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Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels

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Abstract

Eletriptan, a second-generation triptan with high affinity for 5-$HT_{1B/1D}$ receptors, is highly effective in migraine, with or without aura. We compared the effects of eletriptan and sumatriptan on the human isolated middle meningeal and coronary arteries and saphenous vein, used as models for therapeutic efficacy and potential side effects, and have investigated the role of 5-$HT_{1B/1D}$ receptors in contractions induced by these triptans. Concentration–response curves to eletriptan and sumatriptan were constructed in the absence or presence of a selective 5-$HT_{1B/1D}$ receptor antagonist, *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide (GR125743). All three blood vessels constricted in response to eletriptan and sumatriptan, but the middle meningeal artery relaxed following the highest concentration (100 μM) of eletriptan. In the middle meningeal artery, GR125743 antagonised the contractions induced by both eletriptan (pEC_{50}: 7.34 ± 0.13) and sumatriptan (pEC_{50}: 6.91 ± 0.17) to a similar degree (pA_2: 8.81 ± 0.17 and 8.64 ± 0.21, respectively). In the human coronary artery and saphenous vein, sumatriptan-induced contractions (pEC_{50}: 6.24 ± 0.14 and 6.19 ± 0.12, respectively) were also potently antagonised by GR125743 (pA_2: 8.18 ± 0.27 and 8.34 ± 0.12, respectively). The eletriptan-induced contractions of the human saphenous vein (pEC_{50}: 6.09 ± 0.13) were antagonised less effectively by GR125743 (pK_B: 7.73 ± 0.18), and those of the human coronary artery (pEC_{50}: 5.54 ± 0.22) remained unaffected by GR125743 up to a concentration of 100 nM. These results suggest that (i) based on the differences in pEC_{50} values, the cranioselectivity of eletriptan (63-fold) is higher than that of sumatriptan (5-fold) in coronary artery, (ii) the contractile effects of sumatriptan and eletriptan (lower concentrations) in the three blood vessels are mediated via the 5-HT_{1B} receptor, and (iii) additional mechanisms seem to be involved in coronary artery and saphenous vein contractions and middle meningeal artery relaxation following high concentrations of eletriptan.

Keywords: Coronary artery; Eletriptan; GR125743; (Human); Middle meningeal artery; Migraine; Saphenous vein; Serotonin; Sumatriptan

1. Introduction

Sumatriptan, the first of a new class of compounds with an agonist action at 5-$HT_{1B/1D}$ receptors, is effective in the acute treatment of migraine and cluster headaches (Saxena and Tfelt-Hansen, 2000). The high efficacy profile as well as some shortcomings (low oral bioavailability, high headache recurrence and chest symptoms) has prompted the development of several second-generation triptans, including eletriptan. Eletriptan displays a higher affinity than sumatriptan for the 5-HT_{1B} (pK_i: 8.00 and 7.37, respectively) as well as 5-HT_{1D} (pK_i: 8.94 and 8.04, respectively) receptor (Napier et al., 1999). Like sumatriptan, eletriptan constricts porcine carotid arteriovenous anastomoses in vivo (Willems et al., 1998) and canine saphenous vein and basilar artery in vitro (Gupta et al., 1999) and inhibits c-*fos* expression in trigeminal nucleus caudalis following stimulation of the superior sagittal sinus in the cat (Goadsby and Hoskin, 1999). Oral administration of

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the highest dose of eletriptan (80 mg) has been shown to be superior to sumatriptan (100 mg) in the treatment of migraine in three head-to-head clinical trials (Goadsby et al., 2000; Saxena and Tfelt-Hansen, 2000).

It is now generally accepted that the therapeutic action of sumatriptan and other triptans is mainly due to constriction of dilated intra- and extra-cranial blood vessels, although other mechanisms interfering with the trigeminovascular system may also be involved (De Vries et al., 1999a; Saxena and Tfelt-Hansen, 2000). Indeed, the triptans potently contract human isolated middle meningeal (Longmore et al., 1998; Razzaque et al., 1999), temporal (Verheggen et al., 1996), middle cerebral (Hamel and Bouchard, 1991; Jansen et al., 1992) and basilar (Parsons et al., 1998) arteries. However, albeit less profound, the triptans also have the ability to contract non-cranial blood vessels, such as human isolated pulmonary (MacLean et al., 1996) and coronary (Connor et al., 1989; Chester et al., 1990; MaassenVanDenBrink et al., 1998) arteries. Constriction of these blood vessels may lead to cardiovascular adverse events, including myocardial ischaemia and infarction in predisposed individuals (Kelly, 1995; O'Connor and Gladstone, 1995; Ottervanger et al., 1997; Main et al., 1998).

In the present study, we have investigated the contractile effects of eletriptan, in comparison to those of sumatriptan, on human isolated blood vessels used as models with relevance to therapeutic efficacy (middle meningeal artery), coronary adverse events (coronary artery) and peripheral vasoconstriction (saphenous vein). Furthermore, we used the competitive 5-$HT_{1B/1D}$ receptor antagonist *N*-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide (GR125743) (Domenech et al., 1997; Pauwels, 1997) in an attempt to characterise the 5-HT receptors mediating contractions to eletriptan and sumatriptan.

2. Material and methods

2.1. Tissue collection

2.1.1. Human isolated middle meningeal artery

Human middle meningeal arteries were obtained from 10 patients (four males, six females; age 30–69 years) undergoing craniotomy at the neurosurgical unit of the University Hospital 'Dijkzigt', Erasmus University Medical Centre, Rotterdam. During the surgical procedure, a part of the skull is temporarily removed and the dura matter, together with a small piece of the middle meningeal artery, is intentionally cut to obtain access to the brain. This piece of the artery was placed in a plastic tube filled with ice-cold (0–4 °C) physiological saline and immediately transported to the laboratory. Upon arrival at the laboratory, the artery was placed in a cold oxygenated modified Krebs bicarbonate solution of the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11.1; pH 7.4. The cyclooxygenase inhibitor indomethacin (0.1 μM) was added to the Krebs solution to prevent prostaglandin synthesis. Excess tissue surrounding the artery was carefully removed and no attempt was made to remove the endothelium. The middle meningeal artery was used within 2 h of surgery.

2.1.2. Human isolated coronary artery and saphenous vein

The human right epicardial coronary artery was obtained from 11 'heart-beating' organ donors (six males, five females; 17–52 years), who died of non-cardiac disorders (eight to cerebrovascular accidents; three to brain trauma). The hearts were provided by the Heart Valve Bank, Rotterdam, The Netherlands after donor mediation by Bio Implant Services Foundation/Eurostransplant Foundation, Leiden, The Netherlands (for details, see MaassenVanDenBrink et al., 1998). Leftover human saphenous vein was obtained postoperatively from 13 patients (seven males, six females; age 34–81 years) undergoing coronary bypass surgery. Saphenous vein was immediately placed in cold saline and was brought to the laboratory within 15 min.

Upon arrival in the laboratory, the right coronary artery and saphenous vein were cleaned from the surrounding tissue 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, $NaHCO_3$ 25 and glucose 8.3; pH 7.4. No attempt was made to remove the endothelium. The artery and vein were stored overnight in cold oxygenated Krebs solution and were used the following day.

2.2. Experimental protocol

2.2.1. Human isolated middle meningeal artery

The middle meningeal artery was cut into circular 3- to 4-mm-long segments, which were mounted on metal prongs in 10-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37°C. Changes in isometric tension were measured by a force displacement transducer and recorded with IOX 1.203h software (both, EMKA Technology, Paris, France). Segments were stretched to a passive tension of 4 mN and were allowed to stabilise at this level for 60 min (with replacement of Krebs solution every 15 min). All segments were then exposed 2–3 times to 0.1 μM prostaglandin $F_{2\alpha}$ to demonstrate reproducibility of the evoked contractions. Subsequently, the segments were pre-contracted with prostaglandin $F_{2\alpha}$ (1 μM) and the relaxation response to substance P (10 nM) was used to evaluate the presence of a functional endothelium. After washing, the segments were allowed to equilibrate for 60 min, with replacement of buffer every 15 min. The segments were then used in a paired parallel experimental set-up, incubating with the 5-$HT_{1B/1D}$ receptor antagonist

GR125743 (10 and 30 nM) or vehicle for 30 min. Subsequently, a single cumulative concentration–response curve to either eletriptan or sumatriptan (both 1 nM to 100 μM) was constructed in each segment.

2.2.2. Human isolated coronary artery and saphenous vein

Vessels were cut into ring segments of approximately 4-mm length and were suspended on stainless steel hooks in 15-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2 and 5% CO_2 and maintained at a temperature of 37°C. Vessel segments containing macroscopically visible atherosclerotic lesions were not used. Changes in tension were recorded using a Harvard isometric transducer. The segments were allowed to equilibrate for at least 30 min and were washed every 15 min. Segments were stretched to a passive tension of 15 mN (coronary artery) or 10 mN (saphenous vein), respectively. All segments were then exposed to KCl (30 mM) twice, to demonstrate the reproducibility of the evoked contractions. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (coronary artery, 1 nM) or bradykinin (saphenous vein, 0.1 μM) after pre-contraction with prostaglandin $F_{2\alpha}$ (1 μM). After washout, the tissue was exposed to KCl (100 mM) to determine the maximal contractile response to KCl. The tissue was washed and then was allowed to equilibrate for another period of 30 min. After this equilibration period the segments were divided in a paired parallel experimental set-up and were incubated with the 5-$HT_{1B/1D}$ receptor antagonist GR125743 (coronary artery: 3 nM to 1 μM; saphenous vein: 3–30 nM) or with vehicle for 30 min. Subsequently, a single cumulative concentration–response curve to eletriptan or sumatriptan (both 1 nM to 100 μM) was constructed in each segment.

2.3. Data analysis

2.3.1. Concentration–response curves

Contractile responses were expressed as percentage of the contractile response to 1 μM prostaglandin $F_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery and saphenous vein). The occasional spontaneous phasic contractions observed in some coronary artery and saphenous vein segments were not considered in the calculations, but the respective plateau contraction levels were used. Initially, we calculated the mean value of the individual maximum contractile responses (E_{max}) to eletriptan or sumatriptan; in case the contractions did not reach a plateau in an individual experiment, the contraction induced by the highest concentration used (100 μM) was considered as E_{max}. We then analysed mean concentration–response curves with a non-linear regression fitting technique for sigmoidal functions with variable slope using Graphpad Prism 3.0 (Graphpad Software Inc., San Diego, CA, USA) to obtain Hill slopes for the agonists in the absence or presence of different concentrations of GR125743. When mean E_{max} values and Hill slopes were not significantly different, we assumed that GR125743 behaved as a competitive antagonist. The whole data set was then transformed using SPSS 7.5 non-linear regression statistics (SPSS Inc., Chicago, IL, USA) into a dependent fitting model, where the E_{max} values were set to the respective agonist control E_{max}. This model enabled us to obtain valid S.E.M. and 95% confidence intervals of mean pEC_{50} values and dose ratios. Using the pEC_{50} values that were significantly different from their respective controls, Schild regression analysis was performed (SPSS Inc.) to calculate antagonist pA_2 (≥ 3 concentrations of GR125743), apparent pA_2 (2 concentrations of GR125743 with slope set to unity) or pK_B (1 concentration of GR125743 with slope set to unity). When the ratio between EC_{50} values in the absence or presence of antagonist was larger than 1, the dose ratio was calculated.

2.3.2. Statistical analysis

Differences between E_{max}, Hill slopes and pEC_{50} values of vehicle and antagonist-treated groups were analysed according to one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison *t*-test (Graphpad Software Inc.). Differences in potency, efficacy and logarithmically transformed dose ratios between the agonists were analysed according to Tukey's unpaired *t*-test (Graphpad Software Inc.). Cranioselectivity ratios were calculated as the inverse logarithmic difference between pEC_{50} values of agonists in middle meningeal artery and either coronary artery or saphenous vein. The 95% confidence limit (95% CI) of cranioselectivity ratios obtained with eletriptan and sumatriptan were calculated and compared as described by Steel and Torrie (1980). In all cases, statistical significance was assumed when $P < 0.05$. Except for dose ratios and cranioselectivity ratios, where the geometric means with 95% CI are given, all other data are presented as mean ± S.E.M.

2.4. Ethical approval

The Medical Ethics Committee of the Erasmus Medical Centre Rotterdam, which deals with the use of human material for scientific experiments, approved the protocols for this investigation.

2.5. Compounds

Bradykinin acetate, 5-hydroxytryptamine creatinine sulphate (serotonin; 5-HT), indomethacin hydrochloride, prostaglandin $F_{2\alpha}$ Tris salt and substance P acetate were purchased from Sigma (St. Louis, MO, USA). Eletriptan hydrogen bromide, sumatriptan succinate and GR125743 were kindly supplied by Pfizer (Sandwich, Kent, UK). Indomethacin was dissolved in 100% v/v dimethyl

sulphoxide and further diluted in distilled water. All other compounds were dissolved in distilled water.

3. Results

3.1. Human middle meningeal artery

Both eletriptan and sumatriptan contracted the middle meningeal artery in a concentration dependent manner (up to 10 μM) and with similar maxima (E_{max}: 94 ± 8% and 106 ± 17% of contraction to 1 μM prostaglandin $F_{2\alpha}$, respectively), potency (pEC_{50}: 7.34 ± 0.13 and 6.91 ± 0.17, respectively) and Hill slopes (0.86 ± 0.20 and 1.27 ± 0.80, respectively) (Fig. 1; Table 1). However, in contrast to sumatriptan, the highest concentration of eletriptan (100 μM) induced a marked vasorelaxation, which was not affected by GR125743. Incubation with GR125743 caused a parallel rightward shift, with no alteration in Hill slopes, in the concentration–response curves to both eletriptan (contractile part) and sumatriptan; the maximum contractile responses observed with 10 μM were not significantly different after GR125743 compared to the respective control values. The pEC_{50} values and dose ratios (95% CI) of eletriptan after 10 and 30 nM GR125743 were 6.43 ± 0.13 and 8.2 (4.3–19.4), and 5.48 ± 0.14 and 72 (30–172), respectively. For sumatriptan the pEC_{50} values and dose ratios (95% CI) yielded 6.11 ± 0.17 and 6.4 (2.1–19.8) and 5.27 ± 0.18 and 44 (14–137), for 10 and 30 nM GR125743, respectively. Schild regression analysis revealed similar apparent pA_2 values of GR125743 against eletriptan (8.81 ± 0.17) and sumatriptan (8.64 ± 0.21) (Table 1).

3.2. Human coronary artery

The human coronary artery contracted in response to eletriptan and sumatriptan with equal maxima (E_{max}: 17 ± 4% and 12 ± 2% of contraction to 100 mM KCl, respectively) and Hill slopes (0.73 ± 0.26 and 0.96 ± 0.31, respectively). The potency of eletriptan was significantly lower than that of sumatriptan (pEC_{50}: 5.54 ± 0.22 and 6.24 ± 0.14, respectively) (Fig. 2; Table 1). GR125743 (3 nM–1 μM) did not affect the maxima (E_{max}) and Hill slopes of eletriptan and at concentrations up to 100 nM GR125743 there was no shift in the concentration–response curves to eletriptan (data not shown). Due to a non-sigmoidal nature of the concentration–response curve to eletriptan following high concentrations (≥ 300 nM) of GR125743 (Fig. 2, left panel), pEC_{50} values and dose ratios of eletriptan and pA_2 of GR125743 were not determined (see Methods). The concentration–response curve to sumatriptan was shifted in a parallel manner, with no alteration of Hill slopes, by GR125743 (Fig. 2, right panel), yielding a significant decrease in potency (pEC_{50}: 5.44 ± 0.15, 4.71 ± 0.15, 4.66 ± 0.18 and 3.92 ± 0.20 after 30 nM, 100 nM, 300 nM and 1 μM GR125743, respectively, with corresponding dose ratios (95% CI) of 6.3 (2.5–16), 34 (13–87), 38 (14–107) and 209 (70–624), respectively). Schild regression analysis revealed a pA_2 value of 8.18 ± 0.27 for GR125743 against sumatriptan with a slope of 0.89 ± 0.15, which was not significantly different from unity. The dose ratios for eletriptan (2.2)

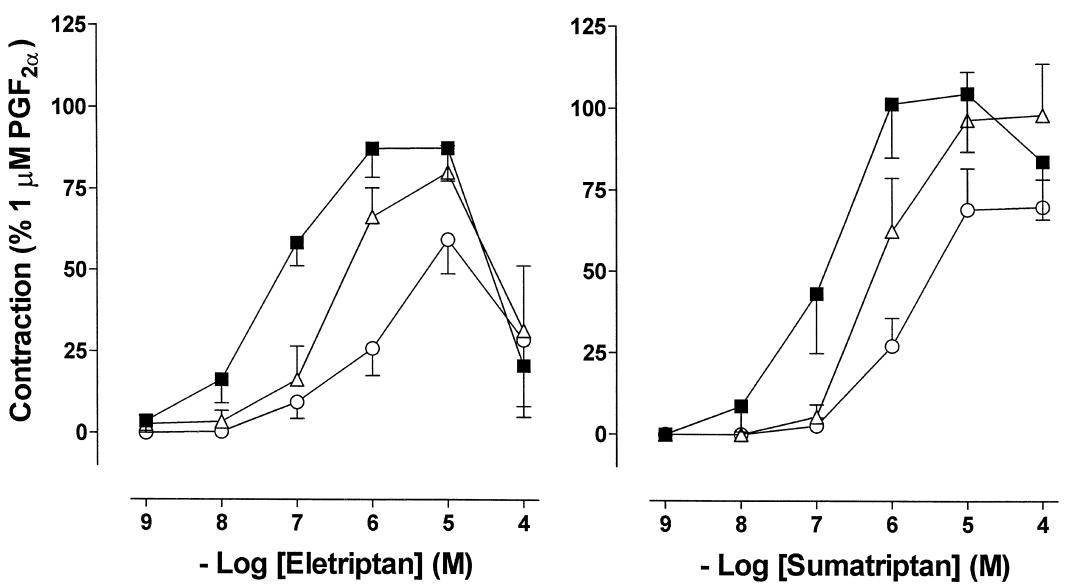

Fig. 1. Cumulative concentration–response curves to eletriptan (left panel; $n = 5$) and sumatriptan (right panel; $n = 5$) in the human isolated middle meningeal artery in the absence (■) or presence of GR125743 (△, 10 nM or ○, 30 nM). Symbols and vertical bars represent the means and S.E.M. $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$.

Table 1
Functional parameters of the agonists eletriptan and sumatriptan, and the antagonist GR125743 in human isolated blood vessels

	Middle meningeal artery		Coronary artery		Saphenous vein	
	Eletriptan	Sumatriptan	Eletriptan	Sumatriptan	Eletriptan	Sumatriptan
E_{max}	94 ± 8	106 ± 17	17 ± 4[a]	12 ± 2[a]	71 ± 10	80 ± 6
pEC_{50}	7.34 ± 0.13[b]	6.91 ± 0.17[b]	5.54 ± 0.22[c]	6.24 ± 0.14	6.09 ± 0.13	6.19 ± 0.12
Cranioselectivity ratio						
Mean (95% CI)[d]			63 (20–199)[c,e]	5 (2–13)[e]	18 (8–41)[e]	5 (2–13)[e]
pA_2 GR125743	8.81 ± 0.17	8.64 ± 0.21	ND	8.18 ± 0.27	7.73 ± 0.18[f]	8.34 ± 0.12

Data are presented as means ± S.E.M. or as 95% CI (n = 4–9). The E_{max} values of eletriptan and sumatriptan are presented as percentage of contraction elicited by either 1 μM prostaglandin $F_{2\alpha}$(middle meningeal artery) or 100 mM KCl (coronary artery and saphenous vein).
ND, not determined because of non-sigmoidal nature of the concentration–response curve.
[a] E_{max} eletriptan and sumatriptan significantly lower than the respective E_{max} in the saphenous vein ($P < 0.05$).
[b] pEC_{50} value significantly higher compared to the respective pEC_{50} values in the coronary artery and saphenous vein (eletriptan $P < 0.01$, sumatriptan $P < 0.05$).
[c] Significantly different from the respective value of sumatriptan ($P < 0.05$).
[d] Cranioselectivity ratio = Inverse logarithm [pEC_{50} (middle meningeal artery) − pEC_{50} (coronary artery or saphenous vein)].
[e] Significantly different from 1 ($P < 0.05$).
[f] pK_B value.

and sumatriptan (6.3) after 30 nM GR125743 were similar, whereas at 100 nM GR125743 the dose ratio for eletriptan (2.7) was significantly lower than for sumatriptan (34).

3.3. Human saphenous vein

Eletriptan and sumatriptan contracted the saphenous vein with equal maxima (E_{max}: 71 ± 10% and 80 ± 6% of contraction to 100 mM KCl, respectively), potency (pEC_{50}: 6.09 ± 0.13 and 6.19 ± 0.12, respectively) and Hill slopes (0.64 ± 0.13 and 0.79 ± 0.14, respectively) (Fig. 3, Table 1). GR125743 (3–30 nM) did not affect the maxima (E_{max}) and Hill slopes of eletriptan and sumatriptan (Fig. 3). The Hill slope of eletriptan control was significantly different from unity, whereas those in the presence of antagonist were not. The concentration–response curves to eletriptan were shifted to the right only after incubation with 30 nM GR125743 (pEC_{50}: 5.56 ± 0.11; dose ratio (95% CI): 3.4 (1.5–7.7)). A parallel rightward shift in the concentration–response curves to sumatriptan was observed with GR125743 (10 and 30 nM), yielding a significant decrease in potency (pEC_{50}: 5.66 ± 0.10 and 4.93 ± 0.10, respectively, with corresponding dose ratios (95% CI) of 3.3 (1.6–7.0) and 18 (9–34), respectively). Schild regression analysis revealed an apparent pA_2 value of 8.34 ± 0.12 for GR125743 against sumatriptan, which was

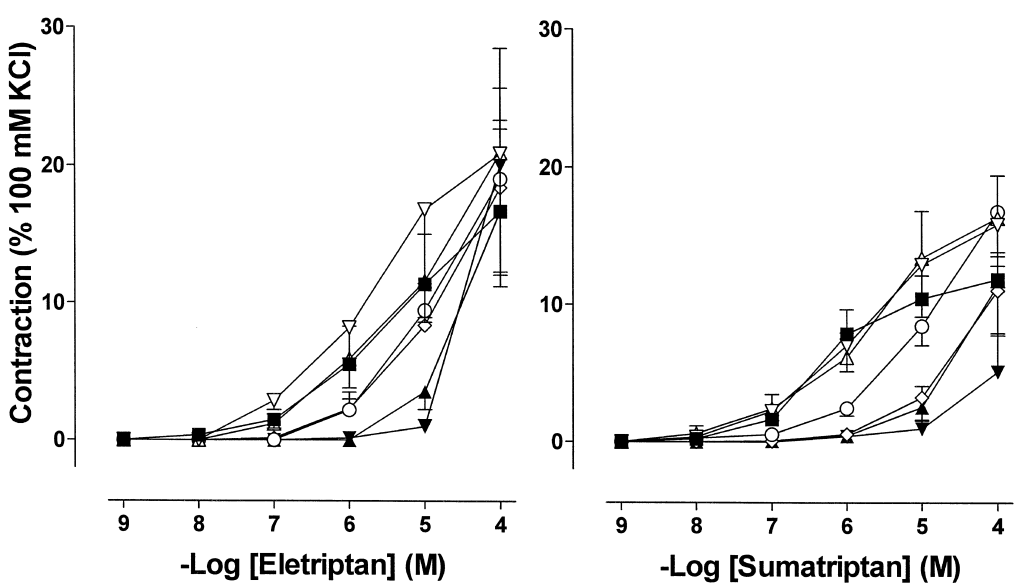

Fig. 2. Cumulative concentration–response curves to eletriptan (left panel; n = 4–8) and sumatriptan (right panel; n = 4–9) in the human isolated coronary artery in the absence (■) or presence of GR125743 (▽, 3 nM; △, 10 nM; ○, 30 nM; ◇, 100 nM; ▲, 300 nM or ▼, 1 μM). Symbols and vertical bars represent the mean and S.E.M.

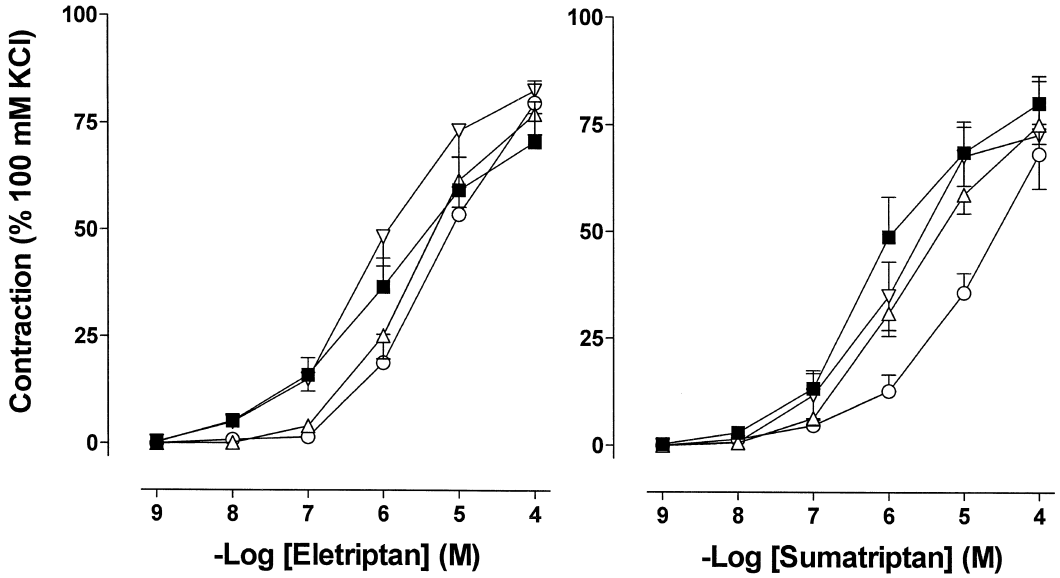

Fig. 3. Cumulative concentration–response curves to eletriptan (left panel; $n = 6$) and sumatriptan (right panel; $n = 6$) in the human isolated saphenous vein in the absence (■) or presence of GR125743 (▽, 3 nM; △, 10 nM or ○, 30 nM). Symbols and vertical bars represent the mean and S.E.M.

similar to the pA_2 value found in both middle meningeal and coronary artery (Table 1). The pK_B of GR125743 obtained at 30 nM against eletriptan (7.73 ± 0.18) appears to be somewhat lower compared to the apparent pA_2 value found against sumatriptan (8.34 ± 0.12). This is also reflected in the fact that the dose ratio obtained at 30 nM GR125743 for eletriptan (3.4) was significantly lower than that for sumatriptan (18).

3.4. Cranioselectivity of eletriptan and sumatriptan

The mean cranioselectivity ratios (inverse logarithm of the difference between the pEC_{50} value in the middle meningeal artery and that in the coronary artery or saphenous vein) and their respective 95% CI are shown in Table 1. Compared to coronary artery and saphenous vein, both eletriptan (63- and 18-fold, respectively) and sumatriptan (5-fold each) were significantly more selective for the meningeal artery. The cranioselectivity for eletriptan in the coronary was significantly higher than that for sumatriptan.

4. Discussion

In the present study, we investigated the effects of eletriptan and sumatriptan on the human isolated middle meningeal artery as a model for anti-migraine activity, and coronary artery and saphenous vein as models for peripheral vascular side-effect potential. In addition, the role of $5\text{-HT}_{1B/1D}$ receptors in the vasoconstrictor responses to the two triptans was evaluated using GR125743, a potent and competitive $5\text{-HT}_{1B/1D}$ receptor antagonist (Domenech et al., 1997; Pauwels, 1997).

4.1. Efficacy profile of eletriptan and sumatriptan in blood vessels

It is well known that acutely acting antimigraine drugs, such as the triptans, have a vasoconstrictor effect, particularly on cranial vessels (De Vries et al., 1999a; Saxena and Tfelt-Hansen, 2000). Indeed, eletriptan contracted human isolated blood vessels used in this investigation with a maximum response similar to that of sumatriptan (see E_{max} values in Table 1). This is in accordance with our previous study in human vessels, where 5-HT also showed a similar E_{max} (MaassenVanDenBrink et al., 2000), but contrasts with observations in the canine basilar artery and saphenous vein, where eletriptan behaved as a partial agonist (Gupta et al., 1999). However, it must be pointed out that in our experiments the efficacy of eletriptan was not studied after receptor alkylation, thus not ruling out the partial agonist nature of eletriptan in human blood vessels.

Unlike sumatriptan, the highest concentration of eletriptan (100 μM) elicited a profound relaxation of the human middle meningeal. The exact reason for this difference between eletriptan and sumatriptan is not known. However, a possible explanation could be that at such high concentrations, which are well beyond the clinically relevant free (i.e., protein unbound) plasma C_{max} values of around 30 and 65–90 nM obtained after 40 and 80 mg oral eletriptan, respectively (Personal communication A.D. McHarg; Milton et al., 1998), the contraction elicited by eletriptan was overruled by vasorelaxation mediated by the 5-HT_7 receptor (Eglen et al., 1997; Saxena et al., 1998;

Terron and Falcon-Neri, 1999). The 5-HT_7 receptor mRNA has been detected in human meningeal vessels (Schmuck et al., 1996) and at this receptor eletriptan (pK_i: 6.70 ± 0.06) has a higher affinity than sumatriptan (pK_i: 5.86 ± 0.11) (Napier et al., 1999). Interestingly, eletriptan relaxed neither the coronary artery nor saphenous vein; the 5-HT_7 receptor mRNA is poorly expressed in the human coronary artery (Nilsson et al., 1999b).

4.2. Effects of GR125743 on contraction elicited by eletriptan and sumatriptan

The contractions elicited by sumatriptan were competitively antagonised by GR125743 in the human middle meningeal and coronary arteries as well as the saphenous vein with similar pA_2 values (8.64, 8.18 and 8.34, respectively; Table 1). These values resemble the affinity estimates of GR125743 at 5-$HT_{1B/1D}$ receptors in radioligand binding (pK_i: 8.2–9.0, Audinot et al., 1997; Domenech et al., 1997) and functional studies against sumatriptan-induced contractions of the human isolated middle meningeal artery (pK_B: 9.1, Razzaque et al., 1999). The results reveal that sumatriptan acts on the same 5-HT receptor (most probably 5-HT_{1B}, Kaumann et al., 1993) in all three blood vessels studied. Indeed, elegant in situ hybridisation and immunohistochemistry experiments have demonstrated that the 5-HT_{1B} but not 5-HT_{1D} receptor mRNA and protein are present in the smooth muscles of human middle meningeal and coronary arteries (Bouchelet et al., 1996; Longmore et al., 1997, 1998; Nilsson et al., 1999a,b). In addition, selective 5-HT_{1D} and 5-HT_{1F} receptor agonists failed to constrict the human cerebral arteries, excluding a role for these receptors in vasomotor responses (Ennis et al., 1998; Bouchelet and Hamel, 1999; Cohen and Schenck, 1999). Finally, the selective 5-HT_{1B} receptor antagonist 2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazo-l-3-yl) biphenyl-4-carbonyl]furo[2,3,*f*]indole-3-spiro-4′-piperidine hydrochloride (SB224289) antagonises sumatriptan-induced vasoconstrictor responses (Verheggen et al., 1998; De Vries et al., 1999b). Thus, there is now overwhelming evidence that the 5-HT_{1B} (not 5-HT_{1D}) receptor mediates vasoconstrictor responses to the triptans.

The contraction of the middle meningeal artery by eletriptan was antagonised by GR125743 with a pA_2 (8.81; Table 1) that was similar to that against sumatriptan-induced contraction in this artery and eletriptan-induced contractions in the dog isolated basilar artery and saphenous vein (pA_2 values: 9.1 and 9.4, respectively) (Gupta et al., 1999). However, in the coronary artery and saphenous vein, GR125743 appeared to block eletriptan-induced contractions in a slightly different manner compared to those by sumatriptan. In the coronary artery, higher concentrations of GR125743 ($\geq$ 300 nM) revealed a non-sigmoidal nature of the concentration–response curves to eletriptan, whereas lower concentrations ($\leq$ 100 nM) of GR125743 failed to block eletriptan-induced contractions (Fig. 2). In the saphenous vein, the Hill slope of eletriptan was significantly different from unity, indicating the involvement of more than one mechanism and, accordingly, GR125743 caused a rightward shift in the concentration–response curve to eletriptan only with the highest concentration used (30 nM; Fig. 3). Moreover, the dose ratio of eletriptan following 30 nM GR125743 (3.4) was significantly lower than that of sumatriptan (18). The discrepancy between antagonism by GR125743 against eletriptan-induced contraction in the coronary artery and saphenous vein and the results obtained in dog basilar artery and saphenous vein (Gupta et al., 1999) may well be species related or could be based on methodological differences, as we have not used the mixed 5-$HT_{2/7}$ receptor antagonist mesulergine in our experiments.

Overall, these data suggest that the sumatriptan-induced contraction in the three vessels examined is mediated by the 5-HT_{1B} receptor. Furthermore, the contractile effects of eletriptan at lower concentrations is largely mediated by the 5-HT_{1B} receptor but an additional receptor/mechanism, unaffected by GR125743, contributes to some extent in the contractions of the human coronary artery and saphenous vein elicited by high concentrations of eletriptan. This is indirectly supported by our experiments in the anaesthetised pigs, where we reported that, unlike sumatriptan (De Vries et al., 1996), the eletriptan-induced constriction of carotid arteriovenous anastomoses was not completely blocked by GR127935, another potent 5-$HT_{1B/1D}$ receptor antagonist (Willems et al., 1998). The exact nature of this additional mechanism involved in eletriptan-induced contraction is unknown, but it is conceivable that a part of the contraction to high concentrations of eletriptan, particularly after blockade of the 5-HT_{1B} receptor by GR125743, may be mediated via the 5-HT_{2A} receptor. In contrast to the human middle meningeal artery that predominantly possesses 5-HT_{1B} receptor population (Jansen et al., 1992; Razzaque et al., 1999), it is known that both human coronary artery and saphenous vein have a mixed population of 5-HT_{1B} and 5-HT_2 receptors (Connor et al., 1989; Bax et al., 1992, 1993; Ishida et al., 1999; Nilsson et al., 1999b). We must, however, concede that eletriptan does not differ from sumatriptan with regard to the affinity at the 5-HT_{2A} receptor (pK_i: both < 5.5, Napier et al., 1999).

4.3. Cranioselectivity of eletriptan and sumatriptan

Eletriptan and sumatriptan contracted the human isolated middle meningeal artery with equal efficacy (E_{max}: 94 ± 8% and 106 ± 17% of the contraction to 1 μM prostaglandin $F_{2\alpha}$, respectively) and potency (pEC_{50}: 7.34 ± 0.13 and 6.91 ± 0.17, respectively). The two drugs exhibited cranioselectivity because they were significantly more potent in contracting the middle meningeal artery than coronary artery (pEC_{50}: 5.54 ± 0.22 and 6.24 ± 0.14,

respectively) or saphenous vein (pEC_{50}: 6.09 ± 0.13 and 6.19 ± 0.12, respectively; Table 1). Eletriptan and sumatriptan were equipotent in the saphenous vein, but in the coronary artery, as we found earlier with another 5-$HT_{1B/1D}$ receptor agonist 3-[2-(dimethylanimo)ethyl]-5-[(trifluoromethyl)sulfonyl]oxy][1*H*]indole oxalate (GMC2021) (Saxena et al., 1996), eletriptan was significantly less potent than sumatriptan. This latter finding appears at variance with the higher binding affinity of eletriptan for the 5 HT_{1B} receptor compared to sumatriptan (Napier et al., 1999), but may involve agonist-specific differences in stimulus-effector coupling (Kenakin, 1987). It can perhaps be argued that the lower potency of eletriptan on the coronary artery may be due to the involvement of the non-5 $HT_{1B/1D}$ mechanism for which eletriptan has a low affinity (see Section 4.2). However, we believe that the contraction elicited via a high affinity site (in this case, the 5 HT_{1B} receptor) would overrule that via a low affinity site.

Based on the difference in pEC_{50} values, sumatriptan proved 5-fold (95% CI: 2–13) more selective for the meningeal artery compared to both coronary artery and saphenous vein. This cranioselectivity ratio of eletriptan in the coronary artery (63, 95% CI 20–200) was significantly higher compared to that of sumatriptan ($P < 0.01$), whereas in the saphenous vein the cranioselectivity of eletriptan, being 18-fold (95% CI: 8–41), was not different from that of sumatriptan ($P = 0.06$). Admittedly, the data obtained with eletriptan are complicated by two factors. Firstly, the coronary artery contraction has a non-5-HT_{1B} receptor component. However, it is the resultant coronary artery contraction and not its underlying mechanism that is important for potential coronary side effects. Secondly, the relaxant response in the middle meningeal artery observed with eletriptan (100 μM) could well reduce its E_{max} and, thus, overestimate its pEC_{50}, compared to sumatriptan. Our results, however, show that the maximal contraction (and Hill slope) by eletriptan is similar to that of sumatriptan as well as 5-HT (see Table 1; MaassenVanDenBrink et al., 2000). Although, the relaxant response manifests at the highest concentration, it cannot be excluded that this response is latently present throughout the concentration range so that the whole concentration–response curve may have been shifted to the right. In this case, it is equally possible that we may have underestimated the pEC_{50} (and cranioselectivity) of eletriptan.

The cranio-coronary selectivity of eletriptan, together with the clinical data showing that the highest dose of eletriptan (80 mg) has a superior efficacy, onset of action and patient acceptability in the acute treatment of migraine when compared with oral sumatriptan (100 mg) (Goadsby et al., 2000), speak in favour of eletriptan. However, it cannot be overemphasised that eletriptan has the capacity to constrict the human coronary artery and, therefore, like the other triptans, must remain contraindicated in patients with coronary artery disease.

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