ELSEVIER

European Journal of Pharmacology 443 (2002) 125–132

ejp

www.elsevier.com/locate/ejphar

Comparison of contractile responses to donitriptan and sumatriptan in the human middle meningeal and coronary arteries

Rémon W.M. van den Broek [a], Antoinette MaassenVanDenBrink [a], Paul G.H. Mulder [b], Ad J.J.C. Bogers [c], Cees J.J. Avezaat [d], Gareth W. John [e], Pramod R. Saxena [a,*]

[a] *Department of Pharmacology, Erasmus University Medical Centre Rotterdam, Post Box 1738, 3000 DR Rotterdam, The Netherlands*
[b] *Department of Epidemiology and Biostatistics, Erasmus University Medical Centre, Post Box 1738, 3000 DR Rotterdam, The Netherlands*
[c] *Department of Cardiothoracic Surgery, Erasmus University Medical Centre, Post Box 1738, 3000 DR Rotterdam, The Netherlands*
[d] *Department of Neurosurgery, Erasmus University Medical Centre, Post box 1738, 3000 DR Rotterdam, The Netherlands*
[e] *Division of Cardiovascular Diseases, Centre de Recherche Pierre Fabre, 17, Avenue Jean Moulin, 81100 Castres, France*

Received 10 January 2002; received in revised form 18 March 2002; accepted 26 March 2002

Abstract

Donitriptan is a potent, high efficacy agonist at 5-$HT_{1B/1D}$ receptors. We investigated the contractile effects of donitriptan and sumatriptan on human isolated blood vessels of relevance to therapeutic efficacy in migraine (middle meningeal artery) and coronary adverse events (coronary artery). Furthermore, using the concentration–response curves in the middle meningeal artery, we predicted the plasma concentration needed for the therapeutic effect of donitriptan. Both donitriptan and sumatriptan contracted the middle meningeal artery with similar apparent efficacy (E_{max}: 103 ± 8% and 110 ± 12%, respectively), but the potency of donitriptan (pEC_{50}: 9.07 ± 0.14) was significantly higher than that of sumatriptan (pEC_{50}: 7.41 ± 0.08). In the coronary artery, the contraction to donitriptan was biphasic with a significantly higher maximal response (E_{max}: 29 ± 6%) than sumatriptan (E_{max}: 14 ± 2%; pEC_{50}: 5.71 ± 0.16), yielding two distinct pEC_{50} values (8.25 ± 0.16 and 5.60 ± 0.24). Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) eliminated the low affinity component of the concentration–response curve of donitriptan and the resultant E_{max} and pEC_{50} were 9 ± 2% and 7.33 ± 0.21, respectively. Ketanserin was without effect on the sumatriptan-induced contraction. Based on the middle meningeal artery contraction, concentrations (C_{max}) of donitriptan that may be expected to have a therapeutic efficacy equivalent to that of 50 and 100 mg sumatriptan are predicted to be around 2.5 and 4.3 nM, respectively. Such concentrations are likely to induce only a small coronary artery contraction of 2.9 ± 1.5% and 3.8 ± 2.0%, respectively; these are not different from those by C_{max} concentrations of sumatriptan (1.7 ± 0.4% or 2.2 ± 0.4%). The present results suggest that, like sumatriptan, donitriptan exhibits cranioselectivity and would be effective in aborting migraine attacks with a similar coronary side-effect profile as sumatriptan.

Keywords: Coronary artery; Donitriptan; Human pharmacology; Middle meningeal artery; Migraine; Sumatriptan

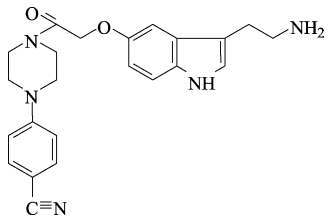
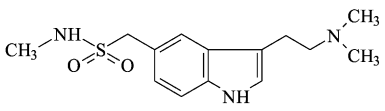
1. Introduction

The introduction of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan (Humphrey and Feniuk, 1991) has been followed by a number of 'second-generation' triptans (Connor et al., 1997; Martin, 1997; Parsons et al., 1998; Gupta et al., 1999), all of which effectively abort migraine headache (Goadsby, 1998; Diener and Limmroth, 1999; Deleu and Hanssens, 2000; Millson et al., 2000; Tfelt-Hansen et al., 2000; Ferrari et al., 2001). Despite the higher affinity at 5-$HT_{1B/1D}$ receptors, better oral bioavailability, increased brain penetration and longer plasma half-life, somewhat surprisingly, the newer triptans show only subtle differences with sumatriptan in the efficacy and tolerability (Goadsby, 1998; Deleu and Hanssens, 2000; Fox, 2000; Millson et al., 2000; Tfelt-Hansen et al., 2000; Ferrari et al., 2001). In the light of the above, Centre de Recherche Pierre Fabre (Castres, France) have reported another triptan, donitriptan (F11356: hydrochloride salt or F12640: mesylate salt), which displays a uniquely high affinity and, more importantly, intrinsic efficacy at recombinant human 5-$HT_{1B/1D}$ receptors with negligible affinity for the 5-HT_{1F} receptor (Table 1). In animal experiments, donitriptan has been shown to have a long duration of action, to gain access to the brain and to be well tolerated (John et al., 1999, 2000).

* Corresponding author. Tel.: +31-10-408-7537/47; fax: +31-10-408-9458.
E-mail address: Saxena@farma.fgg.eur.nl (P.R. Saxena).
URL: http://www.fgg-eur.nl/pharm/.

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01576-5

Table 1
Chemical structure and affinity and efficacy of donitriptan and sumatriptan at human 5-$HT_{1B/1D/1F}$ receptors

Compound	Chemical structure	Inhibition of radioligand binding (pK_i)			Inhibition of cAMP accumulation (pEC_{50})		Enhancement of [^{35}S]GTPγS binding (pEC_{50})	
		5-HT_{1B}	5-HT_{1D}	5-HT_{1F}	5-HT_{1B}	5-HT_{1D}	5-HT_{1B}	5-HT_{1D}
Donitriptan[a]		9.4–10.1	9.3–10.2	5.5	8.91	9.57	8.74	9.08
Sumatriptan		7.8[b]	8.5[b]	7.9[b]	7.16[c]	8.79[c]	6.63[c]	7.75[c]

pK_i and pEC_{50} are expressed as $-\log M$.
[a] John et al. (1999).
[b] Leysen et al. (1996).
[c] Pauwels et al. (1997).

The therapeutic potential of the drug is currently being evaluated in migraine patients.

It is now generally accepted that the therapeutic action of sumatriptan and other triptans is mainly due to constriction of dilated intra- and extracranial blood vessels, although other mechanisms interfering with the trigeminovascular system may also be involved (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001). The constriction of cranial blood vessels by triptans is much more marked than that of the peripheral arteries such as the coronaries (Longmore et al., 1998; Parsons et al., 1998; MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000). Despite this cranioselectivity, the triptans do have a propensity to cause cardiovascular adverse events including myocardial ischaemia and infarction in predisposed individuals (Kelly, 1995; Ottervanger et al., 1997; Main et al., 1998).

In the present study, we have investigated the contractile effects of donitriptan and sumatriptan on human isolated blood vessels of relevance to therapeutic efficacy (middle meningeal artery) and coronary adverse events (coronary artery). Furthermore, using the concentration–response curves in the middle meningeal artery and the basic assumptions about the mechanism of action of triptans, we have made an attempt to predict the free (protein-unbound) plasma concentration needed for therapeutic effect of donitriptan.

2. Material and methods

2.1. Tissue collection

2.1.1. Human middle meningeal artery

The human middle meningeal artery was obtained from seven patients (three male, four female; age 20–72 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 mater, together with a small piece of the middle meningeal artery, is 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, immediately transported to the laboratory and 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 cyclo-oxygenase 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 and macroscopically abnormal segments were rejected. The middle meningeal artery was stored overnight in cold oxygenated Krebs solution and was used the following day.

2.1.2. Human coronary artery

The human right epicardial coronary artery was obtained from seven 'heart-beating' organ donors (five male, two female; 12–65 years), who died of non-cardiac disorders. 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). Upon arrival at the laboratory, the right coronary artery was 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 and

macroscopically abnormal segments were rejected. The artery was stored overnight in cold oxygenated Krebs solution and was used the following day.

2.2. Experimental protocol

2.2.1. Human 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 1.203 h 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 two to three times to 0.1 μM prostaglandin $F_{2\alpha}$ to demonstrate reproducibility of the evoked contractions. If such contractions were less than 3 mM, the vessel segments were not included in the study. Subsequently, the segments were precontracted 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, where possible (i.e. in five out of seven arteries), in a paired parallel experimental setup and a cumulative concentration–response curve to either donitriptan (0.03 nM–1 μM) or sumatriptan (1 nM–10 μM) (both dissolved in 40% v/v polyethylene glycol) was constructed.

2.2.2. Human coronary artery

The artery was 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. Changes in tension were recorded using a Harvard isometric transducer (Harvard Apparatus, South Natick, MA, USA). 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. All segments were then exposed to KCl (30 mM) twice, to prime tissue reproducibility. Subsequently, the functional integrity of the endothelium was verified by observing relaxation to substance P (1 nM) after precontraction 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 allowed to equilibrate for another period of 30 min. After this equilibration period the segments were divided in a paired parallel experimental setup and a cumulative concentration–response curve to either donitriptan, sumatriptan (both dissolved in 40% v/v polyethylene glycol) or sumatriptan (dissolved in distilled water) was constructed. In addition, we incubated half of the segments with the 5-HT_2 receptor antagonist ketanserin (10 μM) for 30 min prior to constructing concentration–response curves to donitriptan and sumatriptan.

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). Initially, we calculated the mean value of the individual maximum contractile responses (E_{max}) to donitriptan and sumatriptan; in case the contractions did not reach a plateau, the contraction induced by the highest agonist concentration was considered as E_{max}. Except for donitriptan in the coronary artery, we analysed all concentration–response curves with a nonlinear regression fitting technique for sigmoidal functions with variable slope using Prism® 3.0 (Graphpad Software, San Diego, CA, USA) to obtain pEC_{50} values for the agonists. The mean concentration–response curve to donitriptan in the coronary artery appeared to be biphasic in nature. Indeed, the curve fitted significantly better to a model of two receptor populations than to a sigmoidal model (goodness of fit: $R = 0.99$ and 0.97, respectively, $P < 0.05$; Prism® 3.0). Thus, two distinct pEC_{50} values of donitriptan-induced contractions in coronary artery were obtained.

Cranioselectivity ratios were calculated as the inverse logarithmic difference between respective pEC_{50} values of donitriptan and sumatriptan in the middle meningeal and coronary arteries (see MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000).

2.3.2. Prediction of plasma concentration of donitriptan required for therapeutic activity

The average free (protein-unbound) maximum plasma concentration (C_{max}) in human volunteers following 50 mg and 100 mg oral sumatriptan is 81 and 135 nM, respectively (see MaassenVanDenBrink et al., 1998, 2000; Fox, 2000). The extent of middle meningeal artery contractions elicited at these concentrations of sumatriptan was derived using individual concentration–response curves (see Fig. 1). Based on the assumption that constriction of dilated cranial blood vessels is the major mechanism of action of triptans in migraine (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001), we have attempted to predict therapeutic C_{max} of donitriptan, i.e. the concentrations of donitriptan that would be needed to elicit a middle meningeal artery contraction equivalent to that caused by 81 or 135 nM of sumatriptan. In two out of seven experiments with donitriptan in the middle meningeal artery, the predicted contraction was higher than the individual E_{max} of donitriptan, thus excluding these experiments from this analysis. Therefore, we chose to analyse the mean concentration–response curve of donitriptan to predict the mean

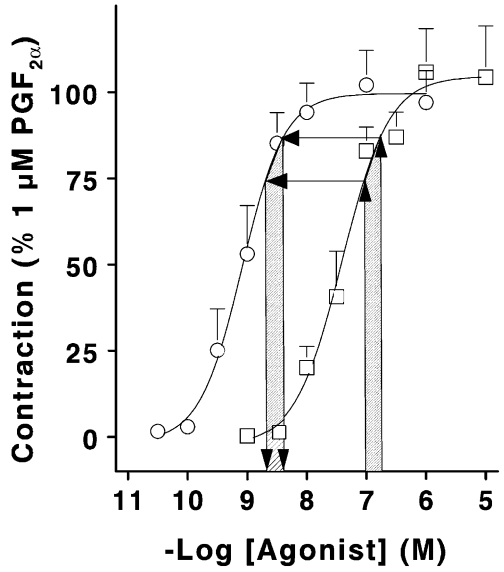


Fig. 1. Cumulative concentration–response curves to donitriptan (○; $n=7$) and sumatriptan (□; $n=5$) in the human isolated middle meningeal artery. Symbols and vertical bars represent the means and S.E.M. $PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$. Arrows represent the prediction of the contraction of the artery at the average free (protein-unbound) C_{max} (81 or 135 nM) after a therapeutic dose of sumatriptan (50 or 100 mg oral tablet, respectively) and the predicted therapeutic C_{max} of donitriptan needed to elicit a contraction equivalent to that caused by 81 or 135 nM sumatriptan.

therapeutic free C_{max} and 95% CI using SPSS 9.0 nonlinear regression statistics (SPSS, Chicago, IL, USA). Lastly, the individual concentration–response curves in the coronary artery were used to calculate the extent of coronary artery contraction that may be expected at the (predicted) therapeutic C_{max} of sumatriptan and donitriptan.

2.3.3. Data presentation and statistical analysis

All data are presented as means ± S.E.M., except where 95% confidence intervals (95% CI) are mentioned. Differences between E_{max} and pEC_{50} values of concentration–response curves as well as the goodness of fit (sigmoidal or biphasic nature of donitriptan in the coronary artery) were analysed according to paired *t*-test or repeated measures one-way analysis of variance (ANOVA), followed where appropriate (coronary artery) by Tukey's multiple comparison *t*-test (Prism® 3.0). The 95% CI of craniose-lectivity ratios obtained with donitriptan and sumatriptan was compared as described by Steel and Torrie (1980). In all cases, statistical significance was assumed when $P<0.05$.

2.4. Ethical approval

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

2.5. Compounds

Indomethacin hydrochloride, ketanserin tartrate, prostaglandin $F_{2\alpha}$ tris salt and substance P acetate were purchased from Sigma (St. Louis, MO, USA). Donitriptan mesylate (F12640) was kindly provided by Centre de Recherche Pierre Fabre. Sumatriptan succinate (batch: C1008/122/1) was a gift from Glaxo SmithKline (Ware, Kent, UK). Indomethacin was dissolved in 100% v/v dimethyl sulphoxide and further diluted in distilled water. Donitriptan mesylate (F12640) was dissolved in 40% v/v polyethylene glycol and sumatriptan was either dissolved in 40% v/v polyethylene glycol or distilled water. All other compounds were dissolved in distilled water.

3. Results

3.1. Relaxation responses to substance P

In vessel segments of the middle meningeal and coronary arteries ($n=7$ each), the relaxation to substance P amounted to 26 ± 8% and 58 ± 10% of precontraction to 1 μM prostaglandin $F_{2\alpha}$, respectively.

3.2. Human middle meningeal artery

As shown in Fig. 1 and Table 2, both donitriptan ($n=7$) and sumatriptan ($n=5$) contracted the middle meningeal artery in a concentration-dependent manner with a similar efficacy (E_{max}: 103 ± 8% and 110 ± 12% of contraction to 1 μM prostaglandin $F_{2\alpha}$, respectively). The potency (pEC_{50}) of donitriptan was significantly higher than that of sumatriptan (9.07 ± 0.14 and 7.41 ± 0.08, respectively; $P<0.0001$). This was also the case when only the five experiments with donitriptan performed in parallel with sumatriptan were considered (pEC_{50}: 9.05 ± 0.17). The Hill slopes of the concentration–response curves to donitriptan (1.2 ± 0.3) and sumatriptan (1.1 ± 0.3) did not significantly differ from each other, nor were they significantly different from unity.

3.3. Human coronary artery

The human isolated coronary artery also contracted in response to donitriptan and sumatriptan in a concentration-dependent manner (Fig. 2 and Table 2). Donitriptan contracted the coronary artery biphasically with an E_{max} of 29 ± 6% of contraction to 100 mM KCl. The concentration–response curves to donitriptan were fitted to a model of two receptor populations and revealed two distinct pEC_{50} values (8.25 ± 0.16 and 5.60 ± 0.24). Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) revealed a sigmoidal curve with a potency (pEC_{50}) of 7.33 ± 0.21 yielding a significant decrease in E_{max} (9 ± 2% of contraction to 100 mM KCl) as compared to donitriptan in the

Table 2
Functional parameters obtained with donitriptan and sumatriptan in the absence or presence of ketanserin (10 μM) in the human isolated middle meningeal and coronary arteries

	Middle meningeal artery		Coronary artery		Cranioselectivity ratio[a] (95% CI)
	E_{max}	pEC_{50}	E_{max}	pEC_{50}	
Donitriptan	103 ± 8%	9.07 ± 0.14[b]	29 ± 6%[b]	8.25 ± 0.16[b,c] 5.60 ± 0.24[c]	7 (3–16)[d,e]
Donitriptan + ketanserin	NP	NP	9 ± 2%	7.33 ± 0.21[b]	55 (19–162)[d]
Sumatriptan	110 ± 12%	7.41 ± 0.08	14 ± 2%	5.71 ± 0.16	50 (22–113)[d]
Sumatriptan + ketanserin	NP	NP	11 ± 3%	5.76 ± 0.14	45 (22–92)[d]

Data are presented as means ± S.E.M. or as mean (95% CI). Except sumatriptan in the middle meningeal artery ($n = 5$), the number of experiments were 7 in each case. The E_{max} values are presented as percentage of contraction elicited by either 1 μM prostaglandin $F_{2\alpha}$ (middle meningeal artery) or 100 mM KCl (coronary artery). NP, Not performed.

[a] Cranioselectivity ratio = Inverse logarithm [pEC_{50} (middle meningeal artery) − pEC_{50} (coronary artery)].

[b] Significantly different from respective values obtained with sumatriptan ($P < 0.05$).

[c] Biphasic concentration response curves yielding two distinct pEC_{50} values.

[d] Significantly different from 1 ($P < 0.05$).

[e] Significantly lower than sumatriptan ($P < 0.05$).

absence of ketanserin ($P < 0.01$). The Hill slope of the concentration–response curves to donitriptan in the presence of ketanserin was 0.8 ± 0.1, which was not significantly different from unity.

Sumatriptan, dissolved in distilled water, contracted the coronary artery with a potency (pEC_{50}) of 5.71 ± 0.16 and maximal effect (E_{max}) of 14 ± 2% of contraction to 100 mM KCl; these were significantly lower as compared to the pEC_{50} (high affinity component) and E_{max} of donitriptan. The Hill slope of concentration–response curves to sumatriptan (0.6 ± 0.1) was significantly different from unity. Incubation with the 5-HT_2 receptor antagonist ketanserin (10 μM) was without effect on either the E_{max} (11 ± 3% of contraction to 100 mM KCl) or the pEC_{50} (5.76 ± 0.14) of sumatriptan, whereas the Hill slope did not differ from unity (0.8 ± 0.3).

Due to the fact that donitriptan was dissolved in 40 v/v polyethylene glycol, we investigated whether the solvent influenced contraction to sumatriptan. Sumatriptan dissolved in 40 v/v polyethylene glycol caused a similar contraction (E_{max}: 10 ± 2% of contraction to 100 mM KCl; pEC_{50}: 6.18 ± 0.29; Hill slope: 0.8 ± 0.3) as compared to sumatriptan dissolved in distilled water.

3.4. Cranioselectivity ratio of donitriptan and sumatriptan

The mean cranioselectivity ratios (inverse logarithm of the difference between the pEC_{50} values in the middle meningeal and coronary arteries) and the respective 95% CI are shown in Table 2. Due to the fact that donitriptan showed a biphasic response in the human coronary artery, the pEC_{50} value obtained in the lower (high affinity) part of the curve was used to calculate the cranioselectivity ratio, since the therapeutic concentration is expected to be in this range (see Section 3.5). Both donitriptan and sumatriptan were significantly more potent in the middle meningeal artery as compared to the coronary artery, whereas the cranioselectivity ratio for donitriptan was slightly lower than that for sumatriptan. After treatment with ketanserin, both donitriptan and sumatriptan had similar cranioselectivity ratios (Table 2).

3.5. Prediction of plasma concentration of donitriptan required for therapeutic activity and predicted contractions

The mean therapeutic free C_{max} following administration of a 50 mg or 100 mg oral tablet of sumatriptan has been reported to be 81 and 135 nM, respectively (see Maassen-VanDenBrink et al., 1998). As shown in Table 3, the contraction to sumatriptan that is predicted to occur at these concentrations was 79 ± 8% and 89 ± 9% of contraction to

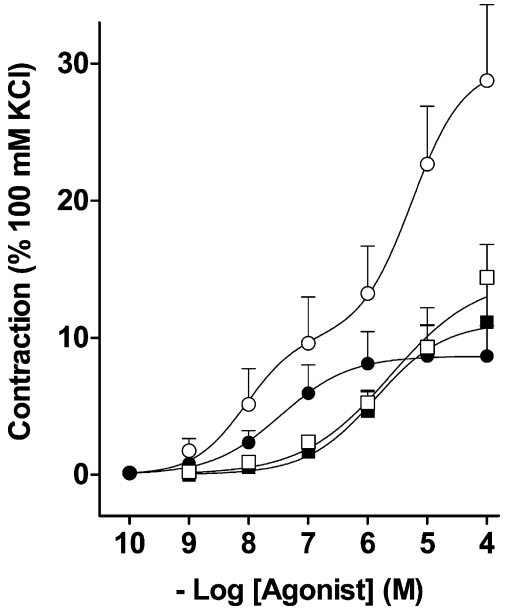


Fig. 2. Cumulative concentration–response curves ($n = 7$ each) to donitriptan in the absence (○) or presence of 10 μM ketanserin (●) and sumatriptan in the absence (□) or presence of 10 μM ketanserin (■). Symbols and vertical bars represent the mean and S.E.M.

Table 3
Predicted therapeutic free plasma C_{max} of donitriptan and predicted contraction of middle meningeal and coronary arteries at free therapeutic C_{max} of sumatriptan and donitriptan

	Free therapeutic C_{max} (nM)		Predicted contraction at free C_{max}	
	Known[a]	Predicted nM (95% CI)	Middle meningeal artery (percent prostaglandin $F_{2\alpha}$)	Coronary artery (percent KCl)
Sumatriptan				
50 mg	81		79 ± 8	1.7 ± 0.4
100 mg	135		89 ± 9	2.2 ± 0.4
Donitriptan				
X mg[b]		2.5 (1.5–6.5)	79	2.9 ± 1.5
Y mg[c]		4.3 (2.4–12.9)	89	3.8 ± 2.0

Data are presented as means ± S.E.M. or as mean (95% CI). Except sumatriptan in the middle meningeal artery ($n = 5$), the number of experiments were 7 in each case.

[a] See MaassenVanDenBrink et al. (1998) and Fox (2000).

[b] Values based on middle meningeal artery contraction induced by free C_{max} after 50 mg sumatriptan.

[c] Values based on middle meningeal artery contraction induced by free C_{max} after 100 mg sumatriptan.

1 μM prostaglandin $F_{2\alpha}$, respectively, in the middle meningeal artery and 1.7 ± 0.4% and 2.2 ± 0.4% of contraction to 100 mM KCl, respectively, in the coronary artery. Interpolating concentration–response curves to donitriptan in the middle meningeal artery, the predicted therapeutic free C_{max} values of donitriptan (i.e. concentration eliciting 79% or 89% of contraction to 1 μM prostaglandin $F_{2\alpha}$) were 2.5 nM (95% CI: 1.5–6.5 nM) and 4.3 nM (95% CI: 2.4–12.9 nM), respectively. At these predicted free C_{max} values, the contraction of donitriptan in the coronary artery may amount to 2.9 ± 1.5% and 3.8 ± 2.0% of contraction to 100 mM KCl, respectively; these were not significantly different from those calculated for sumatriptan (Table 3).

4. Discussion

4.1. Efficacy profile in the middle meningeal artery

It is well known that acutely acting antimigraine drugs (ergots and triptans) have a vasoconstrictor effect, particularly on large cranial arteries and arteriovenous anastomoses (De Vries et al., 1999; Tfelt-Hansen et al., 2000). Donitriptan contracted the human isolated middle meningeal artery with a similar maximal response as sumatriptan (see E_{max} values in Table 2). The results are in accordance with those obtained in the rabbit isolated saphenous vein (John et al., 2000), an established model to detect agonist activity at 5-HT_{1B} receptors (Valentin et al., 1996; Wurch et al., 1997), but are at variance with the high intrinsic activity of donitriptan at 5-$HT_{1B/1D}$ receptors demonstrated in other experimental models (John et al., 1999, 2000). Indeed, like the rabbit saphenous vein model, the human middle meningeal artery preparation apparently does not distinguish between low and high efficacy 5-HT_{1B} receptor agonists, suggesting the preparation is likely to express 5-HT_{1B} receptors in a high density (Longmore et al., 1997, 1998). It is noteworthy that high efficacy agonist activity is not necessarily observed in every model, and models that distinguish high from low efficacy agonists have a low receptor reserve, due to a relatively low receptor density and/or inefficient second messenger coupling (Kenakin, 1993; John et al., 2000). We concede that the E_{max} of donitriptan-induced coronary artery contraction in the presence of ketanserin (mediated by the 5-HT_{1B} receptor) was not higher than that of sumatriptan (see Fig. 2) despite a low 5-HT_{1B} receptor density in this vessel (Longmore et al., 1998), but we do not have data with regard to receptor coupling.

There is now overwhelming evidence that cranial vasoconstrictor response to triptans is mediated via the 5-HT_{1B} receptor (Bouchelet et al., 1996; Longmore et al., 1997, 1998; Van den Broek et al., 2000; Centurión et al., 2001). Indeed, in accordance with the binding affinities at the human 5-HT_{1B} receptor (see Table 1), donitriptan (pEC_{50}: 9.07 ± 0.14) was 48-fold more potent than sumatriptan (pEC_{50}: 7.41 ± 0.08) in contracting the human middle meningeal artery.

4.2. Efficacy profile in the coronary artery

Concentration–response curves to donitriptan in the human isolated coronary artery revealed a biphasic response with a significantly higher maximum contraction as compared to sumatriptan (E_{max}: 29 ± 6% and 14 ± 2% of the response to 100 mM KCl, respectively). This biphasic response and higher response amplitude of donitriptan appears to be at variance with findings in the canine isolated coronary artery, where both donitriptan and sumatriptan showed a similar efficacy and sigmoidal shape of the concentration–response curve (John et al., 2000). However, in contrast to the predominant role of 5-HT_{2A} over 5-HT_{1B} receptors in the human coronary artery (Connor et al., 1989; Kaumann et al., 1994; Ishida et al., 1999; Nilsson et al., 1999), the 5-HT_2 receptor antagonist ketanserin was unable to block 5-HT-induced contractions in the canine coronary artery, suggesting that the 5-HT_{2A} receptor plays little role in this preparation (Cushing and Cohen, 1992). Therefore, we reasoned that the biphasic response to donitriptan in the present experiments could be due to activation of a 5-HT_2-like (possibly the 5-HT_{2A}) receptor at high concentrations of donitriptan (pK_i: 6.7, John et al., 1999). Indeed, the maximal response (E_{max} as percentage of the response to 100 mM KCl) evoked by donitriptan (29 ± 6%) was significantly reduced in the presence of ketanserin (9 ± 2%) to a similar level as that of sumatriptan (11 ± 3%); the latter was resistant to ketanserin (Table 2 and Connor et al., 1989; Kaumann et al., 1994). Furthermore, the concentration–

response curve to donitriptan in the presence of ketanserin was restored to a sigmoidal function and, as expected from their respective affinities at the 5-HT_{1B} receptor (Table 1), yielded a 37-fold higher potency (pEC_{50}: 7.33 ± 0.21) compared to that of sumatriptan (pEC_{50}: 5.76 ± 0.14). It should be mentioned, however, that donitriptan (up to 100 μM) failed to elicit contractile responses in the rat isolated aorta (G.W. John, unpublished observations), which is considered as a typical 5-HT_{2A} receptor preparation. Despite this discrepancy it appears that at low, clinically relevant concentrations, donitriptan constricts the coronary artery via the 5-HT_{1B} receptor and has similar maximal response amplitude as sumatriptan.

One may argue that in the relatively high concentration employed (10 μM), ketanserin can also act as a 5-HT_{1D} receptor antagonist (Zgombick et al., 1995; Pauwels and Colpaert, 1996). This is probably not relevant because 5-HT_{1D} receptor activation is not associated with vasoconstriction (Ennis et al., 1998; Bouchelet et al., 2000; Centurión et al., 2001).

4.3. Cranioselectivity of donitriptan and sumatriptan

It is known that a number of triptans (sumatriptan, rizatriptan, frovatriptan and eletriptan) selectively constrict cranial blood vessels compared to the coronary artery (Longmore et al., 1998; Parsons et al., 1998; MaassenVanDenBrink et al., 2000; Van den Broek et al., 2000). In the present investigation also, donitriptan as well as sumatriptan exhibited cranioselectivity because they were both more potent in contracting middle meningeal artery than coronary artery (see Table 2 for pEC_{50} values). The cranioselectivity ratio, calculated as inverse logarithm of the differences between the respective pEC_{50} values in the middle meningeal and coronary arteries, for donitriptan (sevenfold) was smaller than that for sumatriptan (50-fold). However, it must be emphasised that, in view of the predicted therapeutic concentration range (Table 3), we have used the pEC_{50} value obtained with the high affinity component of the concentration–response curve to donitriptan in the coronary artery. Interestingly, after elimination of the low affinity component of donitriptan by the 5-HT_{2A} receptor antagonist ketanserin, the cranioselectivity ratio was similar to that of sumatriptan (Table 2). This latter finding can be explained by the fact that in this preparation, the 5-HT_{2A} receptor is predominant over the 5-HT_{1B} receptor (Connor et al., 1989; Kaumann et al., 1994; Ishida et al., 1999; Nilsson et al., 1999).

4.4. Prediction of plasma concentration of donitriptan required for therapeutic activity and predicted contractions

Another way to determine cranioselectivity is to calculate predicted contractions at therapeutic free (protein-unbound) C_{max} of donitriptan and compare them with those for sumatriptan (MaassenVanDenBrink et al., 2000). Since therapeutic free C_{max} of donitriptan is currently not known, we have attempted to predict this (see Fig. 1) using two assumptions: (i) cranial vasoconstriction, as reflected by the human isolated middle meningeal artery contraction, is the major mechanism of therapeutic action of triptans (De Vries et al., 1999; Tfelt-Hansen et al., 2000; Feniuk and Humphrey, 2001) and (ii) data obtained in our laboratory setting by and large would apply to the clinical setting. Obviously, we cannot be absolutely sure on either count, but predicted therapeutic C_{max} of donitriptan may also help in determining initial doses to be employed in clinical trials. In any case, a comparison of the predicted and real therapeutic C_{max} values of donitriptan may be useful in the consideration of the mechanism of action of the drug.

On the basis of therapeutic free C_{max} of 50 or 100 mg oral sumatriptan (81 and 135 nM, see MaassenVanDenBrink et al., 1998, 2000) and the predicted contractions of the middle meningeal artery obtained at these concentrations of sumatriptan (79% or 89% of the contraction to 1 μM prostaglandin $F_{2\alpha}$), donitriptan would need a free C_{max} of ~ 3–4 nM to be as effective as sumatriptan (see Table 3). The predicted contractions in coronary artery (expressed as percentage of the contraction to 100 mM KCl) to donitriptan were small (3–4%) and similar to those with sumatriptan at therapeutic concentrations. Thus, based on these predictions, the two drugs have an equal propensity to constrict the coronary artery at therapeutic concentrations.

Acknowledgements

This investigation was partly supported by the Centre de Recherche Pierre Fabre (Castres, France) via a contract with the Erasmus Pharma, which is a full subsidiary of the Erasmus University Holding and is attached to the Department of Pharmacology (Rotterdam, The Netherlands).

References

Bouchelet, I., Cohen, Z., Case, B., Seguela, P., Hamel, E., 1996. Differential expression of sumatriptan-sensitive 5-hydroxytryptamine receptors in human trigeminal ganglia and cerebral blood vessels. Mol. Pharmacol. 50, 219–223.

Bouchelet, I., Case, B., Olivier, A., Hamel, E., 2000. No contractile effect for 5-HT_{1D} and 5-HT_{1F} receptor agonists in human and bovine cerebral arteries: similarity with human coronary artery. Br. J. Pharmacol. 129, 501–508.

Centurión, D., Sanchez-López, A., De Vries, P., Saxena, P.R., Villalón, C.M., 2001. The GR127935-sensitive 5-HT_1 receptors mediating canine internal carotid vasoconstriction: resemblance to the 5-HT_{1B}, but not to the 5-HT_{1D} or 5-HT_{1F}, receptor subtype. Br. J. Pharmacol. 132, 991–998.

Connor, H.E., Feniuk, W., Humphrey, P.P.A., 1989. 5-Hydroxytryptamine contracts human coronary arteries predominantly via 5-HT_2 receptor activation. Eur. J. Pharmacol. 161, 91–94.

Connor, H.E., Feniuk, W., Beattie, D.T., North, P.C., Oxford, A.W., Saynor, D.A., Humphrey, P.P.A., 1997. Naratriptan: biological profile in animal models relevant to migraine. Cephalalgia 17, 145–152.

Cushing, D.J., Cohen, M.L., 1992. Comparison of the serotonin receptors that mediate smooth muscle contraction in canine and porcine coronary artery. J. Pharmacol. Exp. Ther. 261, 856–862.

De Vries, P., Villalón, C.M., Saxena, P.R., 1999. Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur. J. Pharmacol. 375, 61–74.

Deleu, D., Hanssens, Y., 2000. Current and emerging second-generation triptans in acute migraine therapy: a comparative review. J. Clin. Pharmacol. 40, 687–700.

Diener, H.C., Limmroth, V., 1999. Acute management of migraine: triptans and beyond. Curr. Opin. Neurol. 12, 261–267.

Ennis, M.D., Ghazal, N.B., Hoffman, R.L., Smith, M.W., Schlachter, S.K., Lawson, C.F., Im, W.B., Pregenzer, J.F., Svensson, K.A., Lewis, R.A., Hall, E.D., Sutter, D.M., Harris, L.T., McCall, R.B., 1998. Isochroman-6-carboxamides as highly selective 5-HT_{1D} agonists: potential new treatment for migraine without cardiovascular side effects. J. Med. Chem. 41, 2180–2183.

Feniuk, W., Humphrey, P.P.A., 2001. The importance of vasoconstriction in the mechanism of antimigraine action of the triptans. In: Humphrey, P.P.A., Ferrari, M.D., Olesen, J. (Eds.), The Triptans: Novel Drugs for Migraine. Frontiers in Headache Research, vol. 10. Oxford Univ. Press, London, pp. 125–133.

Ferrari, M.D., Roon, K.I., Lipton, R.B., Goadsby, P.J., 2001. Oral triptans (serotonin 5-$HT_{1B/1D}$ agonists) in acute migraine treatment: a meta-analysis of 53 trials. Lancet 358, 1668–1675.

Fox, A.W., 2000. Comparative tolerability of oral 5-$HT_{1B/1D}$ agonists. Headache 40, 521–527.

Goadsby, P.J., 1998. A triptan too far? J. Neurol. Neurosurg. Psychiatry 64, 143–147.

Gupta, P., Scatchard, J., Napier, C., McHarg, A., Wallis, R., 1999. Characterisation of the contractile activity of eletriptan at the canine vascular 5-HT_{1B} receptor. Eur. J. Pharmacol. 367, 283–290.

Humphrey, P.P.A., Feniuk, W., 1991. Mode of action of the anti-migraine drug sumatriptan. Trends Pharmacol. Sci. 12, 444–446.

Ishida, T., Hirata, K., Sakoda, T., Kawashima, S., Akita, H., Yokoyama, M., 1999. Identification of mRNA for 5-HT_1 and 5-HT_2 receptor subtypes in human coronary arteries. Cardiovasc. Res. 41, 267–274.

John, G.W., Pauwels, P.J., Perez, M., Halazy, S., Le Grand, B., Verscheure, Y., Valentin, J.P., Palmier, C., Wurch, T., Chopin, P., Marien, M., Kleven, M.S., Koek, W., Assie, M.B., Carilla-Durand, E., Tarayre, J.P., Colpaert, F.C., 1999. F 11356, a novel 5-hydroxytryptamine (5-HT) derivative with potent, selective, and unique high intrinsic activity at 5-$HT_{1B/1D}$ receptors in models relevant to migraine. J. Pharmacol. Exp. Ther. 290, 83–95.

John, G.W., Perez, M., Pauwels, P.J., Le Grand, B., Verscheure, Y., Colpaert, F.C., 2000. Donitriptan, a unique high efficacy 5-$HT_{1B/1D}$ agonist. Key features and acute antimigraine potential. CNS Drug Rev. 6, 278–279.

Kaumann, A.J., Frenken, M., Posival, H., Brown, A.M., 1994. Variable participation of 5-HT_1-like receptors and 5-HT_2 receptors in serotonin-induced contraction of human isolated coronary arteries. 5-HT_1-like receptors resemble cloned 5-$HT_{1D\beta}$ receptors. Circulation 90, 1141–1153.

Kelly, K.M., 1995. Cardiac arrest following use of sumatriptan. Neurology 45, 1211–1213.

Kenakin, T., 1993. Pharmacologic Analysis of Drug-Receptor Interaction. Raven Press, New York.

Leysen, J.E., Gommeren, W., Heylen, L., Luyten, W.H., Van de Weyer, I., Vanhoenacker, P., Haegeman, G., Schotte, A., Van Gompel, P., Wouters, R., Lesage, A.S., 1996. Alniditan, a new 5-hydroxytryptamine$_{1D}$ agonist and migraine-abortive agent: ligand-binding properties of human 5-hydroxytryptamine$_{1D\alpha}$, human 5-hydroxytryptamine$_{1D\beta}$, and calf 5-hydroxytryptamine$_{1D}$ receptors investigated with [^{3}H]5-hydroxytryptamine and [^{3}H]alniditan. Mol. Pharmacol. 50, 1567–1580.

Longmore, J., Shaw, D., Smith, D., Hopkins, R., McAllister, G., Pickard, J.D., Sirinathsinghji, D.J., Butler, A.J., Hill, R.G., 1997. Differential distribution of 5-HT_{1D}- and 5-HT_{1B}-immunoreactivity within the human trigemino-cerebrovascular system: implications for the discovery of new antimigraine drugs. Cephalalgia 17, 833–842.

Longmore, J., Razzaque, Z., Shaw, D., Davenport, A.P., Maguire, J., Pickard, J.D., Schofield, W.N., Hill, R.G., 1998. Comparison of the vasoconstrictor effects of rizatriptan and sumatriptan in human isolated cranial arteries: immunohistological demonstration of the involvement of 5-HT_{1B}-receptors. Br. J. Clin. Pharmacol. 46, 577–582.

MaassenVanDenBrink, A., Reekers, M., Bax, W.A., Ferrari, M.D., Saxena, P.R., 1998. Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 98, 25–30.

MaassenVanDenBrink, A., Van den Broek, R.W.M., De Vries, R., Bogers, A.J.J.C., Avezaat, C.J.J., Saxena, P.R., 2000. Craniovascular selectivity of eletriptan and sumatriptan in human isolated blood vessels. Neurology 55, 1524–1530.

Main, M.L., Ramaswamy, K., Andrews, T.C., 1998. Cardiac arrest and myocardial infarction immediately after sumatriptan injection. Ann. Intern. Med. 128, 874.

Martin, G.R., 1997. Pre-clinical pharmacology of zolmitriptan (Zomig; formerly 311C90), a centrally and peripherally acting 5$HT_{1B/1D}$ agonist for migraine. Cephalalgia 17, 4–14.

Millson, D.S., Tepper, S.J., Rapoport, A.M., 2000. Migraine pharmacotherapy with oral triptans: a rational approach to clinical management. Expert Opin. Pharmacother. 1, 391–404.

Nilsson, T., Longmore, J., Shaw, D., Pantev, E., Bard, J.A., Branchek, T., Edvinsson, L., 1999. Characterisation of 5-HT receptors in human coronary arteries by molecular and pharmacological techniques. Eur. J. Pharmacol. 372, 49–56.

Ottervanger, J.P., Wilson, J.H., Stricker, B.H., 1997. Drug-induced chest pain and myocardial infarction. Reports to a national centre and review of the literature. Eur. J. Clin. Pharmacol. 53, 105–110.

Parsons, A.A., Raval, P., Smith, S., Tilford, N., King, F.D., Kaumann, A.J., Hunter, J., 1998. Effects of the novel high-affinity 5-$HT_{1B/1D}$-receptor ligand frovatriptan in human isolated basilar and coronary arteries. J. Cardiovasc. Pharmacol. 32, 220–224.

Pauwels, P.J., Colpaert, F.C., 1996. Selective antagonism of human 5-HT_{1D} and 5-HT_{1B} receptor-mediated responses in stably transfected C6-glial cells by ketanserin and GR 127,935. Eur. J. Pharmacol. 300, 141–145.

Pauwels, P.J., Tardif, S., Palmier, C., Wurch, T., Colpaert, F.C., 1997. How efficacious are 5-$HT_{1B/D}$ receptor ligands: an answer from GTP gamma S binding studies with stably transfected C6-glial cell lines. Neuropharmacology 36, 499–512.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics. A Biomedical Approach, 2nd edn. McGraw-Hill Kogakusha, Tokyo.

Tfelt-Hansen, P., De Vries, P., Saxena, P.R., 2000. Triptans in migraine: a comparative review of pharmacology, pharmacokinetics and efficacy. Drugs 60, 1259–1287.

Valentin, J.P., Bonnafous, R., John, G.W., 1996. Influence of the endothelium and nitric oxide on the contractile responses evoked by 5-HT_{1D} receptor agonists in the rabbit saphenous vein. Br. J. Pharmacol. 119, 35–42.

Van den Broek, R.W.M., MaassenVanDenBrink, A., De Vries, R., Bogers, A.J.J.C., Stegmann, A.P.A., Avezaat, C.J.J., Saxena, P.R., 2000. Pharmacological analysis of contractile effects of eletriptan and sumatriptan on human isolated blood vessels. Eur. J. Pharmacol. 407, 165–173.

Wurch, T., Palmier, C., Colpaert, F.C., Pauwels, P.J., 1997. Recombinant saphenous vein 5-HT_{1B} receptors of the rabbit: comparative pharmacology with human 5-HT_{1B} receptors. Br. J. Pharmacol. 120, 153–159.

Zgombick, J.M., Schechter, L.E., Kucharewicz, S.A., Weinshank, R.L., Branchek, T.A., 1995. Ketanserin and ritanserin discriminate between recombinant human 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor subtypes. Eur. J. Pharmacol. 291, 9–15.