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The in vivo pharmacological profile of eletriptan (UK-116,044): a potent and novel 5-$HT_{1B/1D}$ receptor agonist

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Abstract

The anti-migraine drug, eletriptan [(*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole; UK-116,044], is a novel 5-$HT_{1B/1D}$ receptor agonist. In this paper, the regional vasoconstrictor profile of eletriptan, in comparison with sumatriptan, was examined in the anaesthetised dog. The inhibitory actions of eletriptan on neurogenic inflammation in rat dura mater were also assessed. In the anaesthetised dog, eletriptan (1–1000 μg kg^{-1} i.v.) produced a dose-dependent reduction of carotid arterial blood flow with a similar potency and maximum effect to sumatriptan (ED_{50} values: eletriptan and sumatriptan, 12 and 9 μg kg^{-1}, i.v., respectively). However, eletriptan exhibited a significantly lower potency than sumatriptan in reducing coronary artery diameter (ED_{50} values: 63 and 19 μg kg^{-1}, i.v., respectively, $P < 0.05$). In the femoral circulation, sumatriptan caused a significant reduction in arterial blood flow (ED_{50} 35 μg kg^{-1} i.v.) whereas eletriptan (1–1000 μg kg^{-1} i.v.) had no significant effect upon femoral arterial blood flow when compared to vehicle-treated animals. In rats, eletriptan (30–300 μg kg^{-1} i.v.) administered prior to electrical stimulation of the trigeminal ganglion produced a dose-related and complete inhibition of plasma protein extravasation in the dura mater (mean extravasation ratio: control 1.9; eletriptan 1.0, minimum effective dose 100 μg kg^{-1}, $P < 0.05$). The potency and maximum effect of eletriptan was identical to that of sumatriptan in this model. When administered during a period of continual stimulation of the trigeminal nerve, eletriptan (100 μg kg^{-1} i.v.) produced a complete inhibition of plasma protein extravasation. The ability to reduce canine carotid arterial blood flow and inhibit neurogenic inflammation in rat dura mater suggests that vascular and neurogenic mechanisms may contribute to eletriptan's clinical efficacy in migraine patients. In addition, eletriptan exhibits some selectivity for reducing carotid arterial blood flow when compared with femoral arterial blood flow and coronary artery diameter, in the anaesthetised dog.

Keywords: Eletriptan; Sumatriptan; Carotid artery blood flow; Femoral artery blood flow; Neurogenic inflammation; Coronary artery diameter

1. Introduction

The 5-$HT_{1B/1D}$ receptor agonist sumatriptan has been shown to be efficacious in the acute treatment of migraine (Ferrari, 1991; Ferrari and Saxena, 1993; Pilgrim and Blakeborough, 1994). From preclinical studies, sumatriptan has two well-defined peripheral actions on the trigeminovascular system. Firstly, the compound evokes 5-HT_{1B} receptor-mediated contractions of human isolated cerebral and dural arteries (Parsons et al., 1989; Jansen et al., 1992; Razzaque et al., 1999). Secondly, by activation of inhibitory prejunctional 5-HT_{1D} receptors located on perivascular trigeminal nerve endings, sumatriptan prevents the development of a sterile neurogenic inflammation in the dura mater via inhibition of release of vasoactive neuropeptides such as Substance P and calcitonin gene related peptide (CGRP) (Buzzi and Moskowitz, 1990; Buzzi et al., 1991). From clinical studies in migraine patients, there is also evidence that sumatriptan causes both vasoconstriction of intracranial blood vessels (Caekebeke et al., 1992; Friberg et al., 1991), and inhibits the release of the sensory neuropeptide CGRP (Goadsby and Edvinsson, 1993), a major transmitter substance in the firth cranial nerve in man. Although the relative importance of the distinct vascular and neurogenic actions of sumatriptan to its clinical mode of action remains to be determined, it is probably significant that all of the clinically effective 5-$HT_{1B/1D}$ receptor agonists have a common ability to constrict cranial blood vessels and inhibit trigeminal afferents in preclinical animal models (Saito et al., 1988; Buzzi and

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Moskowitz, 1990; Den Boer et al., 1991; Saxena and Ferrari, 1996).

Clinical use of sumatriptan has underwritten the effectiveness of 5-$HT_{1B/1D}$ receptor agonists for the acute treatment of migraine. Nonetheless, clinical use has also highlighted some limitations of sumatriptan in terms of it's adverse event profile and pharmacokinetic properties. For example, adverse cardiovascular events have been reported in response to sumatriptan (Ottervanger et al., 1994; Willett et al., 1992) and the compound is contraindicated in patients with, or at risk of, cardiovascular disease. Furthermore, studies in volunteers have demonstrated that the vasoconstrictor effects of sumatriptan are not restricted exclusively to the cranial vasculature, and marked changes in peripheral haemodynamics have been reported over the therapeutic dose-range (MacIntyre et al., 1993). These data are consistent with the ability of sumatriptan to contract preparations of human isolated saphenous vein (Bax et al., 1992) and coronary artery (Connor et al., 1989; Cocks et al., 1993; Kaumann et al., 1994), probably via activation of contractile 5-HT_{1B} receptors (Kaumann et al., 1993; Nilsson et al., 1999). The aim of our discovery programme was therefore to identify a compound with improved selectivity for the intracranial vasculature over other peripheral blood vessels, such as, the coronary artery.

Eletriptan [(*R*)-3-(1-methyl-2-pyrrolidinylmethyl)-5-[2-(phenylsulphonyl)ethyl]-1*H*-indole], has been shown to be effective in the acute treatment of migraine (Goadsby et al., 2000). Preclinical in vitro studies have demonstrated that eletriptan binds with a high affinity to human recombinant 5-HT_{1B}, 5-HT_{1D} and 5-ht_{1F} receptors (pK_i 8.0, 8.9 and 8.0, respectively), and exhibits a qualitatively similar selectivity profile to sumatriptan when evaluated in a range of 5-HT receptor radioligand binding assays (Napier et al., 1999). In functional studies, eletriptan, unlike sumatriptan, exhibits a partial agonist profile in canine vascular tissues (Gupta et al., 1999). Eletriptan has also been shown to constrict porcine carotid arteriovenous anastomoses in vivo (Willems et al., 1998).

In this paper, we describe the in vivo pharmacological profile of eletriptan in comparison with sumatriptan. There were two aims of the study, the first being to determine if eletriptan exhibits activity in models that may be indicators of clinical efficacy in migraine patients, that is, vasoconstriction of the canine carotid arterial vascular bed and inhibition of neurogenic inflammation in rat dura mater. Secondly, we have measured the regional vasoconstrictor profile of eletriptan, in comparison with sumatriptan, in the anaesthetised dog to establish eletriptan's selectivity profile in vivo.

2. Methods

All in vivo studies were conducted in accordance with the United Kingdom Home Office guidelines and the United Kingdom Animal (Scientific Procedures) Act 1986. All procedures were carried out under full general anaesthesia and animals were humanely euthanased at the end of the experiments.

2.1. Measurement of cardiovascular parameters in the anaesthetised dog

Female Beagle dogs (Pfizer colony, 11–14.5 kg) were premedicated with piritramide (5.0 ml, s.c.) and anaesthetised, via the right cephalic vein, with a mixture of 1:11 volumes of 10% α-chlorarose in PEG300 and 11% urethane in 0.9% saline (3.5 ml kg^{-1}). All dogs were intubated with an endotracheal tube and artificially ventilated with a Bird respirator MK7A (Viamed, Keighley, W. Yorks) to maintain arterial pH, P_{CO_2} and P_{O_2} within normal limits (arterial pH 7.35–7.45; P_{CO_2} 35–45 mm Hg; P_{O_2} 85–120 mm Hg). Body temperature was maintained at 37–39°C using a homeothermic blanket control system. Arterial blood pressure was recorded from a cannulated femoral artery using a pressure transducer (Spectromed Statham, model P231XL, USA). Blood samples for blood-gas analysis were also taken from this arterial line (ABL510, Radiometer, Copenhagen, Denmark). The right femoral vein was cannulated for infusion of a 2nd dose of anaesthetic (3.5 ml kg^{-1}, given approximately 2 h later) and for infusion of sodium bicarbonate (1 M in 0.9% saline) to maintain the acid–base balance. To maintain anaesthesia, the right cephalic vein was used for continuous infusion of anaesthetic (started when the second infusion had been terminated). The left cephalic vein was also cannulated and used for the intravenous administration of test substances. Lead II of the electrocardiogram (ECG) was recorded from sub-epidermal needles, allowing constant monitoring of the cardiac rhythm and derivation of the heart rate. Electromagnetic flow probes (Skalar Medical, Delft, The Netherlands; 2.5 mm diameter) were used to monitor blood flow in the left common carotid artery and in the left femoral artery.

A left thoracotomy was performed by removal of part of the sixth rib and the heart was suspended in a pericardial cradle. The external diameter of the left circumflex coronary artery was measured with a sonomicrometer (ultrasonic transit-time dimension guage, model 120.2, Triton Technology, San Diego, USA). A pair of 5 MHz piezoelectric crystals attached to backing material were sutured to the opposing surfaces of the left circumflex coronary artery, 3–5 cm from its origin. Correct alignment of the crystals was verified by on-line sonomicrometer and oscillosope monitoring. Simultaneous monitoring of blood flow in the left circumflex coronary artery was performed using an electromagnetic flow probe (2.0 mm diameter). Care was taken during instrumentation to limit dissection and damage of visible nerves. All parameters (ECG, blood pressure, coronary artery diameter and blood flows) were

displayed continuously on a Gould TA4000 recorder and simultaneously transferred to an in-house on-line data acquisition system based on the Motorola 68,000 family computer.

After a 60-min stabilisation period, a set of control readings were taken. Cumulative doses of the drug compound (1–1000 μg kg^{-1}) or vehicle equivalent, were administered by bolus injection at 5-min intervals with readings being taken by the computer every 30 s (sampling rate = 250 Hz). For each dose of compound, the response to compound for the measured parameters was reported at the time of the peak change in carotid flow, with the exception of the coronary artery diameter, where maximal reductions generally occurred after the maximal changes in carotid flow.

2.2. Measurement of plasma protein extravasation in rat dura following electrical stimulation of the trigeminal ganglion

Male Sprague–Dawley rats (380–450 g, Charles River, Manston, UK) were kept under diurnal lighting conditions and allowed water ad libitum. Animals were anaesthetised with pentobarbitone (60 mg kg^{-1}, i.p.) and a femoral vein was cannulated for intravenous injections. Animals were placed in a stereotaxic frame (Kopf 900 Instruments, USA) with the incisor bar set at −1.5 mm. Symmetrical burr holes were drilled at 4.0 mm laterally and 4.0 mm anteriorly from bregma for a 400-g rat and adjusted proportionally according to the rat weight. Paired non-concentric bipolar electrodes (5 cm shaft, Clark Electromedical, Pangborne) were lowered 9.5 mm from the dura mater bilaterally into the trigeminal ganglia.

[^{125}I]Radiolabelled human serum albumin (50 μCi kg^{-1}) and Evans Blue (20 mg kg^{-1}) were injected via the femoral vein, followed 5 min later by either drug or vehicle. After a further 10 min the right or left trigeminal ganglion was arbitrarily selected and electrically stimulated (3 min, 2.2 mA, 5 Hz, 2 ms duration; Isostim Stimulus Isolator A320, World Precision Instruments, FL, USA). Immediately after the stimulation, animals were perfused with 0.9% saline via the left cardiac ventricle for a 5-min period at a constant pressure of 120 mm Hg to wash the blood from the head region. Following removal of the brain, the dura mater lining the anterior fossae was removed and dissected bilaterally. Samples of extracranial tissues innervated by the trigeminal nerve, i.e. eyelid, conjunctiva and lower lip, were also removed, weighed, and counted for radioactivity (1277 Gammamaster, LKB, Finland). The recorded counts mg^{-1} wet weight of tissue were then used to calculate an extravasation ratio between the tissues for the stimulated and unstimulated sides.

A second set of experiments, designed to evaluate the effect of eletriptan on an established and ongoing neurogenically-driven inflammatory response were based on a paradigm described originally in guinea-pig by Huang et al. (1993), and adapted by ourselves in rat (Gupta et al., 1995). In rat experiments, the compound or vehicle were administered 5-min after the completion of a 5-min period of electrical stimulation of the trigeminal ganglion and [^{125}I] radiolabelled human serum albumin (50 μCi kg^{-1}) and Evans blue (20 mg kg^{-1}) were administered after a further 5 min. Finally, 10 min after the administration of the radiolabelled albumin, animals were perfused with 0.9% saline (see above for detail), and tissues removed as before.

2.3. Drugs and solutions

The following compounds were purchased: [^{125}I] human serum albumin (Amersham International, UK); Evans blue dye and α-chlorarose (Sigma, Dorset, UK), sodium pentobarbitone (Sagatal, May and Baker, Essex, UK), piritramide (Dipidolor, Janssen Pharmaceuticals, Beerse, Belgium) and urethane (Aldrich Chemicals, Steinhem, Germany).

Eletriptan and sumatriptan were synthesised by the medicinal Chemistry department, Pfizer Central Research, UK. Both compounds were initially dissolved in distilled water and diluted in 0.9% saline to obtain the doses required.

2.4. Statistical analysis and calculations

All data represent the mean ± S.E.M. for n separate experiments. In the anaesthetised dog studies, comparison of drug- and vehicle-evoked effects on mean arterial pressure, heart rate, and femoral circulation was carried out by analysis of covariance, using baseline values as the covariate to adjust mean values to a comparable basis. In studies to measure the vasoconstrictor properties of the tested drugs, contractile potencies are expressed as ED_{50} values, which represent the dose of agonist required to produce 50% of the maximum response attainable for that particular agonist. ED_{50} values were derived by an in-house logistic curve-fitting programme based on ALLFIT (Delean et al., 1978), and are expressed as the geometric mean

Table 1
Baseline control parameters in the anaesthetised dog measured at time zero and at 45 min following completion of the cumulative administration of vehicle equivalent to a cumulative dose of 1000 μg kg^{-1} i.v.

Parameter	Pre-vehicle (0 min)	Post-vehicle (45 min)
MABP (mm Hg)	92 ± 4	92 ± 5
HR (beats/min)	115 ± 18	125 ± 20
Coronary diameter (μM)	3219 ± 320	3244 ± 334
Carotid blood flow (ml/min)	181 ± 15	172 ± 12
Femoral blood flow (ml/min)	114 ± 15	105 ± 15
Coronary blood flow (ml/min)	32 ± 6	35 ± 6

Data are expressed as mean ± S.E.M. They represent the mean value of four separate experiments.

with 95% confidence limits in parentheses. Statistical analysis of the ED_{50} data (carotid blood flow, coronary artery diameter) was carried out by analysis of variance on the log dose scale. Analyses of variance and covariance were carried out using the Genstat statistical package, version 5 (NAG, Oxford, UK).

In plasma extravasation studies, the level of plasma leakage produced in the presence of each dose of test drug was compared with that produced in a separate vehicle-treated control group. Differences between means were analysed for statistical significance by Student's unpaired *t* test. *P* values of less than 0.05 were considered to indicate a significant difference between the responses being compared.

3. Results

3.1. Haemodynamic profile in the anaesthetised dog

To establish the stability of haemodynamic parameters in the anaethetised dog, four control experiments were performed to determine the effect of cumulatively administered saline (0.9% w/v) on all baseline parameters (Table

Table 2
Comparison of baseline values, maximal reductions and potency for eletriptan and sumatriptan on carotid arterial blood flow and coronary artery diameter

	Eletriptan	Sumatriptan
Carotid blood flow		
Baseline flow (ml min^{-1})	171 ± 9	154 ± 12
Maximal reduction (Δ ml min^{-1})	83 ± 7	77 ± 9
ED_{50} (μg kg^{-1}) [95% confidence limits]	12 [10–16]	9 [7–11]
Coronary artery diameter		
Baseline diameter (μm)	3663 ± 259	3355 ± 133
Maximal reduction (Δ μm)	113 ± 13	92 ± 26
ED_{50} (μg kg^{-1}) [95% confidence limits]	63 [37–107][a]	19 [11–31]

Data are expressed as mean ± S.E.M. with the exception of the ED_{50} values, which are expressed as the geometric mean with 95% confidence limits in parenthesis.

[a] $P < 0.05$ when compared to sumatriptan using analyses of variance and covariance tests.

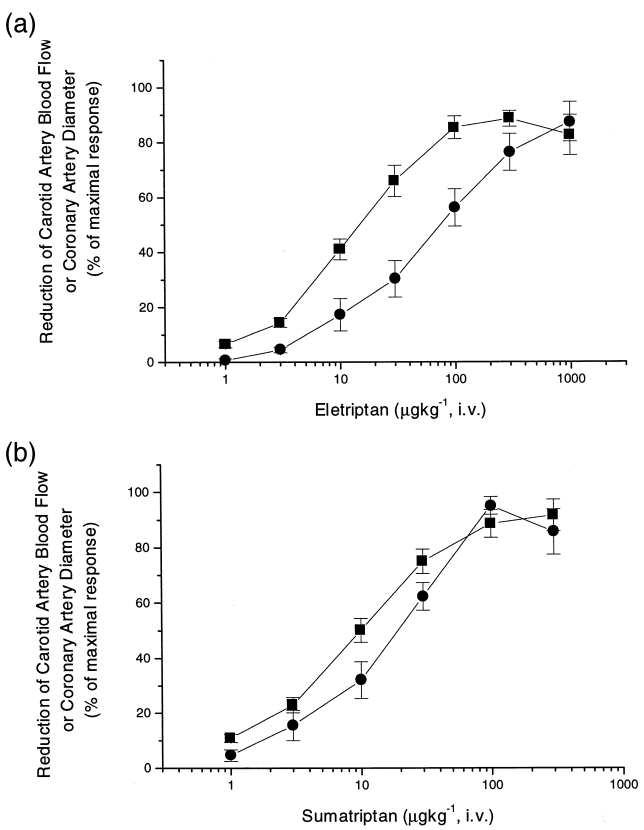

Fig. 1. Effect of (a) eletriptan and (b) sumatriptan on carotid arterial blood flow (■) and coronary artery diameter (●) in the anaesthetised dog. Data are expressed on the ordinate scale as a percentage of the maximum effect produced in that particular vascular bed/vessel, and on the abscissa scale as drug dose (μg kg^{-1} i.v.). Each data point represents the mean response of between 9 and 10 separate experiments, with S.E.M. values shown by the vertical bars.

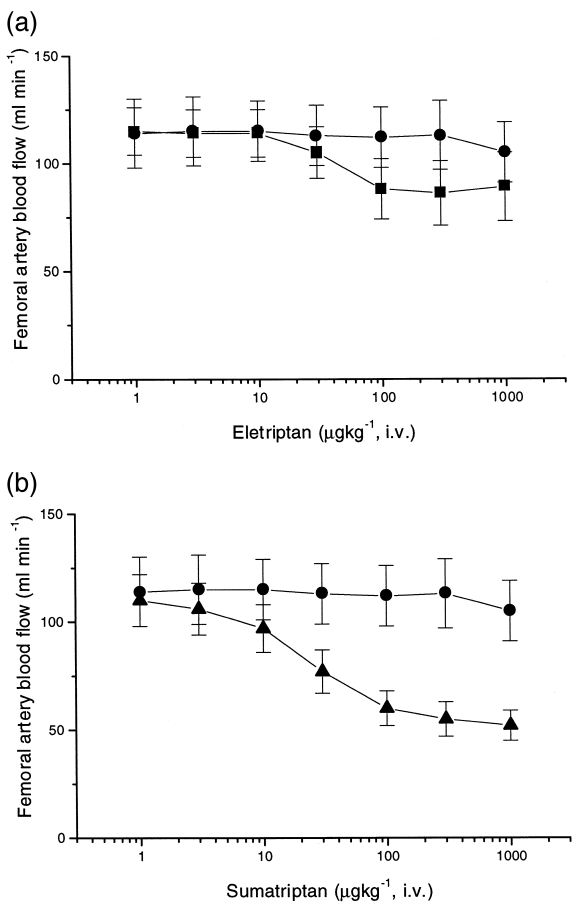

Fig. 2. Comparison of the effect of (a) eletriptan (■) and (b) sumatriptan (▲) compared to vehicle (●) on femoral arterial blood flow in the anaesthetised dog. Data are expressed on the ordinate scale as femoral blood flow (ml min^{-1}), and on the abscissa as drug dose (μg kg^{-1} i.v.). Each data point represents the mean response of between 4 and 10 separate experiments, with S.E.M. values shown by the vertical bars.

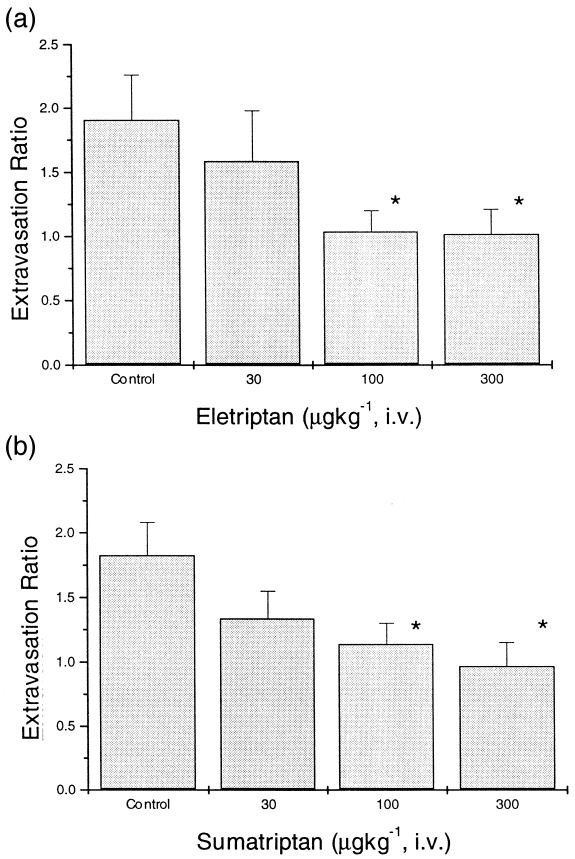

Fig. 3. Effect of (a) eletriptan (30–300 μg kg^{-1} i.v.) and (b) sumatriptan (30–300 μg kg^{-1} i.v.) on plasma protein extravasation produced in the dura mater following electrical stimulation of the trigeminal ganglion in rat. Data are expressed on the ordinate as a mean extravasation ratio in stimulated/unstimulated sides, and on the abscissa as drug dose (μg kg^{-1}). Vertical bars represent the S.E.M. of $n = 7$–10 separate experiments. Statistical analysis was performed using Student's unpaired t test, * $P < 0.05$ when compared to vehicle-treated controls.

1). There were no significant changes in any of the parameters measured over the time period of the experiment.

The intravenous administration of eletriptan or sumatriptan (both 1–1000 μg kg^{-1} i.v.) produced no significant change in heart rate and only at the top dose of 1000 μg kg^{-1} with eletriptan was there a statistically significant increase in mean arterial blood pressure (mean increase of 13.3 mm Hg, $P < 0.05$) and although the magnitude of change was similar for sumatriptan this was not significant (mean increase 7.3 mm Hg).

Eletriptan (1–1000 μg kg^{-1} i.v.) produced a dose-dependent reduction of carotid arterial blood flow with a potency similar to that observed for sumatriptan (mean ED_{50} value μg kg^{-1} i.v. [95% confidence limits]: eletriptan 12 [10–16], $n = 9$; sumatriptan 9 [7–11], $n = 10$; Fig. 1 and Table 2). The maximum reduction in carotid arterial blood flow produced by both drugs was also similar (mean reduction: eletriptan 44% at 1000 μg kg^{-1} i.v.; sumatriptan 42% at 1000 μg kg^{-1} i.v., Table 2). However, over the same dose range, eletriptan exhibited a significantly lower potency to reduce coronary artery diameter when compared with sumatriptan (mean ED_{50} value μg kg^{-1} i.v. [95% confidence limits]: eletriptan 63 [37–107], $n = 9$; sumatriptan 19 [11–31], $n = 10$, $P < 0.05$; Fig. 1 and Table 2), although the maximum reduction in diameter was similar for both compounds (mean reduction: eletriptan 2.6% at 1000 μg kg^{-1} i.v., $n = 9$; sumatriptan 2.5% at 1000 μg kg^{-1}, $n = 10$; Table 2). Neither compound had any significant effect on coronary blood flow compared to vehicle-treated animals (data not shown).

In the femoral circulation, sumatriptan produced a dose-dependent reduction in femoral artery blood flow with an ED_{50} of 35 [17–49] μg kg^{-1} i.v. (mean reduction of 42% at 1000 μg kg^{-1} i.v., $P < 0.05$; $n = 10$) (Fig. 2). In contrast, equivalent doses of eletriptan did not evoke significant changes in femoral flow ($P > 0.05$) when compared with vehicle-treated controls (Fig. 2).

3.2. Trigeminovascular studies in the anaesthetised rat

When administered prior to electrical stimulation of the trigeminal ganglion, eletriptan (30–300 μg kg^{-1} i.v.) produced a dose-dependent inhibition of plasma protein extravasation in the dura mater when compared to that observed in vehicle-treated controls (Fig. 3a). The minimum effective dose (MED) of eletriptan that produced a significant reduction of plasma protein extravasation, when compared to vehicle, was 100 μg kg^{-1} i.v., a dose which also evoked a maximal and complete inhibition of plasma protein extravasation (mean extravasation ratio ± s.e.m: control 1.91 ± 0.35, $n = 10$; eletriptan 100 μg kg^{-1} i.v.

Table 3
Effects of intravenously-administered eletriptan, sumatriptan and vehicle on trigeminal nerve stimulation-induced plasma protein leakage in the anaesthetised rat

(a) Eletriptan

Tissue	Vehicle ($n = 10$)	Dose of Eletriptan (μg kg^{-1} i.v.)		
		30 ($n = 7$)	100 ($n = 8$)	300 ($n = 8$)
Dura	1.91 ± 0.35	1.59 ± 0.39	1.04 ± 0.16[a]	1.02 ± 0.19[a]
Conjunctiva	2.66 ± 0.49	2.20 ± 0.70	1.98 ± 0.46	3.09 ± 0.64
Eye lid	5.04 ± 1.19	4.38 ± 2.12	1.40 ± 0.35[a]	4.77 ± 1.59
Lower lip	3.82 ± 0.92	3.75 ± 0.42	4.47 ± 0.52	3.11 ± 0.26

(b) Sumatriptan

Tissue	Vehicle ($n = 10$)	Dose of Sumatriptan (μg kg^{-1} i.v.)		
		30 ($n = 8$)	100 ($n = 8$)	300 ($n = 7$)
Dura	1.83 ± 0.20	1.34 ± 0.20	1.14 ± 0.16[a]	0.97 ± 0.18[a]
Conjunctiva	2.86 ± 0.48	2.03 ± 0.28	2.54 ± 0.92	1.27 ± 0.30[a]
Eye lid	8.20 ± 2.22	2.62 ± 0.62[a]	4.63 ± 2.92	2.73 ± 1.39
Lower lip	2.20 ± 0.37	1.84 ± 0.23	2.71 ± 0.44	2.02 ± 0.54

Data are expressed as mean ± S.E.M. Number in parenthesis (n) = number of animals per group.

[a] $P < 0.05$ when compared to vehicle-treated group using a Student's t-test for unpaired data.

1.04 ± 0.16, $n = 8$, $P < 0.05$; eletriptan 300 μg kg^{-1} i.v. 1.02 ± 0.19, $n = 8$, $P < 0.05$). In a separate series of experiments, sumatriptan inhibited plasma protein extravasation with a similar potency and maximum effect to eletriptan (mean extravasation ratio ± s.e.m.: control 1.83 ± 0.20, $n = 10$; sumatriptan 100 μg kg^{-1} i.v. 1.14 ± 0.16, $n = 8$, $P < 0.05$; sumatriptan 300 μg kg^{-1} i.v. 0.97 ± 0.18, $n = 7$, $P < 0.05$) (Fig. 3b). This action is selective for the intracranial tissues innervated by the trigeminal nerve as demonstrated by a lack of significant effect of eletriptan on plasma protein extravasation in the facial tissues of lower lip and conjunctiva (Table 3, panel a). A statistically-significant effect was noted in the eye lid following 100 μg kg^{-1} eletriptan, but this was not dose-related and is not considered to be of biological relevance. Sumatriptan also tended to inhibit plasma protein extravasation in the eyelid but significant effects were only achieved at 30 and 300 μg kg^{-1} i.v. in the conjuctiva (Table 3, panel b).

3.2.1. Ongoing and established neurogenically driven response in rat dura mater

In a separate study using a modified protocol, eletriptan was administered during a period of ongoing and established plasma protein extravasation produced by activation of the trigeminal ganglion. Under these conditions eletriptan (100 μg kg^{-1} i.v.) produced a rapid and complete inhibition of plasma protein extravasation in the dura mater (mean extravasation ratio ± s.e.m.: control 1.71 ± 0.2, $n = 10$; eletriptan 0.98 ± 0.1, $n = 10$, $P < 0.05$, Fig. 4). These data demonstrate that a dose of 100 μg kg^{-1} i.v. eletriptan is equally effective against neurogenic inflammation when administered prior to, or during, activation of the trigeminal system.

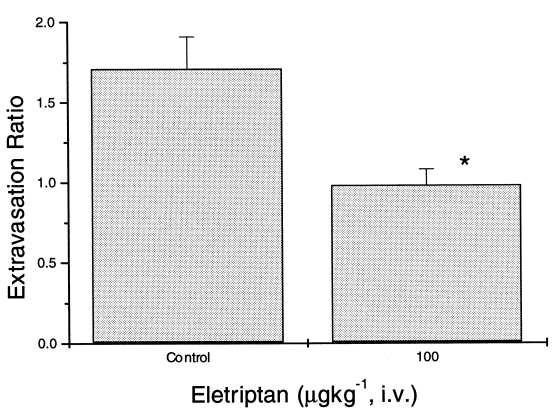

Fig. 4. Effect of eletriptan (100 μg kg^{-1} i.v.) on an established and ongoing neurogenically-driven plasma protein extravasation produced in dura mater. Eletriptan or vehicle were administered 5 min after the completion of a 5-min period of electrical stimulation of the trigeminal ganglion in rat. [^{125}I] human serum albumin leakage in the dura mater was measured after a further 10-min period. Data are expressed on the ordinate, as a mean extravasation ratio in stimulated/unstimulated sides, and on the abscissa as drug dose (μg kg^{-1}). Vertical bars represent the S.E.M. of 10 separate experiments. Statistical analysis was performed using Student's unpaired *t*-test, $^{*}P < 0.05$ when compared to vehicle-treated controls.

4. Discussion

Clinical trials have demonstrated that eletriptan is an effective oral therapy for the acute treatment of migraine (Goadsby et al., 2000). In this paper, we have determined the in vivo pharmacological profile of eletriptan, in comparison with sumatriptan, to establish whether this new agent is efficacious in animal models in which the vascular and neurogenic mechanisms implicated in migraine are measured and to compare the haemodynamic profile of eletriptan with that of sumatriptan in the carotid, femoral and coronary vascular beds.

In evaluating compound effects on perivascular trigeminal afferents in rat, it was demonstrated that when administered prior to electrical stimulation of the trigeminal ganglion, eletriptan has a potency (MED 100 μg kg^{-1} i.v.) and maximum effect equivalent to that seen with sumatriptan. Like sumatriptan and other 5-HT$_1$ receptor agonists such as dihydroergotamine and ergotamine, eletriptan had no effect on plasma protein extravasation in extracranial tissues, which receive inputs from the second and third divisions of the trigeminal ganglion. Thus, it is emerging that the distribution of the receptor(s) which mediates the inhibition of neurogenic inflammation to eletriptan and other agents may be restricted to intracranial perivascular trigeminal afferents, specifically those arising from the first division of the trigeminal nerve, the opthalmic branch. In terms of the pharmacological identity of the target inhibitory receptor, this has not yet been elucidated. However, by use of the polymerase chain reaction, mRNA transcripts for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and more recently the 5-ht$_{1F}$ subtype, have been shown to be located in the trigeminal ganglia of rat (5-ht$_{1F}$ subtype not examined, Bruinvels et al., 1993) and man (Bouchelet et al., 1996). Furthermore, the pharmacology of the prejunctional 5-HT receptor in rat is consistent with the 5-HT$_{1D}$ subtype (Williamson et al., 1997), and the 5-HT$_{1D}$ receptor protein, but not 5-HT$_{1B}$ receptor protein, is expressed on human trigeminal sensory neurones (Longmore et al., 1997). More recently, selective 5-ht$_{1F}$ agonists, such as LY344864 (*N*-[3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluoro-(*R*)-benzamide) and LY334370 (4-fluoro-*N*-(3-(1-methyl-4-piperidinyl)-1*H*-indol-5-yl)benzamide) have also been shown to be effective in animal models of neurogenic dural inflammation (Phebus et al., 1996, 1997). The recent identification of selective pharmacological receptor antagonists for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Price et al., 1997) should help define the functional role of these subtypes in models of neurogenic inflammation.

One criticism of the neurogenic hypothesis of migraine is whether an inhibition of neuropeptide release would translate to a rapid onset of relief of migraine symptoms in

the clinic, and it has been argued that only a vasoconstrictor mechanism would be able to rapidly reverse a pathophysiological event of vascular origin. Thus, we adapted a paradigm described originally by Moskowitz et al., in which it was demonstrated that a 5-min period of electrical stimulation of trigeminal ganglion in guinea-pig could be used to provoke trigeminal fibres which innervate the dura mater to fire continuously, in the absence of electrical stimulation, for up to 45 min (Huang et al., 1993). This experimental protocol has been used to demonstrate that sumatriptan could reverse an established and ongoing neurogenically-driven sterile inflammation when administered during this period of continual neuronal firing (Huang et al., 1993). In the present study eletriptan, when tested at the MED (100 μg kg^{-1} i.v.) defined in pretreatment studies, was able to completely inhibit plasma protein extravasation within minutes. Thus, if the inflammatory environment that develops using this experimental protocol mimics those events which occur pathophysiologically during migraine (Arnold et al., 1998; Pappagallo et al., 1999), it appears that the inflammatory process could be inhibited by eletriptan within a clinically relevant timescale to abort an established migraine attack. Recent data from clinical studies has, however, questioned the importance of inhibiting neurogenic inflammation in the treatment of acute migraine. For example, CP-122,288 ((*R*)-*N*-methyl-[3-(1-methyl-2-pyrrolidinylmethyl)-1*H*-indol-5-yl]methanesulphonamide), a conformationally restricted sumatriptan analogue, was 1000 times more potent than sumatriptan in inhibiting plasma protein extravasation in the dura mater of the rat (Gupta et al., 1995) and so was active at doses that did not have vascular effects. This compound was, however, ineffective in clinical studies of acute migraine (Roon et al., 1997). Similarly, another conformationally restricted zomitriptan analogue, 4991W93 (*trans*-(*S*)-4-[3-(3-dimethylaminocyclobutyl)-1*H*-indol-5-ylmethyl]oxazolidin-2-one) (Giles et al., 1999; Earl et al., 1999), bosentan, an endothelin receptor antagonist (May et al., 1996) and a number of tachykinin NK_1 receptor antagonists (Lee et al., 1994; Goldstein et al., 1997; Connor et al., 1998; Norman et al., 1998), were highly effective in inhibiting plasma protein extravasation but ineffective in the treatment of acute migraine. These data indicate that inhibition of neurogenic inflammation alone is not a prerequisite for an effective acute treatment of migraine.

In anaesthetised dog studies, both eletriptan and sumatriptan produced a dose-dependent reduction of carotid arterial blood flow with a similar potency (ED_{50} 12 μg kg^{-1} i.v. and 9 μg kg^{-1} i.v., respectively) and maximum effect. These data are consistent with findings from an anaesthetised pig study (Willems et al., 1998) in which intravenous administration of eletriptan produced a dose dependent decrease in total carotid blood flow, exclusively by decreasing cephalic arteriovenous anastomotic blood flow. This study also demonstrated that these changes in carotid haemodynamics were predominantly mediated by 5-$HT_{1B/1D}$ receptors. The present study in anaesthetized dogs also showed that doses of eletriptan, which produced a maximal reduction in carotid blood flow, produced no change in heart rate or blood pressure. Eletriptan did, however, produce a significant pressor response at the top dose tested (1000 μg kg^{-1} i.v.) which was similar in magnitude to that observed with sumatriptan. These data are consistent with studies in man in which high oral doses (90 and 120 mg) of eletriptan tended to increase diastolic pressure with no change in heart rate (Milton et al., 1997). When coronary arterial blood flow was measured, no effect was observed to either compound, even at the highest dose tested (1000 μg kg^{-1} i.v.), an observation which is consistent with previous studies using sumatriptan (Feniuk et al., 1989). Although contractile 5-HT_{1B} receptors have been described in canine (Parsons et al., 1992) and human isolated coronary artery (Connor et al., 1989; Cocks et al., 1993; Nilsson et al., 1999), these are conductance rather than resistance vessels, and therefore it was anticipated that blood flow would remain unaffected. However, when coronary arterial diameter was measured using sonomicrometry, both compounds did produce a dose-dependent reduction in vessel diameter. Importantly, eletriptan exhibited a significantly ($P < 0.05$) lower potency than sumatriptan at reducing coronary artery dimensions, and this lower activity was reflected in a wider separation between its carotid:coronary dose–response curves. These results are consistent with findings from in vitro studies with human vascular tissue in which eletriptan had equivalent potency to sumatriptan in contracting the human isolated middle meningeal artery (blood vessel predictive of therapeutic efficacy) but was less potent in the human isolated coronary artery (Maassen VandenBrink et al., 1999). Furthermore, efficacious clinical plasma levels of eletriptan, caused no significant coronary artery constriction in patients without significant obstructive coronary artery disease (Muir et al., 1999). Moreover, in the femoral vascular bed, sumatriptan produced a significant dose-dependent reduction of blood flow (ED_{50} 35 μg kg^{-1} i.v.) when compared with vehicle-treated controls but no significant effect was observed with eletriptan (1–1000 μg kg^{-1} i.v.). Overall, these observations suggest that eletriptan has a different regional vasoconstrictor profile when compared with sumatriptan, exhibiting a selectivity for the carotid arterial bed. The basis for this regional difference is the subject of further investigation.

In conclusion, the work described in the present study demonstrates that eletriptan inhibits intracranial neurogenic inflammation in rat, and reduces carotid arterial blood flow in dog, with a potency and maximum effect equivalent to sumatriptan. The present data support the premise that eletriptan's established efficacy in migraine patients (Goadsby et al., 2000) may be attributed to its ability to inhibit both the vascular and neurogenic mechanisms implicated in migraine albeit that current information would suggest that the neurogenic component may be of lesser

importance. In addition, studies in the anaesthetised dog have demonstrated that eletriptan has a distinct haemodynamic profile being more selective than sumatriptan for reducing carotid arterial blood flow when compared with femoral arterial blood flow and coronary artery diameter. These data suggest that for a given level of efficacy, eletriptan will have a reduced propensity, relative to sumatriptan, to cause changes in coronary artery diameter, however, only clinical experience over a long period of time will confirm or refute this claim.

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