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Augmented contraction of the human isolated coronary artery by sumatriptan: a possible role for endogenous thromboxane

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1 The antimigraine drug, sumatriptan, contracts the human coronary artery and, in some patients, elicits chest symptoms (e.g. pressure and pain), particularly after subcutaneous administration. We studied the effects of the thromboxane A_2 (TxA_2) analogue, U46619 and endothelin-1 on contractile responses to sumatriptan in the human isolated coronary artery as well as the role of endogenously produced TxA_2 and endothelin-1 in contractions evoked by sumatriptan.

2 In the presence of U46619 (1 and 3 nM), mean concentration-response curves to sumatriptan in the human coronary artery were shifted vertically due to the initial contraction by U46619, but when this initial contraction was subtracted from the response to sumatriptan, no significant augmentation was observed. However, analysis of the degree of augmentation in individual arterial segments revealed that the augmentation was variable and related inversely to the E_{max} of sumatriptan in the absence of U46619 ($r=0.78$ and 0.81 for 1 and 3 nM, respectively; $P<0.05$).

3 Treatment with the TxA_2 receptor antagonist, SQ30741 (100 nM), or incubation of vessel segments with aspirin (10 μM), significantly reduced responses to sumatriptan; in aspirin-treated vessel segments, SQ30741 failed to decrease further the contractions to sumatriptan. The decrease in E_{max} of sumatriptan by both SQ30741 and aspirin correlated significantly with the E_{max} of sumatriptan without SQ30741 ($r=0.74$; $P<0.01$) or aspirin ($r=0.94$; $P<0.01$). In aspirin-treated vessel segments, responses to sumatriptan were significantly augmented in the presence of U46619 (3 nM; $P<0.05$).

4 The specificity of SQ30741 was demonstrated by its ability to antagonize coronary artery contractions to U46619 (pA_2: 7.54 ± 0.30), but not endothelin-1. Similarly, incubation with aspirin (10 μM) did not affect contractile responses to endothelin-1, but significantly reduced TxA_2 production in coronary artery segments as judged by a decrease in thromboxane B_2 (TxB_2) from 4.77 ± 0.98 to 1.38 ± 0.36 ng g^{-1} 2 h^{-1}.

5 Endothelin-1 (1 nM) did not significantly augment contractions to sumatriptan; there was also no relationship between the degree of augmentation and the control E_{max} of sumatriptan in the absence of endothelin-1. Furthermore, unlike SQ30741 or aspirin, a high concentration (100 nM) of the non-selective ET_A/ET_B receptor antagonist, SB 209670, failed to affect contractile responses to sumatriptan. However, SB 209670 potently antagonized coronary artery contractions induced by endothelin-1 with a pA_2 of 8.84 ± 0.32.

6 Compared to control vascular segments, endothelial denudation did not reduce TxA_2 production (with endothelium $=2.56\pm1.38$ vs. without endothelium $=12.32\pm4.94$ ng TxB_2 g^{-1} 2 h^{-1}), suggesting that the production of TxA_2 is not confined to the endothelium. The sumatriptan-induced contractions were also unaffected by endothelial denudation.

7 The results of the present study suggest that endogenously produced TxA_2 enhances contractions to sumatriptan in the human isolated coronary artery. Such a mechanism may play a role in causing chest symptoms after sumatriptan by potentiating coronary vascular contraction by sumatriptan *in vivo*.

Keywords: 5-HT_{1D} receptor; aspirin; endothelin-1; human coronary artery; migraine; sumatriptan; thromboxane A_2

Introduction

The antimigraine drug, sumatriptan, a 5-HT_{1D}-like receptor agonist (Humphrey *et al.*, 1988; Ferrari & Saxena, 1993), causes chest symptoms (e.g. pressure and pain) in about 5% of patients in clinical trials (Brown *et al.*, 1991; Willet *et al.*, 1992; Saxena & Tfelt-Hansen, 1993; Plosker & McTavish, 1994). Although extracardiac mechanisms such as increased oesophageal contractions (Houghton *et al.*, 1994) may be involved, sumatriptan has been reported to cause myocardial infarction (see Ottervanger, 1996) due to coronary artery constriction, observed both *in vivo* (MacIntyre *et al.*, 1992) and *in vitro* (Connor *et al.*, 1989; Chester *et al.*, 1990; Bax *et al.*, 1993; Chester *et al.*, 1993b). The response to sumatriptan in human isolated coronary arteries exhibits a high degree of variability (Kaumann *et al.*, 1994), which is not directly related to underlying disease, age or atherosclerosis (Bax *et al.*, 1993; Kaumann *et al.*, 1994). In the human coronary artery (Chester *et al.*, 1993b; Cocks *et al.*, 1993) as well as the guinea-pig iliac (Sahin-Erdemli *et al.*, 1991) and rabbit renal (Tadipatri *et al.*, 1991), iliac (Yildiz & Tuncer, 1995) and femoral (MacLennan & Martin, 1992) arteries, prior exposure to a number of substances enhances responses to sumatriptan by 'unmasking' contractions elicited via stimulation of 5-HT_1 receptors, presumed to be similar or identical to the 5-HT_{1D} type. In particular, this holds true for the thromboxane A_2 (TxA_2)-mimetic, U46619 (Chester *et al.*, 1993b; Cocks *et al.*, 1993), but en-

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dothelin-1 has also been reported to augment contractions to 5-hydroxytryptamine (5-HT) (Yang *et al.*, 1990). However, to our knowledge, no study has been performed on augmentation of sumatriptan-induced contractions of the human coronary artery by endothelin-1.

Kaumann *et al.* (1994) have reported that sumatriptan-induced contractions in the human isolated coronary artery show a wide variability. We hypothesized that endogenous production of TxA_2 by the vessel segments may play a role in the variability of responses to sumatriptan and may be responsible for chest symptoms in some patients after sumatriptan. Indeed, saliva concentrations of TxA_2 were increased in migraine attacks (Tuca *et al.*, 1989) and plasma concentrations of TxA_2 and 5-HT were increased in myocardial infarction and unstable angina (Tada *et al.*, 1981; De Boer *et al.*, 1982; Rubanyi *et al.*, 1987). Therefore, synergistic effects of 5-HT receptor agonists and TxA_2 may be important in these conditions.

In the present study, we investigated augmentation of sumatriptan-induced contractions of the human isolated coronary artery by U46619. Also, the possibility of involvement of endogenously produced TxA_2 in the contraction to sumatriptan was studied by investigating the response to sumatriptan in the presence of the TxA_2 receptor antagonist, SQ30741 (Ogletree *et al.*, 1986) or the cyclo-oxygenase inhibitor, aspirin. The production of the TxA_2 metabolite, thromboxane B_2 (TxB_2) by coronary arteries was investigated in both untreated and aspirin-treated coronary artery segments. Lastly, we studied augmentation of sumatriptan-induced contractions by endothelin-1 and investigated the involvement of endogenously produced endothelin-1 by studying the influence of the ET_A/ET_B receptor antagonist SB 209670 (Ohlstein *et al.*, 1994) on the contraction to sumatriptan.

Methods

Tissue preparation

Right epicardial coronary arteries were obtained from 45 heart-beating organ donors who died of non-cardiac disorders less than 24 h before the tissue was taken to the laboratory (23 trauma, 17 cerebrovascular accident, 4 cerebral hypoxia, 1 hypoglycaemia; 24 male, 21 female; age 1–55 years). In addition, one heart was obtained from a patient undergoing heart transplantation (female suffering from cardiomyopathy, age 63 years). The hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services/Eurotransplant Foundation) after removal of the heart valves for transplantation purposes and stored at 0–4°C in a sterile organ-protecting solution immediately after circulatory arrest. After arrival in the laboratory, the right epicardial coronary artery was removed and placed in a cold, oxygenated Krebs bicarbonate solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_2$ 25 and glucose 8.3; pH 7.4. Vessel segments containing macroscopically visible atherosclerotic lesions were excluded from the study. In some experiments, the endothelium was removed with a cotton swab attached to forceps. Unless mentioned otherwise, the results refer to experiments with endothelium-intact vessel segments. The study was approved by the joint Ethical Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam 'Dijkzigt'.

Functional experiments

Coronary artery segments of approximately 4 mm were suspended between stainless steel hooks in 15 ml organ baths aerated with 95% O_2:5% CO_2, and were maintained at 37°C. Vessel segments were allowed to equilibrate for at least 30 min, and were washed every 15 min. Changes in tissue force were recorded with a Harvard isometric transducer. The vessel segments, stretched to a stable force of about 15 mN, were exposed to 30 mM K^+ twice. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after pre-contraction with prostaglandin $F_{2\alpha}$ (1 μM). The tissue was washed and exposed to 100 mM K^+. After another wash and a 30 min equilibration period, U46619, endothelin-1, SQ30741, SB 209670 or in each case vehicle (paired design) was added, 30 min before a concentration-response curve was constructed. Contractile responses were expressed as percentage of the contraction induced by 100 mM K^+.

To assess the influence of endogenously produced TxA_2 on the response to sumatriptan, vessel segments were kept in Krebs solution containing aspirin (10 μM) overnight before the experiment. Control segments were kept in Krebs solution without aspirin. For the aspirin-treated vessel segments, the experiments were performed in Krebs solution containing 10 μM aspirin, which did not change the pH of the Krebs solution.

Measurement of endogenously produced TxA_2

Production of TxA_2 by human isolated coronary arteries was measured in oxygenated Krebs solution (segment length 1–2 cm in 2 ml during a 2 h period) at 37°C. For aspirin-treated vessel segments, the incubation was performed in Krebs solution containing aspirin (10 μM). After 2 h, a sample of the Krebs solution was removed and indomethacin (30 μM) was added to stop cyclo-oxygenase activity. The samples were centrifuged at 570 *g* (20°C) for 20 min and the supernatant was stored at −20°C until assay. The concentration of TxB_2, the stable metabolite of TxA_2, was measured by radioimmunoassay (Zijlstra & Vincent, 1984; Bax *et al.*, 1994) and expressed as ng TxB_2 per g wet weight of tissue.

Data analysis

All data are presented as mean ± s.e.mean. Concentration-response curves were analysed using the logistic function described by De Lean *et al.* (1978) to obtain pEC_{50} ($-\log EC_{50}$) values. To analyse differences between concentration-response curves to sumatriptan obtained in the presence or absence of either U46619 or endothelin-1, responses to sumatriptan were corrected for the increase in basal tone by the two agents. Differences between the corrected concentration-response curves (in the presence of U46619 or endothelin-1) and the control concentration-response curve were examined by analysis of variance (ANOVA) for repeated measures. The degree of augmentation by U46619 or endothelin-1 was defined as the ratio of the E_{max} of sumatriptan in the presence of either U46619 or endothelin-1 (corrected for the basal contraction by these agents) and that in the absence of these compounds (control E_{max}). Correlation coefficients were calculated according to Pearson (Steel & Torrie, 1980). The degree of variation in a series of experiments under different experimental conditions was expressed by subtracting the contraction in an individual segment from the mean contraction in each series. These values were divided by the mean response to correct for the magnitude of the response. Mean values of the three highest agonist concentrations were used to test differences between experimental conditions with the Wilcoxon signed rank test. The pA_2 values of antagonists were determined by Schild analysis (Schild, 1957). Differences in TxA_2 production, relaxation to substance P, contraction to 100 mM K^+, and differences between pEC_{50} values were analysed using a paired *t* test. *P* values of ≤ 0.05 were considered to indicate significant differences. Data analyses were performed with the SPSS (SPSS Inc., Chicago, Illinois, USA) statistical programme.

Compounds used in this study

Sumatriptan was a gift from Glaxo Wellcome (Dr. H.E. Connor, Ware, Hertfordshire, U.K.); U46619 (9,11-dideoxy-11α,9α-epoxy, methanoprostaglandin $F_{2\alpha}$), prostaglandin $F_{2\alpha}$

(Tris salt), substance P acetate and aspirin (acetylsalicylic acid) were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.); endothelin-1 was purchased from Neosystem Laboratoire (Strasbourg, France); SQ30741 ([1S-[1α,2α(5Z),3α,4α]]-7-[3-[[[[[(1-oxoheptyl) amino]acetyl]amino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) was a gift from Bristol-Myers Squibb (Princeton, NJ, U.S.A.); SB 209670 ((±)-(1S,2R,3S)-3-(2-carboxymethoxy-4-methoxy phenyl)-1-(3,4-methylene-dioxyphenyl)-5-(prop-1-yloxy)-indane-2-carboxylic acid) was a gift from SmithKline Beecham Pharmaceuticals (Dr. E.H. Ohlstein, King of Prussia, Pennsylvania, U.S.A.). Indomethacin was obtained from the pharmacy of University Hospital Rotterdam 'Dijkzigt' (Rotterdam, The Netherlands). [5,6,8,9,11,12,14,15(n)-^{3}H]-thromboxane B_2 (specific activity: 8.21 TBq mmol^{-1}; Batch B65) was purchased from Amersham (Little Chalfont, Buckinghamshire, U.K.); TxB_2 antiserum was purchased from PerSeptive Diagnostics (Cambridge, U.K.).

Results

Basic properties of the preparations: effects of substance P and potassium

Endothelium-intact human coronary artery segments relaxed after addition of substance P (1 nM), the response amounting to 84±5% of the pre-contraction (26.7±2.1 mN) induced by prostaglandin $F_{2\alpha}$ (1 μM; $n=41$). The vessel segments responded to 100 mM K^+ with a contractile response of 34.3±2.3 mN ($n=41$). Vasorelaxation by substance P was related inversely to the age of the heart donors (Pearson's $r=0.40$, $P<0.01$; endothelium-intact vessel segments). On the other hand, the contractile responses to K^+ (100 nM) and prostaglandin $F_{2\alpha}$ (1 μM) were not related to donor's age, nor was there any relationship between the relaxation by substance P or contraction by K^+ and the cause of death or sex of the donors.

Removal of the endothelium resulted in a non-significant reduction of the contractile response to prostaglandin $F_{2\alpha}$ (1 μM; without endothelium = 21.9±3.3 mN vs. with endothelium = 28.3±2.6 mN), but it reduced significantly the relaxant response to substance P (without endothelium = 9±4% vs. with endothelium = 79±9%; $P<0.0001$; $n=7$) as well as the contractile response to 100 mM K^+ (without endothelium = 22.7±3.7 mN vs. with endothelium = 31.2±3.3 mN; $P<0.005$; $n=7$).

Incubation with aspirin (10 μM) did not affect the contractile response to prostaglandin $F_{2\alpha}$ (1 μM; 26.4±2.6 mN vs. 28.9±2.4 mN for control) or the relaxant response to substance P (68±7% of contraction to prostaglandin $F_{2\alpha}$ vs. 73±7% for control segments; $n=17$).

Augmentation of the responses to sumatriptan

In the presence of U46619 (1 or 3 nM), concentration-response curves to sumatriptan were shifted to a higher level due to the initial contraction associated with U46619 (Figure 1a). When this initial contraction was subtracted from the response generated by sumatriptan in the presence of U46619 to allow calculation of the degree of augmentation (see 'data analysis' in the method section), concentration-response curves to sumatriptan in the presence of U46619 were not significantly different from the control curve. However, further analysis of the degree of augmentation in individual arterial segments revealed that the augmentation was variable and related inversely to the E_{max} of sumatriptan in the absence of U46619 (Figure 1b); correlation coefficients were 0.78 (1 nM U46619; $P<0.01$) and 0.81 (3 nM U46619; $P<0.01$).

When experiments in which the E_{max} for sumatriptan was ≤50% of the potassium-induced contraction were analysed separately, a significant augmentation of the response to sumatriptan was found in the presence of both 1 nM and 3 nM U46619 ($n=6$, $P<0.01$).

The pEC_{50} value of sumatriptan (6.5±0.1; $n=13$) was not significantly changed in the presence of U46619 (0.3 nM: 6.6±0.1; 1 nM: 6.6±0.2; 3 nM: 6.9±0.1).

Endogenous production of TxA_2 and contractile responses to sumatriptan

In view of the inverse relationship between the degree of augmentation of sumatriptan responses by U46619 and the E_{max} of sumatriptan, we investigated whether endogenously produced TxA_2 may have already augmented the response to sumatriptan, thus decreasing the margin for further augmen-

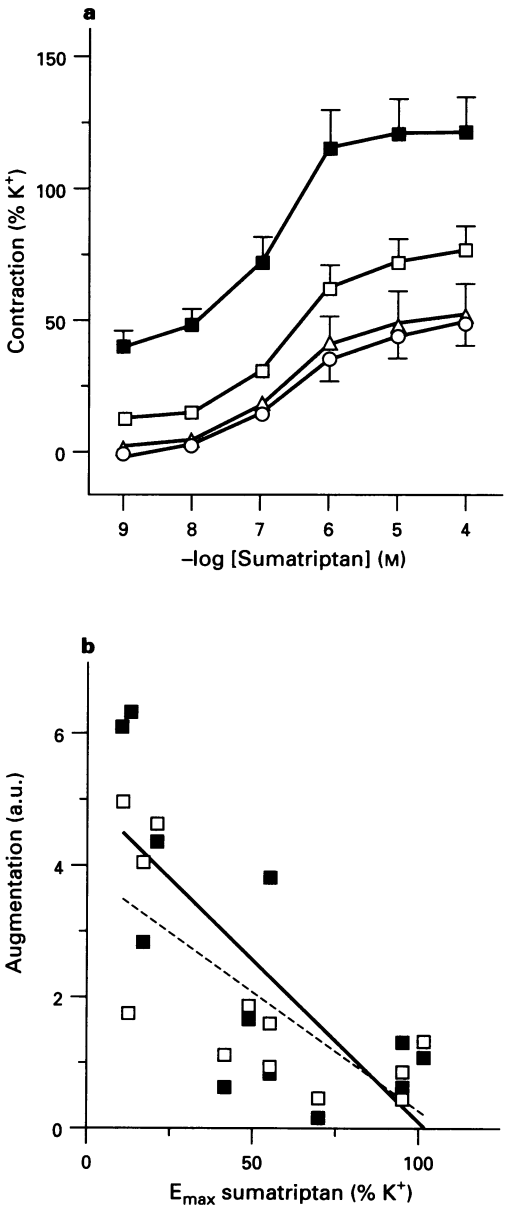

Figure 1 (a) Contractile responses to sumatriptan (expressed as % of the response to 100 mM K^+) in the absence (○, control) or presence of U46619 (△, 0.3 nM; □, 1 nM; ■ 3 nM; $n=12-13$). (b) Augmentation of the contractile response to sumatriptan by U46619 (■, solid line, 3 nM; □, broken line, 1 nM) plotted against the control (without U46619) E_{max} of sumatriptan. The augmentation by U46619, expressed in arbitrary units (a.u.), was calculated as the ratio of E_{max} of sumatriptan in the presence of U46619 (corrected for the basal contraction by U46619) and that in the absence of U46619 (control E_{max}). The degree of augmentation of the response to sumatriptan by U46619 correlated negatively to the control E_{max} of sumatriptan (Pearson's $r=0.78$ for 1 nM U46619 and 0.81 for 3 nM U46619; $P<0.01$; $n=12$).

tation by U46619 in segments with higher endogenous TxA_2 production. In order to test this possibility, we performed experiments using the TxA_2 receptor antagonist, SQ30741 and the cyclo-oxygenase inhibitor, aspirin.

Responses to sumatriptan (in the absence of U46619) were significantly attenuated by 100 nM SQ30741 (E_{max}: $14 \pm 4\%$ vs. $28 \pm 6\%$ in controls; $n = 12$). Incubation with aspirin (10 μM; overnight) also resulted in a decreased response to sumatriptan (E_{max}: $16 \pm 2\%$ vs. $28 \pm 6\%$ in controls; $n = 12$), but in these vessel segments SQ30741 did not further decrease sumatriptan-induced contractions (E_{max}: $13 \pm 3\%$; Figure 2a). The attenuation of the maximal response to sumatriptan by SQ30741 as well as aspirin was significantly correlated to the control E_{max} of sumatriptan ($r = 0.74$ and 0.94 for SQ30741 and aspirin, respectively; $P < 0.01$ for both; Figure 2b). The variability in the concentration-response curves was significantly decreased (for calculation: see 'data analysis'; $P < 0.05$) in the presence of SQ30741 or after treatment with aspirin, indicating that a source of variability had been eliminated under these conditions (s.e.mean/mean response for three highest sumatriptan concentrations; control, 0.241; SQ30741, 0.165; aspirin, 0.111). In addition, as shown in Figure 3, responses to sumatriptan were significantly augmented by 3 nM U46619 ($P < 0.05$; $n = 7$) in aspirin-treated vessel segments, which produce decreased amounts of TxA_2 (see below). However, the production of TxA_2 in vessel segments not treated with aspirin did not correlate with the E_{max} values of sumatriptan ($r = 0.14$; $n = 11$).

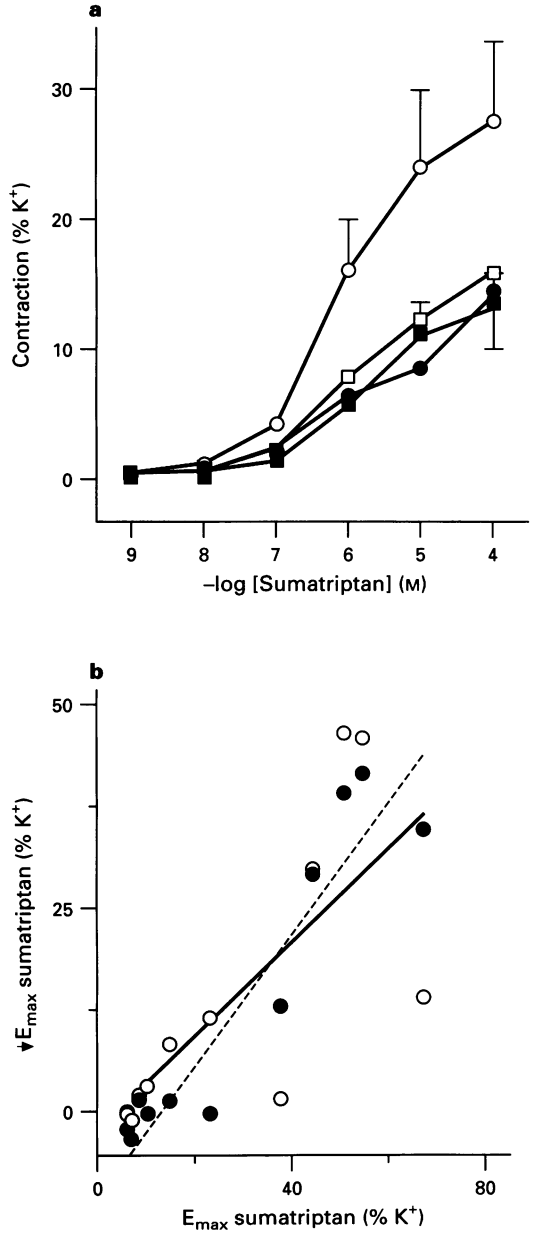

Figure 2 (a) Contractile responses to sumatriptan (expressed as % of the response to 100 mM K^+) in the absence (○,●) or presence (□,■) of SQ30741 (100 nM) in vessel segments with (filled symbols) or without (open symbols) incubation with aspirin (10 μM). The contractile response to sumatriptan was significantly ($P < 0.05$) decreased by SQ30741 in segments not incubated with aspirin. Aspirin incubation also significantly decreased sumatriptan-induced contraction, but no further decrease was noticed with SQ30741 in these vessel segments ($n = 12$ each). (b) Attenuation of the E_{max} of sumatriptan by SQ30741 (100 nM, ○; solid line) or aspirin (10 μM, ●; broken line) plotted against the control E_{max} of sumatriptan (i.e. in the absence of SQ30741 and aspirin). The attenuations by SQ30741 and aspirin were positively correlated to the control E_{max} of sumatriptan (Pearson's $r = 0.74$ and 0.94, respectively; $P < 0.01$; $n = 12$ each).

The potency and selectivity of the TxA_2 receptor antagonist, SQ30741, was studied by comparing concentration-response curves for U46619 and endothelin-1 in the absence or presence of several concentrations (30–300 nM) of SQ30741 (Figure 4). Treatment of the vessel segments with SQ30741 shifted the dose-response curves of U46619 in a parallel manner to the right, but did not affect those to endothelin-1. When data of the experiments with U46619 were represented in a Schild plot, the calculated pA_2 value was 7.54 ± 0.30 ($n = 5$) with a slope (1.39 ± 0.15) not differing from unity, indicating a competitive antagonism.

Incubation of human coronary artery segments with aspirin (10 μM overnight) led to a decreased production of TxA_2. As shown in Figure 5a, the concentrations of TxB_2 (the stable metabolite of TxA_2) in the control and aspirin-incubated segments were, respectively, 4.77 ± 0.98 and 1.38 ± 0.36 ng g^{-1} 2 h^{-1} ($n = 12$ each; $P < 0.005$). The potency as well as the efficacy of endothelin-1 in the aspirin-incubated vessel segments (pEC_{50}: 8.35 ± 0.14; E_{max}: $82 \pm 4.6\%$ 100 mM K^+) was not different from that in the control segments (pEC_{50}: 8.44 ± 0.17; E_{max}: $82 \pm 7.2\%$ 100 mM K^+), indicating that the contractile characteristics of the vessel were not altered by aspirin (Figure 5b).

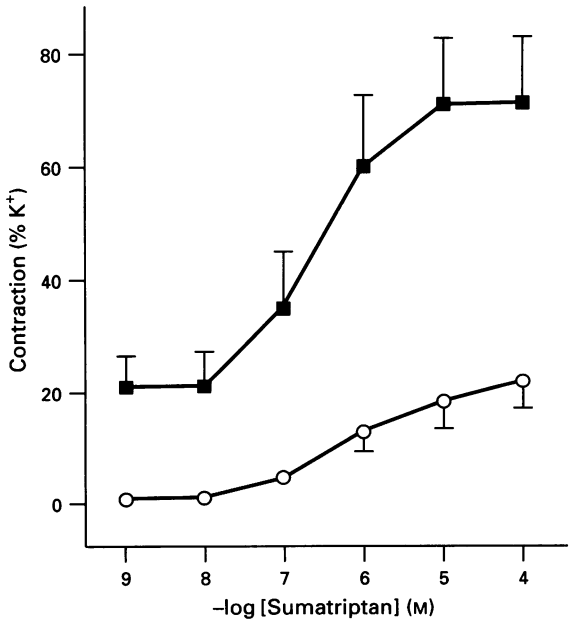

Figure 3 Contractile responses to sumatriptan (expressed as % of the response to 100 mM K^+) in the absence (○, control) or presence (■) of 3 nM U46619 in vessel segments incubated with 10 μM aspirin ($n = 7$ each). The responses to sumatriptan were significantly augmented by U46619 ($P < 0.05$).

Localization of endogenous TxA_2 production

In an attempt to determine whether endogenously produced TxA_2 was derived from the endothelium or other parts of the vascular tissue, a comparison was made between the production of TxA_2 as well as the responses to sumatriptan in endothelium-intact and endothelium-denuded human coronary artery segments. Measurements of TxA_2 production in endothelium-denuded vessel segments revealed a non-significant change in the levels of TxB_2 compared to the vessels where the endothelium was not removed (12.32 ± 4.94 vs. 2.56 ± 1.38 ng $TxB_2\ g^{-1}\ 2\ h^{-1}$, respectively; $n=8$; $P>0.05$). Similarly, there was no significant change in sumatriptan-induced contractile responses in the endothelium-denuded vessel segments (E_{max}: $20 \pm 6\%$ in endothelium-intact vs. $37 \pm 14\%$ in endothelium-denuded segments; $P>0.05$; $n=7$).

Involvement of endothelin-1 in the responses to sumatriptan

In the presence of endothelin-1 (1 nM), the concentration-response curve to sumatriptan yielded higher contractions due to the initial contraction associated with endothelin-1; higher concentrations of endothelin-1 could not be used due to phasic contractions in the majority of vessels. As found with U46619 (see above), the concentration-response curve to sumatriptan in the presence of endothelin-1 was not significantly different from the control curve (Figure 6a). However, unlike U46619, there was no relationship between the degree of augmentation of the response to sumatriptan and the control E_{max} of sumatriptan, in the absence of endothelin-1 ($r=0.26$; $n=10$; $P>0.05$; Figure 6b).

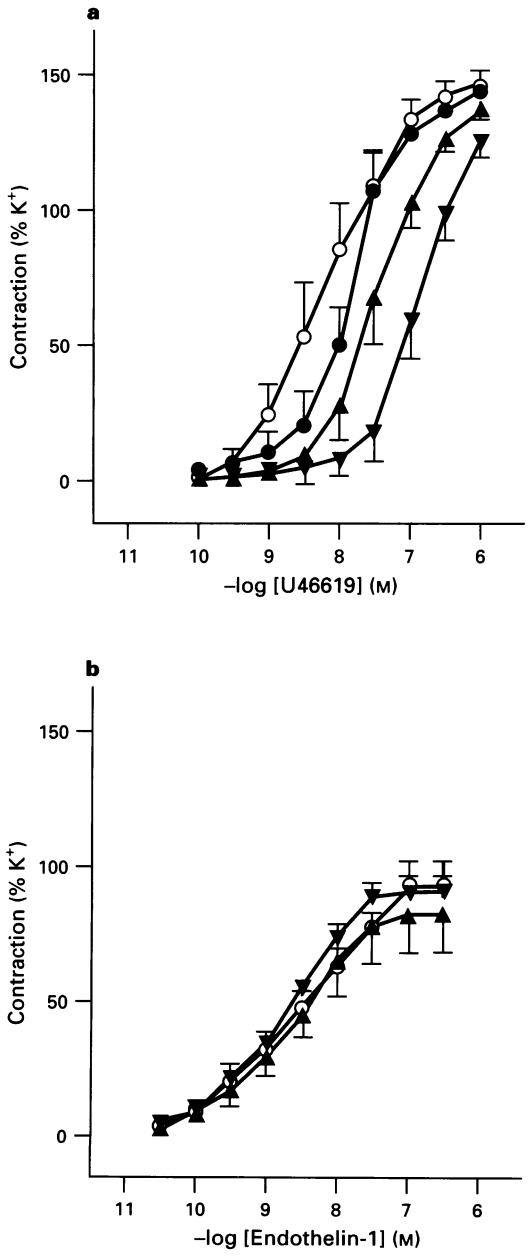

Figure 4 Contractile responses to U46619 (a; $n=5$) and endothelin-1 (b; $n=4$), both expressed as % of the contraction induced by 100 mM K^+, in the absence (○, control) or presence of the TxA_2 receptor antagonist, SQ30741 (●, 30 nM; ▲, 100 mM; ▼, 300 nM). The responses to U46619 were inhibited by SQ30741 (pA_2: 7.54 ± 0.30), while those to endothelin-1 were not modified by SQ30741.

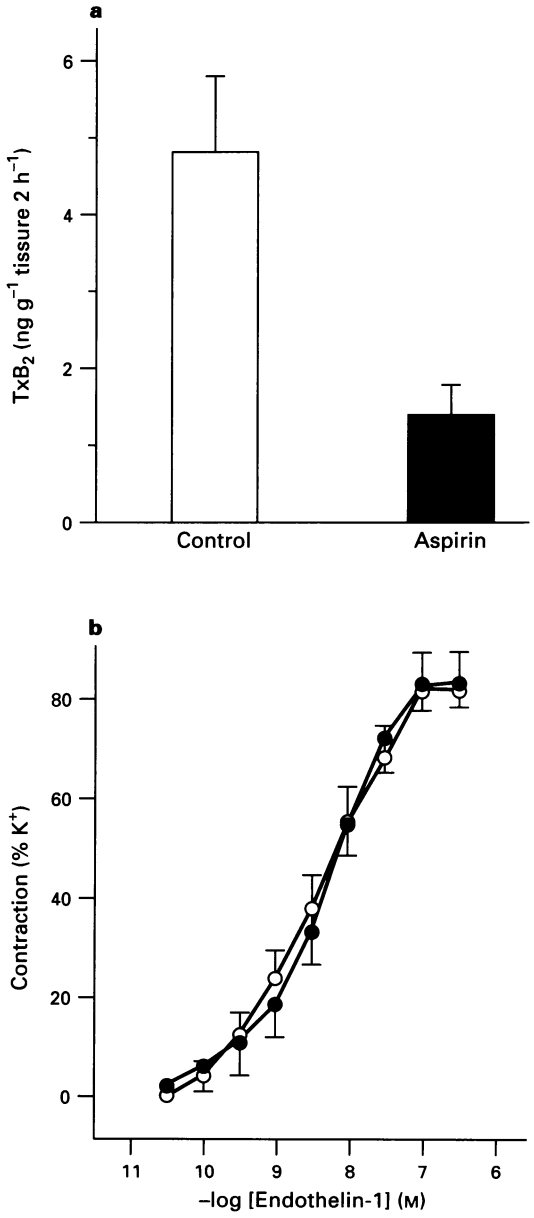

Figure 5 (a) Endogenous production of TxA_2, expressed in terms of its stable metabolite TxB_2 (ng $TxB_2\ g^{-1}\ 2\ h^{-1}$), in human coronary artery segments after incubation with (solid column) or without (open column) aspirin (10 μM). TxA_2 production was significantly decreased in vessel segments incubated with aspirin ($P<0.005$; $n=12$). (b) Contractile responses to endothelin-1 in human coronary artery segments after incubation in the presence (●) or absence (○) of aspirin (10 μM). Incubation with aspirin did not affect the concentration-response curve to endothelin-1.

Concentration-response curves of sumatriptan and endothelin-1 were also generated in the absence or presence SB 209670, a potent non-selective ET_A/ET_B receptor antagonist. A high concentration of SB 209670 (100 nM) failed to affect the concentration-response curve of sumatriptan (E_{max}: $18 \pm 4.2\%$ vs. $20 \pm 7.5\%$ for control; $n=9$; Figure 7a), thus providing little evidence for a role of endothelin-1 in the contractile response to sumatriptan. As expected, SB 209670 shifted the concentration-response curves of endothelin-1 to the right in a parallel manner (Figure 7b). The estimated pA_2 value was 8.84 ± 0.32 ($n=6$) and the slope (0.86 ± 0.23) did not differ from unity, indicating a competitive antagonism.

Discussion

Augmentation of the responses to sumatriptan by U46619

In the present study, the response of the human isolated coronary artery to sumatriptan was not significantly augmented in the presence of the TxA_2 analogue, U46619 (0.3–3 nM). This seems to be at variance with earlier reports of Chester *et al.* (1993b) and Cocks *et al.* (1993), but, in both these studies, the control E_{max} of the sumatriptan response was less than 8% of the K^+-induced contraction. In contrast, several other studies reported a higher E_{max} of sumatriptan (40% of contraction to 90 mM K^+, Chester *et al.*, 1990; 23% of contraction to 100 mM K^+, Bax *et al.*, 1993; 11% of contraction to 0.1 μM U46619, Connor *et al.*, 1989). When, in our study, experiments with a relatively low control E_{max} of sumatriptan ($\leqslant 50\%$ of K^+-induced contractions) were analysed separately, a significant augmentation of the response to sumatriptan was found in the presence of both 1 and 3 nM of U46619. The inverse relationship between the E_{max} of sumatriptan and the degree of augmentation suggested that, in arteries with a high E_{max} of sumatriptan, the response may already have been augmented by an endogenous factor, possibly TxA_2. Differing endogenous production of this factor in the various studies could explain the variation in the obtained control E_{max} of sumatriptan. Also within a study, variable responses to sumatriptan have been reported (Kaumann *et al.*, 1994), but no explanation was forwarded.

Role of endogenous production of TxA_2 in the contractile response to sumatriptan

The hypothesis that endogenously produced TxA_2 may be involved in the response to sumatriptan was investigated in two different ways. First, the TxA_2 receptor was blocked by the use of a TxA_2 receptor antagonist, SQ30741. Second, the formation of endogenous TxA_2 was inhibited by incubation of the tissues with the cyclo-oxygenase inhibitor, aspirin. The findings that (i) sumatriptan-induced contractions were decreased to the same extent by SQ30741 and aspirin, (ii) no further decrease was noticed with SQ30741 in aspirin-treated vessel segments (Figure 2a) and (iii) contractile responses to sumatriptan were augmented by U46619 in aspirin-incubated vessel segments, suggest that an endogenously produced cyclo-oxygenase product, most probably TxA_2, augments the contractile response to sumatriptan. The positive correlation between the attenuation of the response to sumatriptan by SQ30741 or aspirin and the control E_{max} of sumatriptan also supports this contention. Further evidence is provided by the fact that the variability of concentration-response curves of sumatriptan was decreased by both SQ30741 and aspirin; a decreased variability suggests that a source of variation, most likely the presence of differing concentrations of TxA_2, was reduced. However, in contrast, the E_{max} of sumatriptan did not correlate with the concentrations of TxB_2 measured in the medium incubating coronary artery segments not treated with aspirin. There are several factors which may have confounded the relationship between the sumatriptan E_{max} and endogenous TxA_2 production: (i) although these two variables were determined in two segments of the same coronary artery, the functional (E_{max} sumatriptan) and biochemical (TxB_2) assays had to be performed under different conditions; as described in detail in the method section, vessel segments (4 mm length in 15 ml Krebs solution) for functional assays were stretched to a force of about 15 mN and exposed to K^+ (three times), prostaglandin $F_{2\alpha}$ and substance P and washed several times during a period of about 5 h before constructing the sumatriptan curve, whereas the biochemical assays were performed in unstretched, untreated and unwashed vessel segments (1–2 cm length in 2 ml Krebs solution in order to keep the TxB_2 concentration above the detection limit)

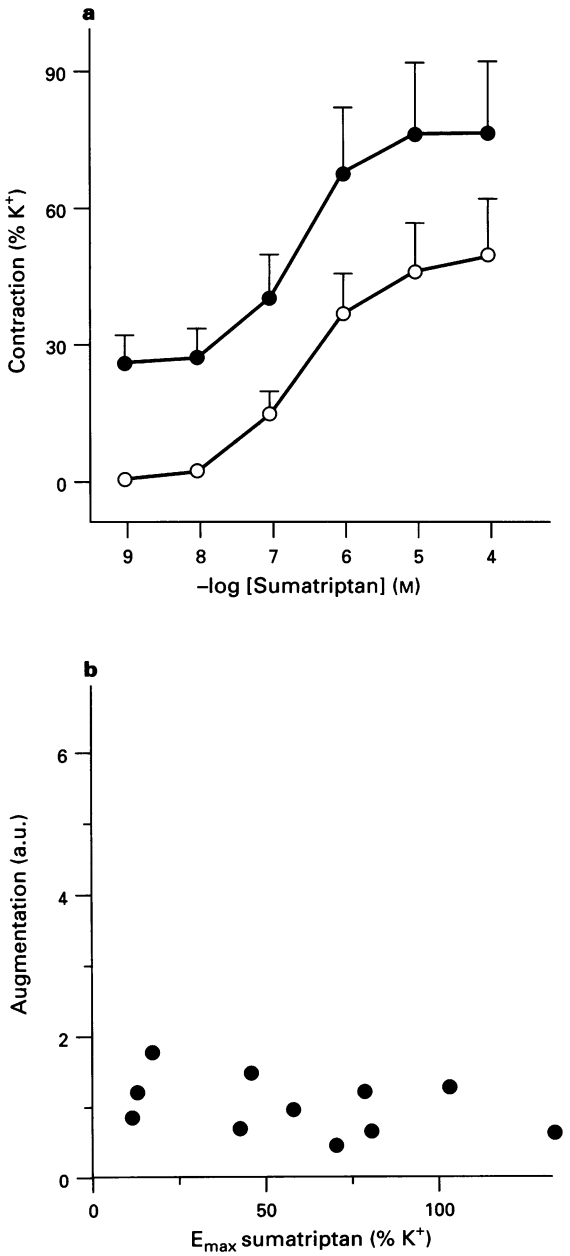

Figure 6 (a) Contractile responses to sumatriptan in the absence (○, control) or presence (●) of endothelin-1 (1 nM). Though the concentration-response curve of sumatriptan was shifted upwards by endothelin-1, there was no augmentation of the responses as can be noted by similar differences in the responses with or without endothelin-1 throughout the concentration-range. (b) The degree of augmentation of the contractile response to sumatriptan, expressed in arbitrary units (a.u.), by endothelin-1 (1 nM) plotted against the control (without endothelin-1) E_{max} of sumatriptan. Note that the augmentation by endothelin-1 was much less than with U46619 (Figure 1) and that there was no significant correlation between the augmentation and the control E_{max} ($r=0.26$; $P>0.05$; $n=10$).

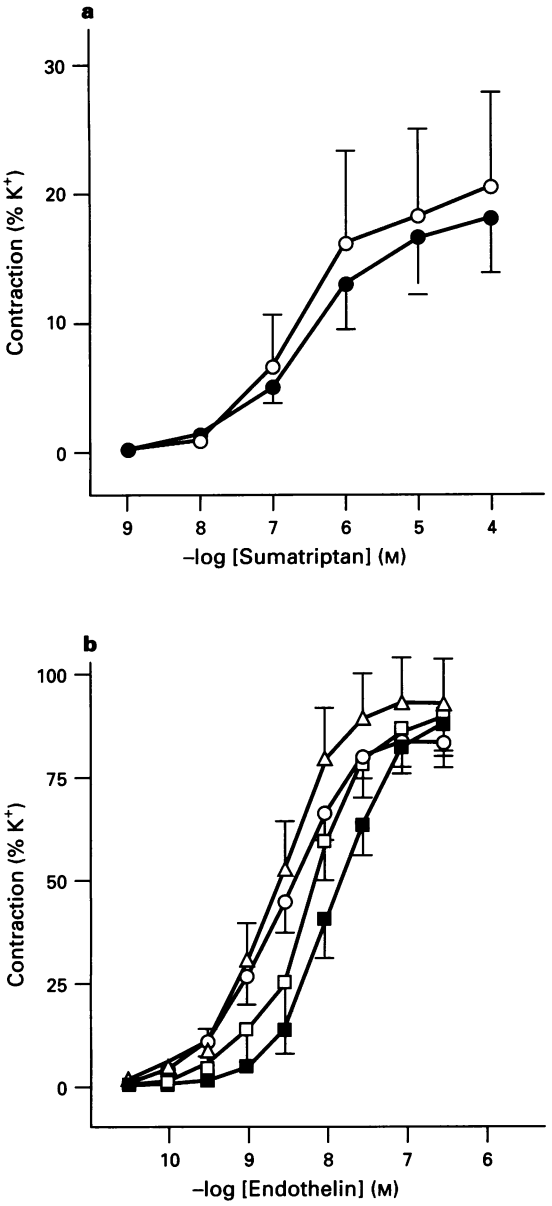

Figure 7 Contractile responses to sumatriptan (a; $n=9$) and endothelin-1 (b; $n=6$), both expressed as % of the contraction induced by 100 mM K^+, in the absence (○, control) or presence of the non-selective ET_A/ET_B receptor antagonist, SB 209670 (△, 0.3 nM; □, 1 nM; ■, 3 nM; and/or ●, 100 nM). Contractile responses to sumatriptan were not affected by SB 209670, but those to endothelin-1 were competitively inhibited (pA_2: 8.84±0.32).

during 2 h, (ii) whereas the E_{max} of sumatriptan is likely to be influenced by TxA_2 concentrations prevailing at that time, the measured TxB_2 concentrations represent cumulative TxA_2 production over a 2 h period, and (iii) as may be expected from earlier investigations (e.g. Sahin-Erdemli *et al.*, 1991; Tadipatri *et al.*, 1991; Chester *et al.*, 1993a), it is possible that a certain threshold concentration of TxA_2 augments the contraction to sumatriptan, without a linear relationship between the amount of endogenous TxA_2 and the potentiation of the response to sumatriptan.

It is conceivable that incubation with aspirin may have affected the ability of the smooth muscle cells to contract non-specifically and, thus, reduced contractile responses to sumatriptan. However, in such a case, it may be expected that the TxA_2 receptor antagonist SQ30741 would have the same effect whether the vessel segments were previously incubated with aspirin or not. Clearly, this was not the case. Furthermore, the response to endothelin-1 was not attenuated by incubation of vessel segments with aspirin. Direct evidence for the fact that TxA_2 was indeed produced by the human isolated coronary artery and that this production was inhibited by aspirin was furnished by the measurements of TxB_2 in the medium surrounding the vessel segments (Figure 5a). Since mechanical removal of the endothelium did not decrease (if anything, tended to increase) the formation of TxB_2 as well as sumatriptan-induced vessel contractions, it would appear that endogenous TxA_2 production is not, or not exclusively, located in vascular endothelium. Although the formation of TxA_2 has been reported to be restricted to the endothelium in the human internal mammary artery (Lin *et al.*, 1993), the media and adventitia of human arteries (gastric, mesenteric, splenic, and renal) have also been reported to produce TxA_2 (Neri Serneri *et al.*, 1983).

Role of endothelin-1 in the responses to sumatriptan

In our study, endothelin-1 did not augment sumatriptan-induced contractions of the human coronary artery. Detailed analysis of the responses in individual vascular segments, in contrast to the experiments with U46619, did not reveal any relationship between the degree of augmentation by endothelin-1 and the magnitude of the control E_{max} of sumatriptan. It must be pointed out that we could use only 1 nM endothelin-1, since a higher concentration (3 nM) caused phasic contractions in the majority of experiments, thus precluding the possibility of obtaining a stable concentration-response curve of sumatriptan. However, with the concentration used (1 nM), the initial contraction of the human coronary artery by endothelin-1 was more than that by 1 nM U46619 (compare Figures 1 and 6), which significantly augmented sumatriptan responses in vessels with low E_{max}. Although Yang *et al.* (1990) have observed that concentrations of 1 nM and, even 0.3 nM of endothelin-1 augment responses to 5-HT, others, in a larger series of experiments, were unable to confirm such a augmentation (Chester *et al.*, 1992). In addition, unlike the TxA_2 antagonist SQ30741, the response to sumatriptan was not influenced by SB 209670, which in the present investigation, as well as in an earlier study (Ohlstein *et al.*, 1994), was shown to be a potent inhibitor of endothelin-1-induced contractions of the human isolated coronary artery. These observations suggest that endothelin-1 did not induce a significant augmentation of the responses to sumatriptan and that the augmentation by U46619 was not merely dependent on increased basal force or augmented levels of PIP_2, as suggested by MacLennan *et al.* (1993). Another argument pleading against the possibility that augmentation may be regulated via increased basal force only, is the fact that responses to sumatriptan in, for example, the guinea-pig iliac artery are augmented by U46619 in threshold concentrations hardly causing any contraction (Sahin-Erdemli *et al.*, 1991; our own unpublished observations).

As mentioned above, endothelin-1 may augment contractile responses to 5-HT in the human isolated coronary artery (Yang *et al.*, 1990). By contrast, U46619 augments the responses to sumatriptan, to an even higher extent than it was found to augment the responses to 5-HT (Chester *et al.*, 1993a,b). Whereas the augmentation of the response to 5-HT by U46619 seems to be regulated most profoundly by the 5-HT_{1D}-like receptor subtype, augmentation of the response to 5-HT by endothelin-1 could be mediated by other 5-HT receptor subtypes, not involved in the contraction to sumatriptan (see MacLennan & Martin, 1990).

In conclusion our results suggest that endogenously produced TxA_2 is able to enhance responses to the 5-HT_{1D}-like receptor agonist sumatriptan in the human isolated coronary artery. This finding could have implications for the synergistic action of 5-HT and TxA_2 in vasospastic diseases, since not only platelet-derived, but also locally produced TxA_2 in the blood vessel wall can augment contractile responses to 5-HT. In addition, although conceivable, it remains to be seen if a similar mechanism exaggerating sumatriptan-induced coronary artery contractions also operates in migraine patients

complaining of chest symptoms. In such a case, prior treatment of patients with aspirin may be able to suppress chest symptoms after sumatriptan, at least in some patients.

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