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Functional profile of almotriptan in animal models predictive of antimigraine activity

Jordi Gras *, Josep Bou, Jesús Llenas, Andrés G. Fernández, José M. Palacios

Pharmacological Development Department, Almirall Prodesfarma, Research Center, Cardener 68-74, 08024-Barcelona, Spain

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Abstract

Almotriptan is a new 5-$HT_{1B/1D}$ receptor agonist whose clinical efficacy for the treatment of migraine attacks has been demonstrated in Phase III clinical trials. We now compare the functional profile of almotriptan (assessed using animal models) with that of sumatriptan. Almotriptan selectively increased carotid vascular resistance in anaesthetised cats after intravenous or intraduodenal administration (ED_{100} = 11 μg/kg, i.v.; ED_{50} = 339 μg/kg, i.d.) and in anaesthetised beagle dogs following intravenous administration (ED_{50} = 116 μg/kg). A study in anaesthetised cats also demonstrated that almotriptan acts by selectively increasing the resistance of the carotid arteriovenous anastomoses without adversely affecting brain irrigation. In addition, almotriptan inhibited meningeal extravasation produced by electrical stimulation of the trigeminal ganglion in anaesthetised guinea pigs in the dose range of 0.3–3 mg/kg, i.v. In conclusion, almotriptan is both a selective constrictor affecting intracranial blood vessels and an inhibitor of neurogenically evoked plasma protein extravasation of the dura mater.

Keywords: 5-$HT_{1B/1D}$ receptor agonist; Migraine; Almotriptan; Preclinical study

1. Introduction

Sumatriptan is a potent and selective 5-$HT_{1B/1D}$ receptor agonist which rapidly relieves migraine and cluster headache attacks (Plosker and MacTavish, 1994). However, the efficacy of sumatriptan may be limited by headache recurrence and contraindication in patients with coronary artery disease (Saxena and Ferrari, 1996). Additionally, even though there is an oral formulation available, the efficacy of sumatriptan also seems to be limited by its low oral bioavailability (Perry and Markham, 1998). Consequently, other 5-$HT_{1B/1D}$ receptor agonists are under study and some of these compounds are already available for clinical use, in late clinical development, or on the market (e.g. zolmitriptan, rizatriptan and naratriptan (Kamali, 2000)).

Although, in recent years, our understanding of the pathogenesis of migraine has greatly improved, neither the origin of migraine nor the mechanism of action of the 5-$HT_{1B/1D}$ receptor agonists is yet fully understood. Migraine premonitory symptoms may reflect hypothalamic dysfunction, whilst aura symptoms may be due to a transient suppression of spontaneous and evoked neuronal activity spreading across the cortex (i.e. cortical spreading depression). The headache that follows is due to cerebral vasodilatation and neurogenic inflammation of the dura mater (Ferrari, 1998). Some authors suggest that neurovascular, rather than purely vascular, mechanisms are involved in triggering migraine (Goadsby, 1994). However, others maintain that only compounds found to induce carotid vasoconstriction in preclinical studies, thus acting through a vascular mechanism, have proven effective in clinical practice (Ferrari and Saxena, 1995).

The present study aimed to establish the in vivo functional profile of almotriptan, a new selective and potent 5-$HT_{1B/1D}$ receptor agonist agent (Bou et al., 2000), in animal models. Due to the existing controversy regarding the relative role of the vascular and the neurogenic components in the etiopathogenesis of the migraine attacks, the contractile effects of almotriptan were tested on both the

* Corresponding author. Tel.: +34-93-291-34-61; fax: +34-93-291-34-45.

E-mail address: jgras@almirallprodesfarma.com (J. Gras).

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carotid vascular bed in cats and dogs and the meningeal extravasation induced by electric stimulation of the trigeminal ganglion in guinea pigs. The effects of almotriptan on carotid arteriovenous anastomoses were also assessed.

2. Material and methods

2.1. Haemodynamics in anaesthetised cats

2.1.1. General setup

Cats (2.1–4.3 kg) of either sex were supplied by Sumont (Torelló, Spain). The animals were fasted for 18 h with water ad libitum before the experiments. The cats were anaesthetised with α-chloralose (70 mg/kg i.p.) and sodium thiopental (20 mg/kg i.p.) and mechanically ventilated (20 strokes/min, 10 ml/kg) by means of a Harvard 552 respiration pump (Harvard Apparatus, Edenbridge, UK). The animals were kept at a constant temperature of 37°C throughout the experiment, using a homeothermic unit (Harvard Apparatus).

All experimental procedures related to animals described in this paper were previously notified to the regulatory authorities, and guidelines approved by the Catalan Parliament were strictly followed.

2.1.2. Experimental protocol for intravenous administration

The right femoral artery and the right femoral vein were cannulated to measure blood pressure and to administer test compounds, respectively. The left carotid and femoral arteries were dissected to implant two blood flow probes. Carotid blood flow was recorded using a Transonic blood flow probe connected to an ultrasonic blood flowmeter (Transonic, model T101, Transonic Systems, Ithaca, NY, USA). Femoral blood flow was recorded using a Valpey–Fisher flow probe connected to a pulsed doppler flowmeter (Valpey–Fisher, model VF1, Hopkinton, USA). All parameters were continuously recorded on a polygraph (Hewlett Packard, 7758A, Palo Alto, CA, USA).

Administration of increasing cumulative intravenous doses of the test substances (i.e. almotriptan or sumatriptan) began after a 30-min stabilisation period. The doses tested were from 0.01 μg/kg to 3 mg/kg. The substances were perfused using a Braun–Melsungen perfusor (Melsungen, Germany). Perfusion time was 3 min, with a 15-min interval between one treatment and the next.

Methiothepin (a 5-HT_1-like receptor antagonist (Humphrey et al., 1989)), 0.1 mg/kg/min, in perfusion up to 1 mg/kg was administered intravenously (femoral vein) 15 min after the last treatment dose, to determine whether this substance would reverse the effects of the test compounds on vascular resistance. The percentage reversal achieved with methiothepin was obtained by taking the maximum effect on vascular resistance of sumatriptan or almotriptan as 100% and the respective baseline values as zero.

The effects of the study compounds on mean blood pressure, heart rate, and carotid and femoral blood flows were evaluated in terms of percentage variation. The vascular resistances of the vascular beds were also calculated. Readings were recorded at the moment of maximum effect and 15 min after administration. The regression line between the logarithm of the dose and the percentage increase of each vascular resistance was calculated. The dose producing a 100% increase (ED_{100}) in the value of this parameter was calculated.

2.1.3. Protocol for intraduodenal administration

The right femoral artery was cannulated for recording of blood pressure, and the right carotid and left femoral arteries each were fitted with a Transonic blood flow probe (Transonic Systems, model T101) to measure blood flow. The abdominal cavity was entered by laparotomy and a cannula tipped with a hook-shaped hypodermic needle was inserted in the lumen of the duodenum and connected to a perfusor to allow intraduodenal administration of the test compounds.

The data were recorded as described in Section 2.1.2. The ranges of doses tested were 0.1, 0.3 and 1 mg/kg for sumatriptan, and 0.03, 0.1, 0.3, 1 and 3 mg/kg for almotriptan. Methiothepin was perfused after the last dose and its percentage reversal was obtained as described above.

The dose producing a 50% increase (ED_{50}) in vascular resistance was calculated.

2.1.4. Protocol to study the effects on carotid arteriovenous anastomoses

The method used was a modification of the techniques described by Perren et al. (1989) and Saxena et al. (1983). Arterial blood pH and CO_2 and O_2 partial pressures were determined at the beginning of the experiment using a Radiometer blood gas analyser (model ABL510, Radiometer, Copenhagen, Denmark).

The right femoral artery and left femoral vein were cannulated to record blood pressure on a polygraph (Lectromed Multitrace 8, Lectromed, Jersey, UK) and to administer the substances, respectively. The right common carotid artery was dissected, and a Transonic flow probe was implanted and connected to an ultrasonic blood flowmeter (Transonic Systems, model T101). The right cranial lingual artery was cannulated for administration of coloured microspheres (Precision coloured microspheres, Dye-Trak™, Triton Technology, San Diego, CA, USA). In addition, surface electrodes to record the electrocardiogram were attached. As the internal carotid artery is not functional in adult cats and as the brain is irrigated by the internal maxillary artery among others, we considered it

suitable to administer the microspheres via the lingual artery, which is a branch of the common carotid (Crouch, 1969). Furthermore, the diameter of most anastomoses (15–150 μm) is greater than that of the capillaries (1–8 μm), so most of the microspheres 15 μm in diameter are trapped in the capillaries, although some reach the venous circulation through the arteriovenous anastomoses and are finally trapped in the lungs (Saxena et al., 1983).

The microspheres were administered 15 min after the test substances or saline solution had been injected. There was an interval of 15 min between treatments. The microspheres were 15 μm in diameter and of five different colours: white, yellow, red, violet and blue. The treatment received by each animal was the vehicle and three different doses of the test substance. The number of microspheres injected varied as follows: 75,000 (red), 330,000 (white), 400,000 (violet), 500,000 (yellow) and 550,000 (blue) to compensate for their different absorbance characteristics. The treatment corresponding to each animal and the colour administered after each dose were randomly chosen.

Upon completion of the experiment, the animals were killed by intravenous injection of KCl, and the brain and the lungs were removed. Samples of the cranial skin and muscle were taken, while the brain and the lungs were extracted whole. The tissues were sectioned into fragments weighing 1 to 2 g, transferred to 16 ml tubes with Teflon® screw stoppers and 7 ml KOH (4 M) containing 2% Tween 80 was added to each tube. The tubes were closed and left overnight at room temperature. The digested tissue was stirred with a Teflon®-coated magnet and then filtered (polypropylene filter: pore size 10 μm and 25 mm in diameter, reference Millipore AN1H02500; filter carrier, reference Millipore XX10-025-40; vacuum pump, reference Millipore XX6022050, Bedford, MA, USA). The tube and the filter were rinsed several times with a solution of 0.05% Tween 80. The microspheres were washed twice with 70% ethanol to dislodge any lipids that might have adhered to them. The filter was withdrawn carefully, placed in a 1.5-ml Eppendorf tube and left to dry ≥ 15 min, after which time 100 μl of dimethylformamide was added. The mixture was stirred for 30 s and then centrifuged at $2000 \times g$ for 3 min. At this point, the dimethylformamide with the colouring in solution was transferred to a spectrophotometer cuvette for absorbance analysis. The absorption spectrum from 320 to 820 nm of the corresponding samples was determined using a spectrophotometer for visible/UV with a wavelength range of 190–820 nm (Hewlett-Packard, model 8452A, Rockville, MD, USA). A final dilution was carried out if the sample gave readings higher than 1.3 units of absorbance.

The carotid flow distribution was obtained from the quantity of colouring in the cerebral and extracerebral tissue, as well as that which had passed through the arteriovenous anastomoses to lodge in the lungs (Saxena et al., 1980; Saxena and Verdouw, 1982).

The following formulae were used in this calculation:

% of carotid flow to the tissue

$$= [\text{colouring in the tissue} / \text{colouring injected}] \times 100$$

Tissue blood flow

$$= [\text{colouring in tissue} / \text{colouring injected}] \times \text{carotid blood flow (ml/min)}.$$

The results were expressed as blood flow in absolute values towards each of the areas considered and as a percentage of carotid blood flow, assuming the latter to be 100%. Finally, vascular resistances were calculated from blood pressure and blood flow values.

2.2. Haemodynamics in anaesthetised beagle dogs

2.2.1. General setup

Beagle dogs (8–16 kg) of either sex were supplied by Almirall (St. Andreu de la Barca, Spain). The animals were fasted for 18 h with water ad libitum before the experiments. The dogs were anaesthetised with sodium pentobarbital (a bolus of 35 mg/kg i.v. plus continuous perfusion of 6 mg/kg/h in the cephalic vein). The animals were connected to a respirator (Harvard, model 607A, Harvard Apparatus) at a constant volume of 10 ml/kg of air at 15 respirations per minute. A homeothermic unit (Harvard Apparatus) kept the animals at a temperature of 37°C throughout the experiment.

2.2.2. Experimental protocol

The right carotid artery and right femoral vein were cannulated to record blood pressure and to administer the compounds, respectively. In addition, the left carotid and femoral arteries were carefully dissected to implant probes for measuring blood flow. The probes were connected to an electromagnetic blood flowmeter (Narco bio-systems, Houston, TX, USA). Haemodynamic parameters were recorded on a polygraph (Hewlett Packard model 7758B).

Almotriptan or sumatriptan was administered in the left femoral vein at increasing cumulative doses from 1 μg/kg to 3 mg/kg, with a perfusion time of 3 min and at 15-min intervals. The effects of the compounds on the vascular resistance of the carotid and femoral beds were evaluated. The dose producing a 50% increase (ED_{50}) in carotid resistance was calculated.

2.3. Effects on meningeal extravasation produced by electrical stimulation of the trigeminal ganglion in anaesthetised guinea pigs

2.3.1. General setup

Male Dunkin–Hartley guinea pigs (350–400 g) were supplied by Biocentre (Sant Feliu de Codines, Spain). The

animals were fasted for 18 h with water ad libitum before the experiments. The animals were anaesthetised with sodium pentobarbital (40 mg/kg i.p.), mechanically ventilated at 50 respirations/min, 10 ml/kg, with a Harvard respirator (model 683) and kept at a constant temperature of 37°C by means of a homeothermic unit (Harvard Apparatus). Both carotid arteries were dissected and the right jugular vein was cannulated.

2.3.2. Experimental protocol

The animals were immobilised in a stereotactic support setup (Kopf Stereotaxic Instruments, Tujunga, CA, USA), and two orifices 1 mm in diameter were made on each side of the cranium (Foredom Drill System, Foredom Electric, Bethel, MN, USA). The coordinates used were AP 2–4, L 4–4, inclination −4.5. Two bipolar electrodes (NE 200X Rhodes Medical Instruments, Woodland Hills, CA, USA) were placed at a depth of 11 mm in the frontal part of the trigeminal ganglions. After 10 min, the study compound, or vehicle, was injected intravenously into the cannulated jugular vein; Evans Blue (50 mg/kg, 1 ml/kg) was injected after a further 5 min. The period of electrical stimulation (3 mA, 5 Hz, 5 ms, Hugo Sachs 215, March-Hugstetten, Germany) began 5 min after administration of the colouring, and lasted for 5 min, after which the electrodes were transferred to the posterior orifices for the same period of time, but with reversed polarity. The animals in the control group underwent the same surgical process but without the electrical stimulation. The two previously dissected carotid arteries were cannulated and connected to a peristaltic pump (Gilson Minipuls 3, Villiers le Bel, France). In order to extract the colouring and the blood from vessels, both jugular veins were sectioned and a carotid constant flow infusion of 100 ml saline containing sodium heparin 0.1 mg/ml was started. In addition, drainage was facilitated through the right auricle.

The temporal–parietal zone of the dura mater covering both cerebral hemispheres was dissected and precisely weighed. The meninges were incubated for 15 h in 2 ml formamide at 55°C.

Absorbance of formamide with the colouring extracted from the tissue was determined using a spectrophotometer (Kontron Uvikon 941, Zürich, Switzerland) at 629 nm. The concentration of Evans Blue was calculated by interpolation on a standard curve. The values were calculated for each animal as the amount of colouring compared to the previously determined weight of the meninges (ng/mg tissue).

2.4. Compounds

Almotriptan maleate or chlorhydrate was synthesised at Ranke (Sant Andreu de la Barca, Spain), naratriptan chlorhydrate was synthesised in the Department of Medicinal Chemistry, Almirall Prodesfarma and sumatriptan as a base was extracted from Imigram® (Glaxo) tablets in the Department of Medicinal Chemistry, Almirall Prodesfarma. Sodium pentobarbital, α-chloralose (base), sodium thiopental and methiothepin were purchased from Grindsted Products (Brabant, Denmark), Merck (Darmstadt, Germany), Abbott (Madrid, Spain) and Roche (Basel, Switzerland), respectively. Other reagents were sodium heparin (Byk Leo, Arganda del Rey, Spain), Evans Blue (Merck) and formamide (Panreac, Montcada i Reixach, Spain).

The compounds were dissolved in 0.9% saline and slightly acidified with 0.5% of 0.1 N HCl for intravenous administration. When administered intraduodenally, the test compounds were dissolved in distilled water, slightly acidified as before, and administered at concentrations between 2.5 and 3 mg/ml.

2.5. Statistics

Depending on the type of data recorded, the statistical test used was a paired or unpaired Student's *t*-test. Significance was considered to be $P < 0.05$.

3. Results

3.1. Haemodynamics in anaesthetised cats

3.1.1. Intravenous route

At the moment of maximum effect on carotid vascular resistance, almotriptan produced a mean increase of 276% (at 1 mg/kg); at 15 min the maximum increase was 189% (at 3 mg/kg) (Fig. 1). A clear increase in vascular resistance in the femoral bed was observed. The effect of almotriptan on carotid resistance was reversed by 78% by methiothepin and by 53% in the femoral zone.

Sumatriptan induced dose-related increases in carotid vascular resistance, both at the moment of maximum effect (271%) and 15 min after administration (198%). As in the case of almotriptan, an increase in femoral vascular resistance was also detected. Methiothepin reversed the effects of sumatriptan on carotid resistance by 71% and almost identically by 72%, in the femoral zone. The administration of vehicle (in the same volume range as the study drugs) had no effect on the parameters studied.

The ED_{100} values for carotid resistance at the moment of maximum effect were 9 and 11 μg/kg, and 15 min after administration were 59 and 52 μg/kg for sumatriptan and almotriptan, respectively. Thus, almotriptan and sumatriptan were equipotent on the carotid vascular bed.

Neither almotriptan nor sumatriptan consistently modified either mean blood pressure or heart rate, although at lower doses almotriptan slightly lowered blood pressure (Fig. 1). Sumatriptan produced a fall in blood pressure in

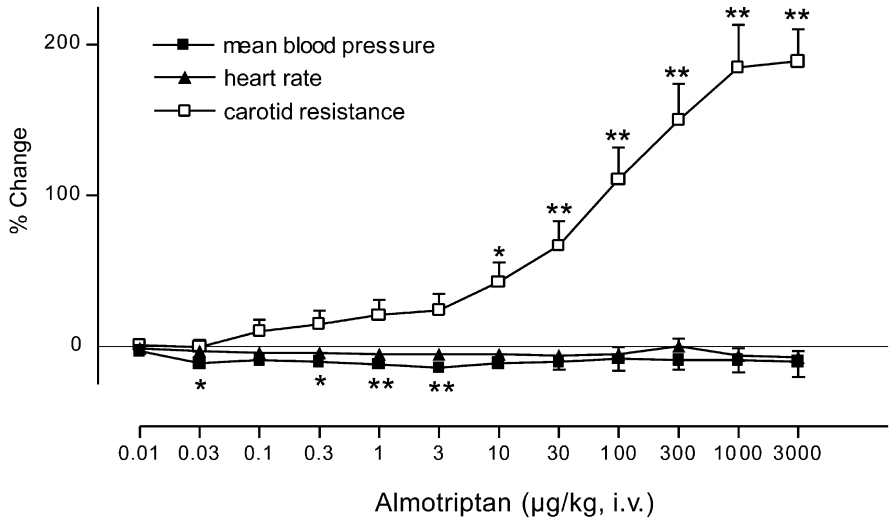

Fig. 1. Effects of intravenous almotriptan on carotid resistance, mean blood pressure, and heart rate in anaesthetised cats ($n = 7$) 15 min after administration. Baseline values (mean ± S.E.M.) are: mean blood pressure = 115 ± 10 mm Hg, heart rate = 171 ± 11 beats/min and carotid resistance = 2.94 ± 0.27 mm Hg min/ml. No statistically significant changes were observed on heart rate. Student's *t*-test for paired data with reference to baseline, $^{*}P < 0.05$, $^{**}P < 0.01$.

some cases at the dose of 3 mg/kg. This effect was reversed when perfusion was stopped.

3.1.2. Intraduodenal route

Intraduodenal almotriptan (0.03–3 mg/kg) induced a dose-dependent fall in carotid flow. As the pressure was hardly altered, this effect was associated with an increase in carotid resistance (maximum effect of 151% at 3 mg/kg) (Fig. 2). A dose-dependent decrease was also observed in femoral flow, over a narrower dose range than with intravenous administration, between 0.03 and 1 µg/kg. An increase in vascular resistance was observed in the same dose range. Intraduodenal administration of vehicle (0.1, 0.3 and 1 ml/kg) caused maximum changes in vascular resistance of −2% and 5% (not significant).

The ED_{50} on vascular resistance for each substance demonstrated that almotriptan (339 µg/kg) and sumatriptan (244 µg/kg) had similar effects on carotid vascular resistance. The increase in vascular resistance caused by almotriptan and sumatriptan was reversed in both carotid

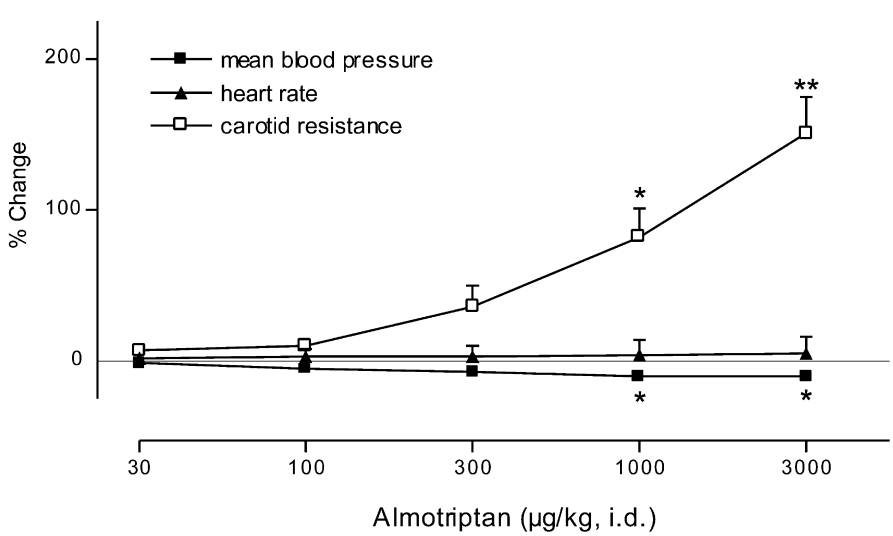

Fig. 2. Effects of intraduodenal almotriptan on carotid resistance, mean blood pressure, and heart rate in anaesthetised cats ($n = 4$) 15 min after administration. Baseline values (mean ± S.E.M.) are: mean blood pressure = 103 ± 15 mm Hg, heart rate = 171 ± 15 beats/min and carotid resistance = 3.10 ± 0.31 mm Hg min/ml. Student's *t*-test for paired data with reference to baseline, $^{*}P < 0.05$, $^{**}P < 0.01$.

and femoral flow by the intravenous perfusion of methiothepin (reversal ranged from 70% to 80%).

The maximum fall in pressure (10%) following almotriptan administration was statistically significant ($P < 0.01$; Fig. 2), and was similar to that caused by sumatriptan. Heart rate was not consistently affected by almotriptan administration (Fig. 2).

3.1.3. Effects on carotid arteriovenous anastomoses

Blood flow through the right common carotid artery was 30 ± 2 ml/min ($n = 17$), of which $79 \pm 4\%$ irrigated the right side of the head. Furthermore, $69 \pm 4\%$ of right common carotid artery flow went to the arteriovenous anastomoses, while $4 \pm 1\%$ went to the brain. The cranium received the remaining $27 \pm 4\%$.

Values for arterial pH and blood gas concentration at the beginning of the experiment were as follows (mean ± S.E.M.): pH = 7.457 ± 0.018, $pCO_2 = 23.2 \pm 1.1$ mm Hg and $pO_2 = 109.6 \pm 2.9$ mm Hg. Body temperature was 36.6 ± 0.2°C.

The three compounds studied (almotriptan, sumatriptan and naratriptan) induced a dose-dependent decrease in blood flow through the arteriovenous anastomoses. At a dose of 1 mg/kg, this flow was reduced from 22.0 ± 5.0, pretreatment, to 6.4 ± 1.8 ml min^{-1} with almotriptan ($P < 0.05$); from 22.7 ± 6.9, pretreatment, to 5.8 ± 1.3 ml min^{-1} with naratriptan; and from 18.6 ± 3.5, pretreatment, to 8.1 ± 2.7 ml min^{-1} for sumatriptan ($P < 0.01$). The increase in cerebral irrigation with almotriptan (from 0.7 ± 0.2 pretreatment to 3.5 ± 0.9 ml min^{-1} at 1 mg/kg) and naratriptan reached statistical significance in both cases, while the increase with sumatriptan was more moderate. Irrigation of the cranial area was slightly and similarly decreased, with all agents, although only the effect of almotriptan reached significance (from 8.6 ± 1.2 pretreatment to 4.3 ± 0.8 ml min^{-1} at 1 mg/kg). A non-significant tendency to a decreased flow through the arteriovenous anastomoses and to the cranium was observed in vehicle-treated animals throughout the experiment, while cerebral irrigation remained unchanged. When the relative flow reaching each of the study areas was considered, qualitatively similar effects were observed with all three substances. The percentage of carotid blood flow perfusing the arteriovenous anastomoses clearly decreased, while that irrigating the brain increased, to reach about a quarter of total carotid flow, while cranial flow was left unchanged. Almotriptan induced a clear dose-related effect on the vascular resistances of arteriovenous anastomoses. In addition, the effects of almotriptan were somewhat greater than those of either sumatriptan or naratriptan (Fig. 3).

The effects on mean blood pressure and heart rate were similar to those observed in the control group. Statistically significant decreases of around 50% in carotid flow were observed with all three treatments. The effects on heart rate with almotriptan reached statistical significance but were too slight to be relevant (maximum fall of 18 beats/min).

3.2. Haemodynamics in anesthetised beagle dogs

Almotriptan (1 mg/kg i.v.) increased carotid vascular resistance by 100% and 83% at the moment of maximum effect and 15 min after administration, respectively. More complex activity was observed in the femoral bed, since doses of up to 0.1 mg/kg induced a decrease in resistance, followed by a subsequent increase. The dose of 1 mg/kg induced a maximal increase of 34% (Fig. 4).

The ED_{50} values for carotid resistance were 116 and 38 μg/kg at the moment of maximum effect for almotriptan and sumatriptan, respectively. Thus, sumatriptan was three times more potent than almotriptan in this model, although their efficacy was similar.

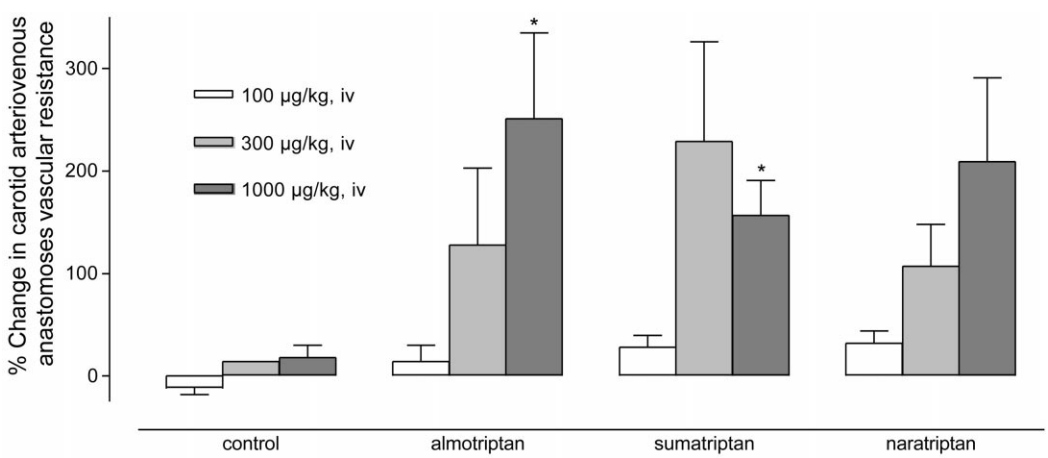

Fig. 3. Effects of almotriptan, sumatriptan and naratriptan on carotid arteriovenous anastomoses vascular resistances in anaesthetised cats. Student's *t*-test for paired data with reference to baseline, $^{*}P < 0.05$.

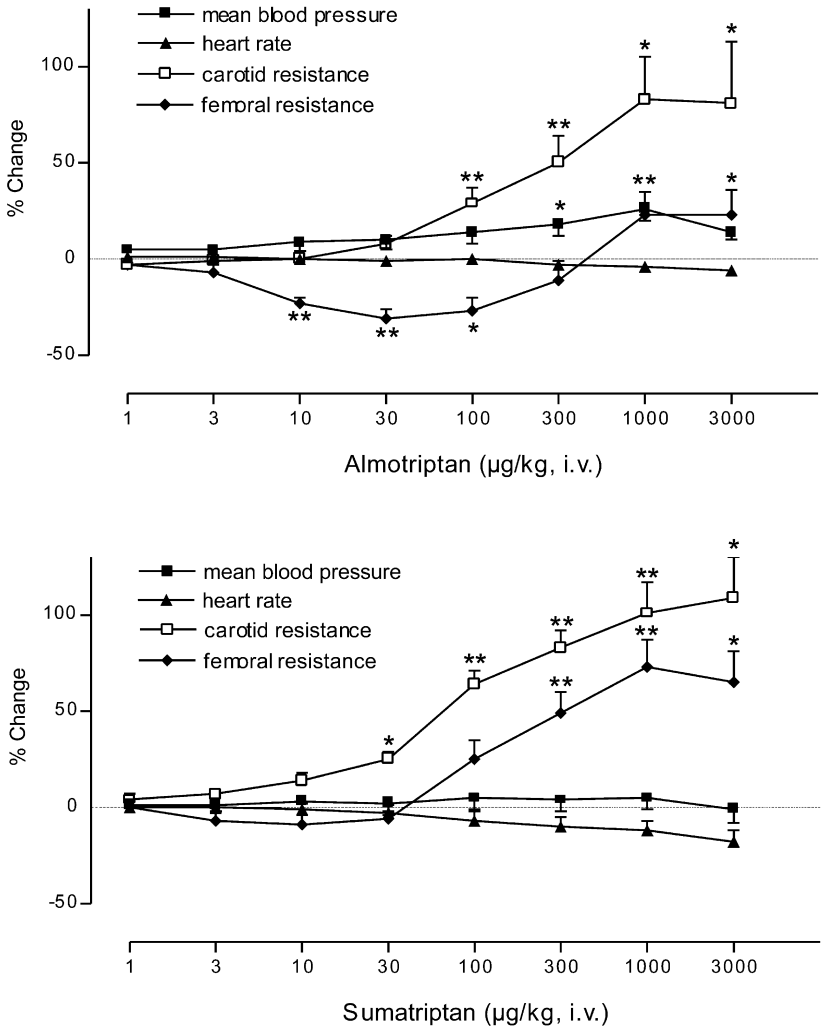

Fig. 4. Effects of intravenous almotriptan (above) and sumatriptan (below) on carotid resistance, femoral resistance, mean blood pressure, and heart rate in anaesthetised beagle dogs ($n = 5$) 15 min after administration. Baseline values (mean ± S.E.M.) for almotriptan and sumatriptan are, respectively: mean blood pressure = 121 ± 8 and 128 ± 6 mm Hg, heart rate = 132 ± 10 and 136 ± 18 beats/min, carotid resistance = 1.50 ± 0.29 and 2.15 ± 0.48 mm Hg min/ml and femoral resistance = 2.83 ± 0.26 and 2.80 ± 0.38 mm Hg min/ml. Student's *t*-test for paired data with reference to baseline, $^{*}P < 0.05$, $^{**}P < 0.01$.

Sumatriptan caused dose-related increases in carotid vascular resistance which reached maximal values of 119% and 109% at the moment of maximum effect and 15 min after administration, respectively. As with almotriptan (see above), a dual effect was observed in the femoral bed, characterized initially by a decrease in vascular resistance up to the dose of 0.01 mg/kg, followed by an increase. At the dose of 1 mg/kg, effects similar to those on the carotid bed were seen (Fig. 4).

Almotriptan at the dose of 1 mg/kg increased blood pressure, reaching a maximum rise of 25% but with no further increase at the dose of 3 mg/kg, and with no effect

Table 1
Meningeal extravasation following electrical stimulation of the trigeminal ganglion in anaesthetised guinea-pigs

Treatment	Dose (mg/kg, i.v.)				
	0.1	0.3	1	3	10
Almotriptan	168 ± 19 (31)	142 ± 13[a] (63)	140 ± 8[a] (69)	118 ± 14[a] (100)	n.a.
Sumatriptan	154 ± 12 (50)	150 ± 17 (56)	145 ± 13[a] (63)	138 ± 13[a] (72)	121 ± 16[a] (96)

Values (means ± S.E.M., $n = 5$–13) are calculated as the amount of Evans blue in ng/weight of the meninges in milligram. Baseline = 118 ± 9 ng of Evans blue/mg tissue and after electrical stimulation = 190 ± 18 ng of Evans blue/mg tissue. Percentage inhibition vs. leakage increase in brackets; n.a., not assessed.

[a] $P < 0.05$ vs. controls (unpaired Student's *t*-test).

on heart rate. Sumatriptan had no effect on mean blood pressure and only a slight effect on heart rate; the dose of 3 mg/kg caused 19% bradycardia, which disappeared after 15 min (Fig. 4).

3.3. Effects against meningeal extravasation produced by electrical stimulation of the trigeminal ganglion in anaesthetised guinea pigs

Almotriptan inhibited the extravasation of Evans Blue induced by bilateral electrical stimulation of the trigeminal ganglion in guinea pigs dose dependently. Although almotriptan and sumatriptan exerted similar effects, almotriptan (0.3 mg/kg) was slightly more potent than sumatriptan (1 mg/kg), based on the first dose with a significant effect (Table 1).

4. Discussion

This study describes the actions of almotriptan, a selective and potent 5-$HT_{1B/1D}$ receptor agonist (Bou et al., 2000), on animal models assumed to be predictive of antimigraine activity. Clinical activity in patients with migraine has already been demonstrated for orally administered almotriptan (Cabarrocas and the Almotriptan Oral Study Group, 1997). Its absolute oral bioavailability in humans is high (70%) (Fernández et al., 1999).

Almotriptan is a potent agonist at the vascular 5-HT_1 receptor, mediating cranial vasoconstriction in vivo. In cats, when given either intravenously or intraduodenally, almotriptan increased carotid vascular resistance, and exhibited the same potency and efficacy as sumatriptan. The effects seen on intraduodenal administration suggest effective enteric absorption. In beagle dogs, intravenous almotriptan also increased carotid vascular resistance, reaching the same maximum effect as sumatriptan. Almotriptan appears to be more selective for the carotid vs. the femoral circulation than sumatriptan. However, almotriptan was less potent than sumatriptan in the carotid vascular bed. The results also demonstrated for almotriptan a higher potency in cats than in dogs, as observed for zolmitriptan (Rolan and Martin, 1998). The contractile responses to 5-HT in intracranial arteries and carotid arteriovenous anastomoses are mediated predominantly by 5-HT_1-like receptors (Hoyer et al., 1994), which may be identical to 5-HT_{1B} receptors (De Vries et al., 1999). The contractile effects of both almotriptan and sumatriptan in the carotid vascular bed were almost completely reversed by methiothepin, consistent with its antagonism of the 5-HT_1 receptor type.

Almotriptan did not produce a non-selective increase in total peripheral resistance as shown by the absence of marked effects on mean blood pressure, even at high doses. However, almotriptan increased vascular resistance in the femoral circuit, a feature shared by other triptans and which is attributable to the presence of 5-$HT_{1B/1D}$ receptors in this vascular bed (Feniuk et al., 1989).

The effects on carotid arteriovenous anastomoses in anaesthetised cats demonstrated that almotriptan acts mainly on the arteriovenous anastomoses, as do the other two antimigraine agents studied. Almotriptan showed a tendency to increase cerebral blood flow in both absolute and relative terms. Almotriptan's effect was comparable to that of naratriptan, but greater than that of sumatriptan. Although the therapeutic implications of this difference, if any, require further study, it appears that almotriptan does not decrease the component of brain blood flow supplied by the carotid artery. The results obtained under these experimental conditions with sumatriptan were similar to those obtained with the radioactive microspheres method in both cats and pigs (Perren et al., 1989; Den Boer et al., 1991).

Neurogenic inflammation of the meninges has been observed in rodent models following electrical stimulation of the trigeminal ganglion (Markowitz et al., 1987). Neurogenic inflammation within cephalic tissues, encompassing vasodilatation and plasma protein extravasation, has been proposed as a putative mechanism in migraine pathogenesis (Buzzi and Moskowitz, 1990). However, evidence such as the lack of clinical efficacy of compounds inhibiting neurogenic plasma protein extravasation (i.e. bosentan, a selective endothelin receptor antagonist and RPR100893, a tachykinin NK_1 receptor antagonist) supports the idea that the tone of the cranial vasculature plays a decisive role in migraine (Schoenen, 1997). In the guinea-pig model now used, almotriptan dose dependently inhibited neurogenic inflammation induced by electrical stimulation of the fifth cranial pair (trigeminal) in the dura mater.

To summarise, the present studies demonstrated that almotriptan selectively contracts the cranial vasculature in cats (intravenous and intraduodenal) and dogs (intravenous) [via selective closure of the carotid arteriovenous anastomoses in cats (intravenous)]. Furthermore, almotriptan inhibits the extravasation of plasma from dural vessels following trigeminal ganglion stimulation in guinea pigs (intravenous). Consequently, in experimental models, almotriptan has the functional profile of a drug effective for the treatment of acute migraine attacks.

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