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Human internal mammary artery contraction by isoprostaglandin $F_{2\alpha}$ type-III (8-*iso*-prostaglandin $F_{2\alpha}$)

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Abstract

Isoprostaglandin $F_{2\alpha}$ type-III (formerly known as 8-*iso*-prostaglandin $F_{2\alpha}$) is produced in large quantities in vivo in clinical situations associated with oxidant stress such as atherosclerosis, hypercholesterolemia, and myocardial reperfusion. Isoprostaglandin $F_{2\alpha}$ type-III may alter smooth muscle and platelet functions. The aim of this study was to evaluate the effects of isoprostaglandin $F_{2\alpha}$ type-III on isolated human internal mammary arteries, and to characterise the signalling underlying mechanisms. In organ baths, concentration-dependent contractions of human internal mammary arteries were obtained in response to isoprostaglandin $F_{2\alpha}$ type-III stimulation. The responses to isoprostaglandin $F_{2\alpha}$ type-III were inhibited in a concentration-dependent manner by the thromboxane A_2 receptor antagonist, GR 32191 ([1*R*-[1 $\alpha(Z)$, 2β,3β,5 $\alpha(+)$-7-[[1,1′-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl) cyclo pentyl]-4-4heptanoic acid], hydrochloride), 3×10^{-9} to 3×10^{-7} M). However, this effect was associated with a decreased maximal contraction. AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid, 10^{-6} to 3×10^{-5} M), an EP_1-DP receptor antagonist had no effect on isoprostaglandin $F_{2\alpha}$ type-III-induced contractions. The maximal responses to isoprostaglandin $F_{2\alpha}$ type-III were significantly reduced in the presence of the cyclooxygenase inhibitor indomethacin (10^{-5} M) (E_{max}: $147\pm20\%$ vs. $213\pm19\%$ in control group, $P<0.05$). Isoprostaglandin $F_{2\alpha}$ type-III stimulated thromboxane B_2 release (5.7-fold increase) from human internal mammary arteries. Baicaleine, a non-specific lipoxygenase inhibitor, (10^{-4} M) and AA 861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4 benzoquinone), a 5-lipoxygenase inhibitor (10^{-5} M) did not affect isoprostaglandin $F_{2\alpha}$ type-III response. In conclusion, this study shows that (1) isoprostaglandin $F_{2\alpha}$ type-III is a vasoconstrictor in human internal mammary arteries, with a potency equivalent to prostaglandin $F_{2\alpha}$, (2) the contractions induced by isoprostaglandin $F_{2\alpha}$ type-III are mediated by TP receptor but not EP_1-DP-receptor activation, (3) thromboxane A_2 but not cysteinyl leukotrienes production is involved in the vascular effects of isoprostaglandin $F_{2\alpha}$ type-III. Isoprostaglandin $F_{2\alpha}$ type-III, produced at sites of free radical generation, may play an important role in internal mammary artery spasm in situations of oxidant stress such as coronary bypass surgery.

Keywords: Isoprostane; Oxidative stress; Mammary artery; Vasoconstriction

1. Introduction

Oxidant stress results from a balance between oxidant production and antioxidant defences in favour of the former. Such a phenomenon has been suggested to contribute to the physiopathology of a variety of diseases such as atherosclerosis (Gniwotta et al., 1997; Pratico et al., 1997), hypercholesterolemia (Reilly et al., 1998), myocardial reperfusion (Delanty et al., 1997), heart failure (Mallat et al., 1998), and diabetes (Davi et al., 1999). One of the main targets of oxygen free radicals is cellular membrane unsaturated fatty acids such as arachidonic acid, leading to lipid peroxidation and cellular injury. Recently, a novel family of prostaglandin F_2 isomers, called F_2-isoprostanes, produced in vivo by free radical peroxidation of arachidonic acid, has been described (Morrow et al., 1990).

Isoprostaglandin $F_{2\alpha}$ type III (formerly known as 8-*iso*-prostaglandin $F_{2\alpha}$ (Rokach et al., 1997)) has been shown to be produced in large quantities in vivo in clinical situations where oxidative stress is thought to occur (Delanty et al., 1997). Isoprostaglandin $F_{2\alpha}$ type-III is bio-

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chemically stable, and may provide a reliable marker of oxidant injury in vivo (Souvignet et al., 2000). Isoprostaglandin $F_{2\alpha}$ type-III may produce physiological or pathological effects due to its ability to alter smooth muscle and platelet functions. It has been shown to be a potent vasoconstrictor of pulmonary, renal and aortic rat vascular beds (Wagner et al., 1997), rat airways (Kang et al., 1993), guinea-pigs airways (Kawikova et al., 1996), porcine carotid and coronary arteries (Mohler et al., 1996), ovine and bovine coronary arteries (Kromer and Tippins, 1996) and human airways (Kawikova et al., 1996). However, the biological activity of isoprostaglandin $F_{2\alpha}$ type-III on human vessels has not been investigated. Internal mammary arteries are currently the best conducts for cardiac revascularisation (Cameron et al., 1996). However, they have a smaller diameter compared with saphenous veins, and a greater tendency to spasm. Therefore, the aims of our study were to both evaluate whether isoprostaglandin $F_{2\alpha}$ type-III, which is released during coronary artery bypass grafting (Delanty et al., 1997), modulates human internal mammary artery tone, and to characterise the signalling mechanisms underlying these effects.

2. Materials and methods

2.1. Preparation of blood vessels

Human internal mammary arteries were obtained during surgery from 59 patients undergoing coronary bypass surgery (53 men and 6 women, median age 65 years, range 41 to 78). The cardiovascular risk factors of the patients were: hypercholesterolemia (44), hypertension (35), cigarette smoking (33), and diabetes (8). The preoperative drug therapy in these patients was as follows: 44 patients were on β-adrenoceptor antagonists, 41 patients were on nitrates, 13 patients were on angiotensin converting enzyme inhibitors, 11 patients were on Ca^{2+} channel antagonists, 6 patients were on angiotensin receptor antagonists, and 1 patient was on a K^+ channel opener.

The discarded distal ends of the arterial grafts were immediately placed in oxygenated HEPES-buffered Krebs solution maintained at 4°C and transferred to the laboratory within 2 h. The HEPES-buffered Krebs solution had the following composition (mM): NaCl (130), KCl (3.8), $CaCl_2$ (2.1), $MgSO_4$ (1.2), KH_2PO_4 (1.2), $NaHCO_3$ (14.8), glucose (10.4) and HEPES (10). Blood vessels were dissected free from connective tissue and cut into 3-mm lengths. The number of rings taken from each artery varied from 2 to 5. The investigation conforms to the principles outlined in the declaration of Helsinki.

2.2. Organ bath technique

Rings were suspended in organ chambers filled with 6 ml of Krebs solution maintained at 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The Krebs solution had the following composition (mM): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1), KH2PO_4 (1), $NaHCO_3$ (25) and glucose (11). Segments were mounted between two stainless steel wires. The upper wire was fixed to an isometric force displacement transducer (UF-1 Pioden Controls limited, Canterbury, UK) and the lower wire was attached to a micrometer (Mitutoyo, Japan). Force measured by the transducer was continuously recorded (Linseis L200E, Bioblock, Illkirch, France).

Following a 30-min equilibration period, the rings were stretched in progressive steps to determine the length-tension curve in order to perform a length-tension normalisation procedure, as previously described (He et al., 1988, 1989). The internal circumference of each ring suspended with an equivalent transmural pressure of 100 mm Hg was determined from its length-tension curve. When the transmural pressure on the rings reached 100 mm Hg the stretch-up procedure was stopped. The rings were released to 90% of their internal circumference at 100 mm Hg (0.9 D100). The mean internal diameter at an equivalent transmural pressure of 100 mm Hg was 1.25 ± 0.05 mm. When the rings were relaxed to a resting diameter of 0.9 D100, the passive preload was 5.13 ± 0.15 g.

Following this normalisation procedure, each ring was stabilised for a further 60-min period, and the Krebs solution was changed at 15-min intervals. The rings were then challenged twice with KCl (90 mM) at a 10-min interval to ensure that responses were reproducible. Following a further 60-min period, concentration–contraction curves were made.

All vessels were tested for a functional endothelium by their ability to relax in response to acetylcholine (10^{-6} M) when pre-contracted with norepinephrine (3×10^{-6} M). No relaxation was observed in response to acetylcholine in any of the tissues tested. Histological examinations were performed on segments taken from six different patients to determine whether the lack of clear-cut relaxation in response to acetylcholine was related to endothelium injury caused by surgery and experimental preparation, or to endothelial dysfunction. Each segment of internal mammary artery was fixed in 4% formaldehyde, processed, and embedded in paraffin for light microscopic evaluation. Each paraffin block was cut, perpendicular to the long axis of the blood vessels, into micrometer sections. The slides were stained with hematoxylin and eosin. In all segments, histological preparations showed an intact endothelium. Therefore, we concluded that the lack of clear-cut relaxation to acetylcholine in these preparations was related to endothelial dysfunction rather than to mechanical damage.

2.3. Vasomotor response to isoprostaglandin $F_{2\alpha}$ type-III

Cumulative dose–response curves for isoprostaglandin $F_{2\alpha}$ type-III, prostaglandin $F_{2\alpha}$ and U46619 (a thromboxane A_2 mimetic (Coleman et al., 1981)) were made. The

effect of endothelium removal on isoprostaglandin $F_{2\alpha}$ type-III responses was studied: endothelium was removed by inserting a cotton thread into the lumen and gently rolling the preparation.

Dose–responses curves for isoprostaglandin $F_{2\alpha}$ type-III were performed 45 min after pretreatment with increasing concentrations of GR 32191 (3×10^{-9} to 3×10^{-7} M), a thromboxane receptor antagonist (Coleman et al., 1994), and AH 6809 (10^{-6} M to 3×10^{-5} M), an EP_1/DP receptor antagonist (Coleman et al., 1994). Doses–responses curves for U46619 were performed 45 min after pretreatment with increasing concentrations of GR 32191 (3×10^{-9} to 3×10^{-7} M). The vasomotor effects of isoprostaglandin $F_{2\alpha}$ type-III were also studied 30 min after pretreatment with indomethacin, a cyclooxygenase inhibitor (10^{-5} M) (Cryer and Feldman, 1998), baicaleine (10^{-4} M), a non-specific 5, 12 and 15 lipoxygenase inhibitor (Cho et al., 1991), and AA 861 (10^{-5} M), a specific 5-lipoxygenase inhibitor (Yoshimoto et al., 1982). In preliminary experiments, neither AH 6809, baicaleine nor AA 861 had any effect on the baseline tension. GR 32191 and indomethacin induced a weak relaxation on basal tone.

All the dose–response curves were made by adding increasing concentrations of the pharmacological agent to the organ bath in 0.5-log unit steps. Only one curve was obtained from each ring. Concentration–response curves were expressed as percentages of KCl 90 mM-induced contraction.

2.4. Thromboxane B_2 and cysteinyl leukotriene determinations

The effects of isoprostaglandin $F_{2\alpha}$ type-III on thromboxane and leukotriene production were also investigated. Thromboxane and leukotriene were measured in internal mammary artery preparations that were not under tension. Rings were placed in glass tubes containing 1 ml of Krebs solution at 37°C gassed with a mixture of 95% oxygen and 5% carbon dioxide. Each ring was stabilised for 60 min, and the Krebs solution changed every 15 min.

The rings were then not stimulated or stimulated with isoprostaglandin $F_{2\alpha}$ type-III (10^{-5} M) for 15 min after pretreatment for 30 min with indomethacin (10^{-5} M) or vehicle, for determination of thromboxane B_2 (a stable thromboxane A_2 metabolite). In another group, the rings were not stimulated or stimulated with isoprostaglandin $F_{2\alpha}$ type-III (10^{-5} M) for 15 min after pretreatment for 30 min with baicaleine (10^{-4} M), AA 861 (10^{-5} M) or vehicle for determination of cysteinyl leukotrienes (leukotriene A_4 metabolites: leukotrienes C_4, D_4 and E_4). In both groups, a solution aliquot was removed and frozen at −80°C. The rings were then dried in an oven to determine dried weight. Thromboxane B_2 and cysteinyl leukotrienes concentrations were determined using a commercially available enzyme immunoassay kit (Cayman, Ann Arbor, USA). Thromboxane B_2 and cysteinyl leukotrienes released into the incubation solution were expressed as pg per mg dry weight of tissue.

A pilot experiment was performed in order to assess the specificity of the anti- thromboxane B_2 serum used for enzyme immunoassay vs. isoprostaglandin $F_{2\alpha}$ type-III, due to their related structure. Cross-reactivity was determined after addition of isoprostaglandin $F_{2\alpha}$ type-III to the antibody-tracer complex. Displacement of 50% initial binding was determined. The relative percentage, expressed as the concentration of thromboxane B_2/concentration of isoprostaglandin $F_{2\alpha}$ type-III $\times$ 100 was less than 0.01, indicating a lack of interference between anti-thromboxane B_2 serum and isoprostaglandin $F_{2\alpha}$ type-III.

2.5. Data analysis

Maximum contraction (E_{max}) and potency (pD_2) were calculated to determine the arterial segment reactivity. E_{max} was expressed as a percentage of KCl 90 mM-induced maximal contraction. The effective concentration of agent that caused 50% of maximum contraction (EC_{50}) was calculated from each curve by a logistic, curve-fitting equation. EC_{50} values were expressed as pD_2 ($-\log EC_{50}$).

Thromboxane B_2 and cysteinyl leukotrienes values were expressed as pg per mg tissue. An unpaired *t*-test was used to compare two means. More than two means were compared using an analysis of variance (ANOVA) (and Bonferroni's test as post-hoc test). Only preplanned comparisons were made. Values of $P<0.05$, corrected by the number of comparisons made, were considered significant. All values are expressed as means ± S.E.M.

2.6. Drugs

The drugs used and their sources were isoprostaglandin $F_{2\alpha}$ type-III (8-*iso*-prostaglandin $F_{2\alpha}$) and prostaglandin

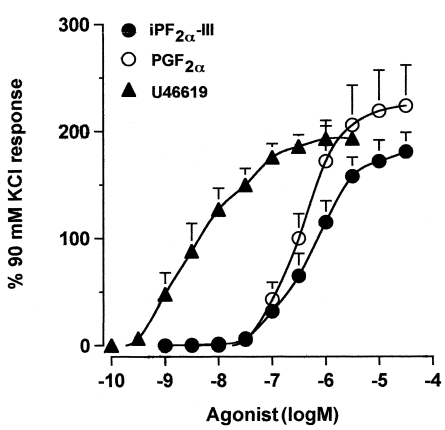

Fig. 1. Concentration–contraction curves for isoprostaglandin $F_{2\alpha}$ type III ($iPF_{2\alpha}$-III), prostaglandin $F_{2\alpha}$ and U46619 (a thromboxane A_2 mimetic) in human internal mammary arteries. Values are means ± S.E.M. ($n=6$ in all groups).

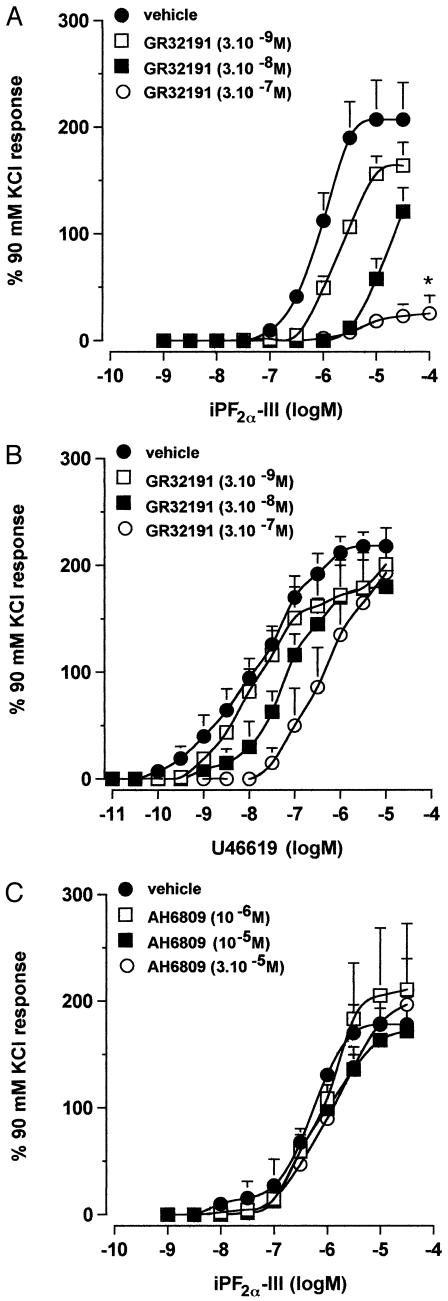

Fig. 2. Mean concentration–effect curves for isoprostaglandin $F_{2\alpha}$ type III ($iPF_{2\alpha}$-III, A) and U46619 (B) in human internal mammary arteries, in the presence of increasing concentrations of GR32191, a thromboxane receptor antagonist. Mean concentration–effect curves for isoprostaglandin $F_{2\alpha}$ type III, in the presence of increasing concentrations of AH 6809, an EP_1 / DP receptor antagonist (C). Values are means ± S.E.M. ($n = 6$ in all groups (A and B), $n = 4$ in all groups (C)). * $P < 0.001$ vs. vehicle and GR32191 (3×10^{-9} M).

$F_{2\alpha}$ from Cayman (Ann Arbor, USA); U46619 (9,11-dideoxy-9α, 11 α-methanoepoxy-prostaglandin $F_{2\alpha}$), indomethacin, baicaleine and AA 861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4 benzoquinone) from Sigma (Saint Quentin Fallavier, France). GR 32191 ([1*R*-[1α(*Z*), 2β,3β,5 α(+)-7-[[1,1′-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl) cyclo pentyl] -4-4heptanoic acid], hydrochlroride) and AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) were kindly provided by GlaxoWellcome (Steventage, UK). Stock solutions of the drugs were held frozen (−20°C) in aliquots and were freshly diluted in distilled water to the appropriate concentrations expressed as final molar concentrations in the organ bath. The enzyme immunoassay kits were purchased from Cayman (Ann Arbor, USA).

3. Results

3.1. Vasoconstrictor effects of isoprostaglandin $F_{2\alpha}$ type-III, prostaglandin $F_{2\alpha}$ and U46619 on human internal mammary arteries

Isoprostaglandin $F_{2\alpha}$ type-III produced a concentration-dependent contraction of human internal mammary artery (Fig. 1). The maximal contraction induced by isoprostaglandin $F_{2\alpha}$ type-III was not statistically different from that of U46619 and prostaglandin $F_{2\alpha}$. The pD_2 values of isoprostaglandin $F_{2\alpha}$ type-III, prostaglandin $F_{2\alpha}$ and U46619 were 6.26 ± 0.15, 6.35 ± 0.15 and 8.33 ± 0.24, respectively ($P < 0.001$ for U46619 vs. isoprostaglandin $F_{2\alpha}$ type-III and prostaglandin $F_{2\alpha}$). The contractile response to isoprostaglandin $F_{2\alpha}$ type-III was not significantly different in endothelium-denuded rings (pD_2 values: 6.34 ± 0.11, E_{max}: $191 \pm 21\%$) from that in endothelium-intact rings.

3.2. Effect of TP and EP_1-DP receptor antagonists on isoprostaglandin $F_{2\alpha}$ type-III-induced contractions

The responses to isoprostaglandin $F_{2\alpha}$ type-III were inhibited in a concentration-dependent manner by GR 32191 (Fig. 2A, Table 1). As shown in Fig. 2A, a right-

Table 1
Effect of increasing concentrations of GR32191, a thromboxane receptor antagonist, on contractions induced by isoprostaglandin $F_{2\alpha}$ type III in internal mammary artery
The responses to isoprostaglandin $F_{2\alpha}$-III obtained in the absence (control) or the presence of GR32191 are expressed as pD_2 values and maximal contraction (E_{max}: % 90 mM KCl response). Results are expressed as means ± S.E.M. $n = 6$ in all groups. N.A.: not applicable (no plateau obtained).

Agonist	pD_2	E_{max} (%)
Control (vehicle)	6.06 ± 0.08	207 ± 35
GR32191 (3×10^{-9} M)	5.71 ± 0.13	164 ± 22
GR32191 (3×10^{-8} M)	N.A.	122 ± 23
GR32191 (3×10^{-7} M)	4.87 ± 0.31[a]	25 ± 16[b]

[a] $P < 0.001$ versus vehicle and $P < 0.01$ versus GR32191 (3×10^{-9})M.

[b] $P < 0.001$ versus vehicle and GR32191 (3×10^{-9} M) (ANOVA and Bonferroni's test as post-hoc test).

ward shift of the concentration–response curve was associated with a decreased maximal contraction. Due to the high cost of isoprostaglandin $F_{2\alpha}$ type-III, one further concentration (10^{-4} M) was tested only in the GR32191 3×10^{-7} M group, confirming the inhibition of the maximal response. GR32191 caused a parallel rightward shift of the concentration–contraction curves for U46619 (Fig. 2B).

AH 6809 had no effect on isoprostaglandin $F_{2\alpha}$ type-III-induced contractions (Fig. 2C).

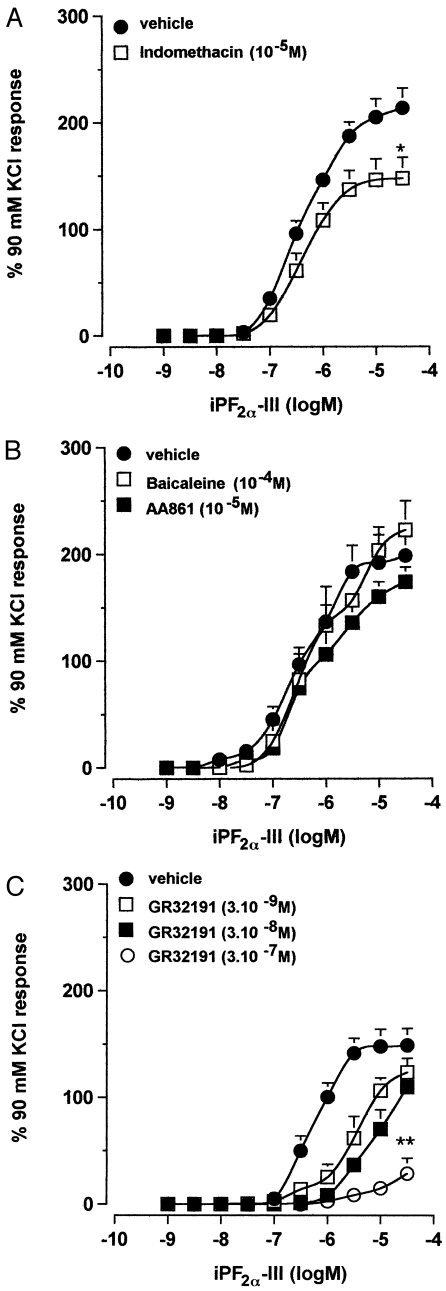

Fig. 3. Mean concentration–effect curves for isoprostaglandin $F_{2\alpha}$ type III (iPF$_{2\alpha}$-III) in human internal mammary arteries, in the presence of indomethacin, a cyclooxygenase inhibitor (A); baicaleine, a non-specific lipoxygenase inhibitor; and AA 861, a 5-lipoxygenase inhibitor (B). Mean concentration–effect curves for isoprostaglandin $F_{2\alpha}$ type III in the presence of increasing concentrations of GR32191, a thromboxane receptor antagonist; 30 min after pretreatment with indomethacin (C). Values are means ± S.E.M. ($n=6$ in all groups (A and B), $n=4$ in all groups (C)). $^{*}P<0.05$ vs. vehicle; $^{**}P<0.01$ vs. vehicle, GR32191 3×10^{-9} and 3×10^{-8} M.

Table 2
Effect of increasing concentrations of GR32191 on contractions induced by isoprostaglandin $F_{2\alpha}$ type III in internal mammary artery, 30 min after pretreatment with indomethacin (10^{-5} M)
The responses to isoprostaglandin $F_{2\alpha}$-III obtained in the absence (control) or the presence of GR32191 are expressed as pD_2 values and maximal contraction (E_{max}: % 90 mM KCl response). Results are expressed as means ± S.E.M. $n=4$ in all groups. N.A.: not applicable (no plateau obtained).

Agonist	pD_2	E_{max} (%)
Control (vehicle)	6.20 ± 0.46	149 ± 16
GR32191 (3×10^{-9} M)	5.45 ± 0.15[a]	124 ± 9
GR32191 (3×10^{-8} M)	N.A.	110 ± 27
GR32191 (3×10^{-7} M)	N.A.	28 ± 14[b]

[a] $P<0.01$ versus vehicle.
[b] $P<0.01$ versus vehicle, GR32191 3×10^{-9} and 3×10^{-9} M (ANOVA and Bonferroni's test as post-hoc test).

3.3. Effect of cyclooxygenase inhibition on isoprostaglandin $F_{2\alpha}$ type-III-induced contractions

The maximal response to isoprostaglandin $F_{2\alpha}$ type-III, but not the pD_2 values, was significantly reduced in the presence of indomethacin (Fig. 3A) (E_{max}: 147 ± 20% vs. 213 ± 19% in the control group, $P<0.05$). In order to test whether the inhibitory effect observed in presence of GR32191 (Fig. 2A) was a consequence of the inhibition of the release of a cyclooxygenase-dependent prostanoid, the responses to isoprostaglandin $F_{2\alpha}$ type-III were evaluated in the presence of indomethacin and GR32191. In the presence of indomethacin, the responses to isoprostaglandin $F_{2\alpha}$ type-III were inhibited in a concentration-dependent manner by GR 32191 (Fig. 3C, Table 2). As shown in Fig. 3C, the rightward shift of the concentration–response curves was associated with a decreased maximal contraction.

3.4. Effect of lipoxygenase inhibitors on isoprostaglandin $F_{2\alpha}$ type-III-induced contractions

Baicaleine (10^{-4} M) and AA 861 (10^{-5} M) did not affect isoprostaglandin $F_{2\alpha}$ type-III dose–response curves (Fig. 3B).

3.5. Effect of isoprostaglandin $F_{2\alpha}$ type-III on thromboxane B_2 and cysteinyl leukotrienes release

To investigate whether the reduction of the contraction induced by isoprostaglandin $F_{2\alpha}$ type-III in indomethacin-treated arteries was due to a decreased release of the

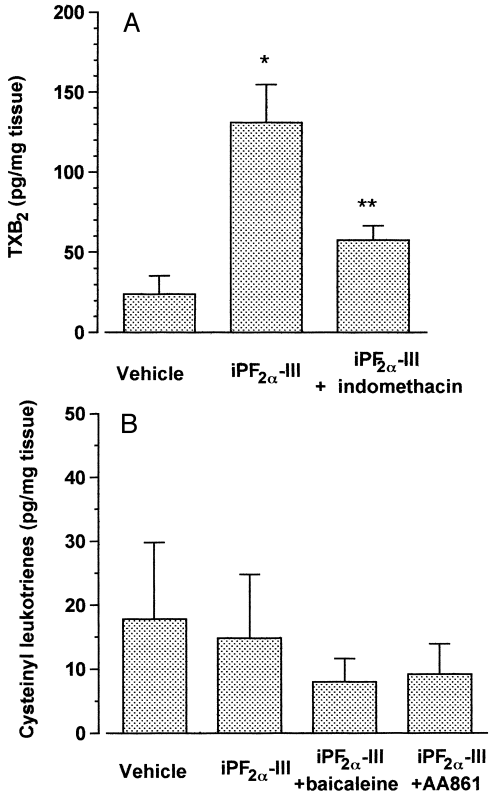

Fig. 4. Effect of isoprostaglandin $F_{2\alpha}$ type III ($iPF_{2\alpha}$-III, 10^{-5} M) on thromboxane B_2 (A) and cysteinyl leukotriene (B) formation in human internal mammary arteries. Tissues were pretreated for 30 min with vehicle, indomethacin (10^{-5} M), baicaleine (10^{-4} M) or AA 861 (10^{-5} M). Values are means ± S.E.M. ($n = 6$ in all groups (A), $n = 4$ in all groups (B)). $^{*}P < 0.001$ vs. vehicle, $^{**}P < 0.01$ vs. isoprostaglandin $F_{2\alpha}$ type III alone.

prostanoid, thromboxane A_2, the effects of isoprostaglandin $F_{2\alpha}$ type-III on thromboxane production were studied. Isoprostaglandin $F_{2\alpha}$ type-III stimulated thromboxane B_2 release (5.7-fold increase) from human internal mammary arteries (Fig. 4A). This effect was inhibited by indomethacin pretreatment.

Basal cysteinyl leukotriene formation was not affected by isoprostaglandin $F_{2\alpha}$ type-III (Fig. 4B). Baicaleine and AA 861 induced a non-significant decrease of cysteinyl leukotriene formation compared with vehicle and isoprostaglandin $F_{2\alpha}$ type-III alone.

4. Discussion

The present findings provide in vitro evidence that isoprostaglandin $F_{2\alpha}$ type-III is a potent vasoconstrictor of human internal mammary artery, with a potency equivalent to that of prostaglandin $F_{2\alpha}$. These effects are mediated at least in part by TP receptor activation and thromboxane A_2 generation.

F_2-isoprostanes are stable oxidation products of arachidonic acid. Free radical oxygenation of arachidonic acid results in the production of numerous F_2-isoprostane isoforms. Rokach has recently proposed a nomenclature for all products formed from free-radical peroxidation of polyunsaturated fatty acids (Rokach et al., 1997). Isoprostaglandin $F_{2\alpha}$ type-III (formerly known as 8-*iso*-prostaglandin $F_{2\alpha}$) has been shown to be generated in large quantities in vivo under conditions of oxidative stress (Morrow et al., 1990; Souvignet et al., 2000). Isoprostaglandin $F_{2\alpha}$ type-III may also be formed by the cyclooxygenase pathway (Pratico et al., 1995) but the contribution of cyclooxygenase-dependent mechanisms to the formation of isoprostaglandin $F_{2\alpha}$ type-III appears negligible under physiological conditions (Wang et al., 1995). Isoprostaglandin $F_{2\alpha}$ type-III may have both physiological and pathological effects, due to its ability to alter smooth muscle and platelet functions.

Isoprostaglandin $F_{2\alpha}$ type-III induced concentration-dependent contractions of human internal mammary artery, with a pD_2 value of 6.26, and maximal contraction similar to those with U46619 and prostaglandin F_2. A similar potency was found with porcine (pD_2 value: 6.16), bovine (pD_2 value: 5.86) coronary arteries (Kromer and Tippins, 1996), and rat aorta (pD_2 value: 6) (Wagner et al., 1997). In contrast with those findings, isoprostaglandin $F_{2\alpha}$ type-III had no effect on ovine coronary arteries (Kromer and Tippins, 1996) and a weak potency was found with rat pulmonary arteries (pD_2 value: 5.1) (Wagner et al., 1997), whereas isoprostaglandin $F_{2\alpha}$ type-III was more potent on piglets retinal arterioles (pD_2 value: 8.2) (Lahaie et al., 1998) and isolated rabbit ear (pD_2 value: 8.15) (Sametz et al., 1999). These data suggest that isoprostaglandin $F_{2\alpha}$ type-III vascular effects depend both on the vessel type and the species studied, with a higher potency on microvessels than on conductance vessels. In the present study, U46619 was approximately 120 times more potent than isoprostaglandin $F_{2\alpha}$ type-III. With the exceptions of piglet retinal vessels (Lahaie et al., 1998), U46619 was also more potent than isoprostaglandin $F_{2\alpha}$ type-III in all studies. Basal levels of free isoprostaglandin $F_{2\alpha}$ type-III in the human circulation have been found to be in an order of magnitude of 10^{-10} M (Morrow et al., 1995). Even in pathological states where oxidative stress is likely to occur, isoprostaglandin $F_{2\alpha}$ type-III concentrations are not likely to induce systemic vasoactive effects. However, at sites of free-radical injury such as reperfusion or inflammation, concentrations may reach sufficiently high levels to induce vascular effects in vivo. As a consequence, isoprostaglandin $F_{2\alpha}$ type-III may have local rather than systemic vascular effects. Indeed F_2 isoprostanes are significantly elevated in situ in atherosclerotic plaques and may promote plaque instability (Pratico et al., 1997).

Endothelium removal did not affect isoprostaglandin $F_{2\alpha}$ type-III-induced contractions. Despite the presence of an intact endothelium, as confirmed by histological examination, no relaxation was observed in response to acetylcholine. The lack of acetylcholine-induced relaxation may

be a consequence of atherosclerosis in patients suffering from coronary heart disease (Förstermann et al., 1988; Ludmer et al., 1986; Reddy et al., 1994). Isoprostaglandin $F_{2\alpha}$ type-III-induced contractions have been shown to be endothelium independent in rat aortic arteries (Wagner et al., 1997). The present observation suggests that endothelium does not modulate isoprostaglandin $F_{2\alpha}$ type-III-induced contractions in human internal mammary arteries from patients suffering from atherosclerosis.

Indomethacin, but not baicaleine or AA 861, reduced the maximal effect of isoprostaglandin $F_{2\alpha}$ type-III, suggesting that prostanoids, but not lipoxygenase derivatives including leukotrienes, were involved in the contractile response. To further explore prostanoid generation, an immunoassay was performed which clearly showed an increase in thromboxane B_2 levels and no change in cysteinyl leukotriene levels in vessels exposed to isoprostaglandin $F_{2\alpha}$ type-III. Cyclooxygenase products have recently been reported to be involved in the vascular effects of isoprostaglandin $F_{2\alpha}$ type-III in rat aorta (Wagner et al., 1997), in porcine retina parenchyma and endothelial cells (Lahaie et al., 1998), however, no such effect was found in rabbit pulmonary vasculature (Barnerjee et al., 1992). Furthermore, phospholipase A_2 inhibition has been shown to inhibit isoprostaglandin $F_{2\alpha}$ type-III induced contractions in porcine retinal arterioles (Lahaie et al., 1998). The present findings suggest strongly an involvement of thromboxane A_2 but not of cysteinyl leukotrienes, in isoprostaglandin $F_{2\alpha}$ type-III-mediated contraction of human internal mammary artery.

Responses to isoprostaglandin $F_{2\alpha}$ type-III were inhibited by GR 32191, a highly potent and selective TP receptor antagonist (Humphrey et al., 1990; Lumley et al., 1989), in a concentration-dependent manner. The GR 32191-induced inhibition was associated with a decrease in maximal response, suggesting non-competitive antagonism, in contrast with U46619. Such a phenomenon has already been described for rat aorta (Wagner et al., 1997), piglet retinal vessels (Lahaie et al., 1998) and porcine coronary arteries (Kromer and Tippins, 1996). This decrease in maximal responses in presence of GR 32191 did not result from thromboxane A_2 production induced by isoprostaglandin $F_{2\alpha}$ type-III since, in presence of indomethacin, GR 32191 caused similar rightward shifts of the concentration–response curves and reductions in the maximal responses to isoprostaglandin $F_{2\alpha}$ type-III. A possible explanation is that isoprostaglandin $F_{2\alpha}$ type-III is acting as a partial agonist at the TP receptor (Kromer and Tippins, 1996; Morrow et al., 1992). In this context, isoprostaglandin $F_{2\alpha}$ type-III may require a high level of receptor occupancy to maximally contract internal mammary artery. Pretreatment with high concentrations of GR32191 may cause sufficient reductions in receptor reserve to prevent isoprostaglandin $F_{2\alpha}$ type-III from occupying a sufficient number of receptors to achieve the maximal effect. Another possibility is that isoprostaglandin $F_{2\alpha}$ type-III may act at a novel receptor, as suggested by Yura et al. (1995), and is inhibited in a non-competitive manner by GR 32191. However, if this was the case, GR 32191 would also have to act at an isoprostane receptor, in contradiction with previous findings suggesting that GR32191 is selective for the TP receptor (Humphrey et al., 1990; Lumley et al., 1989). The present findings therefore do not allow a definitive choice to be made between the two alternative mechanisms by which isoprostaglandin $F_{2\alpha}$ type-III has been hypothesised to contract human internal mammary arteries and further work will be required to determine the exact mechanisms.

AH 6809, an EP_1-DP receptor antagonist, did not alter contractions induced by isoprostaglandin $F_{2\alpha}$ type-III, showing that EP_1 and DP receptors are not involved in the contractile response.

5. Conclusion

The present study showed that isoprostaglandin $F_{2\alpha}$ type-III is a potent vasoconstrictor in human internal mammary arteries, at concentrations that may induce local but not systemic vasoactive effects. Contractile responses were inhibited by a TP receptor antagonist, suggesting that contractions induced by isoprostaglandin $F_{2\alpha}$ type-III are mediated by TP receptor activation. Furthermore, thromboxane A_2, but not cysteinyl leukotriene production is involved in the vascular effects of isoprostaglandin $F_{2\alpha}$ type-III. Isoprostaglandin $F_{2\alpha}$ type-III, produced at sites of free radical generation, may play an important role in internal mammary artery spasm in situations of oxidant stress such as coronary bypass surgery.

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