaglandins. *Arterioscler Thromb Vasc Biol* **25** (8): 1610–1616.

Billups KL (2005). Erectile dusfunction as an early sign of cardiovascular disease. *Int J Impot Res* **1**: S19–24.

Brock TG, McNish RW, Peters-Golden M (1999). Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. *J Biol Chem* **274** (17): 11660–11666.

Christ GJ, Maayani S, Valcic M, Melman A (1990). Pharmacological studies of human erectile tissue: characteristics of spontaneous contractions and alterations in alpha-adrenoceptor responsiveness with age and disease in isolated tissues. *Br J Pharmacol* **101** (2): 375–381.

Daley JT, Brown ML, Watkins T, Traish AM, Huang YH, Moreland RB *et al*. (1996). Prostanoid production in rabbit corpus cavernosum: I. Regulation by oxygen tension. *J Urol* **155**: 1482–1487.

De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM (2000). Endothelial dysfunction in diabetes. *Br J Pharmacol* **130** (5): 963–974.

Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M (2006). Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *J Clin Invest* **116** (4): 1071–1080.

Ermert L, Ermert M, Althoff A, Merkle M, Grimminger F, Seeger W (1998). Vasoregulatory prostanoid generation proceeds via cyclooxygenase-2 in noninflamed rat lungs. *J Pharmacol Exp Ther* **286** (3): 1309–1314.

Espósito K, Giugliano F, Martedì E, Feola G, Marfella R, D'Armiento M *et al*. (2005). High proportions of erectile dysfunction in men with the metabolic syndrome. *Diabetes Care* **28** (5): 1201–1203.

Fonseca VA, Jawa A (2005). Endothelial and erectile dysfunction, diabetes mellitus, and the metabolic syndrome: common pathways and treatments? *Am J Cardiol* **96** (12B): 13M–18M.

Goodwill AG, James ME, Frisbee JC (2008). Increased vascular thromboxane generation impairs dilation of skeletal muscle arterioles of obese Zucker rats with reduced oxygen tension. *Am J Physiol Heart Circ Physiol* **295** (4): H1522–H1528.

Guerre-Millo M (1997). Regulation of ob gene overexpression in obesity. *Biomed Pharmacother* **51** (8): 318–323.

Guo Z, Su W, Allen S, Pang H, Daugherty A, Smart E *et al*. (2005). COX-2 up-regulation and vascular smooth muscle contractile hyperreactivity in spontaneous diabetic db/db mice. *Cardiovasc Res* **67** (4): 723–735.

Hashitani H, Yanai Y, Shirasawa N, Soji T, Tomita A, Kohri K *et al*. (2005). Interaction between spontaneous and neurally mediated regulation of smooth muscle tone in the rabbit corpus cavernosum. *J Physiol* **569** (Pt 3): 723–735.

Henrion D, Dechaux E, Dowell FJ, Maclour J, Samuel JL, Lévy BI *et al*. (1997). Alteration of flow-induced dilatation in mesenteric resistance arteries of L-NAME treated rats and its partial association with induction of cyclo-oxygenase-2. *Br J Pharmacol* **121** (1): 83–90.

Herranz R, Briones AM, Alonso MJ, Vila E, Salaices M (2004). Hypertension alters role of iNOS, COX-2, and oxidative stress in bradykinin relaxation impairment after LPS in rat cerebral arteries. *Am J Physiol Heart Circ Physiol* **287** (1): H225–H234.

Hodnett BL, Dearman JA, Carter CB, Hester RL (2009). Attenuated PGI2 synthesis in Obese Zucker Rats. *Am J Physiol Regul Integr Comp Physiol* **296** (3): R715–R721.

Hsu SM, Raine L, Fanger H (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* **29**: 577–580.

Kawka DW, Ouellet M, Hétu PO, Signer II, Riendeau D (2007). Double-label expression studies of prostacyclin synthase, thromboxane synthase and COX isoforms in normal aortic endothelium. *Biochim et Biophys Acta* **1771**: 45–54.

Keegan A, Cotter MA, Cameron NE (1999). Effects of diabetes and treatment with the antioxidant alpha-lipoic acid on endothelial and neurogenic responses of corpus cavernosum in rats. *Diabetologia* **42**: 343–350.

Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T *et al*. (2000). Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo: a specific vascular action of insulin. *Circulation* **101** (6): 676–681.

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. *Am J Physiol Heart Circ Physiol*. **285** (3): H1254–H1260.

Martínez AC, Prieto D, Hernández M, Rivera L, Recio P, García-Sacristán A *et al*. (2005). Endothelial mechanisms underlying responses to ACh in the horse deep dorsal penile vein. *Eur J Pharmacol* **515**: 150–159.

Matsumoto T, Kakami M, Noguchi E, Kobayashi T, Kamata K (2007). Imbalance between endothelium-derived relaxing and contracting factors in mesenteric arteries from aged OLETF rats, a model of Type 2 diabetes. *Am J Physiol Heart Circ Physiol* **293** (3): H1480–H1490.

Okahara K, Sun B, Kambayashi J (1998). Upregulation of prostacyclin synthesis-related gene expression by shear stress in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* **18** (12): 1922–1926.

Okon EB, Chung AW, Rauniyar P, Padilla E, Tejerina T, McManus BM *et al*. (2005). Compromised arterial function in human type 2 diabetic patiens. *Diabetes* **54** (8): 2415–2423.

Prieto D (2008). Physiological regulation of penile arteries and veins. *Int J Impot Res* **20**: 17–29.

Prieto D, Simonsen U, Hernández M, García-Sacristán A (1998). Contribution of K^+ channels and ouabain-sensitive mechanisms to the endothelium-dependent relaxations of horse penile small arteries. *Br J Pharmacol* **123**: 1609–1620.

Qui Z, Cai H, Morrow JD, Breyer MD (2006). Differentiation of cyclooxygenase 1- and 2-derived prostanoids in mouse kidney and aorta. *Hypertension* **48** (2): 323–328.

Ruiz Rubio JL, Hernández M, Rivera de los Arcos L, Martínez AC, García-Sacristán A, Prieto D. (2004). Mechanisms of prostaglandin E1-induced relaxation in penile resistance arteries. *J Urol* **171**: 968–973.

Saenz de Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA (1989). Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. *N Engl J Med* **320** (16): 1025–1030.

Shi Y, Vanhoutte PM (2008). Oxidative stress and COX cause hyperresponsiveness in vascular smooth muscle of the femoral artery from diabetic rats. *Br J Pharmacol* **154** (3): 639–651.

Simonsen U, Contreras J, García-Sacristán A, Martínez AC (2001). Effect of sildenafil on non-adrenergic non-cholinergic neurotransmision in bovine penile small arteries. *Eur J Pharmacol* **412** (2): 155–169.

Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W *et al*. (1994). Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci USA* **91** (25): 12013–12017.

Topper JN, Cai J, Falb D, Gimbrone MA Jr (1996). Identification of vascular endothelial genes differentially responsive to fluid mechanical stimuli: cyclooxygenase-2, manganese superoxide dismutase, and endothelial cell nitric oxide synthase are selectively

up-regulated by steady laminar shear stress. *Proc Natl Acad Sci USA* **93** (19): 10417–10422.

Vanhoutte PM, Shimokawa H, Tang EH, Feletou M (2009). Endothelial dysfunction and vascular disease. *Acta Physiol (Oxf)* **196** (2): 193–222.

Vickers MA, Wright EA (2004). Erectile dysfunction in patient with diabetes mellitus. *Am J Manag Care* **10** (Suppl. 1): S3–S11.

Villalba N, Martínez MP, Briones AM, Sánchez A, Salaíces M, García-Sacristán A *et al*. (2009). Differential structural and functional changes of penile and coronary arteries from obese Zucker rats. *Am J Physiol Heart Circ Physiol* **297** (2): H696–H707.

Warner TD, Mitchell JA (2004). Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB J* **18** (7): 790–804.

Xiang L, Dearman J, Abram SR, Carter C, Hester RL (2008). Insulin resistance and impaired functional vasodilation in obese Zucker rats. *Am J Physiol Heart Circ Physiol* **294** (4): H1658–H1666.

Yang D, Félétou M, Boulanger CM, Wu HF, Levens N, Zhang JN *et al*. (2002). Oxygen-derived free radicals mediate endothelium-dependent contractions to acetylcholine in aortas from spontaneously hypertensive rats. *Br J Pharmacol* **136** (1): 104–110.