British Journal of Pharmacology (1997) 120, 1039–1048

Mechanism of actions of sumatriptan on coronary flow before and after endothelial dysfunction in guinea-pig isolated heart

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1 The mechanism of action of sumatriptan on coronary flow was examined before and after two different forms of endothelial ablation in guinea-pig isolated hearts. The mechanism was assessed in terms of the influence of the integrity of the coronary endothelium, the role of release of nitric oxide (NO) from the endothelium, and the receptor subtypes mediating the effects.

2 Continuous perfusion with sumatriptan reduced coronary flow, but the concentration-response curve was v-shaped. Sumatriptan (0.001–0.1 μM) caused a concentration-dependent decrease in coronary flow with the maximum effect achieved at 0.23 ± 0.04 μM. The pEC_{50} was 8.49 ± 0.07. At higher concentrations (0.1–10 μM) there was a concentration-dependent diminution of the vasoconstrictor effect. Endothelial ablation by saponin removed the diminution in the vasoconstrictor effect. In contrast, pretreatment with N^G-nitro L-arginine methyl ester (L-NAME) (100 μM, 45 min perfusion) did not affect it. This was despite both saponin and L-NAME being effective in reducing basal release of NO into the coronary effluent (measured by chemiluminescence) to the same extent (71 ± 3 and 73 ± 2%, respectively).

3 GR127935, a selective 5-hydroxytryptamine$_{1D}$ (5-HT$_{1D}$) receptor antagonist (3 and 10 nM), which by itself had no effect on coronary flow or NO release, antagonized the vasoconstrictor response to sumatriptan and unmasked a sumatriptan-induced concentration-dependent increase in coronary flow and NO release. These increases in coronary flow and NO release were abolished by pretreatment with either saponin or L-NAME.

4 Mesulergine, a 5-HT$_2$ receptor antagonist which had no effect by itself on basal coronary flow or NO release, inhibited the vasodilator response to sumatriptan that occurred in the presence of GR127935, and actually enhanced the vasoconstrictor response, increasing the maximum fall in coronary flow from -3.9 ± 0.4 to -5.2 ± 0.4 ml min^{-1} g^{-1} ($P < 0.05$). The diminution of vasoconstrictor effect of sumatriptan was abolished by mesulergine and by pretreatment with saponin, but not by L-NAME.

5 In conclusion, guinea-pig coronary arteries constrict to low concentrations of sumatriptan, causing a reduction in coronary flow. This effect appears to be caused by 5-HT$_{1D}$ agonism with the receptors located on the coronary vascular smooth muscle. With higher concentrations of sumatriptan this is partially offset by a weaker vasodilator effect, which is caused by low affinity 5-HT$_2$ agonism. Although this effect is endothelium-dependent, it is not caused by the release of NO. Interestingly, when the vasoconstrictor effect of sumatriptan was inhibited by the 5-HT$_{1D}$ antagonist GR127935, a high affinity vasodilator effect of sumatriptan was unmasked. This is 5-HT$_2$ receptor mediated and is caused by release of NO from the coronary endothelium.

6 In man, sumatriptan and 5-HT may both be capable of causing pathogenic coronary vasoconstriction. The implications of the present data are that the scope for this may depend greatly on (i) the extent of underlying endothelial dysfunction, (ii) the extent of endothelial 5-HT$_2$ receptor-mediated release of vasodilator autacoids (which include NO) and (iii) the extent of smooth muscle 5-HT$_{1D}$ receptor-mediated vasoconstriction.

Keywords: 5-HT$_{1D}$ receptor; 5-HT$_2$ receptor; coronary flow; endothelial ablation; GR127935; mesulergine; nitric oxide, sumatriptan

Introduction

5-Hydroxytryptamine (5-HT) released by platelets has been suggested to play a role in coronary artery spasm associated with thrombosis (Golino *et al*., 1989; van den Berg *et al*., 1989; Noble & Drake-Holland 1990; Bax *et al*., 1993). 5-HT may constrict human atherosclerotic coronary arteries (McFadden *et al*., 1991; Golino *et al*., 1991) and may precipitate variant angina and myocardial infarction (Hillis & Lange 1991). On the other hand, 5-HT may dilate healthy coronary arteries (Golino *et al*., 1991). Chester *et al*. (1993) demonstrated the conversion of a mild coronary dilatation in response to an intracoronary injection of 5-HT in healthy coronary arteries to a vasoconstriction in patients with atherosclerosis and clinical symptoms of coronary artery disease. Constriction of human coronary arteries has been suggested to occur via the activation of 5-HT$_2$ receptors (Connor *et al*., 1989; Toda & Okomura, 1990). However, the pathogenic relevance of this is questionable since the specific 5-HT$_2$ antagonist, ketanserin, is not effective in the treatment of angina (De Caterina *et al*., 1984; Freedman *et al*., 1984). A ketanserin-resistant 5-HT-induced constriction occurs in human coronary arteries *in vivo* (McFadden *et al*., 1992) and *in vitro* (Kaumann & Brown, 1990), occurring as a consequence of 5-HT$_1$-like agonism ('like' denotes that the receptor subtype is uncertain). In view of these observations, Chester *et al*. (1993) suggested that the relative importance of 5-HT$_2$ and 5-HT$_1$-like receptors in mediating 5-HT-induced coronary constriction may change in coronary artery disease.

In addition to the unresolved role of endogenous 5-HT as mediator of pathogenic coronary vasoconstriction, 5-HT receptor-mediated coronary vasospasm has potential relevance to the safety of receptor type- and subtype-selective synthetic 5-hydroxytryptamine mimetics. The anti-migraine drug su-

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matriptan, a relatively selective 5-HT_{1D} agonist (Humphrey *et al*., 1988; Peroutka & McCarthy, 1989) has been shown to reduce coronary artery diameter during angioplasty (MacIntyre *et al*., 1993), and can cause chest pain and cardiac ischaemia (Willett *et al*., 1992) and even myocardial infarction (Ottervanger *et al*., 1993). Sumatriptan constricts human coronary arteries *in vitro* (Bax *et al*., 1993; Cocks *et al*., 1993; Kaumann *et al*., 1993) and *in vivo* (MacIntyre *et al*., 1992), constricts dog saphenous vein *in vitro* (Humphrey *et al*., 1988) and constricts dog coronary arteries *in vitro* (Parsons *et al*., 1992) and *in vivo* (Fenuik *et al*., 1989; Saxena & Villalon 1990; Humphrey *et al*., 1991). It also constricts guinea-pig iliac artery (Sahin-Erdemli *et al*., 1991; Schoeffter & Sahin-Erdemli, 1992) and rabbit coronary artery (Feletou *et al*., 1994). In contrast, pig coronary arteries and guinea-pig jugular veins relax to sumatriptan via an endothelium-dependent mechanism (Schoeffter & Hoyer, 1990; Gupta, 1992). The cardiac safety of receptor type- and subtype-specific 5-HT mimetics will depend on the scope for receptor type- and subtype-dependent coronary constriction (i.e., the magnitude of the response, its dependence on endothelial integrity, and its receptor subtype dependence). These issues remain contentious.

The demonstration of 5-HT-induced vasoconstriction or vasodilatation in isolated vascular tissue is not necessarily predictive of the response *in vivo* (Cambridge *et al*., 1995). Moreover, 5-HT receptor-mediated responses show a remarkable degree of heterogeneity both between species and between different tissues within the same species. For example, in cat heart, arteries larger than 90 μm constrict to 5-HT, whereas those smaller than 90 μm dilate to 5-HT (Lamping *et al*., 1989). In the rabbit microcirculation the 5-HT_2 receptors appear to be most important in mediating vasoconstriction, whereas in large arteries 5-HT_1-like receptors appear to be more important (Feletou *et al*., 1994).

We have previously shown an increase in coronary flow in guinea-pig isolated heart (endothelium intact) in response to 5-HT. This vasodilatation was abolished, although not converted to a vasoconstriction, by endothelial denudation with saponin (Ellwood & Curtis, 1996a). In view of the need to characterize further the vascular pharmacology of the 5-HT receptor-response system in guinea-pig coronaries, and in order to examine the determinants and mechanism of action of sumatriptan on coronary flow, the aim of the present study was to investigate the effect of endothelial denudation and NO synthase inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) on the responses to sumatriptan in guinea-pig perfused, isolated hearts. The selective 5-HT_{1D} antagonist GR127935 (Skingle *et al*., 1993) and the 5-HT_2 receptor antagonist mesulergine (Closse, 1983; Pazos *et al*., 1985) were used as tools to probe the receptor types responsible for the complex actions of sumatriptan.

Some of the present findings have been presented to the British Pharmacological Society (Ellwood & Curtis, 1996b, c).

Methods

All experiments were performed in accordance with the United Kingdom Home Office 'Guide on the Operation of the Animals (Scientific Procedures) Act 1986'. Male Dunkin Hartley guinea-pigs (Charles River, Kent, 350–400 g) were terminally anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and treated with heparin (sodium heparin, 250 iu, i.p.). The chests were opened and the hearts quickly excised and placed in ice-cold perfusion solution (constituents below). The hearts were cannulated via the aorta (Langendorff mode) within 90 s of removal. Hearts were perfused under constant pressure (100 cmH_2O) with modified Krebs solution containing (mM): glucose 11.1, $CaCl_2$ 1.4, NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2 and KCl 4.0. This was made with ultra pure water (reverse osmosis, Milli RO-50, Watford, U.K.) and was vacuum filtered (5 micron filter paper, Millipore, Watford, U.K.) to remove particulate coronary spasmogens. The modified Krebs solution was gassed with 95% O_2 and 5% CO_2, pH 7.4 and delivered at 37°C. To eliminate any effect that change in heart rate may have on coronary flow, hearts were paced via the left ventricle (stimulus pulse width 0.5 m s^{-1}) at a rate of 275 beats min^{-1}, by a Harvard student stimulator (Edenbridge, U.K.). Heart rate was verified from a unipolar electrogram with one electrode placed in the left ventricle and a second attached to the aortic cannula. This was recorded on a Grass polygraph (model RPS 7C8, Quincy, MA, U.S.A.).

Coronary flow measurement

Coronary flow was measured either by weighing (1 ml = 1 g) coronary effluent with an Ohaus balance (Cambridge, U.K.), accurate to 1 mg (Rees & Curtis, 1993), following timed collection of the effluent, or by the use of an in-line ultrasonic flow probe (Park *et al*., 1992; Transonic Systems, Diss, U.K.). Three consecutive measurements were taken during the last 90 s of drug perfusion and the mean was calculated. Coronary flow increases and decreases, calculated first as positive or negative ml min^{-1} g^{-1} wet weight of the ventricle, were expressed as positive or negative % changes from baseline (%Δ coronary flow).

Following cannulation the hearts were allowed to stabilize for 30 min or until 3 consecutive coronary flow measurements were within 0.4 ml min^{-1}. The study protocol was then commenced. Hearts were excluded from analysis and were replaced if the coronary flow was less than 8 ml g^{-1} min^{-1} at the beginning of the study protocol. All agonists were delivered continuously by perfusion for a set time period (6 min) and cumulative concentration-response curves were constructed by switching between separate perfusion reservoirs.

Endothelial denudation and NO inhibition

The first groups of hearts were perfused with L-NAME, saponin or neither. For L-NAME, 100 μM was delivered for 45 min ($n = 24$). This concentration and duration of perfusion have previously been shown to inhibit substantially the basal release of NO (Pabla & Curtis, 1995). For saponin, 30 μg ml^{-1} was delivered in three cycles of 2 min followed by 2 min Krebs perfusion, followed by a 33 min control perfusion ($n = 24$). This protocol has been shown to reduce substantially the basal release of NO and abolish agonist-induced endothelium-mediated coronary dilatation and NO release without affecting endothelium independent constriction or dilatation in this preparation (Ellwood & Curtis, 1996a; Wiest *et al*., 1989). In hearts perfused with neither L-NAME nor saponin, a time matched perfusion of Krebs solution was delivered for 45 min ($n = 39$). The adequacy of the L-NAME and saponin protocols was tested by 5 min perfusion with either 1 μM ACh or 10 nM substance P (Furchgott, 1983), and hearts were excluded from the study if they exhibited an increase in coronary flow.

All hearts were then perfused for 15 min with one of the following: vehicle (Krebs solution, $n = 39$), 3 nM GR127935 ($n = 13$), 10 nM GR127935 ($n = 36$), 3 μM mesulergine ($n = 18$) or both 3 μM mesulergine and GR127935 (3 nM, $n = 6$ or 10 nM, $n = 18$). In the continued presence of these solutions, the hearts then received incremental concentrations of sumatriptan (0.1 nM to 10 μM). A total of 87 hearts was used (with 4 exclusions).

To test for time-dependent run down in the absence of L-NAME or saponin, time-matched controls ($n = 5$) received vehicle over the same time course (153 min). To test for non-specific effects of 5-HT antagonists, hearts were perfused with 10 nM GR127935 ($n = 6$) or 3 μM mesulergine ($n = 6$) for 153 min, in the absence of L-NAME, saponin or sumatriptan.

Measurement of NO release by chemiluminescence

The NO released from the guinea-pig isolated hearts was measured by the method described by Menon *et al*. (1989), with a Sievers NO analyser (model number 270B, Dyson

Instruments, Hetton, U.K.). It has been used successfully in isolated perfused hearts in our laboratory (Pabla & Curtis, 1995). This method is based on a gas phase chemiluminescence reaction between NO and ozone:

$$NO + O_3 \rightarrow NO_2 + O_2 + h\surd$$

The chemiluminescence was detected by a photomultiplier. The output was processed and recorded by a Mac Lab computer (model 2E, AD Instruments, Hastings, U.K.). A 1 ml aliquot of coronary effluent collected during the last 90 s of drug perfusion was decanted into a plastic Eppendorf tube (BDH Laboratory supplies, Dagenham, U.K.) and immediately frozen in liquid nitrogen, and stored at $-20^{\circ}C$. Subsequently the samples were thawed and analysed. The NO in the coronary effluent undergoes autoxidation to nitrite (Kelm & Schrader, 1990), so the samples were injected into a purge vessel containing a reducing solution (1% potassium iodide and glacial acetic acid). An inert gas (helium) was used to flush the NO under reduced pressure into the NO analyser. The NO content of the samples was calculated by using a standard curve derived from reducing known quantities of sodium nitrite in the purge vessel. Krebs was used to make up the standards to allow for any contaminant nitrite present in the chemical constituents of the perfusion solution. NO content was expressed as pmol g^{-1} min^{-1} wet weight of perfused cardiac tissue (Pabla & Curtis, 1995).

Drugs

Saponin and L-NAME were obtained from Sigma Chemicals, Poole, U.K. Sumatriptan, mesulergine and N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide (GR127935) were gifts from Pfizer Research Ltd. (Sandwich, U.K.).

Data handling and statistics

Concentration-response curves were constructed for individual preparations and, from each, the maximum response, concentration evoking the maximum response and EC_{50} values were read off. Group mean ± s.e.mean were calculated from these values and it is these that have been plotted in the figures. Comparisons were made by an ANOVA, followed by an unpaired *t*-test, modified by Dunnett's correction for multiple comparisons where appropriate. A P value < 0.05 was considered to be statistically significant.

Results

Coronary flow and NO release during drug free perfusion

There was a small time-dependent decline in coronary flow and coronary effluent NO content in the time matched (drug-free perfusion) control hearts. Since this was small compared to drug effects (see below) it has not been taken into consideration when determining drug effects. At time zero (equivalent to the start of the agonist protocol) the mean coronary flow and NO were 11.4 ± 0.2 ml g^{-1} min^{-1} and 638 ± 32 pmol g^{-1} min^{-1}, respectively. At 153 min (equivalent to the end of each experiment) coronary flow and NO release were 10.3 ± 0.2 ml g^{-1} min^{-1} and 471 ± 29 pmol g^{-1} min^{-1}, respectively.

The effect of mesulergine and GR127935 on coronary flow and NO release in the absence of sumatriptan

Continuous delivery of 3 μM mesulergine or 10 nM GR127935 had no effect on basal coronary flow or basal NO release, indicating that these 5-HT antagonists were devoid of non-specific activity in the preparation. In these groups there was a time-dependent fall in coronary flow and NO release which was similar to that seen in time-matched drug-free control hearts (data not shown).

The effect of sumatriptan on coronary flow and NO release

The action of sumatriptan on coronary flow was v-shaped (Figure 1a). At up to 0.1 μM, there was a concentration-dependent decrease in coronary flow. The concentration producing the maximum constrictor response was 0.13 ± 0.04 μM, and the pEC_{50} was 8.49 ± 0.07. At concentrations above 0.1 μM, the decrease in coronary flow diminished (Figure 1a). Thus sumatriptan possessed a high affinity vasoconstrictor action and a lower affinity vasodilator action, with constriction predominating. Neither of these effects was accompanied by

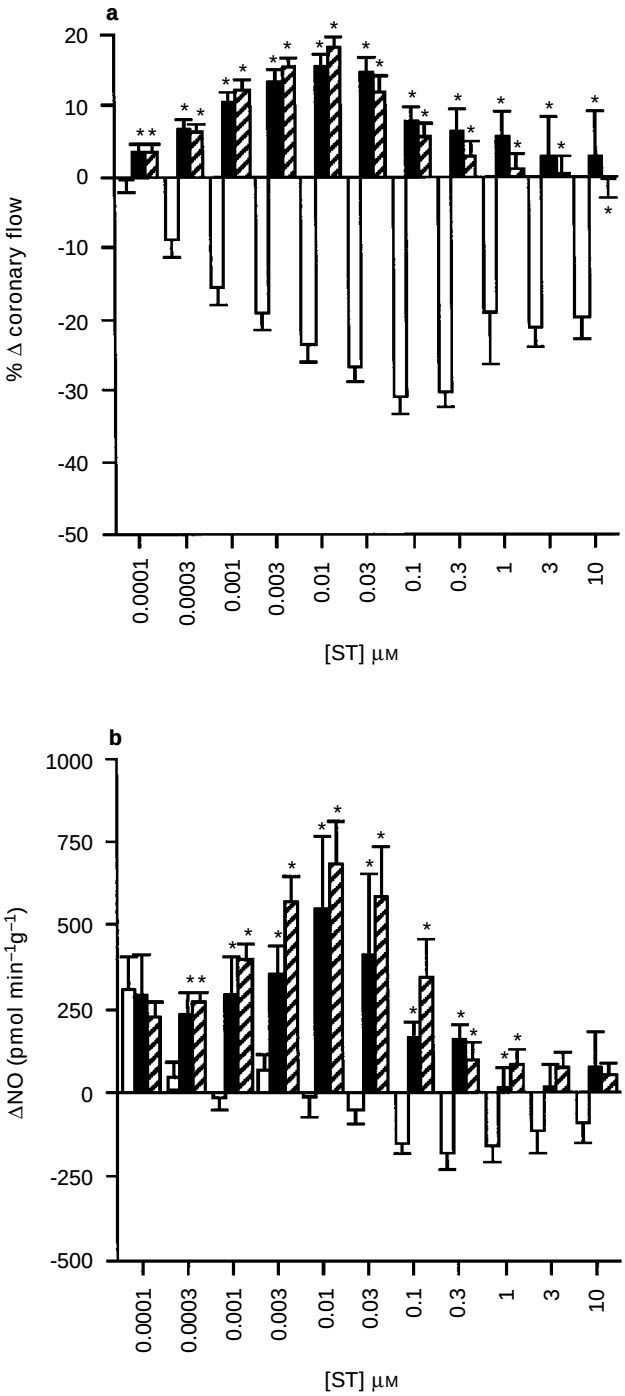

Figure 1 Changes in (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) alone (open columns, $n = 8$) or in the presence of GR127935 3 nM (solid columns, $n = 7$), or 10 nM (hatched columns, $n = 6$). Data are mean ± s.e.mean. $^*P < 0.05$ versus sumatriptan alone.

significant changes in coronary effluent NO content (Figure 1b) and values were similar to those in time matched control hearts.

Effect of GR127935 on responses to sumatriptan

GR127935 abolished the decrease in coronary flow caused by sumatriptan and converted the response into a concentration-dependent flow increase. This newly unmasked coronary dilator action was significant at concentrations of sumatriptan between 0.1 nM and 10 μM (Figure 1a). The concentration evoking the maximum dilator response was 0.02 ± 0.004 μM, and the pEC_{50} was 9.01 ± 0.12. GR127935 also unmasked a concentration-dependent ability of sumatriptan to increase coronary effluent NO content (Figure 1b). This NO release occurred in parallel with the increase in the coronary flow. The effects of 3 nM GR127935 on the actions of sumatriptan were almost indistinguishable from the effects of 10 nM.

Effects of mesulergine on responses to sumatriptan

Mesulergine had the opposite effect to that of GR127935. It exacerbated the vasoconstrictor response to sumatriptan (Figure 2a). Moreover, it abolished the diminution of the constrictor effect previously seen with high concentrations of sumatriptan (0.3 to 10 μM) such that the concentration of sumatriptan producing the maximum constrictor response was significantly increased to 0.73 ± 0.6 μM. Thus the sumatriptan concentration-response relationship became monophasic (Figure 2a). Furthermore, the magnitude of the reduction in coronary flow caused by each concentration of sumatriptan was significantly increased by mesulergine. However, the pEC_{50} remained unchanged. Coronary effluent NO content, unchanged by sumatriptan alone, was again found not to be significantly changed by sumatriptan when administered in the presence of mesulergine (Figure 2b).

Effects of GR127935 plus mesulergine on responses to sumatriptan

PR127935 (3 and 10 μM) reduced, in a concentration-dependent manner, the ability of mesulergine to exacerbate sumatriptan-induced vasoconstriction. Thus at each concentration of sumatriptan, the reduction in coronary flow was approximately halved by 3 nM GR127935 and was abolished completely by 10 nM GR127935 (Figure 2a). The sumatriptan concentration-response relationship, converted from v-shaped to sigmoidal by mesulergine, remained monophasic with the addition of 3 nM GR127935. In the presence of mesulergine and mesulergine combined with GR127935 the pEC_{50}s to sumatriptan were 8.6 ± 0.1 and 7.9 ± 0.1, respectively. Coronary NO content remained unchanged by sumatriptan in the presence of GR127935 plus mesulergine (Figure 2b).

Effects of L-NAME and saponin on basal coronary flow and NO release

Saponin significantly reduced basal coronary flow by a small amount, 1.4 ± 0.3 ml g^{-1} min^{-1} ($P<0.05$, $n=24$, Figure 3a), i.e., $-11 \pm 2\%$. This, nevertheless, was accompanied by a large reduction in basal NO release of $71 \pm 3\%$ ($P<0.05$, Figure 3b). L-NAME reduced basal coronary flow by a more substantial 4.4 ± 0.4 ml min^{-1} g^{-1} ($P<0.05$, Figure 3a), i.e., $-39 \pm 3\%$, which was significantly greater than the reduction caused by saponin ($P<0.05$). L-NAME also reduced basal NO content but only by $73 \pm 2\%$, an effect similar to that caused by saponin ($P<0.05$, Figure 3b).

Effects of sumatriptan following L-NAME and saponin pretreatment

L-NAME pretreatment did not alter the v-shaped coronary constrictor effect of sumatriptan in any way (Figure 4). In contrast, saponin abolished the diminution of the vasoconstriction that occurred with higher concentrations of sumatriptan. In fact, the response to sumatriptan over the 0.3–10 μM concentration range became a monophasic reduction in coronary flow and the concentration producing the maximum response increased from 0.26 ± 0.01 to 2.21 ± 1.4 μM. The maximum reduction in flow in response to sumatriptan increased after saponin pretreatment from -3.9 ± 0.4 ($-30 \pm 2\%$ at 0.3 μM) to -5.2 ± 0.2 ml min^{-1} g^{-1} ($-44 \pm 4\%$

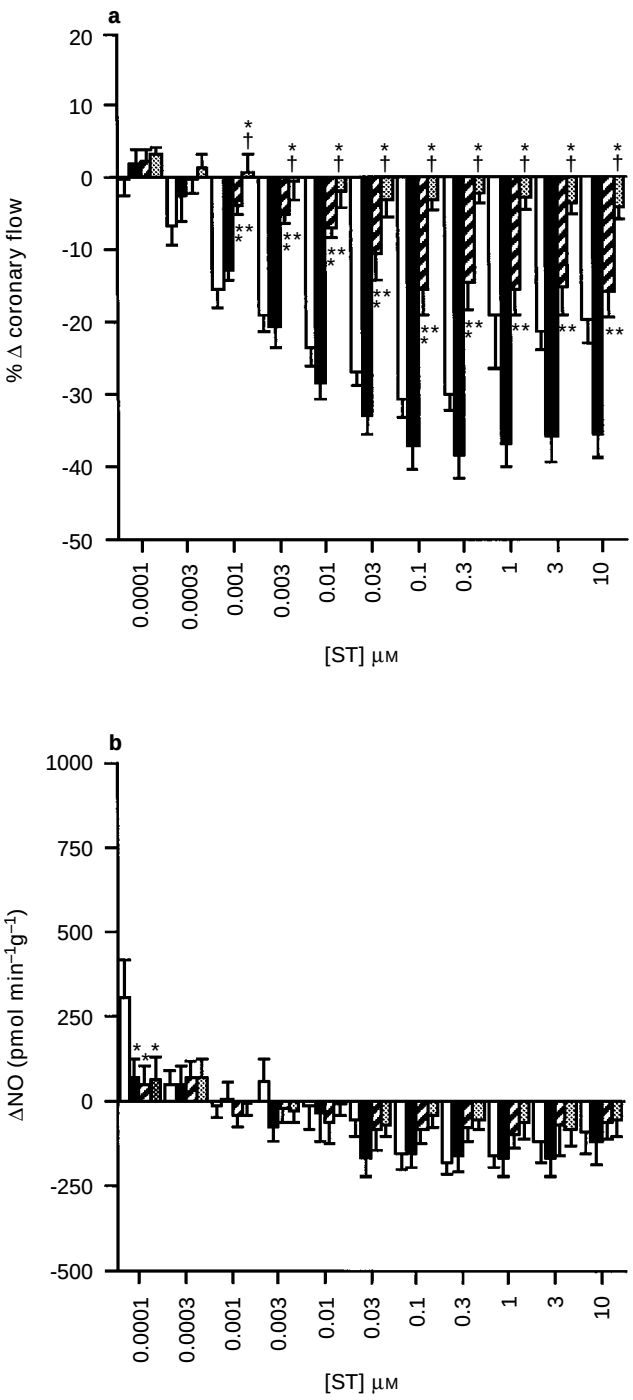

Figure 2 Changes in (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) alone (open columns, $n=8$), in the presence of 3 μM mesulergine (solid columns, $n=8$) or 3 μM mesulergine plus 3 nM GR127935 (hatched columns, $n=7$), or 3 μM mesulergine plus 10 nM GR127935 (stippled columns, $n=6$). Data are mean ± s.e.mean, $*P<0.05$ versus sumatriptan alone, $**P<0.05$ versus sumatriptan plus 3 μM mesulergine, $\dagger P<0.05$ versus sumatriptan plus 3 μM mesulergine and sumatriptan plus 3 μM mesulergine plus 3 nM GR127935.

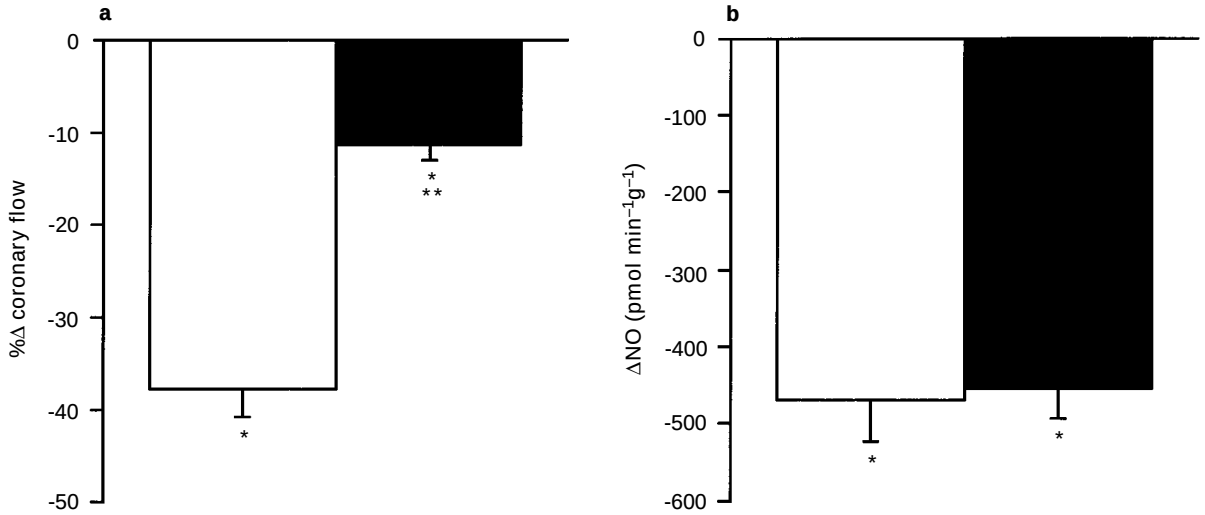

Figure 3 The effect of a 45 min perfusion with 100 μM L-NAME (open columns, $n=24$) or a 30 μg ml^{-1} saponin protocol (solid columns, $n=24$) on (a) coronary flow expressed as % change from baseline (%Δ coronary flow), and (b) coronary effluent NO content expressed as change from baseline (ΔNO). Data are mean±s.e.mean. *$P<0.05$ versus before L-NAME or saponin treatment; **$P<0.05$ versus the L-NAME group.

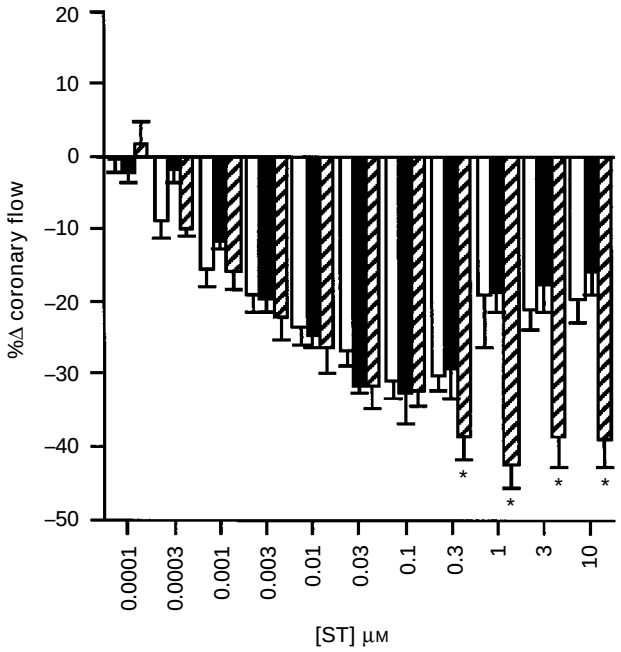

Figure 4 Changes in coronary flow (%Δ coronary flow), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) in hearts that were not pretreated (open columns, $n=6$), or were pretreated with 100 μM L-NAME (solid columns, $n=6$), or a 30 μg ml^{-1} saponin protocol (hatched columns, $n=6$). Data are mean±s.e.mean. *$P<0.05$ versus non-pretreated hearts.

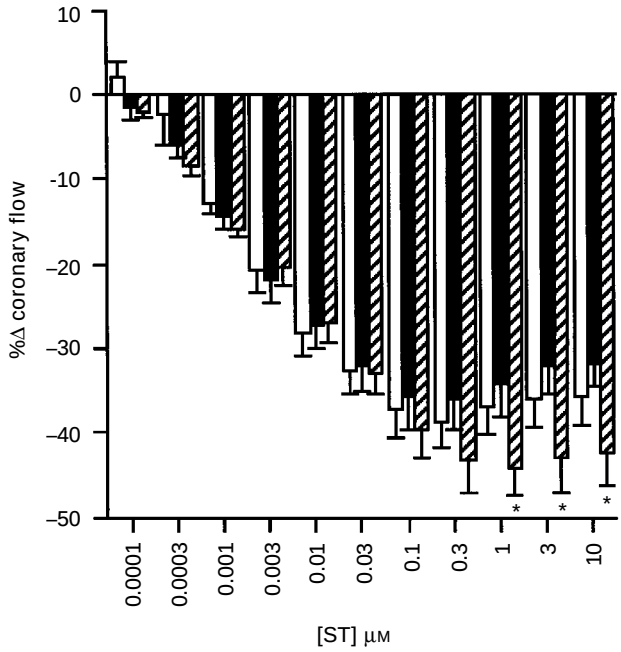

Figure 5 Changes in coronary flow (%Δ coronary flow), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) in the presence 3 μM mesulergine (open columns, $n=6$), following 100 μM L-NAME pretreatment (solid columns, $n=6$), or a 30 μg ml^{-1} saponin protocol (hatched columns, $n=6$). Data are mean±s.e.mean. *$P<0.05$ versus sumatriptan combined with mesulergine.

at 1 μM, $P<0.05$). The pEC_{50}s to sumatriptan following L-NAME and saponin were 8.58±0.12 and 8.52±0.14, respectively.

Effects of mesulergine on responses to sumatriptan following L-NAME and saponin pretreatment

Following pretreatment with L-NAME, there was no change in the coronary constrictor response to sumatriptan administered in the presence of mesulergine (Figure 5). Thus, the concentrations of sumatriptan (in the presence of mesulergine) producing the maximum constrictor responses were 0.29±0.12 μM and 0.28±0.09 μM, respectively. In contrast, saponin caused a significant enhancement (at the top end of the concentration-response curve) of the contractile response to sumatriptan in the presence mesulergine (Figure 5). The concentration of sumatriptan producing the maximum constrictor response (in the presence of mesulergine) following saponin was 0.97±0.11 μM ($P<0.05$, versus no saponin). Saponin abolished the diminution of the reduction in coronary flow with high concentrations of sumatriptan. This caused an increase in the concentration of sumatriptan producing the maximum constrictor response to 6.7±1.3 μM.

Effect of GR127935 on responses to sumatriptan following L-NAME and saponin

L-NAME pretreatment abolished the vasodilatation caused by sumatriptan in the presence of GR127935 at the lower end of the sumatriptan concentration-response curve (0.3 nM to 0.1 μM; Figure 1a), revealing a small vasoconstriction (Figure 6a). This was accompanied by inhibition of sumatriptan-induced NO release (Figure 6b). At concentrations of sumatriptan above 0.3 μM in the presence of GR127935, there was a significant, though insubstantial, vasodilatation (Figure 6a).

Pretreatment with saponin (Figure 7a) completely abolished the vasodilatation caused by sumatriptan in the presence of GR127935 (Figure 1a). In contrast to L-NAME, this effect was found over the full range of sumatriptan concentrations. The effect was accompanied by an inhibition of NO release (Figure 7b), although, interestingly, saponin did not cause a significant decrease in NO release with the lowest concentration of sumatriptan (0.1 nM) compared to control. This effect was partially inhibited by GR127935 (Figure 7b).

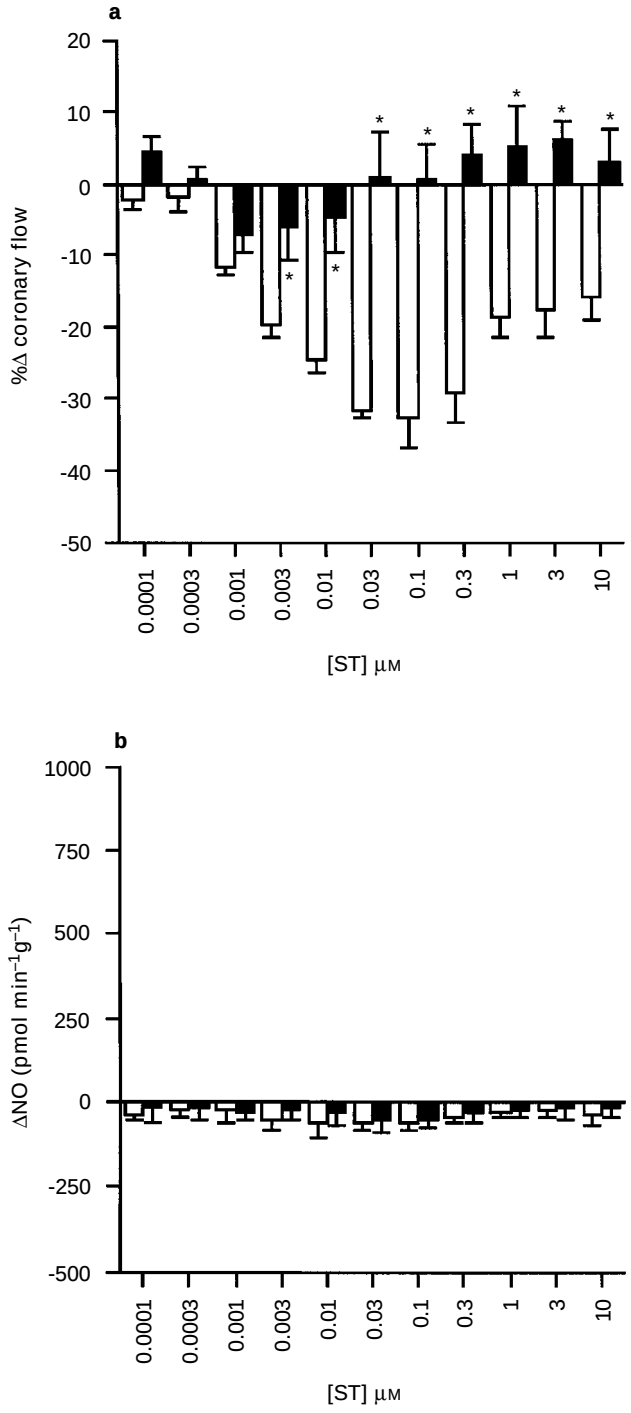

Figure 6 Effects of increasing concentrations of sumatriptan (ST) alone (open columns, $n=6$) or sumatriptan in the presence of 10 nM GR127935 (solid columns, $n=6$) on (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), each measured during the last 90 s of 6 min periods of perfusion in hearts pretreated with 100 μM L-NAME. Data are mean ± s.e.mean. $^*P<0.05$ versus sumatriptan.

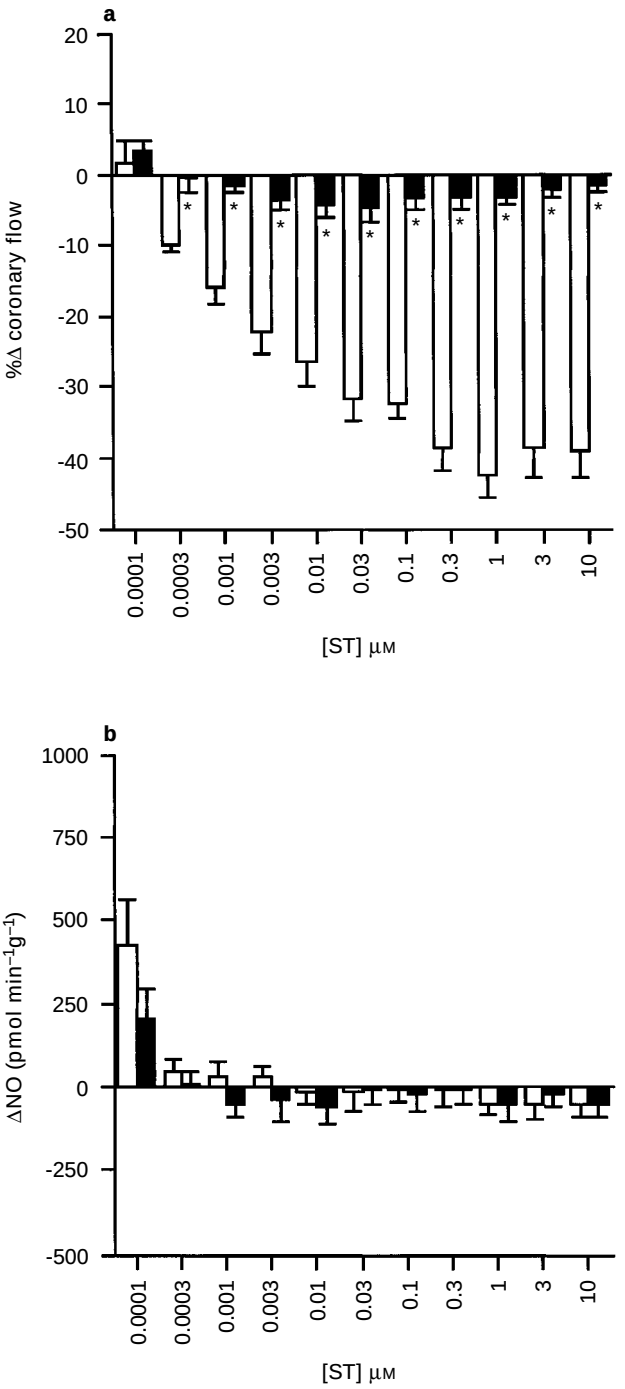

Figure 7 Effects of increasing concentrations of sumatriptan (ST) alone (open columns, $n=6$) or sumatriptan in the presence of 10 nM GR127935 (solid columns, $n=6$) on (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), each measured during the last 90 s of 6 min periods of perfusion in hearts pretreated with a 30 μg ml^{-1} saponin protocol. Data are mean ± s.e.mean. $^*P<0.05$ versus sumatriptan.

Effects of GR127935 plus mesulergine on responses to sumatriptan following L-NAME and saponin

Sumatriptan, when administered in the presence of 10 nM GR127935 and 3 μM mesulergine had been found previously to have no effect on coronary flow (Figure 2a) or NO (Figure 2b). This response was not affected by pretreatment with either L-NAME (Figure 8) or saponin (Figure 9).

Discussion

We examined the actions of sumatriptan on coronary flow and NO release in the guinea-pig isolated heart. Sumatriptan has been shown to be a selective 5-HT_{1D} agonist (Peroutka & McCarthy, 1989; Schoeffter & Hoyer, 1989) but has also been shown to act as a full agonist at 5-HT_{1A} and 5-HT_{1B} receptors, although with lower potency (pEC_{50}s of 5.6 and 6.0, respec-

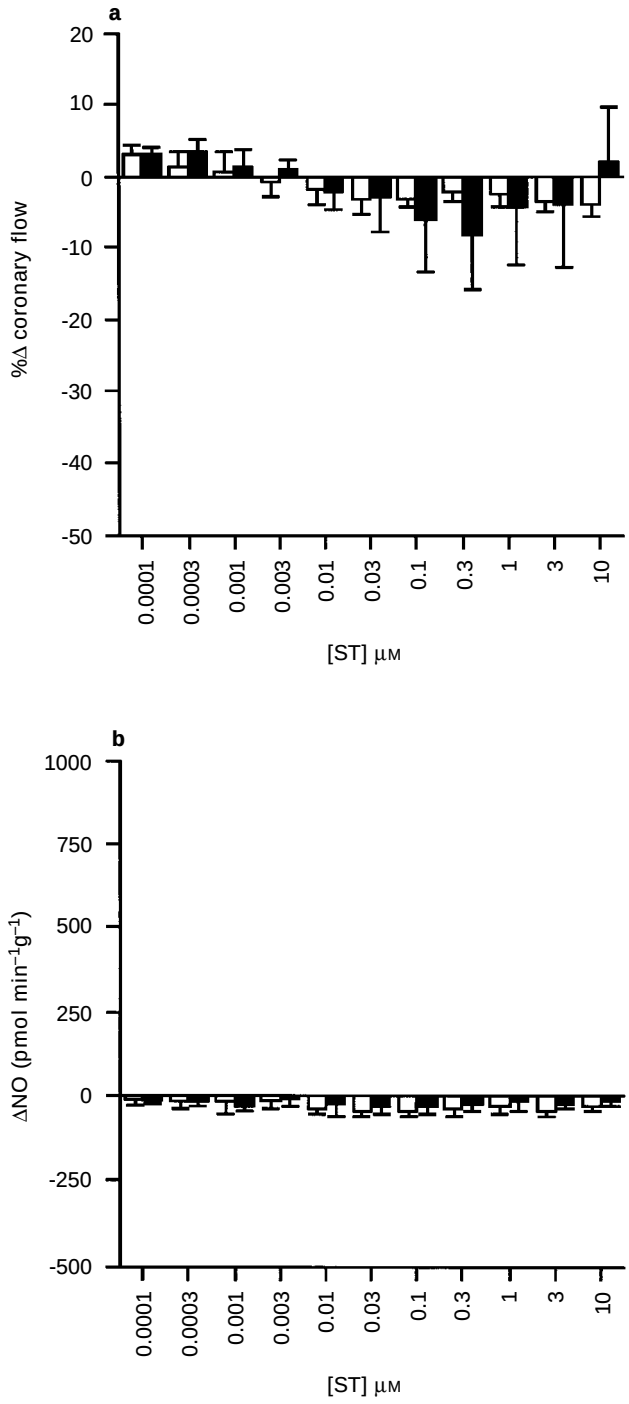

Figure 8 Changes in (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) in the presence of 3 μM mesulergine and 10 nM GR127935 combined, in hearts that were not pretreated (open columns, $n=6$) or were pretreated with 100 μM L-NAME (solid columns, $n=6$). Data are mean ± s.e.mean.

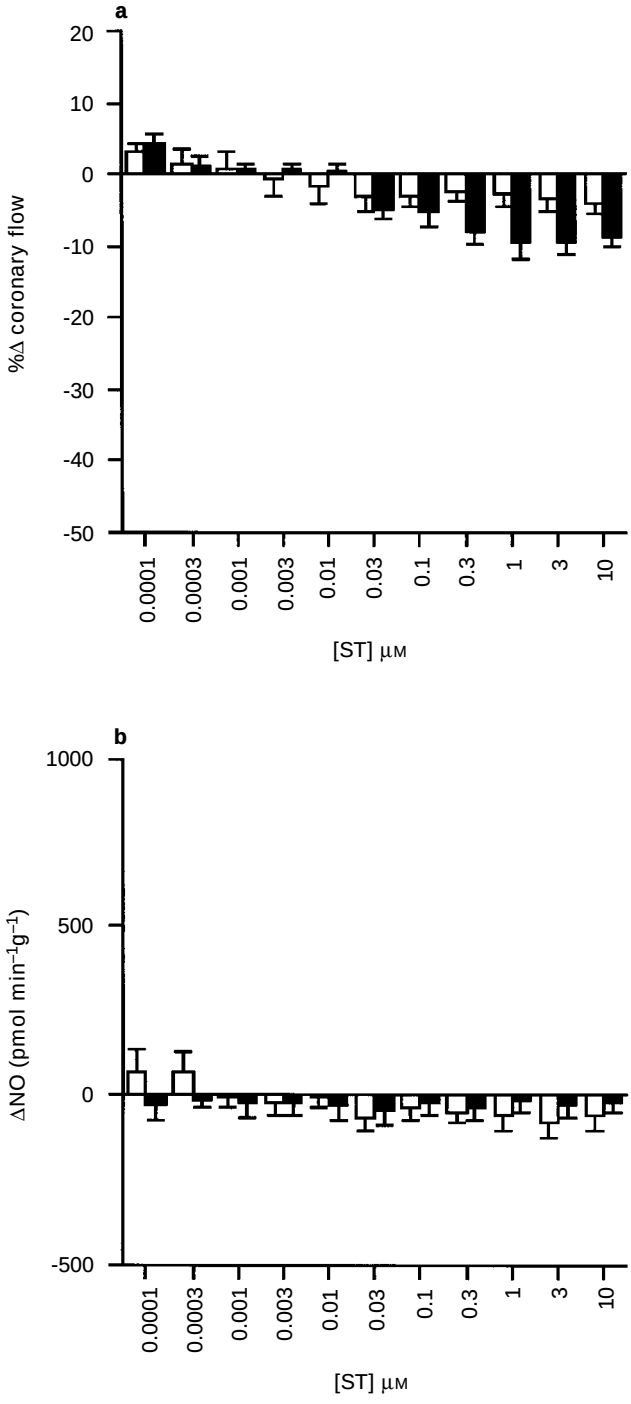

Figure 9 Changes in (a) coronary flow (%Δ coronary flow) and (b) coronary effluent NO content (ΔNO), measured during the last 90 s of 6 min periods of perfusion with increasing concentrations of sumatriptan (ST) in the presence of 3 μM mesulergine and 10 nM GR127935 combined in hearts that were not pretreated (open columns, $n=6$) or were pretreated with a 30 $\mu g\,ml^{-1}$ saponin protocol (solid columns, $n=6$). Data are mean ± s.e.mean.

tively; Hoyer *et al*., 1994). In addition, sumatriptan has some activity at 5-HT_{2C} receptors (pEC_{50} = 4.3, Hoyer *et al*., 1994). The nature of the receptors responsible for the action of sumatriptan on coronary vasculature and the role of the endothelium in modulating these actions were examined in the present study. There is evidence that there are two distinct 5-HT receptors involved in endothelium-dependent vasodilatation. In the pig coronary artery and the guinea-pig jugular vein the receptor involved appears to be of the 5-HT_{1D} subtype (Schoeffter & Hoyer, 1990; Gupta, 1992). In other tissues such as the rabbit jugular vein, pig vena cava, pig pulmonary artery and rat jugular vein the receptors involved appear not to be of the 5-HT_1-like subtype (Leff *et al*., 1987), but share some pharmacological features in common with the 5-HT_2 receptor class (Martin *et al*., 1993), and may be of the 5-HT_{2B} or 5-HT_{2C} subtype (Martin *et al*., 1993; Glusa & Richter, 1993; Bodelsson *et al*., 1993; Ellis *et al*., 1995). At least two subtypes of receptor, and at least two endothelial autacoids (one of which is NO) were found to regulate the nature and extent of the actions of sumatriptan in the present study. The predominant effect was vasoconstriction, but this was made worse when two different endothelium-dependent vasodilator mechanisms were inhibited by endothelial ablation.

Actions of sumatriptan on coronary flow and NO release

In endothelium-intact guinea-pig hearts, sumatriptan caused a high affinity concentration-dependent reduction in coronary flow. This constrictor response was v-shaped and, at higher concentrations, the vasoconstriction diminished, indicative of a low affinity vasodilator effect (that was not mediated by NO). The v-shaped contractile response profile was similar to that shown for dog isolated coronary arteries (Parsons *et al*., 1992).

GR127935, which is a potent highly selective 5-HT_{1D} receptor antagonist (Skingle *et al*., 1993), inhibited the reduction in coronary flow caused by sumatriptan. In addition GR127935 unmasked a high affinity coronary vasodilator action of sumatriptan which was accompanied by an increase in coronary effluent NO content.

Mesulergine removed the diminution of the vasoconstrictor effect of high concentrations of sumatriptan and blocked sumatriptan-induced NO release. Mesulergine has 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} antagonist activity (Hoyer *et al*., 1994), so sumatriptan may have activity on coronary 5-HT_2 receptors (Schoeffter & Hoyer, 1989). The present data support this suggestion and reveal that 5-HT_2 agonism contributes to the effects of sumatriptan on coronary flow.

The present data suggest that in endothelium-intact hearts, sumatriptan initiates a high affinity 5-HT_{1D}-receptor-mediated coronary constriction (blocked by GR127935) that masks (and apparently prevents the operation of) a high affinity, NO-mediated vasodilator action. The constrictor action appears to be attenuated slightly by a low affinity, NO-independent 5-HT_2 receptor-mediated vasodilator action (blocked by mesulergine). However, both the high and low affinity vasodilator actions of sumatriptan were effectively overwhelmed by the predominant constrictor action of 5-HT, and were unmasked only after administration of the 5-HT_{1D} receptor antagonist, GR127935. To examine these complex actions further, we used two techniques to isolate the role of the endothelium and NO release in modulating the actions of sumatriptan.

Role of endothelium and NO in mediating the latent vasodilator action of sumatriptan

The low affinity vasodilator action of sumatriptan was not affected by L-NAME (Figure 4), confirming that it was not mediated by NO. However, saponin abolished this vasodilatation. The saponin protocol used has previously been found to abolish vasodilatation to substance P without affecting the response to sodium nitroprusside (Ellwood & Curtis, 1996a). Thus, the low affinity vasodilator action of sumatriptan that is blocked by the 5-HT_2 antagonist, mesulergine, requires an intact coronary endothelium, but is not mediated by NO. Additionally, saponin (though not L-NAME) exacerbated the ability of mesulergine to facilitate sumatriptan-induced vasoconstriction over the concentration range at which sumatriptan alone induced a low affinity coronary vasodilatation. This further supports the evidence that the low affinity sumatriptan-induced vasodilatation is caused by agonism at 5-HT_2 receptors that are present on the coronary endothelium. However, it also demonstrates that NO is not the intercellular autacoid responsible for mediating this effect.

In contrast, the high affinity vasodilator action of sumatriptan, revealed in the presence of GR127935, was almost completely abolished by L-NAME and saponin (Figures 6 and 7), confirming that it was mediated by endothelium-dependent NO release. Interestingly, the high affinity vasodilator action of sumatriptan was also abolished by mesulergine. This suggests that 5-HT_2 agonism elicits both a high affinity NO release and a low affinity release of a different vasodilator substance from the guinea-pig coronary endothelium. Presumably different 5-HT_2 receptor subtypes are involved in these responses, although we have no evidence yet to discriminate between the three options (2A, 2B and 2C).

Possible limitations of the present study

The predominant action of sumatriptan on coronary flow in the guinea-pig endothelium-intact heart is a GR127935-sensitive coronary vasoconstriction. This action is not blocked by saponin and thus represents a direct action on coronary smooth muscle rather than an indirect effect mediated by the release of an autacoid from the endothelium. However, to deduce that this action is mediated by 5-HT_{1D} receptors depends on the selectivity of GR127935. GR127935 has been shown to be a partial agonist of 5-$HT_{1D\alpha}$ receptors at concentrations above 10 nM in cultured cell lines (Watson *et al*., 1995; Pauwels *et al*., 1995; Pauwels & Palmier, 1995; Pauwels & Colpaert, 1995). However, Skingle *et al*. (1996) argued that the transfection of high numbers of 5-HT_{1D} receptors (5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes) in to cell lines may lead to overestimation of the extent to which GR127935 is a partial agonist. In the present study, 10 nM GR127935 had no effect alone on coronary flow or NO release, which would suggest that GR127935 does not act as a partial agonist in guinea-pig coronary artery. Thus, there is no evidence that GR127935 does anything other than block 5-HT_{1D} receptors over the concentration range that possessed activity in the present study. On this basis, it would appear reasonable to deduce that the predominant constrictor action of sumatriptan was mediated by agonism at 5-HT_{1D} receptors located on the coronary smooth muscle.

The predominant effect of sumatriptan in saponin-treated hearts was a monophasic coronary constriction larger than that observed in endothelium-intact hearts. This effect was also blocked by GR127935. Endothelial injury is therefore likely to enhance any potentially hazardous coronary constriction that sumatriptan may elicit, by shifting the balance in favour of the 5-HT_{1D} receptor-mediated actions. However, we are tempted to conclude that this is likely to be mediated not simply by the physical exposure of smooth muscle 5-HT_{1D} receptors in the coronary lumen. There are two reasons for this deduction. First, the mechanism is evidently extant when the endothelium is intact. Second, it is enhanced by manoeuvres that reduce endothelial autacoid release. Thus the impairment of 5-HT receptor-mediated endothelially-derived vasodilator autacoid release is likely to contribute to the greater scope for sumatriptan to constrict coronary arteries after endothelial injury. We have suggested that this involves loss of endothelial release of NO and loss of release of another vasodilator autacoid. Studies with mesulergine suggested that 5-HT_2 receptors in the endothelium mediate the release of both these vasodilator autacoids. However, the accuracy of this conclusion is dependent on the selectivity of mesulergine. Mesulergine is certainly a potent 5-HT_2 antagonist; the pEC_{50}s at 5-HT_{2A} and 5-

HT_{2C} receptors are both 9.19 (Hoyer *et al*., 1994). However, it is inactive at 5-HT_4 receptors (Hoyer *et al*., 1994) and there is no evidence of activity at 5-HT_1-like receptors. Thus it seems reasonably safe to deduce that inhibition of actions of sumatriptan by mesulergine represents evidence of 5-HT_2 receptor modulation. This is perhaps surprising since sumatriptan has been shown to be a selective 5-HT_1 agonist. Thus it would appear that sumatriptan is less selective than initially thought. The pEC_{50} for sumatriptan at 5-HT_{2C} receptors has been found to be 4.3 (Hoyer *et al*., 1994), so the much higher affinity interaction that we observed between mesulergine and sumatriptan presumably results from 5-HT_{2A} receptor modulation.

The study is also limited by the lack of information on the nature of the second autacoid released from the endothelium that mediates sumatriptan-induced vasodilation. Prostacyclin and other cyclo-oxygenase products may be excluded since indomethacin has no effect on the endothelium-dependent NO-independent vasodilator effects of sumatriptan (unpublished observations from our laboratory). Further work is necessary to determine the identity of the second autacoid.

Finally, although sumatriptan did not cause endothelial NO release by itself, it did so in the presence of GR127935. This implies that the coupling of the mesulergine-sensitive endothelial receptors to endothelial nitric oxide synthase (NOS) is suppressed if endothelial 5-HT_{1D} agonism is extant. This suggests that the transductional linkage between mesulergine-sensitive 5-HT_2 receptors and NOS may be uncoupled by 5-HT_{1D} agonism. The study is limited to the extent that the nature of this uncoupling mechanism was not examined.

Conclusions

Sumatriptan has complex actions on coronary flow in the guinea-pig isolated heart. The predominant effect is a 5-HT_{1D} receptor-mediated vasoconstriction. However, this effect is greatly enhanced if NO-dependent and NO-independent vasodilator effects, which appear to be mediated by 5-HT_2 receptors located on the coronary endothelium, are vitiated. This suggests that any tendency that sumatriptan may have to evoke pathogenic coronary constriction may be worsened by endothelial injury, through the loss of these actions. We found no evidence of sumatriptan-induced endothelium-independent coronary vasodilatation. Thus, in species in which vasoconstriction is not the predominant effect of sumatriptan in endothelium-intact hearts, the extent to which endothelial injury converts sumatriptan to a hazardous coronary vasoconstrictor is likely to be greater than was observed in guinea-pig hearts. In addition, in the guinea-pig heart, endothelial 5-HT_2 receptor-mediated NO release appears to be suppressed by extant 5-HT_{1D} agonism suggesting the possibility that 5-HT_{1D} agonism may uncouple the transductional linkage between 5-HT_2 receptors and nitric oxide synthase.

We thank Dr Paul Gupta (Pfizer UK, Sandwich, Kent) for providing us with mesulergine, GR127935 and sumatriptan, and the BBSRC and Pfizer UK for financial support. A.J.E. is an SERC-CASE Award student.

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(Received August 7, 1996
Revised December 4, 1996
Accepted December 9, 1996)