



The vasoconstrictor effect of 8-epi prostaglandin F_{2α} in the hypoxic rat heart

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1 8-epi prostaglandin (PG) F_{2α}, a vasoconstrictor isoprostane, is synthesized under conditions of oxidative stress. This study was undertaken to investigate the vasoconstrictor effect of 8-epi PGF_{2α} in the coronary circulation before and after a period of oxidative stress.

2 The effects of the isoprostane 8-epi PGF_{2α} and the thromboxane mimetic U46619 were compared in the isolated rat heart perfused in the Langendorff mode at a constant pressure of 80 mmHg.

3 In normal hearts U46619 caused a dose-related reduction in coronary flow (ED₅₀ 4.7 ± 2.2 nmol). In contrast, 8-epi PGF_{2α} had no effect.

4 After reducing perfusion pressure to 20 mmHg for 30 min and reperfusing at 80 mmHg, the dose-response curve to U46619 was unaffected. In contrast, 8-epi PGF_{2α} caused a dose-dependent drop in coronary flow (ED₅₀ 52.6 ± 12.7 nmol), producing a similar maximal reduction to U46619.

5 Similarly, after perfusion with xanthine and xanthine oxidase for either 15 or 30 min there was little change in the response to U46619 in comparison to control hearts. In contrast, 8-epi PGF_{2α} caused a reduction in coronary flow similar to that produced by U46619, the magnitude of the response being related to the length of xanthine/xanthine oxidase perfusion.

6 Responses to both U46619 and 8-epi PGF_{2α} after xanthine/xanthine oxidase perfusion were blocked by the selective thromboxane receptor antagonist SQ29548 10⁻⁷ M.

7 These results show that oxidative stress in the isolated perfused rat heart reveals a potent vasoconstrictor effect of the isoprostane 8-epi PGF_{2α} by an action on the thromboxane receptor.

8 The data also suggest that, since 8-epi PGF_{2α} is a partial agonist at the thromboxane receptor, thromboxane receptor reserve is increased by oxidative stress.

Keywords: Isoprostane; thromboxane; prostaglandin; coronary circulation; oxidative stress; hypoxia

Abbreviations: 8-epi PGF_{2α}, 8-epi prostaglandin F_{2α}; HR, heart rate; LVP, left ventricular pressure; TP, thromboxane receptor

Introduction

Isoprostanes, like the prostaglandins, are metabolites of arachidonic acid. However, unlike the prostaglandins, they are principally derived by free radical attack on membrane phospholipids (Morrow *et al.*, 1990) and are produced *in vivo* in conditions of oxidative stress (Delanty *et al.*, 1996; 1997). They are formed *in situ* esterified to phospholipids and are thought to be released by the action of a phospholipase (Morrow *et al.*, 1992). We have previously reported that one of the isoprostanes, 8-epi prostaglandin (PG) F_{2α}, has a vasoconstrictor effect on isolated arterial rings (Kromer & Tippins, 1996; 1998). In porcine coronary artery 8-epi PGF_{2α} has a partial agonist action on the thromboxane (TP) receptor (Kromer & Tippins, 1996). Increased 8-epi PGF_{2α} production has been reported in ischaemic heart disease (Kromer *et al.*, 1997), and urinary levels of 8-epi PGF_{2α} increase in acute myocardial infarct patients after thrombolytic therapy or coronary artery bypass surgery (Delanty *et al.*, 1997) and after percutaneous transluminal coronary angioplasty (Reilly *et al.*, 1997).

The aim of this study was to investigate the vasoconstrictor properties of 8-epi PGF_{2α} in comparison to the thromboxane mimetic U46619 in isolated perfused rat hearts and in hearts subjected to oxidative stress.

Methods

Male Sprague-Dawley rats weighing 200–250 g were anaesthetized with Hypnorm and Hypnovel (2 mg ml⁻¹) in the ratio of 2:5:1 part of water at a dose of 2.7 ml kg⁻¹ i.p. and 400 units sodium heparin given i.v. *via* the tail vein. The heart was removed and immediately placed into ice-cold Krebs (mM) NaCl 118, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 24, Glucose 11 and sodium pyruvate 2.0. The ascending aorta was then cannulated rapidly and retrogradely perfused with Krebs solution (pH 7.4, bubbled with 95% O₂-5% CO₂, 37°C) at a constant pressure of 80 mmHg measured with an Elcomatic pressure transducer (EM750) attached to a side arm of the aortic perfusion cannula. Left ventricular pressure (LVP) was measured by a fluid-filled Clingfilm balloon inserted into the left ventricle (Curtis *et al.*, 1986) and connected to a second pressure transducer (Bell and Howell Ltd. Type 4-422-0001). Left ventricular end diastolic pressure was set to approximately 15 mmHg (Curtis *et al.*, 1986) which was optimal for maximal left ventricular developed pressure. Heart rate (HR) was derived from the left ventricular pressure trace by a cardiometer. The perfusion flow was continuously measured with a Gould blood flowmeter (SP2202) by positioning a flow probe in-line with the cannula. Data were recorded on a six channel recorder (Lectromed; MX-6). In xanthine/xanthine oxidase perfusion experiments hearts were electrically paced at 350 beats min⁻¹.

After a 25 min stabilization period, responses to U46619 or to 8-epi PGF_{2α} were recorded. Agonists were added as 10 μl bolus doses into the aortic perfusion cannula. The hearts were left for 10 min between doses to allow values to return to

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baseline or until flow and systolic LVP stabilized. In separate experiments, oxidative stress was created either by reduction of perfusion pressure to 20 mmHg for 30 min and then reperfusion at 80 mmHg, or by perfusion with Krebs containing 0.23 mM xanthine and 0.5 mU ml⁻¹ xanthine oxidase for 15 or 30 min and then reperfusion with normal Krebs (Basu & Karmazyn, 1987). In both cases responses to U46619 or to 8-epi PGF_{2α} were then repeated.

Drugs

8-epi PGF_{2α}, U46619 (11, 9, epoxy-methano PGH₂) and the thromboxane antagonist SQ29548 ((1S-[1,2b(5Z),3b,4]]-7-[3-[[2-(phenylamino) carbonyl hydrazino]methyl]-7-oxabicyclo [2.2.1]-hept-2-yl]-5-heptenoic acid) were purchased from Cayman Chemicals (Ann Arbor, MI, U.S.A.). All compounds were dissolved in ethanol and subsequently diluted to the required concentrations in Krebs solution. The highest ethanol concentration in the perfusate was approximately 0.2% which had no effect on the heart. Xanthine was dissolved in 5 M NaOH and diluted to the appropriate concentration in Krebs solution. The final concentration of NaOH in the perfusate was approximately 0.001 M which on its own had no effect on any measured parameter.

Statistical analysis

Results are expressed as mean ± s.e.mean. ED₅₀ values and E_{max} values were calculated using Prism software (GraphPad Inc., San Diego, CA, U.S.A.). Student's *t*-test was used to investigate differences in responses before and after oxidative stress. A *P* value < 0.05 was taken to be the minimum level of statistical significance with *n* number of animals tested.

Results

Under resting conditions mean coronary flow at a constant pressure of 80 mmHg was 17.5 ± 3.5 ml min⁻¹ (*n* = 6), systolic LVP 86 ± 7 mmHg and HR 246 ± 36 beats min⁻¹. U46619 caused a dose-related reduction in flow (ED₅₀ 4.7 ± 2.2 nmols, *n* = 5, Figure 1). HR was unaffected by U46619 and systolic and developed LVP only decreased when there was a marked reduction in coronary flow (maximal decrease in systolic LVP of 26.5 mmHg after the administration of 32 nmols U46619). 8-epi PGF_{2α} had no effect on any parameter measured in control rat hearts, even up to a dose of 256 nmols.

Perfusion at 20 mmHg resulted in a drop in flow to between 2 and 5 ml min⁻¹. After 30 min of 20 mmHg perfusion followed by reperfusion at 80 mmHg, flow increased briefly (29.3 ± 6.4 ml min⁻¹) and then reached a steady rate of 11.9 ± 6.3 ml min⁻¹. After reperfusion the dose-response curve to U46619 shifted slightly to the left, but this was not statistically significant (ED₅₀ 3.1 ± 2.7 nmols; *P* > 0.05, Student's *t*-test, Figure 1). However, 8-epi PGF_{2α} caused a dose-dependent vasoconstriction after reperfusion with a threshold dose of 16 nmols and an ED₅₀ of 52.6 ± 12.7 nmols (Figure 1). 8-epi PGF_{2α} had a similar effect to U46619 on other parameters measured, a large decrease in coronary flow associated with a decrease in systolic and developed LVP, but no effect on HR.

In hearts perfused with xanthine and xanthine oxidase, resting coronary flow was 11.8 ± 1.2 ml min⁻¹ (*n* = 5) and systolic LVP was 112 ± 19 mmHg. U46619 caused a dose-dependent decrease in flow with an ED₅₀ of 0.8 ± 0.1 nmol (*n* = 5, Figure 2). After perfusion with xanthine and xanthine

oxidase for either 15 or 30 min, the U46619 dose-response curves gave ED₅₀ values of 0.8 ± 0.1 nmol (*n* = 4) and 0.6 ± 0.1 nmol (*n* = 5) for 15 and 30 min perfusions respectively (*P* > 0.05; Student's *t*-test). As in the low flow experiments, 8-epi PGF_{2α} had no effect on normal rat coronary vasculature even up to doses of 256 nmols. However, after xanthine and xanthine oxidase perfusion, 8-epi PGF_{2α} caused a marked coronary constriction (E_{max} 2.1 ± 0.2 ml min⁻¹ g⁻¹, ED₅₀ 26.7 ± 4.8 nmols after 15 min and E_{max} 3.3 ± 0.3 ml min⁻¹ g⁻¹, ED₅₀ 32.8 ± 6.7 nmols after 30 min perfusion, Figure 2).

After perfusion of the heart with xanthine and xanthine oxidase for 15 min, the reduction in coronary flow produced by submaximal doses of both U46619 (8 nmols) and 8-epi PGF_{2α} (56 nmols) was virtually abolished by perfusion of the tissue with Krebs containing 10⁻⁷ M SQ29548, a TP receptor antagonist (4.7 ± 0.9 ml min⁻¹ g⁻¹ control, 0.6 ± 0.3 ml min⁻¹ g⁻¹ SQ29548 and 2.5 ± 0.1 ml min⁻¹ g⁻¹ control, 0.1 ± 0.1 ml min⁻¹ g⁻¹ SQ29548, for U46619 and 8-epi PGF_{2α} respectively).

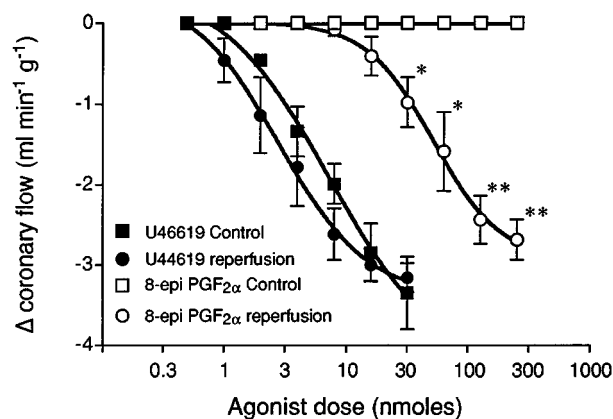


Figure 1 Effect of low flow on vasoconstrictor responses to U46619 and 8-epi PGF_{2α} in the isolated rat heart. **P* < 0.05, ***P* < 0.005, unpaired *t*-test, *n* = 5.

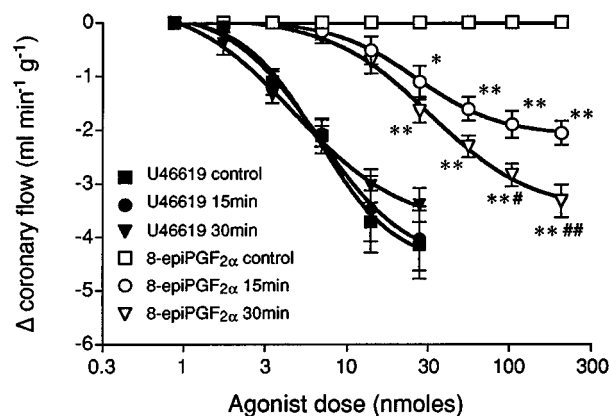


Figure 2 Effect of xanthine/xanthine oxidase perfusion on vasoconstrictor responses to U46619 and 8-epi PGF_{2α} in the isolated rat heart. **P* < 0.005 and ***P* < 0.001 compared to control; #*P* < 0.005 and ##*P* < 0.001 compared to perfusion for 15 min, unpaired *t*-test, *n* = 5.

Discussion

This study shows that constriction of the rat coronary vasculature occurs in response to 8-epi PGF_{2α} following oxidative stress induced either by low flow perfusion or by perfusion with xanthine and xanthine oxidase, with the isoprostane being completely devoid of constrictor activity before the tissue has been subjected to oxidative stress. The response to the thromboxane mimetic U46619 is unaffected by oxidative stress. Secondly, responses to both U46619 and 8-epi PGF_{2α} after oxidative stress were blocked by the TP receptor antagonist SQ29548, suggesting that they were mediated by an action on the TP receptor. To our knowledge, no other study has reported a similar increase in reactivity to other mediators in response to oxidative stress.

It is widely, though not universally, accepted that the action of 8-epi PGF_{2α} is mediated by an action on the TP receptor (Banerjee *et al.*, 1992; Crankshaw, 1995; John & Valentin, 1997; Kang *et al.*, 1993; Kromer & Tippins, 1996; Takahashi *et al.*, 1992; Zhang *et al.*, 1996). We have reported previously that 8-epi PGF_{2α} acts as a partial agonist at the TP receptor (Kromer & Tippins, 1996). In the present study, the response to 8-epi PGF_{2α} increased to 80 ± 7% of the response to U46619 after ischaemia and to 50 ± 5% and 80 ± 7% of the response to U46619 after 15 or 30 min respectively of perfusion with xanthine and xanthine oxidase. This increase in intrinsic activity to the isoprostane may be explained therefore by an increase in TP receptor density (Kenakin, 1993) after oxidant stress. The response to a partial agonist is dependent upon receptor density and the stimulus response coupling. As we showed previously, when TP receptor reserve is low 8-epi PGF_{2α} acts only as an antagonist, whereas a high efficacy agonist such as U46619 induces a maximum response (Kromer & Tippins, 1996). Conversely, when receptor reserve is high 8-epi PGF_{2α} will produce a maximum response similar to that evoked by U46619 (Kromer & Tippins, 1996). The present data support the suggestion that TP receptor reserve primarily determines the degree of intrinsic activity exhibited by 8-epi PGF_{2α} in a given preparation (John & Valentin, 1997).

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