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Enhanced response of pig coronary arteries to endothelin-1 after ischemia–reperfusion. Role of endothelin receptors, nitric oxide and prostanoids

Belén Climent, Nuria Fernández *, Elena Sanz, Ana Sánchez, Luis Monge, Angel Luis García-Villalón, Godofredo Diéguez

Departamento de Fisiología, Facultad de Medicina, Universidad Autónoma de Madrid, Arzobispo Morcillo 2, 28029 Madrid, Spain

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Abstract

To analyse the coronary effects of endothelin-1 after ischemia–reperfusion, the left anterior descending coronary artery of anesthetized pigs was subjected to 30-min occlusion followed by 60-min reperfusion. Then, rings distal (ischemic arteries) and proximal (control arteries) to the occlusion were taken from this artery and prepared for isometric tension recording. The sensitivity of the contraction in response to endothelin-1 (3×10^{-10}–3×10^{-7} M) and the endothelin ET_B receptor agonist IRL-1620 (3×10^{-10}–3×10^{-7} M) was greater in ischemic vessels. The endothelin ET_A receptor antagonist BQ-123 (10^{-7}–3×10^{-6} M) decreased the sensitivity of the response to endothelin-1 similarly in ischemic and control arteries. The endothelin ET_B receptor antagonist BQ-788 (10^{-6} M), endothelium removal or the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME 10^{-4} M) potentiated the response to endothelin-1 and IRL-1620 in control arteries only. The cyclooxygenase inhibitor meclofenamate (10^{-5} M) augmented the maximal response to endothelin-1 in control arteries, and reduced it in ischemic arteries. In precontracted arteries, IRL-1620 (3×10^{-11}–3×10^{-10} M) relaxed control but not ischemic arteries, and L-NAME or meclofenamate abolished this relaxation. Therefore, ischemia–reperfusion increases the coronary vasoconstriction in response to endothelin-1 probably due to impairment of endothelin ET_B receptor-induced release of nitric oxide and prostacyclin, augmentation of the contractile response to activation of endothelin ET_B receptors, and increased release of vasoconstrictor prostanoids.

Keywords: Coronary circulation; Coronary vasoconstriction; Myocardial ischemia; Endothelin receptor; Endothelial dysfunction

1. Introduction

Ischemia–reperfusion is a clinical and experimental event that can produce dysfunction of the myocardium and coronary vasculature, and several lines of evidence suggest that endothelium dysfunction and endothelin-1 are involved in these effects. The increased production of endothelin-1 and the increased coronary vasoconstrictor effects that it induces are thought to be partially responsible for the damage that is caused during this condition (Pernow and Wang, 1997).

The mechanisms that underlie the increased coronary response to endothelin-1 after ischemia–reperfusion remain to be elucidated. It has been attributed to an upregulation of endothelin ET_A receptors (Watts et al., 1992; Neubauer et al., 1991; Thompson et al., 1995; Wang et al., 1995; Lockowandt et al., 2001), to a reduced endothelin ET_B receptor-mediated release of nitric oxide or prostacyclin as a result of endothelial dysfunction (Watts et al., 1992), to an increased endothelin-1 binding sites or loss of counteracting vasodilator mechanisms (Neubauer et al., 1991), and to a loss of modulatory role of nitric oxide (Fernández et al., 2003). The relative importance of endothelin ET_A and ET_B receptors in the coronary vascular actions of endothelin-1 can be altered in some pathological states and endothelin ET_B receptors may mediate coronary vasoconstrictor responses not observed under normal conditions (Cannan et al., 1996; Hasdai et al., 1997; Wackenfors et al., 2004). Most of the studies show that selective antagonists for

* Corresponding author. Tel.: +34 91 497 5490; fax: +34 91 497 5478.
E-mail address: nuria.fernandez@uam.es (N. Fernández).

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endothelin ET_A receptors and mixed antagonists for endothelin ET_A/ET_B receptors improve the recovery of myocardial and coronary endothelium dysfunction after ischemia–reperfusion (Wang et al., 2002). Also, it has been reported that selective antagonists for endothelin ET_B receptors provide similar protection of the myocardium and coronary vasculature than selective antagonists for endothelin ET_A receptors against ischemia–reperfusion (Szabó et al., 1998). Therefore, we hypothesize that an alteration of endothelin ET_B receptors function could be also involved in the increased coronary response to endothelin-1 after ischemia–reperfusion.

Defining the relative contribution of endothelin ET_A and ET_B receptors to the coronary vasoconstrictor effects of endothelin-1 during ischemia–reperfusion has important clinical implications in order to select the proper endothelin receptors antagonists as a treatment for this condition. As the endothelium is very sensitive to ischemia–reperfusion (Kim et al., 1992), it could be also of interest to further investigate the role of nitric oxide and prostanoids in the coronary action of endothelin-1 during ischemia–reperfusion.

The present work was performed to evaluate the role of endothelin ET_A and ET_B receptors, and their interaction with nitric oxide and prostanoids in the coronary response to endothelin-1 after ischemia–reperfusion. This was achieved by analysing the effects of this peptide in isolated coronary arteries that had been previously exposed to 30 min of ischemia followed by 60 min of reperfusion in anesthetized pigs. The effects in these arteries were compared to those obtained in control (non-ischemic) coronary arteries from the same pigs.

2. Materials and methods

2.1. Experimental preparation

In this study, 29 young pigs (15 males and 14 females) were used (4–5 months old, 35–40 kg). The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the experimental procedure in the present study was approved by the local Animal Research Committee. Anesthesia was induced with an intramuscular injection of 25 mg/kg ketamine hydrochloride, 1 mg atropine sulphate and 1 mg/kg diazepam. After orotracheal intubation, ventilation with a mixture of oxygen and isoflurane was adjusted to maintain normocapnia and a stable level of anesthesia. A median sternotomy was performed, and the pericardium was opened. The distal segment of the left anterior descending coronary artery was dissected, and a snare-type occluder was placed around this artery. With this occluder, a 30-min coronary occlusion was induced in 26 pigs (12 males and 14 females) and the subsequent gradual release of this occlusion to induce 60 min of reperfusion. During coronary occlusion and reperfusion each animal received the i.v. administration of ~60 mg lidocaine 2%. In every animal systemic arterial pressure was measured through a polyethylene catheter placed in one femoral artery and connected to a Statham transducer. Systemic arterial pressure and heart rate were simultaneously recorded on a Grass model 7 polygraph. Blood samples from the femoral artery were taken periodically to measure pH, pCO_2 and pO_2 by standard electrometric methods (Radiometer, ABLTM5, Copenhagen, Denmark). At the 60 min of reperfusion, the pigs were killed with an overdose of i.v. thiopental sodium and potassium chloride, and the heart was removed. Then, branches of the left anterior descending coronary artery, 5 mm distal (ischemic arteries) and 20 mm proximal (control arteries) to the occluder, were dissected out and cut into cylindrical segments 3 mm in length. Both types of coronary artery segments had a similar external diameter of about 1 mm. Each arterial segment was prepared for isometric tension recording in a 4-ml organ bath at 37 °C, containing modified Krebs–Henseleit solution with the following composition (millimolar): NaCl, 115; KCl, 4.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. Briefly, the method consists of passing through the lumen of the vascular segment of two fine stainless steel pins, 90 μm in diameter. One pin is fixed to the organ bath wall, while the other is connected to a strain gauge for isometric tension recording, thus permitting the application of passive tension in a plane perpendicular to the long axis of the vascular cylinder. The recording system included a Universal Transducing Cell UC3 (Statham Instruments) and a Statham Microscale Accessory UL5 (Statham Instruments, Inc.). Changes in isometric force were recorded on a Macintosh computer by use of Chart v3.6/s software and a MacLab/8e data acquisition system (AD Instruments). A previously determined optimal passive tension of 1.5 g was applied to the vascular segments, and they were allowed to equilibrate for 60–90 min.

Another 3 male pigs were sham operated and were not subjected to ischemia–reperfusion. These animals were then also killed with an overdose of i.v. thiopental sodium and potassium chloride, and their hearts were removed. Branches of the left anterior descending coronary artery, proximal and distal to the occluder, were dissected out and prepared for isometric tension recording in order to compare their responses to endothelin-1 and IRL-1620.

2.2. Experimental protocol

The responses to endothelin-1 (3×10^{-10}–3×10^{-7} M) and the selective endothelin ET_B receptor agonist IRL-1620 (3×10^{-10}–3×10^{-7} M) were obtained under resting conditions in the intact proximal and distal arteries of the sham operated pigs, and in the ischemic and control arteries of the pigs subjected to ischemia–reperfusion. These latter experiments were performed in intact arteries, in arteries without endothelium, and in arteries pretreated with either the inhibitor of nitric oxide synthesis N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M) or the cyclooxygenase inhibitor meclofenamate (10^{-5} M). The effects of endothelin-1 were also recorded in the absence and in the presence of the endothelin ET_A receptor antagonist BQ-123 (10^{-7}–3×10^{-6} M) and of the endothelin ET_B receptor antagonist BQ-788 (10^{-6} M). To determine whether endothelin ET_A receptors might be involved

Table 1
Values of pD_2 and maximal contraction (E_{max}) obtained with endothelin-1 and IRL-1620 in resting control and ischemic pig coronary arteries

	pD_2		E_{max}, mg	
	Control	Ischemic	Control	Ischemic
Endothelin-1				
Intact arteries (20)	7.98±0.06	8.25±0.05[a]	4184±427	3864±267
Arteries treated with 10^{-6} M				
BQ-123 (6)	7.67±0.04[b]	7.66±0.05[b]	3836±286	3588±242
Arteries treated with 10^{-6} M				
BQ-788 (6)	8.13±0.13	7.90±0.08	5125±396[a]	2646±391[a]
Arteries without endothelium (6)	8.31±0.10[a]	8.34±0.12	5484±609	3716±411
Arteries treated with 10^{-4} M				
L-NAME (11)	8.22±0.09[a]	8.06±0.07	4855±470[a]	4129±500
Arteries treated with 10^{-5} M meclofenamate (8)	8.04±0.09	8.01±0.08	4846±530[a]	2764±121[a]
IRL-1620				
Intact arteries (10)	7.47±0.08	7.85±0.10[a]	461±125	1067±157[a]
Arteries without endothelium (10)	7.70±0.09[a]	7.57±0.11	902±209[a]	910±128
Arteries treated with 10^{-4} M				
L-NAME (8)	7.88±0.11[a]	7.44±0.09	1180±163[a]	1540±286
Arteries treated with 10^{-5} M meclofenamate (7)	7.31±0.10	7.25±0.04[b]	398±52	449±77[b]

Values are means±S.E.M.
[a] $P<0.05$.
[b] $P<0.01$ compared with its corresponding control. In parentheses, number of segments from control or ischemic arteries.

in the contraction following exposure to IRL-1620, the effects of BQ-123 (10^{-6} M) were recorded in ischemic and control arteries during the plateau of the IRL-1620-induced contraction.

The response to IRL-1620 (3×10^{-11}–3×10^{-7} M) was also obtained in ischemic and control arteries precontracted with the thromboxane analogue U46619 (3×10^{-7}–10^{-6} M). This was performed in intact arteries, in arteries without endothelium and in arteries pretreated with L-NAME (10^{-4} M) or meclofenamate (10^{-5} M).

The response to sodium nitroprusside (10^{-8}–10^{-4} M) was also assayed in ischemic and control arteries precontracted with U46619 (3×10^{-7}–10^{-6} M).

The responses to endothelin-1 and IRL-1620 were evaluated as cumulative dose–response curves. To eliminate the endothelium, the lumen of the arteries was rubbed mechanically before mounting the arteries in the organ baths. L-NAME, meclofenamate, BQ-123 or BQ-788 were applied to the organ bath for 30–35 min before the responses to endothelin-1 or IRL-1620 were tested.

Each arterial segment was used to determine only one concentration–response curve for endothelin-1 or for IRL-1620. The values of the contraction in response to endothelin-1 and to IRL-1620 are shown as a percentage of the maximal contraction obtained with the corresponding peptide. The relaxation with IRL-1620 and sodium nitroprusside is expressed as a percentage of the active tone achieved with U46619. The EC_{50} values for the concentration–response curves to endothelin-1 and IRL-1620 were calculated as the concentration producing 50% of the maximum effect by geometric interpolation. The pA_2 for BQ-123 was determined by Schild analysis.

2.3. Statistical analysis

The data are expressed as mean±S.E.M. The effects of coronary occlusion and reperfusion on mean arterial pressure, heart rate, and blood gases and pH were evaluated as changes in absolute values and percentages by applying one-way, repeated-measures analysis of variance (ANOVA) followed by Student's *t*-test for paired data. To evaluate the sensitivity of control and ischemic arteries to endothelin-1 and IRL-1620, the pD_2 of each dose–response curve for these peptides was calculated as the negative logarithm of the EC_{50}. Statistical comparisons of E_{max} and pD_2 values between ischemic and control arteries, and between untreated and treated arteries were made using unpaired Student's *t* test. Comparisons of the effects of endothelin-1 and IRL-1620 obtained in control arteries, as well as in ischemic arteries under the different conditions tested were made using analysis of variance (ANOVA) followed by Dunnett test. In each case, $P<0.05$ was considered statistically significant.

2.4. Drugs used

Endothelin-1 (human, porcine) was from Peninsula Laboratories; IRL-1620 (N-Suc-[Glu9,Ala11,15]-Endothelin-

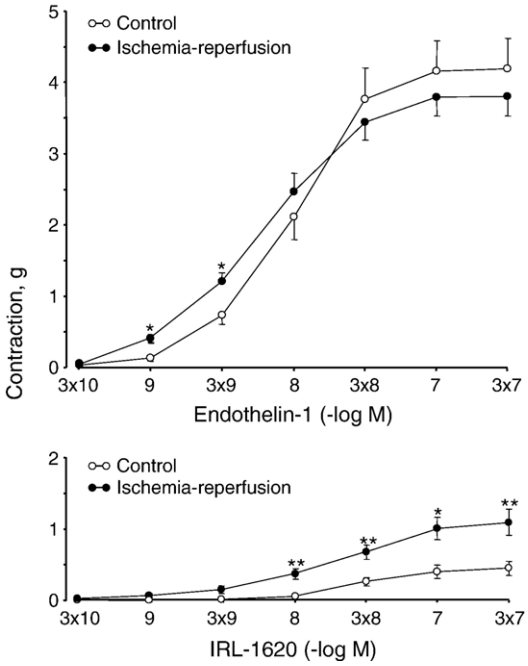

Fig. 1. Summary of the contractile responses to endothelin-1 (top) and to the ET_B receptor agonist IRL-1620 (bottom) in pig coronary arteries under control conditions and after ischemia–reperfusion. *$P<0.05$; **$P<0.01$ compared to control.

1); BQ-123 (*cyclo*-(D-Asp-Pro-D-Val-Leu_D-Trp)); BQ-788 (2,6-Dimethylpiperidinecarbonyl-γ-Methyl-L-leu-N_{in}-1-(Methoxycarbonyl)-D-Trp-D-Nle sodium salt); N^{ω}-nitro-L-arginine methyl ester (L-NAME), meclofenamate (2[1,6-Dicloro-3-methylphenyl-amino]benzoic acid sodium salt), U46619 (9, 11-Dideoxy α, 9α-Epoxymethanoprostaglandin $F_{2\alpha}$) and sodium nitroprusside were from Sigma. All the drugs were dissolved in distilled water and then diluted in isotonic NaCl immediately before use.

3. Results

3.1. Hemodynamic changes during ischemia and reperfusion

In 26 animals, mean systemic arterial pressure decreased from 79±2 mm Hg (control) to 70±2 mm Hg (at the end of occlusion, $P<0.01$) during coronary occlusion, and it decreased further during reperfusion to 66±3 mm Hg (at 60 min after reperfusion, $P<0.01$). Thus, mean arterial pressure was lower after 60 min of reperfusion than following the 30 min of occlusion ($P<0.01$). Heart rate during coronary occlusion (126±7 beats/min) and reperfusion (124±6 beats/min) was not significantly distinct from the control (127±7 beats/min). Systemic blood gases and pH did not change significantly during ischemia and reperfusion when compared to control conditions.

In all the animals during occlusion, and more frequently during reperfusion, episodes of cardiac arrhythmias were observed, the frequency and duration of which varied between animals. Ventricular fibrillation during coronary occlusion in 2 animals, and during reperfusion in another 5 animals commenced, and these animals died; as a result, none of these 7 animals were included in the study.

3.2. In vitro arterial response

3.2.1. Contractile responses to endothelin-1 and IRL-1620

Endothelin-1 (3×10^{-10}–3×10^{-7} M) and IRL-1620 (3×10^{-10}–3×10^{-7} M) produced a concentration-dependent contraction in proximal and distal coronary vascular segments from sham operated pigs. For the two peptides, the sensitivity was similar in both types of arteries whereas the maximal contraction was higher ($P<0.05$) in proximal arteries. For endothelin-1, the maximal contraction and the pD_2 values in proximal arteries were 3713±709 mg and 8.26±0.07, and in distal arteries they were 1810±345 mg and 8.15±0.12, respectively. For IRL-1620, the corresponding maximal contraction and pD_2 values in proximal arteries were 508±77 mg and 7.65±0.07, and in distal arteries were 284±46 mg and 7.54±0.05, respectively.

The mean values of pD_2 and the maximal contraction obtained with endothelin-1 and IRL-1620 in resting ischemic and control arteries in the different conditions tested are

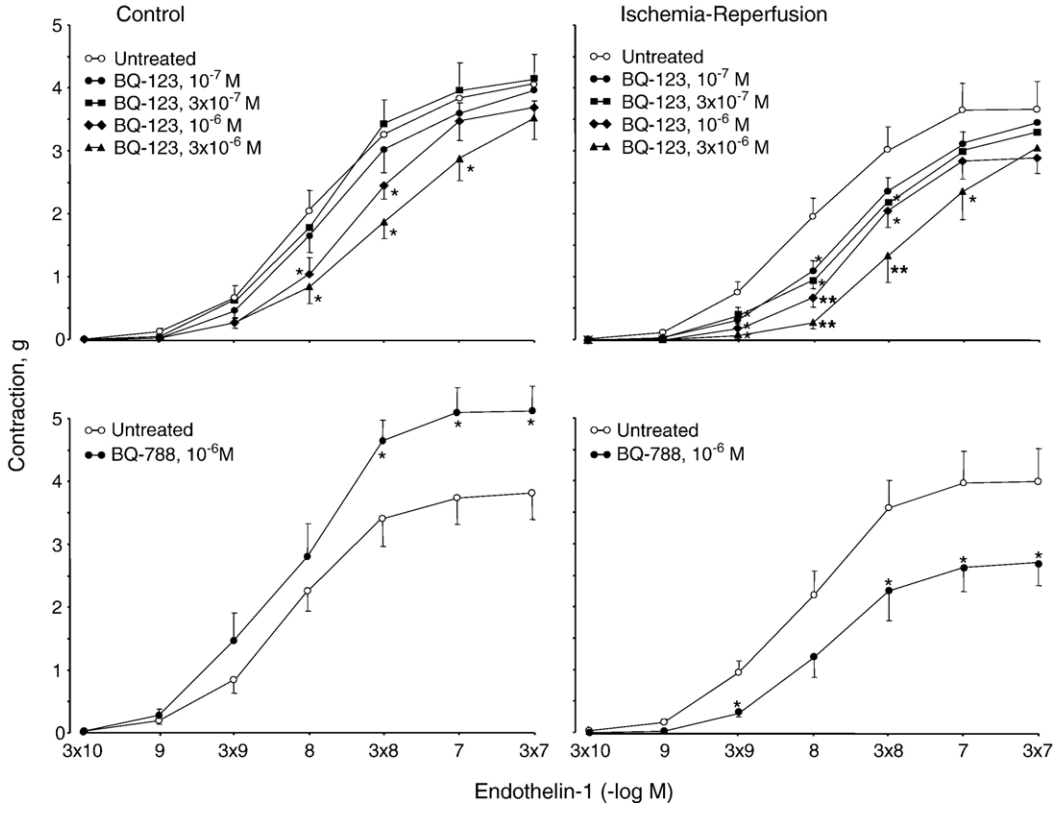

Fig. 2. Summary of the contractile responses of pig coronary arteries to endothelin-1 under control conditions (left) and after ischemia–reperfusion (right) in arteries untreated and treated with the endothelin ET_A receptor antagonist BQ-123 (10^{-7} M; 3×10^{-7} M; 10^{-6} M; and 3×10^{-6} M) (top) or treated with the endothelin ET_B receptor antagonist BQ-788 10^{-6} M (bottom). *$P<0.05$; **$P<0.01$ compared to untreated.

summarized in Table 1. While endothelin-1 (3×10^{-10}–3×10^{-7} M) produced concentration-dependent contraction in both ischemic and control coronary vascular segments, the sensitivity to endothelin-1 was greater in ischemic segments, where a similar maximal response was observed in both types of arteries (Table 1 and Fig. 1). IRL-1620 (3×10^{-10}–3×10^{-7} M) also produced concentration-dependent contraction in ischemic and control coronary vascular segments, but both the sensitivity and maximal contraction were higher in ischemic arteries (Table 1 and Fig. 1).

The endothelin ET_A receptor antagonist BQ-123 (10^{-7}–3×10^{-6} M) produced a parallel rightward displacement of the concentration–response curve to endothelin-1 in control arteries with a pA_2 of 6.31 and a slope of 0.69 (different from 1) (Fig. 2). In ischemic arteries, BQ-123 also shifted the concentration–response curve for endothelin-1 to the right in a parallel way, with a pA_2 (6.47) and slope (0.62) similar to that found in control arteries (Fig. 2). Table 1 shows the pD_2 values and the maximal contraction for endothelin-1 in the presence of 10^{-6} M BQ-123.

The endothelin ET_B receptor antagonist BQ-788 (10^{-6} M) did not change the sensitivity to endothelin-1, but it increased the maximal contraction in control segments while reducing it in ischemic arteries (Table 1 and Fig. 2).

Application of BQ-123 (10^{-6} M) during the plateau of the IRL-1620-induced contraction, relaxed this contraction by a similar magnitude in control arteries (24±2%, 25 segments) and ischemic arteries (23±2%, 21 segments). However, when BQ-788 (10^{-6} M) was applied during the plateau of the contraction produced by IRL-1620, it completely abolished this contraction in both control and ischemic arteries (6 segments for each type of arteries).

Endothelium removal increased the sensitivity of control arteries to endothelin-1 without significantly altering the maximal contraction to endothelin-1 (Table 1 and Fig. 3). Furthermore, the absence of endothelium increased both the sensitivity and maximal contraction in response to IRL-1620 (Table 1 and Fig. 4). In contrast, it did not modify either the sensitivity or the maximal response to both peptides in ischemic arteries.

L-NAME increased the sensitivity as well as the maximal effect of endothelin-1 and IRL-1620 in control arteries (Table 1, Figs. 3 and 4). However, this treatment did not modify the response to endothelin-1 or IRL-1620 in ischemic arteries (Table 1, Figs. 3 and 4).

Meclofenamate augmented the maximal response without changing the sensitivity to endothelin-1 in control arteries (Table 1 and Fig. 3), and did not affect the response to IRL-1620 (Table 1 and Fig. 4). However, in ischemic arteries this treatment decreased the maximal response evoked by both endothelin-1 and IRL-1620, and also decreased the sensitivity to IRL-1620.

3.2.2. Relaxing responses to IRL-1620

After inducing contraction with U46619, a similar level of active tone was reached in both groups of arteries (996±492 mg

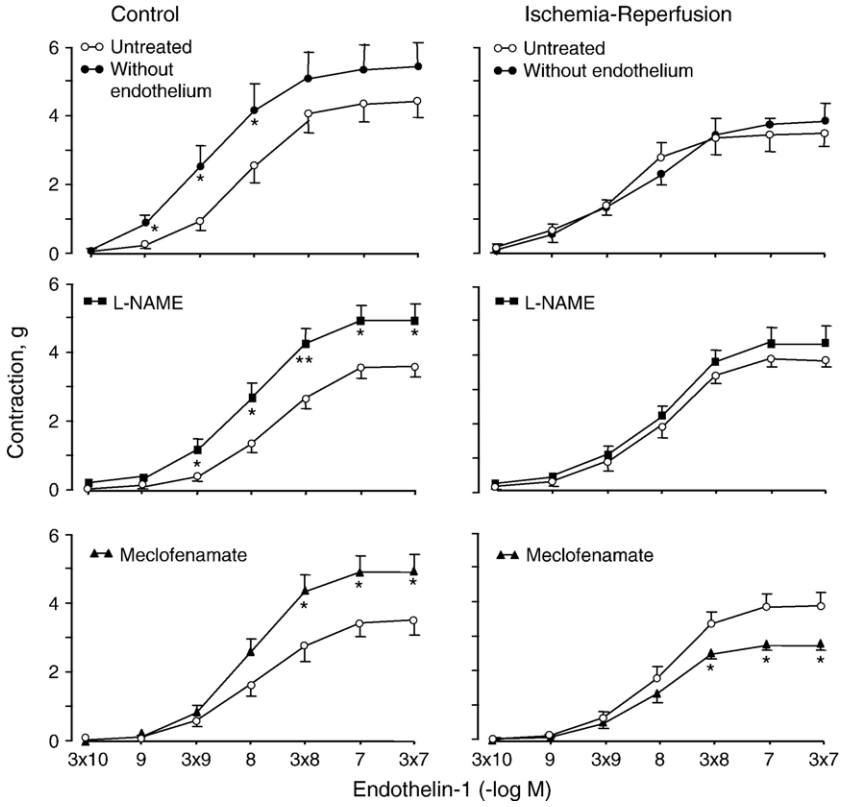

Fig. 3. Summary of the contractile responses to endothelin-1 in pig coronary arteries under control conditions (left) and after ischemia–reperfusion (right): arteries untreated, without endothelium, treated with the inhibitor of nitric oxide synthesis L-NAME (10^{-4} M) or treated with the inhibitor of cyclooxygenase meclofenamate (10^{-5} M). *$P<0.05$; **$P<0.01$ compared to untreated.

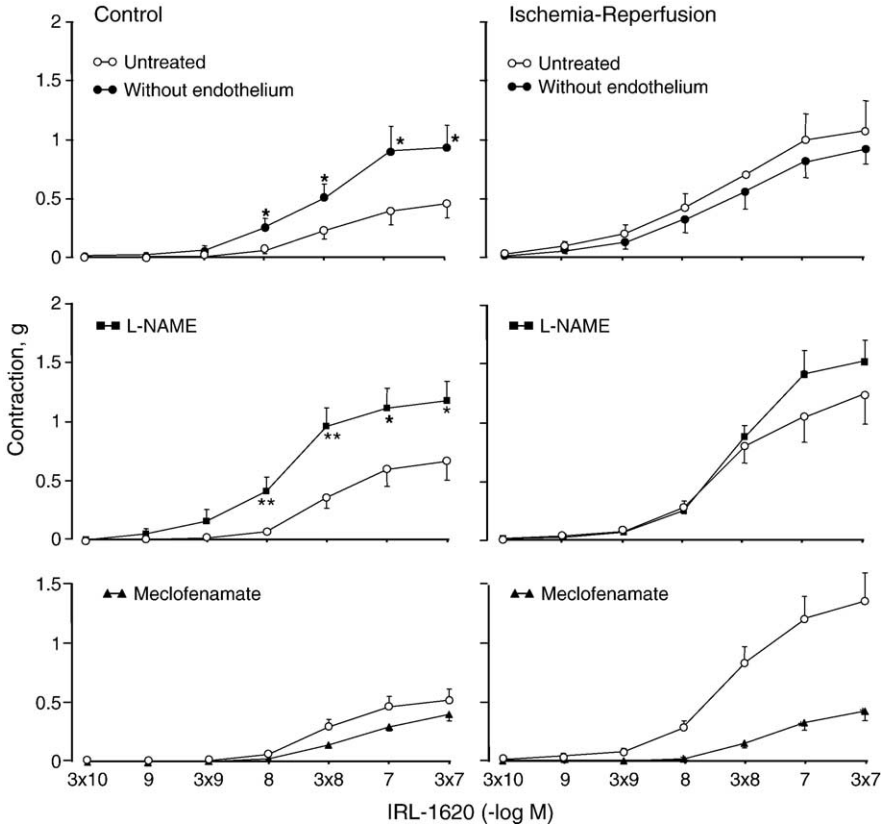

Fig. 4. Summary of the contractile responses to IRL-1620 in pig coronary arteries under control conditions (left) and after ischemia–reperfusion (right): arteries untreated, without endothelium, treated with the inhibitor of nitric oxide synthesis L-NAME (10^{-4} M) or treated with the inhibitor of cyclooxygenase meclofenamate (10^{-5} M). *$P<0.05$; **$P<0.01$ compared to untreated.

for 20 ischemic arterial segments vs. 1020±317 mg for 20 control arterial segments). In control arteries, IRL-1620 at lower doses (3×10^{-11}–3×10^{-10} M) induced a small relaxing effect that was not observed in ischemic arteries (Fig. 5). This relaxation of control arteries was abolished by treatment with L-NAME (10^{-4} M) or meclofenamate (10^{-5} M) (Fig. 5). Higher doses of IRL-1620 (10^{-9}–3×10^{-7} M) caused contraction in both ischemic and control segments, but this contractile effect has not been analyzed.

3.2.3. Relaxing responses to sodium nitroprusside

After contraction with U46619, the degree of active tone reached was similar in both ischemic and control arteries (1066 ±185 mg for 21 ischemic arterial segments vs. 884±138 mg for 16 control arterial segments). Sodium nitroprusside (10^{-8}–10^{-4} M) induced a dose-depending relaxation that was similar in both types of arteries. In control and ischemic arteries, the maximal relaxation was 100% and the pD_2 values were 7.13±0.10 and 7.10±0.08, respectively ($P>0.05$).

4. Discussion

The present study was performed to analyse the role of endothelin ET_A and ET_B receptors, as well as the interaction of these receptors with nitric oxide and prostanoids in the response to endothelin-1 in coronary vessels after ischemia–reperfusion. The results show that after ischemia–reperfusion the sensitivity of the coronary response to endothelin-1 is increased by mechanisms that may include: (1) endothelial dysfunction with an impairment of endothelin ET_B receptor-induced release of nitric oxide and prostacyclin; (2) an augmented contraction provoked by activation of endothelin ET_B receptors probably located in smooth musculature; and (3) an increase in the release of vasoconstrictor prostanoids.

In control arteries, we found that endothelin-1 produces a marked pig coronary vasoconstriction, which may be mainly mediated by activation of endothelin ET_A receptors. This is in line with results obtained by others in coronary vessels of several species, including humans (Yanagisawa et al., 1988; Franco-Cereceda, 1989; Ihara et al., 1991; Davenport et al., 1993; Wang et al., 1994; García et al., 1996). The present results also suggest that the activation of endothelin ET_B receptors may release nitric oxide and prostacyclin from the endothelium, which may counteract the contractile response to endothelin-1, or relax precontracted arteries in response to IRL-1620. It has been reported that both nitric oxide and prostacyclin may mediate coronary vasodilatation after activation of endothelin ET_B receptors (Ushio-Fukai et al., 1992; D'Orleans-Juste et al., 2002) or may counteract the coronary vasoconstriction produced by endothelin-1 (García et al., 1996; Wang et al., 1994; Thompson et al., 1995). Therefore, our present results suggest that the normal coronary response to endothelin-1 may

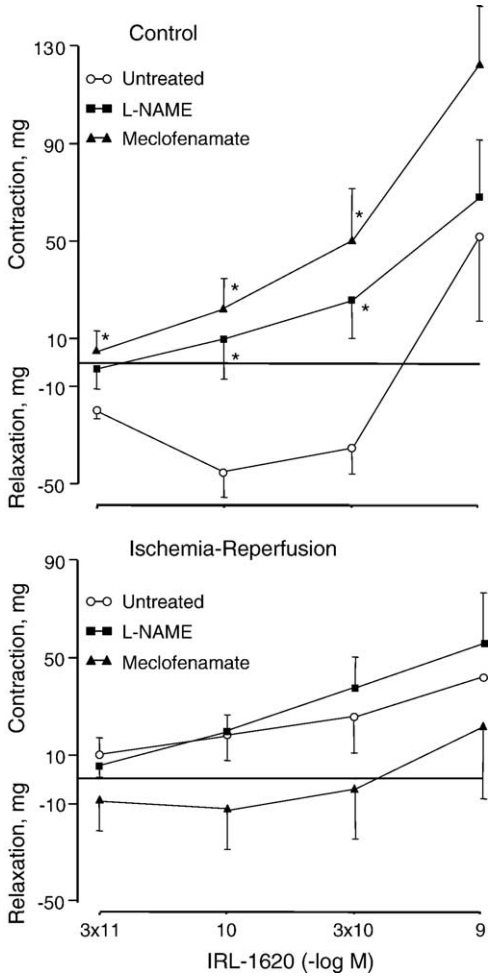

Fig. 5. Summary of the responses to IRL-1620 in precontracted pig coronary arteries under control conditions (top) and after ischemia–reperfusion (bottom): arteries untreated, treated with the inhibitor of nitric oxide synthesis L-NAME (10^{-4} M), and treated with the inhibitor of cyclooxygenase meclofenamate (10^{-5} M). *$P<0.05$; **$P<0.01$ compared to untreated.

be mainly the result of its interaction with endothelin ET_A receptors producing contraction, and with endothelial endothelin ET_B receptors modulating this contraction by releasing nitric oxide and prostacyclin.

The sensitivity to endothelin-1 and IRL-1620 was comparable in normal proximal and distal branches of the pig left anterior descending coronary artery and the maximal response was higher in proximal arteries. This feature was altered by ischemia–reperfusion as the sensitivity to endothelin-1 of distal arteries, which had been exposed to ischemia–reperfusion, was higher than that of proximal, non ischemic arteries, and the maximal response was similar in both types of arteries. Therefore, our results suggest that ischemia–reperfusion increases the coronary response to endothelin-1, a feature that has been reported previously by others (Neubauer et al., 1991; Watts et al., 1992; Thompson et al., 1995; Wang et al., 1995; Lockowandt et al., 2001).

The mechanisms that underlie the increased coronary response to endothelin-1 after ischemia–reperfusion remain to be elucidated. It seems to be that the endothelium is very sensitive to ischemia–reperfusion (Nicklas and Gips, 1989; Kim et al., 1992; Fernández et al., 2002,2003). Endothelial dysfunction after ischemia–reperfusion might be a basic mechanism to provoke the augmented coronary response to endothelin-1 in this condition by decreasing the release of endothelial vasodilators after activation of endothelin ET_B receptors located in the endothelium, as well as by unmasking this type of receptors located in smooth musculature and mediating vasoconstriction. We observed that the endothelin ET_A receptor antagonist BQ-123 inhibited the sensitivity of the concentration–response curve for endothelin-1 to a similar extent in ischemic and normal arteries. The endothelin ET_B receptor antagonist BQ-788 reduced the maximal response to endothelin-1 in ischemic vessels, in contrast to that found in control arteries. This suggests that the role of endothelial endothelin ET_B receptors in counteracting the normal coronary response to endothelin-1 is depressed after ischemia–reperfusion. This suggestion is supported by the fact that endothelium removal or L-NAME did not affect the contractions induced by endothelin-1 and IRL-1620 in ischemic arteries whereas they potentiated these contractions in control arteries. We also found that precontracted ischemic arteries did not relax in response to IRL-1620, but they preserved their ability to relax in response to sodium nitroprusside, suggesting that the lack of relaxation in response to IRL-1620 is probably due to endothelial dysfunction and not to the impaired response of vascular smooth muscle. On the other hand, the depressant effect of BQ-788 on the maximal response to endothelin-1, and the augmented action of IRL-1620 in ischemic arteries may also suggest that the function of endothelin ET_B receptors in mediating coronary vasoconstriction is increased after ischemia–reperfusion. Therefore, ischemia–reperfusion may impair the function of the endothelium which may cause diminution of nitric oxide release after activation of endothelin ET_B receptors, as well as upregulation of endothelin ET_B receptors in mediating vasoconstriction. This would result in increased coronary response to endothelin-1 during this pathological condition. In ischemic arteries, meclofenamate inhibited the maximal response to endothelin-1 and IRL-1620, whereas it augmented this response in control arteries. This suggests that after ischemia–reperfusion coronary vessels may release vasoconstrictor prostanoids in place of vasodilatory prostanoids when stimulated with endothelin-1 and the agonist of endothelin ET_B receptors. This could also contribute to the increased coronary response to endothelin-1 after ischemia–reperfusion.

From studies in isolated rat hearts, it has been suggested that the increased coronary response to endothelin-1 after ischemia–reperfusion is mainly due to the diminished capacity of endothelial cells to modulate or limit the constriction in response to endothelin-1 (Watts et al., 1992), or that it is likely due to the combined effect of upregulation of endothelin ET_A receptors and reduction of opposing endothelin ET_B receptors-mediated vasodilatation (Thompson et al., 1995). Studies using isolated pig coronary arteries suggest that the increased response to endothelin-1 after ischemia–reperfusion is probably

related to upregulation of endothelin ET_A receptors but unrelated to alteration of endothelial cells to release vasodilator substances (Wang et al., 1995; Lockowandt et al., 2001). The study of Wang et al. (1995) was performed in coronary arteries from pigs exposed to ischemia after coronary thrombosis and reperfusion after coronary thrombolysis, and the authors observed that the response to a selective agonist for endothelin ET_B receptors did not change after ischemia–reperfusion, and that BQ-123 antagonized the endothelin-1-induced contraction in the ischemic–reperfused and control arteries to the same extent. From these results the authors suggest that the enhanced coronary vasoconstriction in response to endothelin-1 is related to endothelin ET_A receptors activation, probably due to an increased number of these receptors (Wang et al., 1995). Our data with endothelin-1 and BQ-123 or BQ-788, and those with IRL-1620 suggest that the interaction of endothelin-1 with endothelin ET_B receptors rather than with endothelin ET_A receptors may be involved in the increased coronary vasoconstrictor action of endothelin-1 during ischemia–reperfusion. The main difference between our study and that of Wang et al. (1995) is that, in addition to the procedure to induce ischemia and reperfusion, the duration of both ischemia and reperfusion was more prolonged in our model, and this feature may influence the behaviour of endothelin ET_B receptors. The presence or degree of the augmented coronary response to endothelin-1 after ischemia–reperfusion may depend on ischemia duration and severity (Neubauer et al., 1991; Lockowandt et al., 2001; Fernández et al., 2002, 2003).

In conclusion, we found that after ischemia–reperfusion, coronary vasoconstriction in response to endothelin-1 is increased. This abnormal endothelin-1 response may be attributable to endothelial dysfunction associated with the impairment of endothelin ET_B receptor-induced release of nitric oxide and prostacyclin, to augmented contraction in response to activation of endothelin ET_B receptors probably located in smooth musculature; as well as through the increased release of vasoconstrictor prostanoids. Our data support the idea that endothelin-1 may play a role in the detrimental effects of ischemia–reperfusion, and suggest that blocking endothelin ET_B receptors, as well as endothelin ET_A receptors, should be considered when treating ischemia–reperfusion.

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