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The selectivity of MDL 74,721 in models of neurogenic versus vascular components of migraine

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Abstract

MDL 74,721 (*R*)-2-(*N*1,*N*1-dipropylamino)-8-methylaminosulfonylmethyl-1,2,3,4-tetrahydronaphthalene, a sulfonamidotetralin, has been found to exhibit a 10 000-fold greater potency in neurogenic versus vascular models of migraine. Sumatriptan, a relatively pure 5-HT_{1D}/5-HT_{1B} receptor agonist, also showed higher potency versus neurogenic inflammation. However, for sumatriptan the potency difference (100-fold) in the two pathophysiological models was less pronounced than seen for MDL 74,721. The affinity profile of MDL 74,721 at 5-HT_1 receptor subtypes may in part explain its ability to differentiate these two physiological responses. MDL 74,721 demonstrated nanomolar affinity for 5-HT_{1A} (12.7 ± 0.3 nM) and 5-HT_{1D} (41.3 ± 10.9 nM) but considerably lower affinity for 5-HT_{1B} receptors (> 1000 nM). Serotonin-like activity was seen in in vitro functional assays including inhibition of forskolin-stimulated cAMP accumulation in human 5-HT_{1D} receptor-transfected fibroblasts or eliciting vasoconstriction in isolated human pial arteries. The intrinsic activity (relative to $5-HT_{E_{Amax}}$) and affinity (pD_2) for the human cerebrovascular 5-HT receptors were: 5-HT (100%, 7.51 ± 0.09), sumatriptan (94%, 6.85 ± 0.1) and MDL 74,721 (66%, 5.70 ± 0.23). In anaesthetised cats, treatment with MDL 74,721 resulted in a dose-related reduction in the percentage of carotid flow going through the arteriovenous anastomoses to the lungs, with an ED_{50} of 0.3 mg/kg i.v., the same as sumatriptan. However, in the guinea-pig neurogenic model, MDL 74,721 inhibited plasma protein extravasation with an ED_{50} of 0.023 μg/kg compared to 2.5 μg/kg for sumatriptan. MDL 74,721 was also effective in this model (in rats) after oral administration. In conclusion, MDL 74,721 demonstrates a preclinical profile consistent with anti-migraine efficacy. Its marked preference for inhibiting neurogenic inflammation makes this compound a useful tool for assessing the relative contribution of this pathophysiological mechanism to the human disease state.

Keywords: 5-HT receptor pharmacology; Migraine model; Aminotetralin; Cerebrovascular regulation; Inflammation, neurogenic

1. Introduction

Migraine is a periodic, hemicranial, throbbing headache often accompanied by nausea and vomiting. Migraine usually begins in childhood, adolescence or early adult life and recurs in diminishing number and intensity during advancing years. The complete clinical picture of a migraine attack suggests a series of processes including vascular, neuronal and biochemical elements occurring at different sites. Early theories, such as Wolff's vascular theory (Graham and Wolff, 1938) and Heyck's arteriovenous anastomosis theory (Heyck, 1958), focused on vascular events as the primary mechanism in migraine.

The spreading depression theory for the aura of migraine was first proposed by Leao (1944), and the role of neurotransmitters, particularly 5-hydroxytryptamine (5-HT, serotonin) as a central mediator of a migraine attack have received much attention (Humphrey et al., 1990). More

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recently, the trigeminovascular theory has moved to the forefront of current research (Moskowitz, 1990, 1992), together with Olesen's vascular-supraspinal-myogenic model for migraine pain (Olesen, 1990). Although there are many hypotheses which attempt to explain the inappropriate vascular and/or neural system changes that occur during a migraine attack, there is still no explanation for the causes of the disease.

Sumatriptan is the most recent treatment for an acute migraine attack to have reached the market. It has a high degree of selectivity for 5-HT_1 receptors with notable affinity for the 5-HT_{1D} subclass (Feniuk et al., 1991). The efficacy of sumatriptan has been attributed to constriction of the large cranial blood vessels, which are believed to become distended during an attack giving rise to the headache (Friberg et al., 1991). The afferent nociceptive transmission is conducted to the central nervous system by the trigeminal nerve. Moskowitz (1990, 1992) has suggested that sumatriptan can also act presynaptically on trigeminovascular afferents to inhibit the release of vasodilatory peptides such as substance P and calcitonin gene-related peptide (CGRP) thereby attenuating migraine pain. In accordance with this proposal, sumatriptan has been demonstrated to attenuate the increase in plasma levels of CGRP during a migraine attack (Goadsby and Edvinsson, 1993).

The present study examined the effects of MDL 74,721, a sulfonamidotetralin, in animal models of the putative neurogenic and vascular components of migraine. The characterization has been extended to include sumatriptan to determine the relative neuroinhibitory and vasoconstrictor activities of the two agents. The neurogenic component has been examined in vivo, in both rat and guinea-pig plasma protein dural extravasation models. The vasoconstrictor components have been investigated in isolated human pial arteries and in vivo in the cat arteriovenous anastomoses model. The binding affinities of MDL 74,721 and sumatriptan at selected 5-HT_1 receptor subtypes have also been compared since sumatriptan is believed to exert its therapeutic effects through 5-HT receptor activation.

2. Materials and methods

2.1. Chemistry

MDL 74,721 ((*R*)-2-(*N*1,*N*1-dipropylamino)-8-methylaminosulfonylmethyl-1,2,3,4-tetrahydronaphthalene; Fig. 1) has been prepared as previously described by (Hibert, M., Petty, M.A., Jones, C.R., Novel 8-sulfamethylene-2-amino tetralins. EP 0451008. Issued June 28, 1995). The compound was studied as the methanesulfonate salt.

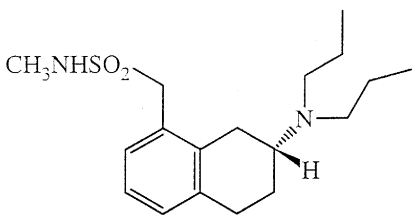

Fig. 1. The chemical structure of MDL 74,721 ((*R*)-2-(*N*1,*N*1-dipropylamino)-8-methylaminosulfonylmethyl-1,2,3,4-tetrahydronaphthalene).

2.2. Binding studies

2.2.1. Human 5-HT_{1D} receptor binding assay

HeLa cells stably transfected with human 5-HT_{1D} receptors were harvested by scraping into 0.9% phosphate buffered saline, containing 0.5 mM EDTA. The cell suspension was centrifuged at $300 \times g$ and lysed by freezing the pellet in 50 mM Tris–HCl (pH 7.6). The lysate was homogenized using a Polytron and centrifuged at $40\,000 \times g$ for 10 min at 40°C. The resulting pellet was resuspended in 50 mM Tris–HCl (pH 7.6). Membrane homogenate (appro. 150 μg) was incubated during 30 min at 37°C in 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, 0.5 mM EDTA, 0.1% ascorbic acid, 10 μM pargyline, 5 nM [^{3}H]5-HT and 0.1 nM–10 μM MDL 74,721 or sumatriptan. Nonspecific binding was defined as that remaining in the presence of 100 μM 5-HT. Incubation was terminated by rapid filtration through Whatman GF/B glass fiber filters, 0.1% ice-cold polyethylenimine, using a Brandel cell harvester. The filters were rinsed with Tris–HCl (50 mM, pH 7.4) and counted in a liquid scintillation counter. Protein content was determined according to Lowry et al. (1951).

2.2.2. Rat 5-HT_{1B} receptor binding assay

Young male rats (OFA, Iffa Credo, L'Arbresle, France) were killed by decapitation, the brains were immediately removed and the frontal cortex dissected. The fresh tissue was homogenized in ice-cold incubation buffer (50 mM Tris, pH 7.7) using a Polytron (setting 6 for 15 s). The homogenate was centrifuged at $40\,000 \times g$ for 15 min at 4°C. The supernatant was discarded and the pellet was resuspended in the same buffer and centrifuged as before. The centrifugation and wash was repeated twice more with a 15 min 37°C incubation between the second and third centrifugation. The final pellet was resuspended in 50 mM Tris, pH 7.7, 4 mM $CaCl_2$. 100 nM 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), 100 nM mesulergine, 0.1% ascorbic acid, 10 μM pargyline. Membrane homogenate (10 mg original wet weight) was incubated for 60 min at 20°C in 1.5 nM [^{3}H]5-HT, the buffer described above, and 0.1 nM–10 μM MDL 74,721, or sumatriptan. Nonspecific binding was defined as that remaining in the presence of 10 μM 5-HT. Incubation was terminated by rapid filtration through Whatman GF/B glass fiber filters, presoaked in 0.1% polyethenimine. The filters were rinsed with Tris–HCl (50 mM, pH 7.4) and counted in a liquid scintillation counter. Protein content was determined according to Lowry et al. (1951).

2.2.3. Rat 5-HT_{1A} receptor binding assay

The method of Gozlan et al. (1983) was utilized with minor modifications. Young male rats (OFA, Iffa Credo)

were killed by decapitation, the brains were immediately removed and the hippocampus dissected. The fresh tissue was homogenized in ice-cold incubation buffer (50 mM Tris, pH 7.4) using a Polytron (setting 6 for 15 s). The homogenate was centrifuged at $40\,000 \times g$ for 15 min at 4°C. The supernatant was discarded and the pellet was resuspended in the same buffer and centrifuged as before. The final pellet was resuspended in 14 vol of 50 mM Tris, pH 7.4. Membrane homogenate (7.8 mg original wet weight) was incubated for 10 min at 37°C in 50 mM Tris–HCl pH 7.4, 0.4 nM [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), and 0.1 nM–10 μM MDL 74,721, or sumatriptan. Nonspecific binding was defined as that remaining in the presence of 10 μM 5-HT. Incubations were terminated by rapid filtration through Whatman GF/B glass fiber filters, presoaked in water. The filters were rinsed with Tris–HCl (50 mM, pH 7.4) and counted in a liquid scintillation counter. Protein content was determined according to Lowry et al. (1951).

2.2.4. Data analysis for receptor binding assays

IC_{50} values and Hill coefficients for competition binding experiments were calculated using non-linear regression to a 4 parameter logistic equation using INPLOT (Graphpad Software, San Diego, CA, USA) or Excel (Microsoft) spreadsheet software. IC_{50} values were converted to K_i values using the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

2.3. Functional activity at the human 5-HT$_{1D}$ receptor: inhibition of forskolin-stimulated cAMP accumulation

Cyclic AMP accumulation was measured using methods described previously (Baron and Siegel, 1989). Briefly, fibroblasts stably expressing the human 5-HT$_{1D}$ receptor were loaded with [^{3}H]adenine, washed, suspended, and then challenged with 10 μM forskolin in the presence of various concentrations of serotonin, sumatriptan, or MDL 74,721. After 10 min at 37°C, [^{3}H]cAMP was isolated and quantitated as described previously.

2.4. In vitro studies in human cerebral arteries: responses to 5-HT$_{1B/1D}$ agonists

Ramifications (about 1 mm outside diameter) of human middle cerebral artery were obtained at surgery from patients undergoing temporal lobe surgery. Upon removal from the cortical surface, the pial vessels were immediately used for isometric tension measurements as previously described (Hamel and Bouchard, 1991). For this purpose, vessel segments (3–4 mm in length) were mounted between two L-shaped metal prongs in temperature-controlled (37°C) tissue baths containing an oxygenated Krebs-solution. Vessels were allowed to stabilize at 0.3–0.4 g for 60 min. They were then exposed to a depolarizing Krebs solution containing 124 mM K^+ to determine their maximal contractile capacity, followed by several washes in normal Krebs and a 60 min recovery period. Log concentration–response curves were generated for 5-HT, sumatriptan and MDL 74,721 in vessels under resting tension. Relative potencies of the agonists were determined according to their respective pD_2 values and maximal contractile responses (E_{Amax} expressed as a percentage of the $5-HT_{E_{Amax}}$ in the same vessel segments; for details see Hamel and Bouchard, 1991).

2.5. In vivo preparations

Male Sprague-Dawley rats (Iffa Credo, 200–250 g or Charles River Laboratories, Wilmington, DE, USA, 260–350 g), male Hartley guinea pigs (Charles River Laboratories) weighing 200–250 g and male cats (Harlan CPB, Zeist, Netherlands) weighing 3.5–4.6 kg were used. Animals were kept under controlled conditions, with respect to temperature, humidity and light. Before any intervention commenced animals were fasted overnight, but had free access to water.

2.6. Carotid haemodynamics

2.6.1. Surgery

Cats were anaesthetised with chloralose (80 mg/kg administered intraperitoneally; i.p.) and pentobarbitone (10 mg/kg i.p.). Body temperature was maintained around 37°C by means of an electric heating pad. The cats were ventilated and arterial blood gases monitored and kept within normal limits (pH 7.35–7.45; Pa_{O_2} 90–150 mmHg; Pa_{CO_2} 35–45 mmHg). Systemic blood pressure and heart rate were measured from a femoral artery catheter and blood flow in both common carotid arteries was measured with electromagnetic flow probes (1.5–2 mm; Skalar, Delft, Netherlands). The signals were recorded on a Gould RS 3400 recorder and also by a digital acquisition analysis program (PONEMAH, Storrs, CT, USA). A catheter was placed in the left lingual artery to allow retrograde injection of the microspheres into the carotid artery.

2.6.2. Distribution of carotid blood flow

The distribution of common carotid flow was determined using 15 ± 1 μm diameter radioactive microspheres labelled with ^{141}Ce, ^{113}Sn, ^{95}Nb, or ^{113}Sn (NEN, Du Pont de Nemours, Paris, France). For each measurement, 5×10^4 microspheres, labeled with one of the nuclides, was sonicated, mechanically agitated and injected, via the lingual artery, into the carotid artery. In these studies, the effects of 3 doses of each compound were compared to responses obtained with the particular vehicle. After a 30 min stabilization period, vehicle (1 ml) was injected intravenously over a 1 min period and baseline measurements of carotid haemodynamics were made 15 min later by injecting microspheres. The interval between successive vehicle or compound administrations was therefore 18 min

including a 2 min control period. No more than four microsphere injections were made in any experiment. At the end of the experiment, the animal was killed and the kidneys, lungs and the various tissues of the head were removed, placed into vials and weighed. The radioactivity in each vial was counted for 1 min in a gamma counter, using suitable windows for discriminating the different nuclides. In all studies the radioactive microspheres were injected in a randomized order, and because there was complete entrapment of microspheres passing through the cranial circulation and in the capillaries of the lungs (no radioactivity was detected in the kidneys), the values determined for the lungs provide an index of the arteriovenous anastomotic fraction of the common carotid blood flow (Saxena and Verdouw, 1982; Johnston and Saxena, 1987). Thus, carotid blood flow was distributed according to the amounts of radioactivity in cerebral (brain) and extracerebral tissues (tissues other than brain in the head) as well as the amount of radioactivity in the lungs.

Therefore:

$$\%\ \text{Carotid Blood Flow to Tissue} = \frac{\text{Tissue Radioactivity}}{\text{Injected Radioactivity}} \times 100$$

and

$$\text{Tissue blood flow} = \frac{\text{Tissue Radioactivity}}{\text{Injected Radioactivity}} \times \text{Carotid blood flow (ml/min)}$$

An ED_{50} value was defined as the drug dose attenuating carotid flow to 50% of the maximal drug-induced reduction.

2.7. Neurogenic model of plasma protein extravasation

Electrical stimulation of the trigeminal ganglion was performed in guinea pigs (Matsubara et al., 1992; Huang et al., 1993) and pentobarbitone (60 mg/kg, i.p.) anaesthetised rats as described previously (Limmroth et al., 1996). In brief, rats were aligned into a stereotaxic frame with the incisor bar set at −1.5 mm from horizontal and the calvarium was exposed by a midline incision. Symmetrical burr holes (2 mm in diameter) were drilled at 3.7 mm posterior to bregma, 3.2 mm lateral to the sagittal suture to expose the dura mater. Bipolar electrodes were placed into the right and left trigeminal ganglia to a depth of 9.5 mm from the overlying dura mater. Ten min (i.v., femoral vein) or 20 min (p.o.) after compound injection and 5 min after injection of [^{125}I]bovine serum albumin (50 μCi/kg, femoral vein), the right trigeminal ganglion was stimulated (0.6 mA, 5 ms, 5 Hz, 5 min). The animal was perfused transcardially with saline via the left cardiac ventricle for 2 min at constant pressure of 100 mm Hg and the dura mater was dissected bilaterally, rinsed, weighed and counted for radioactivity as previously described (Markowitz et al., 1987). An ED_{50} value was defined as the drug dose attenuating albumin extravasation to 50% of the maximal drug-induced reduction.

MDL 74,721 was evaluated for its ability to prevent neurogenic extravasation after oral administration. MDL 74,721 (1 and 3 mg/kg) was administered orally (p.o.) to rats 20 min before the i.v. injection of the tracer [^{125}I]bovine serum albumin (50 μCi/kg) and 25 min before stimulation of the right trigeminal ganglion as described above. After perfusing the animal, the dura mater was removed bilaterally, rinsed and radioactivity measured as an index of plasma extravasation.

2.8. Inhibition of raphe nucleus cell firing

Male Sprague-Dawley rats (260–350 g) were anaesthetized with chloral hydrate (400 mg/kg i.p.) initially. Additional injections were given i.v. as required to maintain anaesthesia throughout each experiment. Cisternal drainage was performed to prevent tissue swelling by puncturing the dura over the cerebellomedullary cistern, and a burr hole 3 mm square was then drilled into the skull over the lamda suture. A stereotaxic apparatus in conjunction with the Burleigh Inchworm microdrive was used to position the electrode at the midline, 0.4–0.7 mm anterior to lamda, and 4.5–5.5 mm ventral to the exposed tissue to reach the dorsal raphe nucleus. MDL 74,721 was dissolved in water and administered intravenously via the lateral tail vein.

Recordings were made using single barrel micropipettes (Radnoti Starbore, 2 mm O.D.) with tip diameters of 1–2 μm filled with 2 M NaCl to obtain electrodes with in vitro impedances ranging from 2–8 MΩ at 60 Hz. The electrode signal was fed through a high-impedance amplifier and monitored on an oscilloscope. Serotonergic neurons were characterized by wide-duration action potentials (1–2 ms, positive-negative spikes), a metronome-like rhythm, and a slow firing rate (0.5–3.0 Hz). The firing rate was computed from an electronic counter triggered by individual neuronal spikes in 10 s samples.

The baseline rate of cell firing in the period prior to injection was compared to the maximal drug response to calculate percent suppression. Multiple injections were given to the same animal following a suitable interval to allow recovery of the cell firing rate. Recordings were then made from the same or a different cell at the next dose level. A total of 5 animals were used for these measurements involving 13 cells at the 3 dose levels. Data were analyzed using non-linear regression according to the logistic equation $f(X) = B + M(X^n)/(K^n + X^n)$ where B is the basal firing rate, X is the dose of drug administered, M is the percent suppression of firing rate, n is a slope factor, and K is the ED_{50}.

3. Results

3.1. Binding profile

Binding affinity was measured to rat 5-HT_{1A}, human 5-HT_{1D} and rat 5-HT_{1B} receptors using competition binding techniques and quantified with non-linear regression to a 4 parameter, logistic equation. Results are presented in Table 1. MDL 74,721 exhibited high affinity for rat 5-HT_{1A} and human 5-HT_{1D} receptors, with K_i values of 12.7 and 41.3 nM, respectively. MDL 74,721 showed low affinity for the rat 5-HT_{1B} receptor with $IC_{50} > 1$ μM. In contrast, sumatriptan was roughly equipotent at the structurally-related human 5-HT_{1D} and rat 5-HT_{1B} subtypes, but displayed lesser affinity for rat 5-HT_{1A} receptors. MDL 74,721 was examined at several other CNS receptors and showed IC_{50} values which were greater than 1 μM at dopamine D_2 receptors, $5HT_3$ receptors, α_1-adrenoceptors, α_2-adrenoceptors and $5HT_2$ receptors (data not shown).

3.2. Effects on cAMP accumulation

The 5-HT_{1D} receptor is negatively coupled to adenylate cyclase and therefore agonism is reflected in inhibition of forskolin-stimulated cAMP accumulation. Cyclic AMP levels were measured in HeLa cells stably transfected with the human $5HT_{1D}$ receptor by prelabeling the adenine nucleotide pool and monitoring its conversion into radiolabeled cAMP. In the absence of pharmacological stimulation (basal), cAMP accounted for 0.188 ± 0.009% of the cell-associated radioactivity. Forskolin (10 μM) increased cAMP levels 17 ± 3-fold over basal levels. Serotonin produced a concentration-dependent inhibition of the response to forskolin which, at maximally-effective concentrations, reduced the response by 67 ± 2%. The response to serotonin was taken as a reflection of full agonism and used to calculate the intrinsic activity of other agonists in activation of the recombinant receptor. Both sumatriptan and MDL 74,721 mimicked the response to serotonin and in this preparation were full agonists. The respective values for the EC_{50}, Hill slope, and maximal inhibition for these agonists is shown in Table 2.

Table 1
The binding affinity of MDL 74,721 and sumatriptan at various 5-HT_1 receptor subtypes

Ligand	K_i (nM) rat 5-HT_{1A} [^{3}H]8-OH-DPAT	K_i (nM) rat 5-HT_{1B} [^{3}H]5-HT	K_i (nM) human 5-HT_{1D} [^{3}H]5-HT
MDL 74,721	12.7 ± 0.3	>1000	41.3 ± 10.9
Sumatriptan	476 ± 3	55.1 [a]	15.5 ± 4.1

Affinities of MDL 74,721 and sumatriptan for the rat cortical 5-HT_{1A}, rat cortical 5-HT_{1B} and the cloned human 5-HT_{1D} receptors. Results are expressed as inhibition constants ± S.E.M. ($n = 3$–7 experiments) except for rat 5-HT_{1B} which was a single determination.
[a] Peroutka and McCarthy (1989) have reported similar results ($K_i = 27$ nM).

Table 2
Receptor-mediated inhibition of cAMP accumulation in fibroblasts stably expressing the human 5-HT_{1D} receptor

Agonist	EC_{50} (nM)	Hill slope	Inhibition at maximum (%)
5-HT	23.3 ± 13.0	1.44 ± 0.45	67 ± 2
MDL 74,721	37.1 ± 16.9	0.78 ± 0.14	68 ± 2
Sumatriptan	30.4 ± 15.8	1.17 ± 0.09	66 ± 3

Inhibition of forskolin- (10 μM) stimulated cAMP accumulation was measured in [^{3}H]adenine-labeled fibroblasts expressing the human 5-HT_{1D} receptor. The results shown are means ± S.E.M. of 4 independent experiments. Parameters were calculated using non-linear regression analysis as described by Baron and Siegel (1989).

3.3. Effects on human isolated pial arteries

Fig. 2 presents the efficacy of 5-HT, sumatriptan and MDL 74,721 in isolated human cerebral arteries. The rank order of potency expressed as pD_2 values was: 5-HT (7.51 ± 0.09; mean ± S.E.M., $n = 14$) > sumatriptan (6.85 ± 0.1, $n = 11$) > MDL 74,721 (5.70 ± 0.2, $n = 7$). Sumatriptan was a full agonist relative to 5-HT (relative E_{Amax} was 94 ± 3%), whereas MDL 74,721 was slightly less effective in inducing vasoconstriction, even at concentrations as high as 10^{-4} (relative E_{Amax} of 66 ± 8%). Contractile responses elicited by 5-HT, sumatriptan and MDL 74,721 were readily reversible upon removal of the agonist.

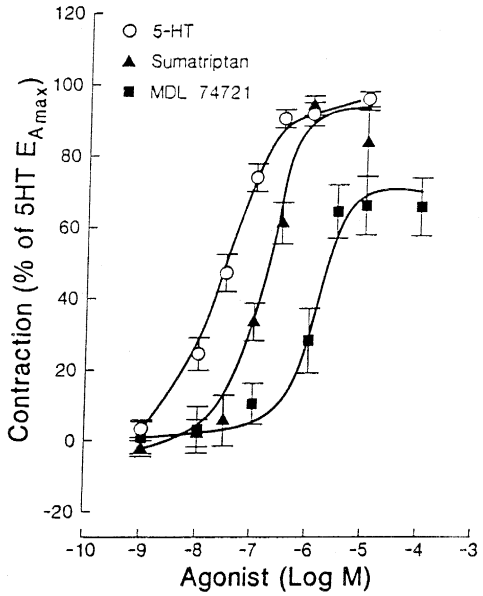

Fig. 2. Log concentration–response curves of 5-HT ($n = 14$), sumatriptan ($n = 11$) and MDL 74,721 ($n = 7$) generated in isolated human pial arteries. The response of the vessel segments to the agonists was compared to the maximal vasoconstriction elicited by 5-HT in the same preparation, and is expressed as a percentage of the maximal 5-HT response. The results are expressed as the mean ± S.E.M.

3.4. Effects on the arteriovenous anastomotic fraction of common carotid flow

The arteriovenous anastomotic fraction of carotid flow was assessed in anaesthetised cats by measuring the percentage of radioactive microspheres appearing in the lungs after their introduction via the carotid circulation. Treatment with MDL 74,721 (0.1–1.0 mg/kg i.v.) resulted in a dose-related reduction in the percentage of carotid flow going through arteriovenous anastomoses to the lungs with an ED_{50} value of 0.3 mg/kg (Fig. 3). This reduction was associated with a decrease in carotid blood flow and an increase in flow in the cerebral and extracerebral circulations. Similarly sumatriptan (0.03–1.0 mg/kg i.v.) decreased the arteriovenous anastomotic fraction of carotid flow (Fig. 4) accompanied by a reduction in carotid blood flow and an increase in cerebral and extracerebral blood flows. The ED_{50} of sumatriptan was 0.3 mg/kg. Heart rate and blood pressure decreased in a dose-dependent fashion with both MDL 74,721 and sumatriptan.

3.5. Effect on neurogenic inflammation in the guinea pig

MDL 74,721 and sumatriptan were compared for their ability to attenuate plasma protein extravasation (^{125}I-labelled bovine serum albumin) within the dura mater following unilateral electrical trigeminal ganglion stimulation (0.6 mA, 5 ms, 5 Hz, 5 min). The threshold for MDL 74,721 (0.0032 μg/kg) was significantly lower than that for sumatriptan (1.1 μg/kg). Similarly the ED_{50} was 0.023 μg/kg for MDL 74,721 versus 2.5 μg/kg for sumatriptan (Fig. 5). Maximal responses were elicited with doses of 3.2 and 85 μg/kg for MDL 74,721 and sumatriptan, respectively. The resulting dose–response curves differed markedly in their shape with those of sumatriptan being considerably steeper than those describing MDL 74,721. Additionally, there may be a difference in the extent of the inhibition of extravasation by the two compounds. The curves for MDL 74,721 appear to plateau at extravasation ratios greater than 1 indicating a residual extravasation response. In animals receiving the highest dose of MDL 74,721 (3.2 μg/kg), a ratio of 1.15 (95% confidence limits: 1.25–1.05; df = 6) consistent with residual extravasation was observed.

The potential contribution of post-junctional mechanisms to the effect of MDL 74,721 on the trigeminovascular response was evaluated by measuring the ability of MDL 74,721 to inhibit substance P-mediated extravasation. Substance P (1 nmol/kg i.v.) significantly increased dural radioactivity by 50.6 ± 7.0% ($n = 7$) relative to vehicle-treated ($n = 5$) guinea pigs. Pre-treatment with MDL 74,721 (0.32 or 3.2 μg/kg i.v.) was without significant effect on the extravasation response. In the presence of the

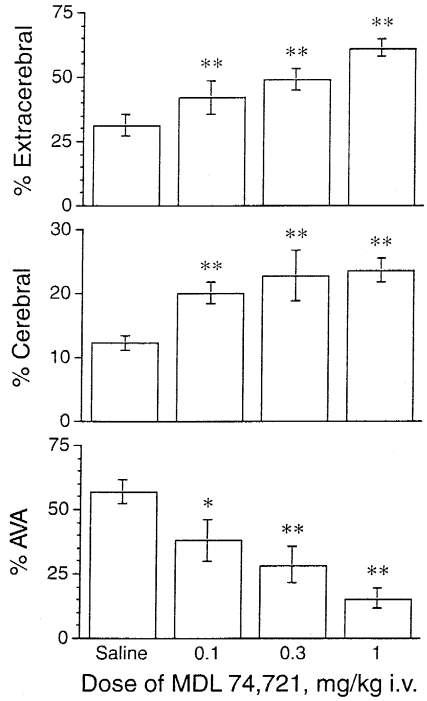

Fig. 3. The effects of increasing doses of MDL 74,721 (i.v.) on the percentage of carotid flow passing through the arteriovenous anastomoses to the lungs, going to the brain and to the other head tissues as assessed by the radioactive microsphere method. The results are expressed as the mean ± S.E.M. of 5 cats and were compared to saline treatment by a Duncan's multiple comparison test.

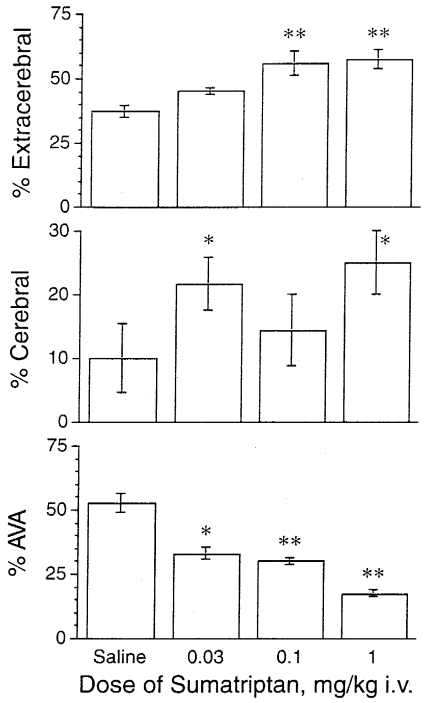

Fig. 4. The effects of increasing doses of sumatriptan (i.v.) on the percentage of carotid flow passing through the arteriovenous anastomoses to the lungs, going to the brain and the other head tissues as assessed by the radioactive microsphere method. The results are expressed as the mean ± S.E.M. of 5 cats and were compared to saline treatment by a Duncan's multiple comparison test.

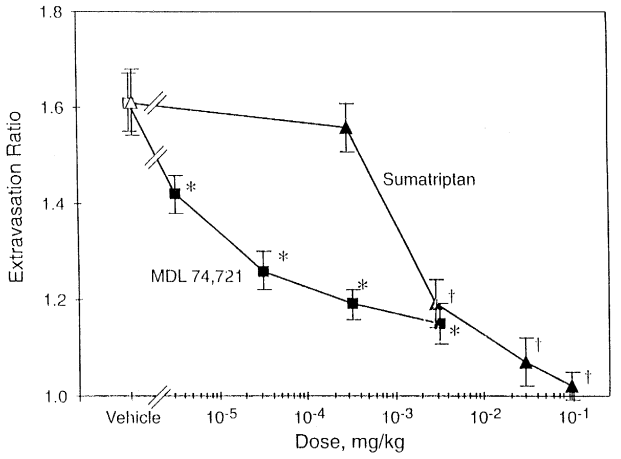

Fig. 5. Dose–response relationship for inhibition of dural protein extravasation following trigeminal ganglion stimulation. Plasma protein extravasation was measured in guinea-pig dura mater following right trigeminal ganglion stimulation. Animals ($n = 4–9$/group) received intravenous MDL 74,721 (closed squares), sumatriptan (closed triangles), or vehicle (open symbols) 10 min before electrical stimulation. [^{125}I]BSA was used as a marker of plasma protein extravasation and was given i.v. 5 min before electrical stimulation. Data are expressed as the ratio:

$$\frac{\text{cpm/mg wet weight (stimulated side)}}{\text{cpm/mg wet weight (unstimulated side)}}$$

and are presented as means ± S.E.M. Asterisks represent statistical differences (ANOVA followed by contrast analysis; $P < 0.05$) relative to vehicle treatment.

two doses of MDL 74,721, substance P increased dural radioactivity by 42.7 ± 9.5% ($n = 6$) and 36.9 ± 7.0% ($n = 7$), respectively.

Oral administration of MDL 74,721 (1 and 3 mg/kg) to rats induced a significant and dose-related attenuation of neurogenic dural plasma extravasation. The ratio of stimulated side to non-stimulated side was 1.72 ± 0.07 in vehicle-treated rats which was reduced to 1.16 ± 0.03 ($P < 0.01$) and 1.04 ± 0.01 ($P < 0.01$) after p.o. administration of either 1 or 3 mg/kg MDL 74,721, respectively.

3.6. Effect of intravenous MDL 74,721 on raphe cell firing

Serotonin 5HT$_{1A}$ agonists are known to inhibit raphe cell firing through activation of somatodendritic autoreceptors (Sprouse and Aghajanian, 1987). As MDL 74,721 has potent 5HT$_{1A}$ receptor affinity, this provided an electrophysiological endpoint for assessing central pharmacodynamics of the compound. Raphe firing rate was monitored in chloral hydrate anaesthetized rats. After determination of baseline firing rate, MDL 74,721 was administered intravenously and peak changes in firing rate were measured. Dose-dependent suppression of raphe cell firing was observed with 0.1, 0.3 or 1 mg/kg MDL 74,721. Firing rate was suppressed by 45 ± 17% ($n = 7$ cells), 90 ± 8% ($n = 4$ cells) and 100% (one cell), respectively. An ED$_{50}$ of 0.11 mg/kg i.v. was derived from these data.

4. Discussion

Migraine has been proposed to arise from either alterations in cerebrovascular tone (Heyck, 1958) or from an inflammatory process involving neurovascular regulation (Moskowitz, 1990, 1992). While preclinical models exist for both aspects of the proposed pathophysiology, clinical verification of their relative importance in disease manifestation will require development of pharmacological tools with improved selectivity for the individual components. MDL 74,721 is a novel aminotetralin analogue of sumatriptan with unexpected potency for inhibiting the neuronal inflammation versus its vasoconstrictor potency. The vasomotor effects of MDL 74,721 have been studied in vitro using isolated, human, pial arteries and in vivo by measuring the ability of the compound to regulate flow through arteriovenous anastomoses, possibly in the feline carotid rete (Edvinsson and Uddman, 1983). Effects on prejunctional trigeminovascular receptors were also studied by monitoring neurogenic inflammation elicited by trigeminal ganglion stimulation in the guinea pigs and rats. These types of pharmacological comparisons have suggested that MDL 74,721 shows greater potency for dural extravasation versus vasoconstriction and thus at low doses could differentiate the contributions of neural versus vascular processes in migraine.

Ergot alkaloids and 5-HT derivatives are known to display high affinity interactions with a large number of distinct serotonin recognition sites. However, as confirmed by the clinical benefit shown by the relatively subtype-selective 5-HT receptor agonist, sumatriptan, more specific biochemical targets for migraine therapeutics are afforded by specific 5-HT receptor subtypes. Sumatriptan was originally described as a 5-HT$_{1D}$ selective agonist. However, increases in the appreciation of the 5-HT receptor family diversity and in the affinity of sumatriptan for the newly described subtypes underscores that its anti-migraine action could be due to multiple pharmacological interactions. For example, 5-HT$_{1D}$ receptors are comprised of at least two related molecular species. The most recent nomenclature (Hartig et al., 1996) is based on genomic information and denotes the subtypes as 5-HT$_{1D}$ and 5-HT$_{1B}$ replacing the respective previous conventions of 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. Studies of the distribution of 5HT$_1$ receptor mRNA indicate that human cerebral arteries exclusively express 5-HT$_{1B}$ receptor mRNA (Hamel et al., 1993b; Bouchelet et al., 1996), whereas human trigeminal ganglia express mRNA species encoding not only 5-HT$_{1D}$ receptors, as originally suggested (Rebeck et al., 1994), but also the 5-HT$_{1B}$ receptor subtype (Bouchelet et al., 1996).

In the present study, MDL 74,721 and sumatriptan were shown to be approximately equipotent ligands at the human 5-HT$_{1D}$ receptor both as assessed using radioligand binding methods and functional assays. Moreover, both compounds were full agonists as reflected in their ability

to achieve levels of inhibition of the cAMP response like those exhibited by the natural agonist, 5-HT. MDL 74,721 exhibits at least 25-fold selectivity for the human 5-HT_{1D} versus the rat 5-HT_{1B} binding site. In contrast, sumatriptan is equipotent at both of these binding sites. It should be noted, that while rat and human 5-HT_{1B} receptors are greater than 90% identical in amino acid sequence, the presence of a single asparagine residue in the putative seventh transmembrane domain of the rat orthologue has led to situations where affinity measurements in the rat were poorly predictive of those in higher species (Parker et al., 1993). These results indicate that very small changes in the amino acid sequences can have large effects on the pharmacology associated with the receptor.

The affinity of a potential antimigraine agent for 5-HT_{1B} versus 5-HT_{1D} receptors has potential therapeutic importance since both the coronary artery and saphenous vein have 5-HT_1-like receptors which mediate contraction (Cushing et al., 1994). 5-HT_1 agonists which have a greater affinity for the vascular receptor may have a greater propensity to cause coronary vasoconstriction. Consistent with both vascular responses utilizing a common pharmacological mechanism, sumatriptan is equipotent in constricting saphenous vein and coronary artery preparations (Cushing et al., 1994). In the present experiments, the ability of compounds to constrict human cerebral arteries was measured as an indication of its antimigraine potential. Pharmacological and molecular biological studies indicate that the contractile response is mediated by the 5-HT_{1B} receptor subtype (Hamel et al., 1993a,b; Bouchelet et al., 1996). Consistent with the results of the binding studies described above, MDL 74,721 was at least 10-fold less potent than sumatriptan in eliciting this 5-HT_{1B}-mediated response. Furthermore, MDL 74,721 exhibited maximal responses which were about 70% of those obtained with 5-HT or sumatriptan, suggesting that it may behave as a partial agonist at 5-HT_{1B} receptors in this vascular preparation.

The neurogenic hypothesis of migraine proposes a cascade of events involving an inflammatory process in which there is a retrograde firing of the trigeminal nerve, release of inflammatory mediators, and changes in vascular permeability. Successful treatment would limit the release of these mediators through a mechanism involving prejunctional 5-HT receptors. As mentioned above, molecular biological studies indicate the presence of message encoding both 5-HT_{1D} and 5-HT_{1B} receptor subtypes in human trigeminal ganglia (Bouchelet et al., 1996). MDL 74,721, although having a similar affinity to sumatriptan for the human 5-HT_{1D} receptor, was much more potent at reducing the plasma extravasation resulting from trigeminal ganglion stimulation, both following i.v. administration to guinea pigs and after oral administration to rats. Hence, simple 5-HT_{1D} or 5-HT_{1B} receptor affinity does not seem to be the explanation behind this enhanced potency of MDL 74,721, though it could contribute to the apparent selectivity of MDL 74,721 for neurogenic relative to the vascular components of the disease.

Potency differences could also be produced through additional interactions involving post-junctional elements of the inflammation cascade. MDL 74,721, at doses producing complete inhibition of the electrically-induced extravasation response were without significant effect on the post-junctional, substance P-mediated response. Thus, the observed activity of MDL 74,721 versus the trigeminovascular response is mediated by prejunctional receptors. As indicated by the raphe firing studies, MDL 74,721 appears to readily and efficiently penetrate the central nervous system. This is in contrast to sumatriptan, which has been reported to have very poor CNS bioavailability (Shepheard et al., 1995). As central mechanisms may play an important role in migraine (e.g. Weiler et al., 1995), the therapeutic effects of a centrally-active agent like MDL 74,721 could be different than those of a purely peripherally-acting agent.

Recently, it was reported that CP-122,288, a constrained sumatriptan analogue, inhibited neurogenic plasma protein extravasation in guinea-pig (Lee and Moskowitz, 1993) and rat dura (Beattie and Connor, 1995), with an enhanced potency compared to sumatriptan, through either a 5-HT_{1D}/5-HT_{1B}-independent mechanism or a non-5-HT-mediated event. Additional confirmation has come from studies employing knock-out mice lacking the 5-HT_{1B} receptor. In these animals, CP-122,288 administration produces potent inhibition of extravasation, whereas sumatriptan is ineffective (Yu et al., 1996). Thus, while sumatriptan appears to utilize the prejunctional 5-HT_{1B} receptors to achieve its pharmacological response in the extravasation model, related chemical structures (CP-122,288 and possibly MDL 74,721) may achieve similar efficacy through other 5-HT receptor species or potentially through non-5-HT receptor-mediated mechanisms. Beattie and Connor (1995) have suggested that the 5-HT_{1E}, 5-HT_{1F} or 5-HT_7 receptor subtypes may be involved in the inhibition of dural plasma protein extravasation. In this regard, it should be noted that expression of 5HT_{1F} receptor message has recently been found in human trigeminal ganglia (Bouchelet et al., 1996). The binding affinity of MDL 74,721 for the 5-HT_{1E}, 5-HT_{1F} or 5-HT_7 receptor subtypes has not been assessed.

Although a comprehensive description of MDL 74,721 binding to 5-HT receptor subtypes is not available, it was found that the compound displayed potent binding affinity for 5-HT_{1A} receptors. This in vitro activity, although potent, is unlikely to contribute significantly to the efficacy observed in animal models of the vascular and inflammatory components of migraine. Hamel and Bouchard (1991) found no evidence for a direct role of 5-HT_{1A} receptors in cerebral vasoconstriction elicited by 5-HT in man and therefore 5-HT_{1A} receptor activation would not be expected to alter potency in the human pial artery preparation and possibly in the cat arteriovenous anastomoses model.

Buzzi et al. (1991) found that the potent 5-HT_{1A} agonist 8-hydroxydipropylaminotetralin, was approximately 4-fold less potent than sumatriptan in inhibiting dural extravasation in rats. Thus, while 5-HT_{1A} receptor activity may contribute to efficacy against extravasation it is unlikely to explain the much greater potency of MDL 74,722 relative to sumatriptan seen in the present study. Central 5-HT_{1A} receptors have been reported to be involved in the regulation of systemic blood pressure and sympathetic tone (DiFrancesco, 1994), stimulation of which may lead to vasodilatation and perhaps aggravation of the migraine symptoms. The potential for such a response would depend on the particular vascular bed involved and its relative proportion of 5-HT_{1A} and 5-HT_{1D} receptors.

In conclusion, MDL 74,721 is a centrally-active sulfonamidotetralin derivative with an affinity equivalent to that of sumatriptan for human 5-HT_{1D} receptors. Unlike sumatriptan, MDL 74,721 has a low affinity for the closely-related rat 5-HT_{1B} receptor. MDL 74,721 exhibits a much higher potency in the neurogenic model of migraine as opposed to vascular models in which it is comparatively less potent than sumatriptan. Potent, oral activity was demonstrated in the neurogenic model and initial studies characterizing the oral absorption kinetics have indicated that MDL 74,721 is very rapidly absorbed (J. Dow, personal communication). These findings suggest that MDL 74,721 may be efficacious in the acute treatment of a migraine attack. Furthermore, the results indicate that MDL 74,721 may target preferentially the prejunctional receptors mediating neurogenic inflammation. Further pharmacological characterization is needed to determine whether this prejunctional response is mediated through the 5-HT_{1D} receptor or another receptor subtype.

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