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Cyclooxygenase inhibitors attenuate endothelin ET_B receptor-mediated contraction in human temporal artery

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Abstract

It is well documented that endothelin ET_B receptor-mediated contraction develops in artery segments incubated in culture and that the reaction is augmented by proinflammatory cytokines, but little is known of the mechanisms involved. Segments of human temporal artery were incubated in organ culture for 2 days in the absence or presence of interleukin-1β (IL-1β), with or without nonsteroidal anti-inflammatory drugs, glucocorticoids or a nitric oxide synthase inhibitor. Thereafter, contractions were induced by the selective endothelin ET_B receptor agonist, sarafotoxin S6c. Acetylsalicylic acid, indomethacin, nimesulide and rofecoxib were all effective in eliminating the increase in endothelin ET_B receptor-mediated contraction induced by interleukin-1β, but only indomethacin and rofecoxib significantly reduced the spontaneous development of this reaction in cultured arteries. Dexamethasone and methylprednisolone augmented the reaction, and the nitric oxide synthase inhibitor had no effect. The results clearly indicate a role for cyclooxygenase, most likely cyclooxygenase-2, in endothelin ET_B receptor-mediated contraction in this preparation. © 2002 Published by Elsevier Science B.V.

Keywords: Endothelin ET_B receptor; Cyclooxygenase; Interleukin-1β; NSAID (nonsteroidal anti-inflammatory drug); Rofecoxib; Glucocorticoid

1. Introduction

Endothelin ET_A receptors are found in smooth muscle cells in a variety of vascular beds and predominantly mediate the potent vasoconstrictor effects of endothelin-1 (ET-1) in human arteries (Riezebos et al., 1994; Davenport et al., 1995). Endothelin ET_B receptors are found in endothelial cells where they mediate dilatation (Masaki et al., 1991), as well as in smooth muscle cells where they mediate contraction (Seo et al., 1994; Haynes et al., 1995). It is not yet clear how interaction between the receptors is coordinated in vivo.

In human pial arteries in vitro, contraction is mediated through endothelin ET_A receptors and no endothelin ET_B receptor-mediated vasoactivity is usually observed (Pierre and Davenport, 1995, 1998). However, further examination of endothelin-induced vasoactivity in this model has indicated that ET_B receptor-mediated contractile ability exists, but tends not to be demonstrable under the circumstances usually employed experimentally (Touzani et al., 1997). Similarly, fresh segments of human temporal artery demonstrated only strong endothelin ET_A receptor-mediated contraction and transient endothelin ET_B receptor-mediated dilatation in vitro (Lucas et al., 1996). Nevertheless, mRNA for endothelin ET_B receptors was found in this artery even in endothelium-denuded vessels, suggesting that these receptors are present in the smooth muscle (White et al., 1998).

It is now well documented that endothelin ET_B receptor-mediated contractile ability becomes demonstrable in animal and human arteries following organ culture, though not observed in fresh preparations (Adner et al., 1996, 1998; Möller et al., 1997; White et al., 1998). Furthermore, this endothelin ET_B receptor-mediated contractile activity is enhanced by proinflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (Leseth et al., 1999; Uddman et al., 1999; White et al., 1999, 2000). While the use of a nonphysiological in vitro model limits extension of the results to situations in vivo, the mechanisms involved

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may nevertheless have relevance (Adner et al., 1996). Interleukin-1β is known to increase production of endothelin-1 from endothelial cells by enhancing transcription of preproendothelin-1 (Yoshizumi et al., 1990). A role in pathological conditions where both endothelin and proinflammatory cytokines have been implicated potentially exists, such as heart disease (Sharma et al., 2000), septic shock (Pittet et al., 1991; van Deuren et al., 1995) and stroke (Ziv et al., 1992; Mathiesen et al., 1997; Zimmermann and Seifert, 1998; Zuccarello et al., 1998). Indeed, endothelin ET_B receptors have been implicated in the induction of fever in rats (Fabricio et al., 1998) and fever in subarachnoid haemorrhage has now been shown to be associated with vasospasm and poor outcome (Oliveira-Filho et al., 2001). Other inflammatory mediators implicated in cerebral ischaemia include prostanoids and nitric oxide (del Zoppo et al., 2000). Anti-inflammatory therapies are now being explored, including the inhibition of inducible nitric oxide synthase and cyclooxygenase-2, to reduce ischaemic damage (Iadecola and Alexander, 2001).

It is as yet unknown what mechanisms are involved in endothelin ET_B receptor-mediated contraction in artery preparations following organ culture. In the present work, segments of human temporal artery have been cultured in the presence of anti-inflammatory cyclooxygenase inhibitors, glucocorticoids, or a nitric oxide synthase inhibitor, and endothelin ET_B receptor-mediated contractions compared to the reactions in control segments.

2. Materials and methods

2.1. Organ culture and vasomotor responses in vitro

Branches of human superficial temporal artery were obtained during excisions of brain tumours under conditions approved by the Regional Committee for Medical Research Ethics. After removal, arteries were immediately placed in ice-cold buffer (composition given below). Segments approximately 1.5 mm long were either tested at once, or placed in serum-free Dulbecco's minimal essential medium (DMEM) (Gibco BRL Life Technologies, Roskilde, Denmark) supplemented with 6 mmol l^{-1} glucose, 2.5 mmol l^{-1} glutamine, 40 mg l^{-1} gentamycin (Gibco) and 1 mg ml^{-1} human serum albumin, in 24-well plates (Nunc Products, Denmark) containing 1 ml medium per well. Where required, drugs were added at various concentrations, and interleukin-1β at a concentration of 10 ng ml^{-1}. No more than one segment was incubated per well. Incubation was carried out at 37 °C and pH 7.4 for 2 days.

For experiments, segments were mounted on two L-shaped tungsten prongs, diameter 0.1 mm, in small reaction baths with 5 ml buffer solution containing (in mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 12, NaH_2PO_4 1.2, glucose 5.5. The baths were kept at a temperature of 37 °C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2 to maintain physiological pH. The holders were connected to Grass FT03C force-displacement transducers, linked to a MacLab analog–digital convertor (AD Instruments, London, UK), through a preamplifier (Transbridge TBM4, World Precision Instruments, New Haven, CT, USA). The digitalized tension was continuously monitored and stored in an Apple Macintosh computer. Artery segments were equilibrated for 1 h (passive tension 5–6 mN) during which time the buffer was changed every 10 min to achieve a stable tension.

A test contraction was initially induced by 10 μM prostaglandin $F_{2\alpha}$, and after a washout period to remove this contraction, the selective ET_B receptor agonist sarafotoxin S6c was added in logarithmically increasing concen-

Table 1
Maximal contraction (E_{max}) to sarafotoxin S6c expressed as a percentage of the contraction induced by 0.1 μM ET-1, and sensitivity expressed as pEC_{50} (negative logarithm of peptide concentration inducing half-maximal response) in segments of organ-cultured human temporal artery exposed to various drugs, (a) without or (b) with 10 ng ml^{-1} interleukin-1β

Drug	E_{max} (% 0.1 μM ET-1)	pEC_{50}	n
A. Spontaneous reactions			
Acetylsalicylic acid (10 μM)	28.8 ± 7.2	9.03 ± 0.23	6
Indomethacin (10 μM)	10.5 ± 2.3[a]	8.57 ± 0.22	6
Control	32.1 ± 5.3	9.01 ± 0.13	10
Nimesulide (10 μM)	21.5 ± 5.5	9.09 ± 0.26	6
Rofecoxib (10 μM)	13.8 ± 4.5[b]	8.98 ± 0.16	6
Control	35.9 ± 7.8	8.42 ± 0.15	6
Dexamethasone (10 μM)	58.4 ± 8.7[b]	8.59 ± 0.15	6
Methylprednisolone (10 μM)	62.0 ± 10.0[b]	8.94 ± 0.19	6
Control	35.1 ± 8.0	9.08 ± 0.12	6
B. Reactions with 10 ng ml^{-1} interleukin-1β (IL-1β)			
IL-1β	72.8 ± 4.7[a]	8.59 ± 0.11	9
Acetylsalicylic acid (10 μM) + IL-1β	26.3 ± 8.1	8.82 ± 0.16	5
Indomethacin (10 μM) + IL-1β	35.4 ± 7.9	8.42 ± 0.15	6
Control	26.0 ± 6.6	8.89 ± 0.17	10
IL-1β	71.2 ± 5.9[a]	8.60 ± 0.14	7
Nimesulide (10 μM) + IL-1β	30.4 ± 5.9	8.96 ± 0.23	6
Rofecoxib (10 μM) + IL-1β	30.0 ± 6.0	9.10 ± 0.22	7
Control	36.4 ± 6.1	8.85 ± 0.22	7
IL-1β	76.3 ± 5.3[b]	8.60 ± 0.13	6
Dexamethasone (1 μM)	48.0 ± 13.4[b]	8.85 ± 0.27	6
Dexamethasone (1 μM) + IL-1β	68.2 ± 4.8[b]	9.02 ± 0.14	6
Control	25.8 ± 9.2	8.62 ± 0.25	6
IL-1β	74.4 ± 5.9[a]	8.62 ± 0.16	6
L-NAME (10 μM)	34.6 ± 6.8	8.94 ± 0.25	6
L-NAME (10 μM) + IL-1β	74.5 ± 10.7[a]	9.00 ± 0.16	6
Control	30.4 ± 6.4	9.09 ± 0.21	7

Values are given as the mean ± S.E.M. There were no significant differences between any of the control groups.

[a] Significantly different from corresponding control, $P < 0.05$: one-way ANOVA + Student–Newman–Keul.

[b] Significantly different from corresponding control, $P < 0.05$: repeated-measures ANOVA + Student–Newman–Keul.

trations. At the end of this reaction, 0.1 μM endothelin-1 was added to produce a maximum contraction to endothelin in this preparation (White et al. 1998). Maximal contraction with sarafotoxin S6c (E_{max}) was calculated as a percentage of the endothelin-1 induced contraction, and contraction at the level of EC_{50} (the concentration inducing 50% response) was calculated as pEC_{50} (the negative logarithm of the concentration of agonist eliciting half-maximal response). Maximal contractions induced by 10 μM prostaglandin $F_{2\alpha}$ and 0.1 μM endothelin-1 are not significantly different, nor are they significantly affected by organ culture for 2 days (White et al., 2000).

Various drugs were tested for their effect on the sarafotoxin-induced contraction. These included the anti-inflammatory glucocorticoids dexamethasone and methylprednisolone, as well as nonsteroidal anti-inflammatory drugs (acetylsalicylic acid, indomethacin, nimesulide and rofecoxib), and the nitric oxide synthase inhibitor L-NAME (*N*ω-nitro-L-arginine methyl ester). The number of experiments (*n*) denotes results obtained with artery preparations from *n* patients. No experiment was carried out more than once on the artery preparation from any patient.

2.2. Drugs and other chemicals

Drugs were prepared fresh and most were used at a concentration of 10 μM, except for dexamethasone, which was also tested at 1 μM, and L-NAME, which was used at 100 μM. Stock solutions of acetylsalicylic acid were prepared in pure water, and indomethacin, nimesulide and rofecoxib in 5% sodium bicarbonate. All reagents were diluted to working concentrations in 0.9% NaCl and were added to the reaction baths in 50-μl aliquots. Sarafotoxin S6c and endothelin-1 were purchased from Novabiochem, Läufelfingen, Switzerland. Recombinant human interleukin-1β was from R&D Systems Europe, Abingdon, England.

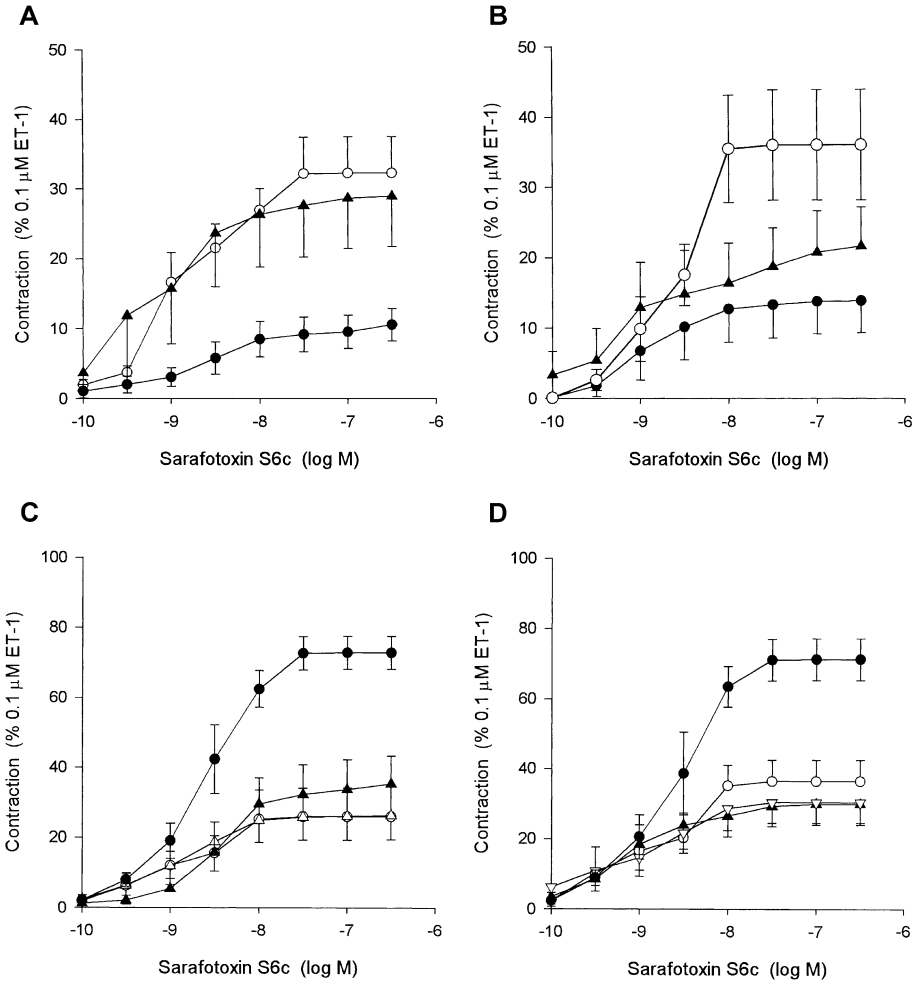

Fig. 1. Concentration–response curves for contractions induced by sarafotoxin S6c in human temporal artery after 2 days in organ culture: (A) control segments (○, $n=10$) and segments incubated with 10 μM acetylsalicylic acid (▲, $n=6$) or 10 μM indomethacin (●, $n=6$); (B) control segments (○, $n=6$) and segments incubated with 10 μM nimesulide (▲, $n=6$) or 10 μM rofecoxib (●, $n=6$); (C) control segments (○, $n=10$) and segments incubated with 10 ng ml^{-1} interleukin-1β (●, $n=9$), 10 μM acetylsalicylic acid and 10 ng ml^{-1} interleukin-1β (△, $n=5$) or 10 μM indomethacin and 10 ng ml^{-1} interleukin-1β (▲, $n=6$); (D) control segments (○, $n=7$) and segments incubated with 10 ng ml^{-1} interleukin-1β (●, $n=7$), 10 μM nimesulide and 10 ng ml^{-1} interleukin-1β (▽, $n=6$) or 10 μM rofecoxib and 10 ng ml^{-1} interleukin-1β (▲, $n=7$).

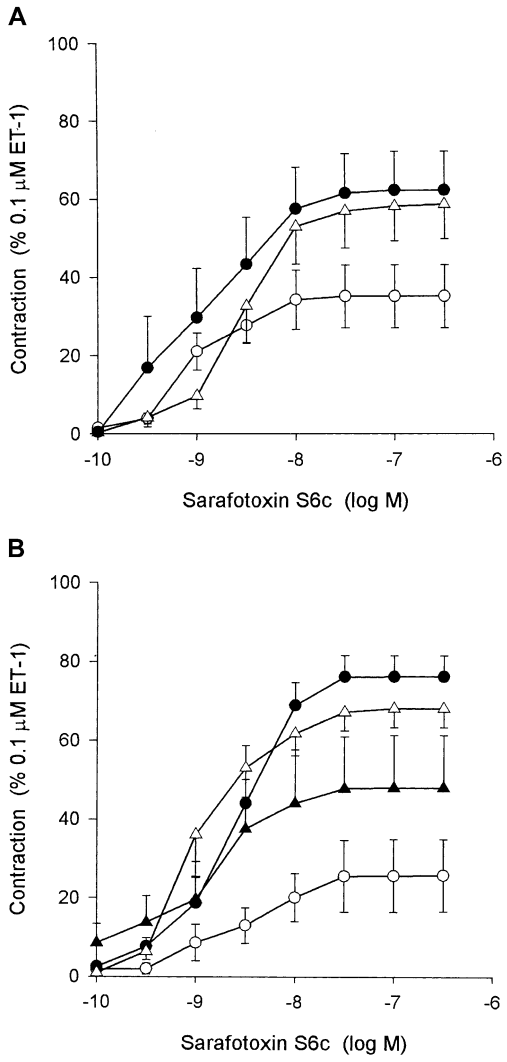

Fig. 2. Concentration–response curves for contractions induced by sarafotoxin S6c in human temporal artery after 2 days in organ culture: (A) control segments (○, $n=6$) and segments incubated with 10 μM dexamethasone (△, $n=6$) or 10 μM methylprednisolone (●, $n=6$); (B) control segments (○, $n=6$) and segments incubated with 1 μM dexamethasone (▲, $n=6$), 10 ng ml^{-1} interleukin-1β (●, $n=6$) or 1 μM dexamethasone and 10 ng ml^{-1} interleukin-1β (△, $n=6$).

Prostaglandin $F_{2\alpha}$, human serum albumin, acetylsalicylic acid and indomethacin were from Sigma, St. Louis, MO, USA. Other drugs were obtained from commercial preparations; dexamethasone (Decadron®, Merck, Sharp and Dohme, Netherlands), and methylprednisolone (Solu-Medrol®, Upjohn, USA). Rofecoxib (Chan et al., 1999) was a kind gift from MSD (Norge). All other chemicals used were of analytical grade.

2.3. Statistics

Results are expressed as the mean ± S.E.M. Statistical analysis was carried out using analysis of variance (ANOVA) and Student–Newman–Keul's test for multiple comparisons. Values of $P<0.05$ were considered significant.

3. Results

The spontaneous development of endothelin ET_B receptor-mediated contraction in cultured segments of human temporal artery compared to fresh segments has been well documented (White et al., 1998, 1999, 2000), as has the augmentation of this reaction by proinflammatory cytokines such as interleukin-1β (White et al., 1999, 2000). Values for E_{max} in the various reactions are shown in Table 1. Levels for the spontaneous (control) endothelin ET_B-mediated contractions were not significantly different between the various groups and the levels are in accordance with those previously reported (White et al., 1998, 1999, 2000). No significant differences in mean values for pEC_{50} were found in any test group (Table 1A and B).

When the nonselective cyclooxygenase inhibitors acetylsalicylic acid or indomethacin were added to the culture medium, only indomethacin significantly reduced endothelin ET_B receptor-mediated contraction at sarafotoxin S6c concentrations above 3 nM (Table 1A, Fig. 1A). Similarly, the selective cyclooxygenase-2 inhibitor rofecoxib significantly reduced the spontaneous sarafotoxin-mediated contraction at concentrations over 10 nM. Nimesulide was only significantly different to the control at 10 nM sarafotoxin S6c (Table 1A, Fig. 1B). However, when endothelin ET_B receptor-mediated contraction was augmented by 10 ng ml^{-1} interleukin-1β, all the cyclooxygenase inhibitors tested eliminated the effect of the cytokine, reducing the level to the same range as for the spontaneous reaction (Table 1B, Fig. 1C,D).

These results were in contrast to those obtained with glucocorticoids. The spontaneous endothelin ET_B receptor-mediated contraction was not reduced by these substances. On the contrary, the contraction was significantly increased

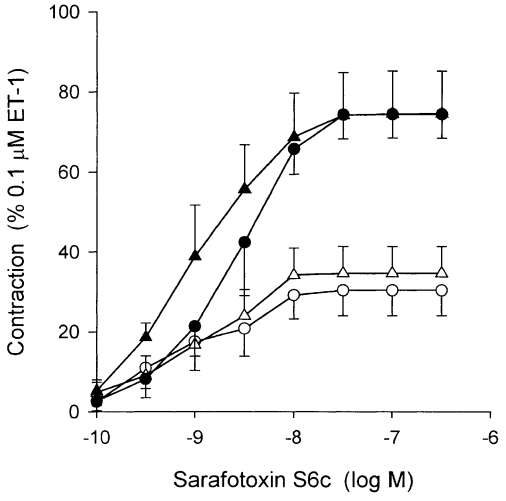

Fig. 3. Concentration–response curves for contractions induced by sarafotoxin S6c in human temporal artery after 2 days in organ culture: control segments (○, $n=7$) and segments incubated with 100 μM L-NAME (△, $n=6$), 10 ng ml^{-1} interleukin-1β (●, $n=6$) or 100 μM L-NAME and 10 ng ml^{-1} interleukin-1β (▲, $n=6$).

by both dexamethasone and methylprednisolone (10 μM) at sarafotoxin S6c concentrations above 10 nM (Table 1A, Fig. 2A). Dexamethasone (1 μM) also significantly augmented the spontaneous contraction, but had no significant effect on the increase induced by 10 ng ml^{-1} interleukin-1β. All the reactions in this group were significantly increased relative to the control at sarafotoxin concentrations above 30 nM (Table 1, Fig. 2B). The nonselective nitric oxide synthase inhibitor L-NAME (100 μM) had no significant effect on either the spontaneous or the interleukin-augmented endothelin ET_B receptor-mediated contraction (Table 1, Fig. 3).

4. Discussion

The present results suggest that increased endothelin ET_B receptor-mediated contraction, whether spontaneous or cytokine-enhanced, is probably mediated via cyclooxygenase activity. All the cyclooxygenase inhibitors tested eliminated the enhancement of endothelin ET_B receptor-mediated contraction induced by interleukin-1β, though only indomethacin and rofecoxib significantly reduced the spontaneous induction of the reaction. The inhibitors acetylsalicylic acid and indomethacin can produce full inhibition of both cyclooxygenase-1 and cyclooxygenase-2, but have a greater relative selectivity for cyclooxygenase-1. Similarly, nimesulide has greater selectivity for cyclooxygenase-2 (Mitchell and Warner, 1999). At the dose employed in the present study (10 μM), all these substances probably inhibited both isoforms. On the other hand, rofecoxib has considerably increased selectivity for the cyclooxygenase-2 isoform. In other assays in vitro (Warner et al., 1999; FitzGerald and Patrono, 2001), rofecoxib exerted little inhibition of cyclooxygenase-1 at 10 μM, but inhibition of cyclooxygenase-2 was near maximal. The results therefore infer that the cyclooxygenase isoform most likely to be involved in the endothelin ET_B receptor-mediated contraction is cyclooxygenase-2. This is not unlikely, since cyclooxygenase-2, though found constitutively in some tissues, is expressed after tissue injury, stimulated by inflammatory factors like interleukin-1β and is believed to mediate the enhanced prostanoid release in the inflammatory response (Mitchell and Warner, 1999).

However, the results give a clear indication that glucocorticoids were not effective in curtailing endothelin ET_B receptor-mediated contraction; on the contrary, they produced an increase in the reaction in the absence of interleukin-1β, and did not reduce the reaction in the presence of the cytokine. This is surprising if cyclooxygenase-2 mediates the development of endothelin ET_B receptor-mediated contraction as glucocorticoids like dexamethasone have been shown to inhibit expression of cyclooxygenase-2 (but not cyclooxygenase-1) at concentrations similar to those employed in the present study (Masferrer et al., 1992; Mitchell et al., 1994). It is possible that cyclooxygenase-1 alone is able to mediate the reaction. It is perhaps more likely that the release of endogenous inflammatory factors during removal and preparation of the artery segments already upregulates the expression of cyclooxygenase-2 and that subsequent addition of glucocorticoids is ineffective in blocking the reaction.

It is also known that dexamethasone inhibits the induction of inducible nitric oxide synthase (Liu et al., 1993), so the increased endothelin ET_B receptor-mediated contraction in the presence of glucocorticoids might have been an indicator that the endogenous production of nitric oxide normally limited the degree of the reaction. However, the nonselective nitric oxide synthase inhibitor L-NAME was ineffective towards the endothelin ET_B receptor-mediated contraction. Although nitric oxide is known to be involved in the dilatation mediated by endothelin ET_B receptors on endothelial cells (Kitazono et al., 1995; Verhaar et al., 1998), the present results did not suggest a role for nitric oxide in endothelin ET_B receptor-mediated contraction in this model.

Many reactions constitute the inflammatory cascade, including proinflammatory cytokine production, increased expression of cyclooxygenase-2 and increased expression of endothelin receptors. It has long been known that endothelin activates phospholipase A_2 in vascular smooth muscle cells (Resink et al., 1989; Reynolds et al., 1989), leading to the release of arachidonic acid metabolites that subsequently can be transformed by cyclooxygenase. Previous results have shown that the development of endothelin ET_B receptor-mediated contraction in cultured human temporal artery is accompanied by upregulation of endothelin ET_B receptor mRNA and de novo synthesis of endothelin ET_B contractile receptors. However, the increased reaction seen in the presence of interleukin-1β was not accompanied by further upregulation (White et al., 1999). The present results may provide an explanation for this. The mechanisms associated with endothelin ET_B receptor-mediated contraction are not yet fully understood, but a prostanoid product of cyclooxygenase could be involved, as has been demonstrated for endothelin ET_A receptor-mediated contraction (Abdel-Latif et al., 2000). The present results do not indicate whether this putative prostanoid would be produced by the endothelial or smooth muscle cells, though the latter would seem more likely. Interestingly, interleukin-1β has been shown to stimulate cyclooxygenase-2 expression and increase prostanoid production in human smooth muscle cells (Pang and Knox, 1997; Beasley, 1999). If the presence of interleukin-1β in our assay system increases expression of cyclooxygenase, then more efficient, cyclooxygenase-mediated prostanoid production could result in the enhanced contraction seen following stimulation of endothelin ET_B receptors by sarafotoxin S6c. Both the spontaneous and the interleukin-enhanced reactions would be expected to be sensitive to cyclooxygenase inhibitors, as shown here. Indeed, Vila et al. (2001) recently demonstrated that endothelin-1 potentiates noradrenaline-induced contractions in rabbit pulmonary artery and this effect was mediated via endothelin ET_B

receptors. Their results suggested that the potentiation was effected by production of a cyclooxygenase-generated factor, probably thromboxane A_2. It is possible that part of the therapeutic ability of cyclooxygenase inhibitors may be associated with a reduction of endothelin-mediated prostanoid production in certain inflammatory conditions.

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