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Comparison of contractile responses to 5-hydroxytryptamine and sumatriptan in human isolated coronary artery: synergy with the thromboxane A_2-receptor agonist, U46619

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1 The interaction between the thromboxane A_2 receptor agonist, U46619 and two 5-hydroxytryptamine (5-HT) receptor agonists, the non-selective, naturally occurring agonist, 5-HT and the selective 5-HT_1-like agonist, sumatriptan were studied in human epicardial coronary arteries *in vitro*.

2 Coronary artery rings (2–4 mm in diameter) were prepared from epicardial arteries from explant hearts of patients undergoing heart transplant (cardiomyopathy, $n = 13$; ischaemic heart disease, $n = 10$) and unused donor hearts ($n = 5$). Each ring of artery was set at optimal resting conditions to record changes in isometric force.

3 The majority of artery rings developed phasic, rhythmic contractions either spontaneously or in response to all vasoconstrictor agonists tested. Both the spontaneous and agonist-induced phasic contractions were abolished by nifedipine (0.1 μM).

4 Concentration-contraction curves to 5-HT-receptor agonists and noradrenaline (NA), were first constructed in artery rings that did not develop phasic activity. 5-HT and ergometrine were the most potent agonists with EC_{50} values of 6.8 ± 0.2 and 7.7 ± 0.2 ($-\log$ M) respectively. Potencies (EC_{50}'s) to sumatriptan, methysergide and noradrenaline could not be determined due to their poor ability to contract the coronary artery. Maximum contractions (E_{max}; normalized as a percentage of the contraction to a maximum-depolarizing concentration of K^+ in physiological salt solution (KPSS)) for 5-HT, ergometrine, sumatriptan, methysergide and noradrenaline were 40 ± 10, 9 ± 3, <5, <5 and $<5\%$ respectively.

5 In arteries without phasic activity, U46619 (1 nM) caused an increase in force of $3.8 \pm 1\%$ KPSS. With U46619 present, the E_{max} values for 5-HT, ergometrine, sumatriptan and methysergide were all markedly increased. For 5-HT and sumatriptan, E_{max} values were $92 \pm 4\%$ and $49 \pm 14\%$ KPSS respectively. The presence of U46619 did not significantly change the sensitivity (EC_{50}) to 5-HT.

6 In a separate series of arteries, nifedipine (0.1 μM) was used to block phasic, contractile activity. The synergy observed between U46619 and 5-HT or sumatriptan still occurred although the E_{max} values for each agonist were depressed but the EC_{50} values were again unaffected.

7 In conclusion, these *in vitro* studies indicate that the normally poor contractions to sumatriptan, in human coronary arteries are significantly enhanced when active force is induced with a thromboxane A_2-receptor agonist, U46619. The enhanced response is not specific for either sumatriptan or 5-HT_1-like receptors since contractions to 5-HT, ergometrine and methysergide were also potentiated by U46619.

Keywords: Human coronary artery; sumatriptan; thromboxane A_2; 5-hydroxytryptamine; synergy

Introduction

Sumatriptan (GR43175), first characterized as a selective 5-HT_1-like receptor agonist (see Bradley *et al.*, 1986; Van Heuven-Nolsen, 1988) in the contraction assay of dog saphenous vein (Humphrey *et al.*, 1988), has been developed as a treatment for migraine (Humphrey *et al.*, 1990 and see Perrin *et al.*, 1989; Ferrari *et al.*, 1991). Whilst the precise mechanism by which sumatriptan relieves migraine is unknown, it may involve stimulation of a population of 5-HT_1-like receptors located on intracerebral vessels. During a migrainous headache, distension, oedema and extravasation of the intracranial vessels occurs partly due to the nociceptive impulses from the Vth cranial nerve. Sumatriptan may constrict these vessels, therefore reducing the release of the inflammatory mediators or it may have a separate action on neuropeptide release from sensory nerve terminals (Humphrey & Feniuk, 1991; Moskowitz, 1992). In other vasculature studied, sumatriptan has been shown to constrict cranial arteriovenous anastomoses (AVA shunts) of anaesthetized cats (Feniuk *et al.*, 1987; Perren *et al.*, 1989) and pigs (den Boer *et al.*, 1990), constrict the carotid arterial bed of the anaesthetized dog (Brittain *et al.*, 1987; Feniuk *et al.*, 1989a), cause a small dilatation of the coronary vasculature in the anaesthetized dog (Feniuk *et al.*, 1989b), contract human (Parsons *et al.*, 1989), canine and primate (Connor *et al.*, 1989a) basilar arteries and cause a general lack of contraction of peripheral arteries in man (Nielsen & Tfelt-Hansen, 1989).

Sumatriptan contracts human isolated large coronary arteries via 5-HT_1-like receptors (Chester *et al.*, 1990). However, these arteries are thought to possess only few 5-HT_1-like receptors since compared with 5-HT, the contractions to sumatriptan are poor (Connor *et al.*, 1989b; Chester *et al.*, 1990). Also, contractions to 5-HT in this tissue were found to be substantially antagonized by the selective 5-HT_2-receptor antagonist, ketanserin (Connor *et al.*, 1989b; Toda & Okamura, 1990). Furthermore, *in vivo* evidence in man to support this came from a clinical study by Golino *et al.* (1991). They found that ketanserin blocked completely both the decrease in large coronary diameter and the increase in flow to the distal bed to intracoronary infusions of 5-HT in patients with and without angiographic evidence of atherosclerosis. In a similar study, however, McFadden *et al.* (1991) included patients with Prinzmetal's angina (diagnosed previously by intracoronary challenge with ergometrine (ergonovine)) and

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found that intracoronary infusions of 5-HT caused spasm (zero flow) which was resistant to ketanserin (McFadden *et al.*, 1992). Ketanserin does not prevent either ergometrine-induced ischaemia (Freedman *et al.*, 1984) or spontaneous attacks of variant angina (De Caterina *et al.*, 1984) in man and although ergometrine activates many different types of receptors including α-adrenoceptors (Feniuk *et al.*, 1989b), both it (Muller-Schweinitzer, 1980; Sakanashi & Yonemura, 1980; Brazenor & Angus, 1981; Holtz *et al.*, 1982), and the antimigraine drug methysergide (Saxena, 1974; Brazenor & Angus, 1981), cause contractions via receptors other than α-adrenoceptors. Thus, in the dog isolated coronary artery, ergometrine was found to be a potent agonist at unspecified 5-HT receptors (Brazenor & Angus, 1981) whilst in the rabbit saphenous vein, ergometrine was demonstrated to be a potent 5-HT_1-like receptor agonist (MacLennan & Martin, 1990). Also, Kawachi *et al.* (1984), found selective hyperreactivity to ergometrine in canine coronary arteries *in vivo* with intimal thickening induced by balloon catheter denudation followed by high cholesterol diet. In addition, Egashira *et al.* (1992) found that ergometrine mediated hyperconstriction of endothelium-denuded coronary arteries in the conscious dog was resistant to block by ketanserin, prazosin and indomethacin and suggested that non-5-HT_2 receptors may play a role in ergometrine-induced hyperconstriction.

In the present study, we compared the concentration-contraction curves to 5-HT and sumatriptan in the presence and absence of another vasoconstrictor agent, the thromboxane A_2-receptor agonist, U46619. This agent was chosen for three reasons. First, since conditions of organ bath experiments are necessarily devoid of blood and extrinsic factors such as circulating hormones and neural control, addition of one putative factor (U46619) in a concentration-controlled manner may in part resemble conditions within the artery wall *in vivo* and thus influence contractility of other vasoconstrictor agents (Angus, 1989). Second, thromboxane A_2 and 5-HT are released from aggregating platelets and intracoronary thrombus formation has been shown to result in focal vasoconstriction in patients with coronary artery disease (Zeiher *et al.*, 1991b). Finally, MacLennan & Martin (1992) showed marked synergy between U46619 and 5-HT contractile responses in the rabbit femoral artery with evidence that 5-HT_1-like rather than 5-HT_2 receptors were involved. Our results show that the normally poor contractions to the selective 5-HT_1-like agonist sumatriptan and the non-selective agonist 5-HT in human, isolated, coronary arteries, are markedly enhanced in the presence of U46619 which is in close agreement with the findings of MacLennan & Martin (1992) in the rabbit femoral artery.

Methods

Tissue source

Human large coronary arteries were obtained from 28 hearts, 23 of which were from patients undergoing heart transplantation and 5 unused normal donor hearts (3 from patients undergoing heart-lung transplants for cystic fibrosis and primary pulmonary hypertension). The diseased hearts were obtained from patients diagnosed with cardiomyopathy (13 patients) and ischaemic heart disease (10 patients).

Artery preparation

Approximately 10–20 min after removal of the heart, the large distal right coronary artery, left anterior descending artery, circumflex artery and the first branches of these vessels were dissected free of adhering connective tissue and fat and placed in cold, oxygenated Krebs solution (see below). After dissection, arteries were cut into 3 mm ring segments with a fixed double-bladed scapel. Ring segments were suspended on stainless steel wire hooks, 500 or 350 μm in diameter, in 25 ml jacketed glass organ baths. The upper wire hook was suspended from a force transducer (Grass FT03C) via which isometric force was amplified and monitored on a single-channel, flat-bed chart recorder. The lower hook was fixed to an inert support leg attached to a micrometer. The tissues were maintained in modified Krebs solution of the following composition (in mM): Na^+ 144, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, glucose 11, pH 7.4, at 37 ± 0.1°C and saturated with 5% CO_2 in oxygen. Up to 30 rings from the same heart were set up simultaneously.

Normalization

In order to compare the reactivity of arteries with different internal diameters, each ring segment was set to the same passive stretch conditions by a normalization procedure prior to construction of concentration-response curves. The normalization procedure was adapted from that developed by Mulvany & Halpern (1977) and involves setting the arteries at a passive tension equivalent to 90% of their internal circumference (0.9 L_{100}) if they had been relaxed and perfused with a transmural pressure of 100 mmHg in the absence of constrictor tone. This wall tension (T) (at 0.9 L_{100}) can be used to estimate the equivalent transmural pressure (P) assuming a thin walled sphere from Laplace, $T = r.P$ where radius (r) is circumference $(L)/2\pi$. P is a useful measure of whether the arteries of different size have been normalized to similar equivalent distending pressures. The procedure has been described previously (Angus *et al.*, 1986). During the normalization stretch procedure, some coronary artery rings developed spontaneous contractions, making normalization difficult (see Figure 1). Consequently, sodium nitroprusside (SNP; 10 μM) was added 5 min prior to normalization to reduce this activity. SNP was washed out once the tissues had been normalized at 0.9 L_{100}.

Experimental protocols

Two experimental protocols were followed. Initially, artery rings were normalized and left resting at 0.9 L_{100} for 1 h before they were contracted with a potassium depolarizing solution (potassium physiological salt solution, KPSS, 124 mM K^+). Once the contraction to KPSS had plateaued, the tissues were washed with normal Krebs solutions and the force allowed to return to baseline. Only one concentration-contraction curve to a single agonist was obtained for any one ring of artery by cumulative addition of the agonist. The 5-HT receptor agonists tested were 5-HT, sumatriptan, methysergide and ergometrine as well as the α-adrenoceptor agonist, noradrenaline. In some experiments, the responses to the agonists were obtained in the presence of low concentrations of the thromboxane A_2-mimetic, U46619, added 5–10 min previously. The contraction curves to noradrenaline were always generated in the presence of propranolol (1 μM) to prevent β-adrenoceptor-mediated smooth muscle relaxation.

In the second protocol, ring segments were normalized to 0.9 L_{100} and contracted with KPSS followed by washout as previously described. To prevent any phasic contractile activity developing either spontaneously or in response to constrictor agonists, nifedipine (0.1 μM) was added and concentration-contraction curves to the 5-HT receptor agonists were generated in the absence or presence of U46619 (1 nM).

Drugs

Drugs used and their sources were: sumatriptan (Glaxo Group Research, Ware, U.K.); U46619 ([1,5,5-hydroxy-11, 9-(epoxymethano)prosta-5z, 13E-dienoic acid], Upjohn, Kalamazoo, MI, U.S.A.); (−)-noradrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.); 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma, St. Louis, MO, U.S.A.); methysergide

maleate (Sandoz S.A., Basle, Switzerland); sodium nitroprusside dihydrate (Roche, Dee Why, NSW, Australia); ergometrine maleate (David Bull Laboratories, Mulgrave, Victoria, Australia); propranolol HCl (ICI, Villawood, NSW, Australia) and nifedipine (Bayer A.G., Wuppertal, Germany). All drugs were diluted in distilled water with the exception of U46619 and nifedipine which were made up as 1 mM and 10 mM stock solutions respectively in absolute ethanol. Further dilutions of these drugs were made in distilled water.

Data analysis

Parameters at normalization and maximum contractions to KPSS of vessels obtained from hearts with different aetiologies were analyzed in a subset of 19 patients. The maximum response to KPSS in each artery ring was normalized for diameter and expressed as F_{max}/unit diameter (g mm^{-1}; see Table 1). All contractile responses were measured as a percentage of the maximum increase in force to KPSS. When phasic activity occurred, the response was taken as the peak of each contraction. When vessels were precontracted with low concentrations of U46619, the contractile responses to the additional agonist were measured above the U46619-induced contraction and expressed as a percentage of the total KPSS contraction in the absence of U46619.

The individual contraction curves were fitted to the sigmoidal logistic equation, $Y = P_1 + P_2/[1 + e^{P3(\log X - P4)}]$, where X = agonist concentration, P_1 = lower plateau response, P_2 = range between the lower and the maximal plateau of the concentration-response curve, P_3 = a negative curvature index indicating the slope independently of the range and P_4 = log dose required to produce a half maximal response (EC_{50}) (Elghozi & Head, 1990). From this relationship, computer estimates of the concentrations required to give, 10, 30, 50, 70 and 90% (EC_{10} – EC_{90}) of the maximum response were determined. The individual EC_{50} values were averaged and the mean and standard error of the mean calculated.

Student's unpaired *t* tests were used to compare the EC_{50} and maximum values of the agonists in the absence and presence of U46619. For the comparison of passive vessel parameters and KPSS (see Table 1), a one-way analysis of variance was used to make multiple comparisons of EC_{50} values and maximum responses between the independent groups. When the ANOVA indicated that differences existed between all groups, Scheffe's test was applied to determine the source of variation (Wallenstein *et al.*, 1980). Statistical significance was accepted at $P<0.05$ for both tests and values are given as mean ± s.e.mean.

Results

A summary of vessel parameters grouped for hearts with different aetiologies is given in Table 1. The vessels obtained from ischaemic hearts were approximately 20% smaller in internal diameter than those from cardiomyopathic hearts. They also had lower transmural pressures, levels of resting force, levels of force developed 60 min following normalization and maximal contractions to KPSS (F_{max}) compared with the latter two groups (Table 1). When F_{max} was normalized for vessel diameter, no difference was noted between the ischaemic and cardiomyopathy groups (3.1 vs 3.4 g mm^{-1}, $P>0.05$). The normalized F_{max} value for the ischaemic group was significantly smaller than that obtained for unused donor hearts (3.1 vs 4.0 g mm^{-1}; $P<0.05$; see Table 1). In addition, normalization of rings from cardiomyopathic hearts in the presence of SNP to prevent development of phasic activity during this procedure resulted in a significant increase in internal diameter, resting force and, level of force 60 min after normalization (Table 1).

Phasic activity

In 6 of the 28 hearts, spontaneous phasic activity developed during the normalization procedure in 8% of all rings of artery (Fgiure 1). In 10 of the hearts, a further 5% of rings developed phasic contractions after the vessels had been set at their optimal resting passive force (Figure 1). This activity consisted of large all-or-none type rhythmical contractions each of which were maintained for 1–2 min. SNP (10 μM)

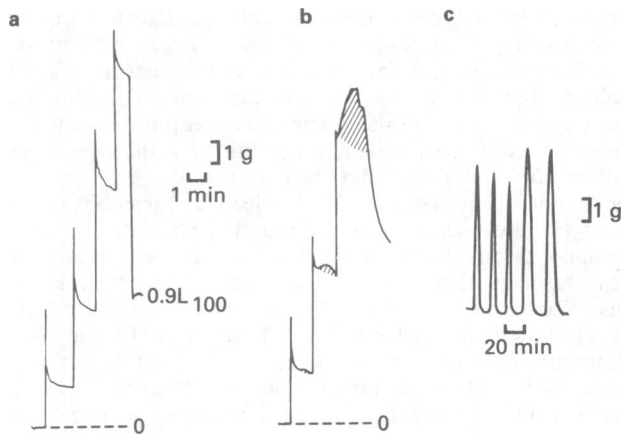

Figure 1 Original chart recordings depicting changes in passive and active isometric force during (a and b) and after (c) the stretch procedure at normalization. The shaded areas in (b) represent development of active force during the passive stretch procedure at normalization (see Methods). The artery in (c) shows a typical pattern of spontaneous phasic contractile activity following normalization. 0 represents zero force on the tissue prior to stretch. 0.9 L_{100} is the normalised internal circumference of each artery (see Methods).

Table 1 Human coronary artery – summary of initial vessel parameters at normalization and contraction in response to K^+ depolarization

Disease	*Number of patients*	*Number† of rings*	*D* (mm)	*P* (mmHg)	*Resting force* F_1(g)	*Force at 60 min* (g)	*KPSS* (F_{max}) (g)	F_{max}/*Diam* (g mm^{-1})
Cardiomyopathy	6	41	3.84 ± 0.15[1]	59.0 ± 1.3[1]	5.6 ± 0.3[1]	6.5 ± 0.5[1]	12.5 ± 0.5[1]	3.42 ± 0.17[1,2]
Ischaemic heart disease	6	55	3,03 ± 0.09[2,3]	53.4 ± 1.1*	4.3 ± 0.2[2]	4.6 ± 0.2[2]	9.3 ± 0.6[2]	3.09 ± 0.19[1]
Unused donor hearts	4	35	3.47 ± 0.12[1,2]	59.5 ± 0.7[1]	5.9 ± 0.3[1]	6.3 ± 0.3[1]	13.3 ± 0.8[1]	4.02 ± 0.32[2]
Cardiomyopathy (– SNP)	3	38	2.83 ± 0.11[3]	60.6 ± 1.4[1]	4.1 ± 0.3[2]	4.3 ± 0.3[2]	11.5 ± 0.7[1,2]	4.12 ± 0.24[2]

D is internal diameter at 0.9 L_{100} (see text). P is equivalent transmural pressure at 0.9 L_{100} (see text).
*Mean significantly different from means of all other groups ($P<0.05$, Scheffe's test).
Values marked with the same number are not significantly different ($P>0.05$, Scheffe's test).
†Minimum of 4 rings per heart.
– SNP: ring segments normalized in the absence of sodium nitroprusside.

was added prior to normalization in artery rings from 16 of the hearts in an attempt to inhibit this spontaneous phasic activity during the normalization procedure. It was successful in 92% of rings but of these, 33% then developed phasic contractions once sodium nitroprusside had been washed out. In 66% of all artery rings from 28 hearts, phasic contractions were also triggered by addition of constrictor agonists such as U46619, sumatriptan, 5-HT and noradrenaline. Generally then, vasoconstrictors increased the level of tonic, active force in the tissues until an apparent threshold level was reached at which point phasic activity was induced (Figure 2). Consequently, concentration-contraction curves with maintained plateaus could only be generated in rings from a small number of patients (see below).

Agonist responses in the absence of phasic activity

5-HT (0.001–30 μM), sumatriptan (0.003–30 μM), methysergide (0.003–30 μM), ergometrine (0.001–30 μM) and noradrenaline (0.001–30 μM) caused concentration-dependent contractions in the human coronary artery with an apparent order of potency of ergometrine > 5-HT > noradrenaline = methysergide > sumatriptan (Figure 3). Ergometrine and 5-HT were the most potent agonists tested with EC_{50} values of 7.7 ± 0.2 (− log M; 7 rings from 3 patients) and 6.8 ± 0.2 (− log M; 7 rings from 3 patients) respectively. In this subgroup of rings, 5-HT also generated the highest maximal response of 40 ± 10% KPSS. The remaining agonists studied were weak constrictors. Ergometrine contracted the vessels to 7–21% of the maximum contraction to KPSS and noradrenaline, in the presence of propranolol (1 μM), generated maximal responses ranging from 3–18% KPSS. Sumatriptan and methysergide either failed to contract the vessels or contracted them to less than 5% of the maximum KPSS contraction (see Table 2 and Figures 3 and 4).

Phasic contractions induced by agonists

In most rings of artery that did not develop phasic activity after normalization, 5-HT and the less efficacious agonists like sumatriptan, ergometrine and methysergide were able to cause marked contraction of the coronary artery due to their induction of phasic activity. For example, 5-HT and sumatriptan generated peak phasic responses of 82 ± 7% KPSS (12 rings, 6 patients) and 71 ± 18% KPSS (6 rings, 3 patients) respectively (see Figure 2). In one patient, methysergide and ergometrine caused maximum phasic contractions of 75% and 78% KPSS respectively and in a further two patients, noradrenaline generated maximal phasic contractions of 42% and 75% KPSS.

Nifedipine abolished the phasic contractions that developed following normalization (data not shown) and in response to exogenously applied constrictor agonists. Thus, in the presence of nifedipine, agonists like 5-HT always caused concentration-dependent contractions with maintained plateaus.

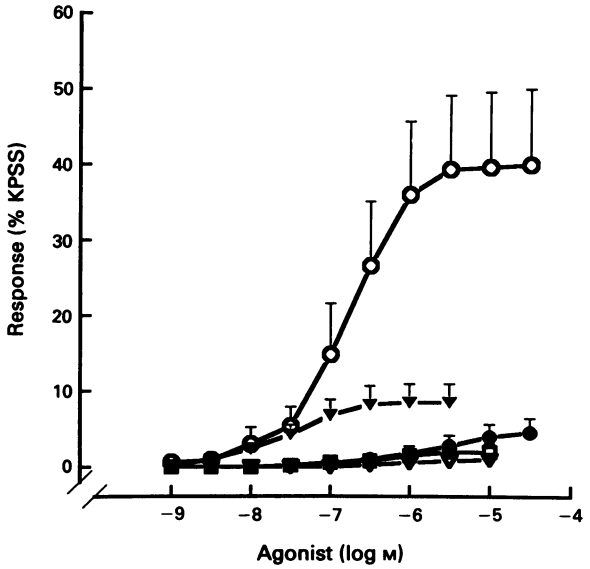

Figure 3 Concentration-contraction curves to 5-hydroxytryptamine (5-HT, ○, $n = 7$), sumatriptan (▽, $n = 7$), methysergide (●, $n = 5$), ergometrine (▼, $n = 7$) and noradrenaline (□, $n = 4$) in isolated rings of human coronary artery that did not develop phasic contractile activity either spontaneously or in response to the added agonists. Values are mean ± 1 s.e.mean and are normalized to the maximal contraction to KPSS.

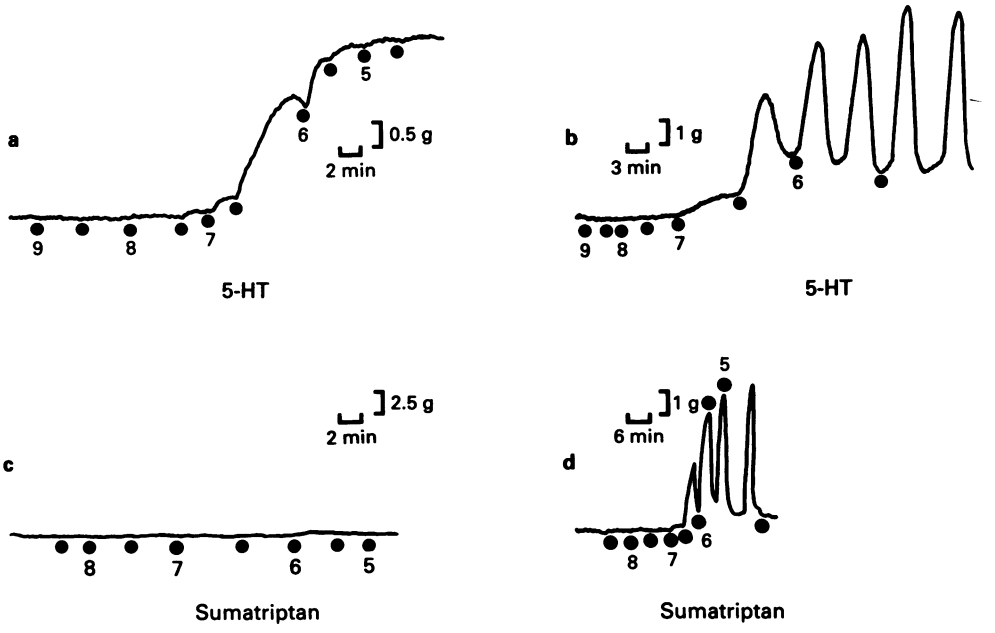

Figure 2 Representative chart recordings from four rings of coronary artery from the same patient. Rings were exposed to cumulative concentrations (− log M) of 5-hydroxytryptamine (5-HT) (a and b) and sumatriptan (c and d). Traces on the right (b and d) developed phasic contractile activity during the addition of each agonist.

Agonist responses in the presence of U46619

U46619 (1 nM) caused a contraction of 3.8 ± 1.1% KPSS (24 rings from 12 patients) in arteries that did not develop phasic contractile activity in response to constrictor agonists. Under these conditions, the maximal contraction responses to 5-HT, sumatriptan, ergometrine and methysergide were markedly increased (Figure 4). A quantitative comparative study was conducted to examine the effect of U46619 on responses to 5-HT and sumatriptan in these rings without phasic activity. U46619 (1 nM) generated a contraction of 6.6 ± 2.4% KPSS (6 rings, 3 patients) and caused a significant increase ($P < 0.05$, Student's unpaired t test) in the maximum contraction response to 5-HT from 40.2 ± 10% KPSS (7 rings, 3 patients) to 92.3 ± 4% KPSS (3 rings, 3 patients; see Figure 5 and Table 2). The maximum response to sumatriptan was also significantly increased from 4.6 ± 1.9% KPSS (10 rings, 5 patients) to 48.5 ± 14% KPSS (3 rings, 3 patients) in the presence of U46619 (Figure 5 and Table 2). Sensitivity to 5-HT was unaffected by U46619 (see Table 2).

In the presence of nifedipine (0.1 μM), U46619 (1.0 nM) caused a contraction of 2.6 ± 0.7% KPSS (7 rings, 3 patients) that was not significantly different from the group not treated with nifedipine (Table 2). Under these conditions U46619 increased significantly the maximum contractions to 5-HT from 22.8 ± 6.4% KPSS (8 rings, 4 patients) to 49.0 ± 6.5% KPSS (3 rings, 3 patients) and sumatriptan from 2.5 ± 1.1% KPSS (6 rings, 3 patients) to 11.4 ± 3.5% KPSS (4 rings 3 patients; see Figure 5 and Table 2). No change in sensitivity to either agonist was observed in the presence of U46619 and nifedipine as compared with U46619 alone (Table 2).

Discussion

The main finding from this study was that in the presence of U46619, human epicardial coronary arteries *in vitro* showed a marked increase in contractility to both 5-HT and the selective 5-HT_1-like receptor agonist, sumatriptan. This synergistic

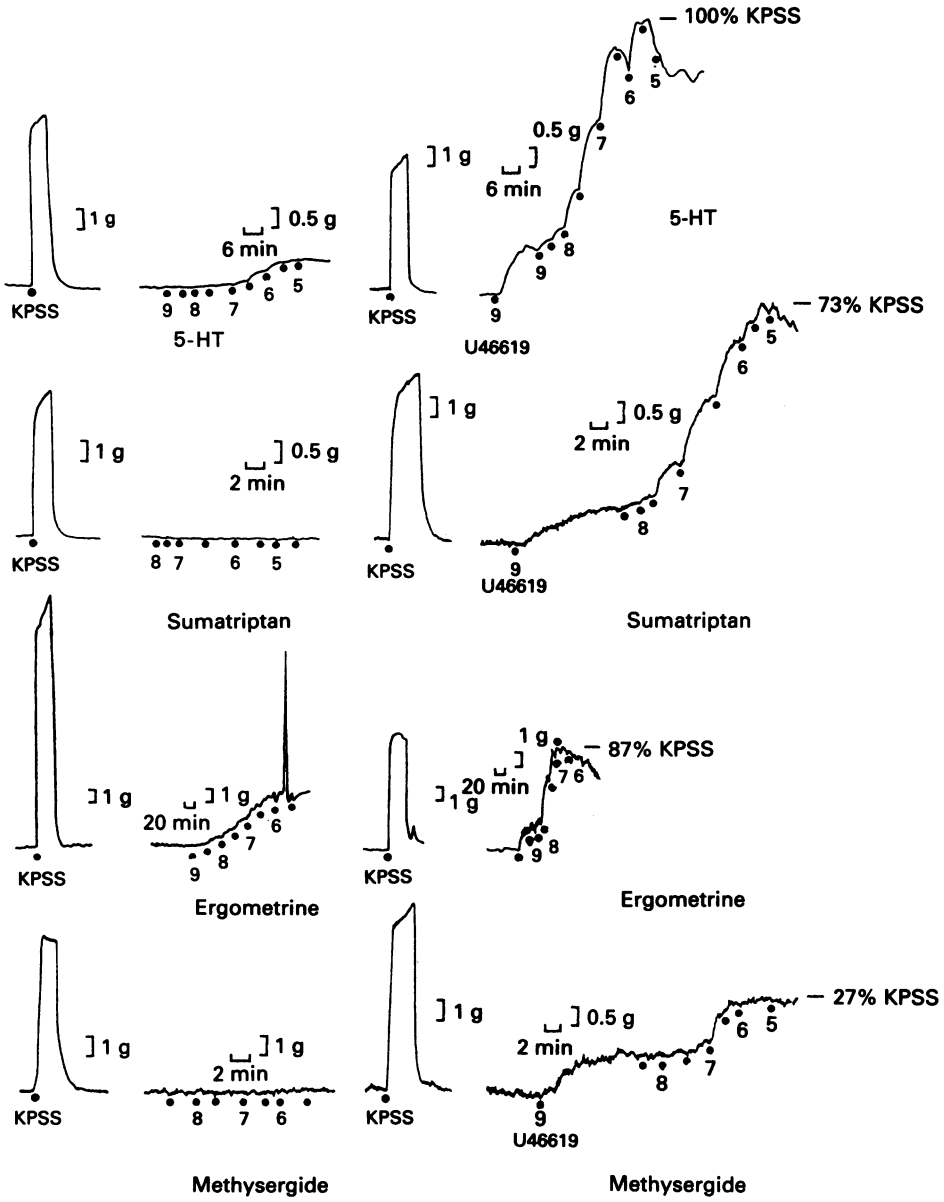

Figure 4 Original chart recordings from rings of coronary artery from a single patient showing synergistic effects of U46619 (right panels) on contractions to cumulative additions (− log M) of 5-hydroxytryptamine (5-HT), sumatriptan, ergometrine and methysergide (left panels) in rings of artery without phasic activity (except note the one phasic contraction to ergometrine in the absence of U46619). Maximal contractions to each agent were compared to the KPSS maximum in each case. Note the gain change in some of the traces in the presence of U46619 (right panels).

interaction did not appear to be specific for either sumatriptan or 5-HT_1-like receptors since contractions to 5-HT, ergometrine, and methysergide were also potentiated by U46619. Nor was the synergy likely to have been specific for the thromboxane A_2 analogue (U46619) as the agonist used to induce threshold levels of active force, since Yang *et al.* (1990) showed that threshold concentrations of endothelin-1 (Yanagisawa *et al.*, 1988) enhanced the contractions to noradrenaline and 5-HT in human isolated coronary and internal mammary arteries. Indeed there are numerous reports in the literature where subthreshold or threshold concentrations of one constrictor agonist can readily amplify the response to a second agent. For example in rabbit femoral artery, MacLennan & Martin (1992) reported anecdotally that both histamine and angiotensin II amplified the contraction to 5-HT, and in rat mesenteric small arteries contraction to sympathetic nerve stimulation was enhanced by as much as 500% by threshold concentrations of vasopressin, neuropeptide Y, endothelin-1 and methoxamine (Lew & Angus, 1992).

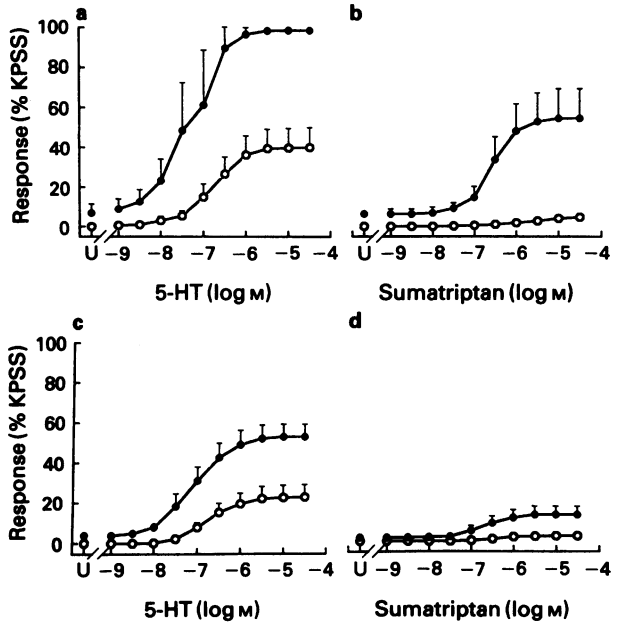

Figure 5 Effects of nifedipine (0.1 μM) on the synergism between U46619 (U: 1 nM) and 5-hydroxytryptamine (5-HT) or sumatriptan in isolated rings of human coronary artery: (○) absence and (●) presence of U46619. Cumulative contraction curves to 5-HT and sumatriptan were constructed in the absence (a, b) and presence (c,d) of nifedipine. All contraction curves in the absence of nifedipine were from artery rings without phasic activity. Values are means ± 1 s.e.mean and are normalized to the tissues' maximal contraction to KPSS.

In the absence of both U46619-induced tone and phasic contractile activity (see below), we found similar results as those reported by Connor *et al.* (1989b) and Chester *et al.* (1990) who found 5-HT to be more potent and cause significantly greater contractions compared with sumatriptan in human, isolated, coronary arteries. Unlike these studies, we did not attempt to determine the EC_{50} for sumatriptan in vessels without any U46619-induced tone because of the poor contractions generated under these conditions. With tone increased with U46619, however, the potency of 5-HT was unaltered and that to sumatriptan was approximately an order of magnitude less than that for 5-HT in the presence of U46619 which agrees with previous studies (Connor *et al.*, 1989b). Although our studies were not performed in the presence of ketanserin, other studies have shown that 5-HT activates both 5-HT_2 and 5-HT_1-like receptors (see Connor *et al.*, 1989b; Chester *et al.*, 1990) and sumatriptan 5-HT_1-like receptors only (Chester *et al.*, 1990).

Whilst the synergy observed between U46619 and 5-HT receptor agonists may not be specific for these two classes of constrictor agents, our results highlight the importance of vascular tone when comparing absolute contractions to vasoconstrictor agonists with apparently different efficacies. Martin *et al.* (1986) also pointed this out in the rabbit isolated aorta where they showed that removal of the endothelium resulted in a much greater enhancement of contractions to partial as compared to full adrenoceptor agonists, presumably due to removal of basal EDRF release (see Moncada *et al.*, 1991). We used the thromboxane A_2-mimetic, U46619, to increase the level of tone since not only is thromboxane A_2 released together with 5-HT and other compounds upon activation of platelets (Golino *et al.*, 1989) but it has also been reported to potentiate contractions to 5-HT in a variety of isolated blood vessels including the rabbit femoral artery (MacLennan & Martin, 1992), guinea-pig iliac artery (Sahin-Erdemli *et al.*, 1991) and human digital arteries (Young *et al.*, 1986). Also, thromboxane A_2 and 5-HT have been postulated to be the mediators of coronary vasoconstriction triggered by local platelet activation in the dog *in vivo* (Golino *et al.*, 1989) and in man (Zeiher *et al.*, 1991b). Furthermore, Ashton *et al.* (1987) found that thromboxane A_2 and 5-HT acted cooperatively to induce cyclic flow variations in anaesthetized dogs with severe coronary artery stenosis and Quillen *et al.* (1991) found that long-term cholesterol feeding in adult cynomolgus monkeys caused coronary artery hyperreactivity to both U46619 and 5-HT. Therefore, the *in vitro* conditions of assay used in our study may represent conditions that in part occur within the artery wall *in vivo*.

Table 2 Sensitivity and maximum contractions to 5-hydroxytryptamine (5-HT) and sumatriptan in human coronary artery in the absence and presence of U46619

Agonist	*U46619* (nM)	*Δ U46619* (% KPSS)	n	*EC_{50} value* (− log M)	*Maximum response* (% KPSS)
5-HT	0	–	7	6.80 ± 0.17	40.2 ± 10.1
	1	6.8 ± 4.6	3	7.41 ± 0.26	92.3 ± 4.0*
5-HT +	0	–	8	6.67 ± 0.10	22.8 ± 6.4
nifedipine (0.1 μM)	1	3.8 ± 0.4	3	7.08 ± 0.16	49.0 ± 6.5*
Sumatriptan	0	–	10	†	4.6 ± 1.9
	1	6.3 ± 1.8	3	6.51 ± 0.09	48.5 ± 13.9*
Sumatriptan +	0	–	6	†	2.5 ± 1.1
nifedipine (0.1 μM)	1	1.7 ± 0.9	4	6.65 ± 0.11	11.4 ± 3.5*

n = number of rings; values are given as mean ± s.e.mean.
*Mean significantly different from control ($P<0.05$, Student's unpaired t test)
†The EC_{50} value for sumatriptan was not calculated in the absence of U46619 as the maximum contraction to the agonist was less than 5% KPSS.
ΔU46619: increase in force to U46619 (1 nM) expressed as a % of the contraction to KPSS.

Other local and circulating substances may also contribute to the synergy observed here between 5-HT receptor agonists and U46619. These include endothelin-1, vasopressin, angiotensin II, kinins and the level of neuronal activity, which for large arteries as those considered here, would be expected to be minimal. Also, the endothelium's ability to release endothelium-derived relaxing factors like prostacyclin and nitric oxide (see Moncada *et al.*, 1991) is another important consideration not only in terms of the inhibitory effects of these dilators on constrictor activity, but also in terms of their antithrombotic effects since both factors themselves synergize to inhibit platelet aggregation (see Moncada *et al.*, 1991). Conditions that have been reported to enhance reactivity of human coronary arteries both *in vitro* and *in vivo* include the presence and location of atherosclerotic lesions (Golino *et al.*, 1991; McFadden *et al.*, 1991), intracoronary thrombus formation (Zeiher *et al.*, 1991b) and endothelial cell dysfunction (Berkenboom *et al.*, 1991; Drexler & Zeiher, 1991; Zeiher *et al.*, 1990a).

The human coronary artery exhibited a tendency to develop large all-or-none type phasic contractions either spontaneously or in response to vasoconstrictors such as U46619, 5-HT and sumatriptan (see also Golenhofen, 1978; Ross *et al.*, 1980; Kalsner, 1985; Kimura *et al.*, 1989) although Connor *et al.* (1989b) and Chester *et al.* (1990) did not report this type of activity. Whilst the reasons for the discrepancy between the studies is unclear, the type and duration of the patients' drug therapy prior to transplantation may be important. Drugs prescribed to patients in this study included diuretics, anticoagulants, cardiac glycosides, angiotensin converting enzyme inhibitors and antiarrhythmic drugs. It was noted, however, that the cardiomyopathic, ischaemic and unused donor hearts exhibited the same trends and degree of reactivity and were therefore comparable.

The inhibition of phasic contractile activity by nifedipine in this study suggests voltage-operated Ca^{2+} channels may be involved both in the spontaneous and agonist-induced phasic activity. Nifedipine also reduced the tonic maximum contractions to 5-HT and sumatriptan. Sumner *et al.* (1992) also showed a similar depression of maximum contractions to 5-HT and sumatriptan in the dog isolated saphenous vein, a tissue that is known to contract sensitively and maximally to sumatriptan (Humphrey *et al.*, 1988). Interestingly, the tonic contractions to other constrictor agonists like U46619 and endothelin-1 in the human coronary artery were unaffected by nifedipine (Cocks & Stork, unpublished data). This is an important point, given that the ability to demonstrate synergy between U46619 and 5-HT in the presence of nifedipine relied on the tonic response to U46619 being unaffected.

A possible explanation for the synergy between 5-HT receptor agonists and U46619 may be due to the absence or dysfunction of the endothelium, since earlier studies in pig and dog coronary arteries indicated that contractile responses to 5-HT and noradrenaline are enhanced in the absence of endothelium (Cocks & Angus, 1983; Cohen *et al.*, 1983). This is unlikely, however, as histological examination of selected vessels revealed no disruption of endothelium integrity and endothelium-dependent vasodilators such as substance P and histamine caused potent relaxation (Cocks & Kemp, unpublished data). A more likely explanation is that U46619 facilitates the transducer mechanisms of these agonists. Angus & Brazenor (1983) found that the constrictor response to U46619 in dog isolated coronary arteries, were not altered by the Ca^{2+}-entry blockers, nifedipine, D600 and verapamil, therefore suggesting that the contractions generated by U46619 are largely dependent on release of intracellular calcium. In addition, Young *et al.* (1986), have shown that the potentiation of the contractile effect of 5-HT by U46619 in human digital arteries was a result of increased mobilization of intracellular calcium stores. In the present study, low concentrations of U46619 did not always cause contraction yet in all cases contractions to other agonists were potentiated. Release of intracellular calcium may account for these observations. Thus, in the human coronary artery there may be a threshold level of calcium at which contractions are triggered. Pretreatment with concentrations of U46619 that failed to cause contraction, could bring the cellular levels of calcium closer to threshold, such that other agonists now cause release of more intracellular Ca^{2+} from the same or different stores or influx of extracellular Ca^{2+} through receptor or voltage-operated channels. Even with concentrations of U46619 that caused significant contractions, the increase in reactivity to 5-HT and sumatriptan probably reflected sensitization to further increases in intracellular calcium. Thus, in the rabbit femoral artery, MacLennan & Martin (1992) showed marked potentiation of the contractions to 5-HT in the presence of 5-HT_2 receptor blockade even when U46619 itself caused a large contraction.

An important question that arises from the present study relates to the possible clinical relevance of coronary 5-HT_1-like receptors, particularly in instances of advanced atherosclerosis and vasospastic disease. McFadden *et al.* (1992) showed that in patients with variant angina where 5-HT induced spasm at the sites of stenoses as well as in patients with stable angina where 5-HT constricted the distal epicardial coronary arteries, these responses to 5-HT were resistance to ketanserin. While McFadden *et al.* (1992) discussed the limitations of their study, they speculated that more direct evidence for a role of 5-HT_1-like receptors in coronary vasospasm may be determined by the use of selective 5-HT_1-like agonists such as sumatriptan. In fact, a recent study in man found that intravenous infusion of sumatriptan significantly reduced coronary artery diameter (MacIntyre *et al.*, 1992). This issue, however, will be more convincingly resolved with the development of a selective 5-HT_1-like antagonist. Nevertheless, there is a case report of a patient with migraine who, in response to subcutaneous sumatriptan, developed severe chest pain and ECG changes (marked ST elevation) characteristic of coronary vasospasm (Willett *et al.*, 1992). In addition, there have been two case reports of ventricular arrhythmias (Curtin *et al.*, 1992) associated with subcutaneous sumatriptan. These isolated reports, however, should be considered against the international post marketing experience of treating 3 million attacks of migraine. Nevertheless, in their replies to these case reports, the company has acknowledged that there are circumstances where sumatriptan should be avoided (Castle & Simmons, 1992) and emphasised that sumatriptan should be contraindicated in patients with ischaemic heart disease and related cardiac disorders (Pilgrim *et al.*, 1992).

In conclusion, our study demonstrates that inherent active force or tone as well as receptor populations are crucial in determining the reactivity to vasoconstrictor agents in human coronary artery *in vitro*. It also highlights the importance of *in vitro* assay conditions when comparing contractile activities of agonists with different apparent efficacies (see also Martin *et al.*, 1986; MacLennan & Martin, 1992). Thus, given appropriate conditions, 5-HT_1-like receptor agonists like sumatriptan can cause relatively potent and powerful contractions in human large coronary arteries *in vitro*.

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