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Receptor specificity and trigemino-vascular inhibitory actions of a novel 5-$HT_{1B/1D}$ receptor partial agonist, 311C90 (zolmitriptan)

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1 311C90 (zolmitriptan zomig: (*S*)-4[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-2-oxazolidinone) is a novel 5-$HT_{1B/1D}$ receptor agonist with proven efficacy in the acute treatment of migraine. Here, we describe the receptor specificity of the drug and its actions on trigeminal-evoked plasma protein extravasation into the dura mater of the anaesthetized guinea-pig.

2 At the '5-HT_{1B}-like' receptor mediating vascular contraction (rabbit saphenous vein), the compound was a potent ($p[A_{50}] = 6.79 \pm 0.06$) partial agonist achieving $77 \pm 4\%$ of the maximum effect to 5-hydroxytryptamine (5-HT). In the same experiments, sumatriptan ($p[A_{50}] = 6.48 \pm 0.04$) was half as potent as 311C90 and produced $97 \pm 2\%$ of the 5-HT maximum effect. Studies in which receptor inactivation methods were used to estimate the affinity (pK_A) and efficacy relative to 5-HT ($\tau_{rel.}$) for each agonist confirmed that 311C90 exhibits higher affinity than sumatriptan ($pK_A = 6.63 \pm 0.04$ and 6.16 ± 0.03, respectively) and that both drugs are partial agonists relative to 5-HT ($\tau_{rel} = 0.61 \pm 0.03$ and 0.63 ± 0.10, respectively, compared to 5-HT = 1.0).

3 Consistent with its effects in rabbit saphenous vein, 311C90 also produced concentration-dependent contractions of primate basilar artery and human epicardial coronary artery rings. In basilar artery, agonist potency ($p[A_{50}] = 6.92 \pm 0.07$) was similar to that demonstrated in rabbit saphenous vein, again being 2–3 fold higher than for sumatriptan ($p[A_{50}] = 6.46 \pm 0.03$). Both agonists produced about 50% of the maximum response obtained with 5-HT in the same preparations. In rings of human coronary artery, the absolute potency of 311C90 and sumatriptan was higher than in primate basilar artery ($p[A_{50}] = 7.3 \pm 0.1$ and 6.7 ± 0.1, respectively), but maximum effects relative to 5-HT were lower ($37 \pm 8\%$ and $35 \pm 7\%$, respectively). In both types of vessel, the inability of 5-$HT_{1B/1D}$ agonists to achieve the same maximum as the endogenous agonist 5-HT is explained by the additional presence of 5-HT_{2A} receptors.

4 311C90 displayed high affinity at human recombinant 5-HT_{1D} (formerly 5-$HT_{1D\alpha}$) and 5-HT_{1B} (formerly 5-$HT_{1D\beta}$) receptors in transfected CHO-K1 cell membranes (pIC_{50} values $= 9.16 \pm 0.12$ and 8.32 ± 0.09, respectively). In intact cells, the drug produced concentration-dependent inhibition of forskolin-stimulated adenylyl cyclase ($p[A_{50}] = 9.9$ and 9.5, respectively) achieving the same maximum effect as 5-HT. Excepting human recombinant 5-HT_{1A} and 5-ht_{1F} receptors at which the drug behaved as an agonist with modest affinity ($pIC_{50} = 6.45 \pm 0.11$ and 7.22 ± 0.12, respectively), 311C90 exhibited low, or no detectable affinity (pK_i or $pK_B \leqslant 5.5$) at numerous other monoamine receptors, including other 5-HT receptor subtypes.

5 When administered to anaesthetized guinea-pigs ten minutes before unilateral electrical stimulation of the trigeminal ganglion (1.2 mA, 5 Hz, 5 ms, 5 min), 311C90 ($3-30\ \mu g\ kg^{-1}$, i.v.) caused a dose-dependent inhibition of [^{125}I]-albumin extravasation within the ipsilateral dura mater. At the same doses, the drug also produced dose-dependent falls in cranial vascular conductance ($32.3 \pm 7.5\%$ at $30\ \mu g\ kg^{-1}$), as measured in the ear by laser doppler flowmetry.

6 These results show that 311C90, a novel member of the 5-$HT_{1B/1D}$ agonist drug class, exhibits a high degree of pharmacological specificity. Its potent partial agonist action at '5-HT_{1B}-like' receptors in intracranial arteries, coupled with potent agonism at 5-HT_{1D} and 5-HT_{1B} receptors and an ability to inhibit neurogenic plasma protein extravasation in the dura, are consistent with its utility as an effective acute treatment for migraine.

Keywords: 5-HT receptors; 311C90, zolmitriptan; zomig; migraine; neurogenic extravasation

Introduction

The pathophysiology of migraine and related vascular headaches remains obscure, yet it is clear that certain drugs effective in acute treatment, specifically sumatriptan and the ergot alkaloids, share discrete pharmacological properties which account for their therapeutic effect. The most pertinent properties appear to be a selective constrictor action on intracranial blood vessels coupled with an ability to inhibit neurogenically-evoked plasma protein extravasation in the

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dura mater (Humphrey & Feniuk, 1991; Moskowitz, 1992). Although cranial vasoconstriction has been vigorously defended as the most important action relevant to the therapeutic effect of sumatriptan and the ergots, there is now convincing evidence to show that these drugs inhibit trigeminal neuronal activity independently of vessel constriction (see Humphrey & Goadsby, 1994). Furthermore, pharmacological studies indicate that both effects are mediated by receptors with the operational characteristics of the '5-HT_{1D}-like' receptor identified on smooth muscle and sympathetic nerve terminals in the saphenous vein of dog (Feniuk *et al*., 1979; 1985) and rabbit (Martin & MacLennan, 1990; Martin *et al*., 1991).

Recent studies have shown that in addition to an apparently peripheral location on cranial blood vessels and the terminals of the sensory nerves innervating them, 5-HT_{1B} and/or 5-HT_{1D} receptors are also present within the trigeminal nucleus caudalis where they serve to modulate cranial nociceptive input (Goadsby & Edvinsson, 1994; Mills & Martin, 1995). These central sites may represent an additional important target for drug action, but are not accessible to sumatriptan unless the blood-brain barrier is first disrupted (Kaube *et al*., 1993). By contrast, the novel, selective 5-$HT_{1B/1D}$ receptor agonist 311C90 accesses each of these sites and thus inhibits trigemino-vascular activation centrally as well as peripherally. Ongoing clinical studies have confirmed the excellent efficacy and favourable safety profile of this new drug (Klein, 1995; 1997; Earl, 1995; 1997). Here we describe its receptor pharmacology, its effects on vascular smooth muscle and its actions at peripheral elements of the trigemino-vascular system.

Methods

Isolated intact tissue studies

Animal tissue Smooth muscle and cardiac tissues were removed from animals killed in accordance with the Home Office Animals (Scientific Procedures) Act 1986, and prepared for the measurement of tissue isometric force by use of standard, published methods (vascular rings: Martin *et al*., 1987; tracheal strips: Leff & Martin, 1988; oesophageal strips: Baxter *et al*., 1991; cardiac tissues: Collis, 1983). Briefly, preparations were suspended in 5, 10 or 20 ml organ baths containing physiological buffer (Krebs or Tyrode solution), aerated with 95% O_2: 5% CO_2 and maintained at 37°C (cardiac tissue, 34°C), pH 7.4. The composition of the Tyrode buffer was (mM): NaCl 136.9, $NaHCO_3$ 11.9, KCl 2.7, $NaH_2PO_4.2H_2O$ 0.4, $MgCl_2.6H_2O$ 1.1, $CaCl_2.6H_2O$ 2.5 and glucose 11.1. The Krebs buffer comprised (mM): NaCl 118.4, $NaHCO_3$ 25.0, KCl 4.8, $NaH_2PO_4.2H_2O$ 1.2, $MgCl_2.6H_2O$ 1.2, $CaCl_2.6H_2O$ 2.5 and glucose 11.1. Possible oxidative deamination of 5-hydroxytryptamine (5-HT) and the test agonists was always prevented by exposing tissues at the start of the experiment to the irreversible monoamine oxidase inhibitor pargyline (500 μM for 30 min). Single or paired cumulative concentration-effect (E/[A]) curves were then constructed with 0.5 $\log_{10}$ [agonist] increments. The effect of antagonists was studied after 60 min incubation (120 min for methiothepin).

Human vascular tissue Epicardial coronary vessels (circumflex or proximal left anterior descending arteries) were obtained from the hearts of five transplant recipients (see below) and stored in Tyrode buffer at 4°C for 5–8 h before use. After gradual rewarming to room temperature (20–23°C), each vessel was cut into rings ($6 \times 3-4$ mm) and suspended in 20 ml organ baths containing gassed Tyrode buffer at 37°C. After a stabilisation period (~30 min), pargyline (500 μM) was added to the bath and the ring segments progressively stretched over a period of 30 min by 4 successive applications of 1 g force. Tissue resting force was finally adjusted to 3 g and excess pargyline removed by two exchanges of the organ bath buffer. Viability was assessed by addition of a depolarising concentration of KCl (80 mM). After washout and restoration of tissue resting force, prazosin (0.3 μM) idazoxan (1.0 μM), mepyramine (0.3 μM) and cimetidine (30.0 μM) were added to block α_1- amd α_2-adrenoceptors as well as histamine H_1 and H_2 receptors, respectively. After 60 min, a single cumulative E/[A] curve was constructed. When the maximum of each curve established, a super-maximal concentration of 5-HT (30 μM) and, subsequently, U46619 (0.1 μM) was added to provide independent measures of tissue contractility.

Coronary artery: patient details

(1) Female, 36 yrs: cardiomyopathy, macroscopically normal. (2) Male, 5 yrs: congenital defect, macroscopically normal. (3) Male, 60 yrs: ischaemic heart disease, widespread atheromatous lesions. (4) Male, 17 yrs: cardiomyopathy, macroscopically normal. (5) Female, 9 yrs: cardiomyopathy, macroscopically normal.

Operational activity at human recombinant 5-HT receptors

CHO-K1 cells stably transfected with human recombinant 5-HT_{1B}*, 5-HT_{1D}*, 5-HT_{1A} or 5-ht_{1F} receptors (*current NC-IUPHAR sanctioned nomenclature for 5-HT receptors (see Hartig *et al*., 1996)) were grown to confluency in 12-well plates, washed with phosphate buffered saline and incubated at 37°C in HEPES buffered Dulbecco's Modified Eagle's Medium containing 10 μM pargyline and 100 μM isobutylmethylxanthine, to inhibit phosphodiesterase activity. E/[A] curves were constructed by addition of agonist, immediately followed by forskolin at a concentration sufficient to elicit a 10 to 20 fold increase in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (5-HT_{1D}, 5-HT_{1A}, 5-ht_{1F}: 1 μM; 5-HT_{1B}: 10 μM). Reactions were terminated after 10 min by removal of medium and addition of ice-cold ethanol to extract the cyclic AMP. Pilot experiments (data not shown) confirmed that cyclic AMP accumulation was on a linear part of the time-course at the 10 min time point. After 2 h the ethanol samples were evaporated to dryness and cyclic AMP concentrations were measured by scintillation proximity assay (SPA, Amersham).

Table 1 Receptors at which 311C90 affinity was determined by use of radioligand binding displacement

Receptor	*Radiolabel*	K_D (nM)	*Receptor source*
5-HT_{1A}	[^{3}H]-5-HT (1.8 nM)	1.8	Human recombinant receptor, CHO-K1 cells
5-HT_{1D}	[^{3}H]-5-HT (1.5 nM)	1.5	Human recombinant receptor, CHO-K1 cells
5-HT_{1B}	[^{3}H]-5-HT (1.58 nM)	1.5	Human recombinant receptor, CHO-K1 cells
5-ht_{1F}	[^{3}H]-5-HT (10 nM)	7.8	Human recombinant receptor, CHO-K1 cells
5-HT_{2C}	[^{3}H]-mesulergine (0.7 nM)	0.6	Guinea-pig cortex
α_2-Adrenoceptor	[^{3}H]-idazoxan (7 nM)	7.0	Rat cortex
Dopamine D_1	[^{3}H]-SCH23390 (0.3 nM)	0.3	Rat striatum
Dopamine D_2	[^{3}H]-spiperone (0.2 nM)	0.04	Rat striatum

The radiolabel and the concentration used in the assay, its K_D at the target receptor, and the source of membranes containing the receptor are shown.

Radioligand binding studies

Competition binding assays were performed to determine drug affinity (pK_i or pIC_{50}) at certain receptors. The concentration and type of radiolabel used, together with the source to target receptor, is shown in Table 1. Briefly, the appropriate radioligand (at a concentration approximating K_D) and a wide range of test drug concentrations (in duplicate) were incubated with the relevant receptor preparation (brain homogenate or cell membranes) for 30 min at 27°C – conditions determined previously to satisfy mass-action behaviour. The assay buffer comprised: 50 mM Tris-HCl, 5 mM $CaCl_2$, 0.1% w/v ascorbate and 10 μM pargyline. Non-specific binding was defined by an excess of unlabelled 5-HT. Incubations were terminated by rapid filtration and washing with ice-cold buffer. Specifically bound radiolabel was measured by scintillation spectroscopy.

Anaesthetized guinea-pigs: extravasation of [^{125}I]-bovine serum albumin in dura mater and ear blood flow

Male Dunkin-Hartley guinea-pigs (200–250 g) were anaesthetized with pentobarbitone (50 mg kg^{-1}, i.p.) and rectal temperature maintained at 37°C by a homeothermic blanket. Animals were artificially respired (80 breaths min^{-1}, 10 ml kg^{-1}) via a tracheotomy and the right external jugular vein and common carotid artery cannulated to permit drug administration and measurement of arterial blood pressure (and derived heart rate), respectively.

Extravasation studies Preparation of animals for unilateral trigeminal ganglion stimulation and measurement of plasma protein extravasation within the ipsilateral dura mater was essentially as described by Markowitz *et al.* (1987). Briefly, animals were placed in a stereotaxic frame with the incisor bar at 4 mm from the horizontal. Burr holes were drilled 4 mm laterally and 4 mm posteriorly from bregma for electrode placement. Test drug (or vehicle: 0.9% w/v saline) was then injected via a venous cannula, followed 5 min later by [^{125}I]-BSA (bovine serum albumin; 50 μCi kg^{-1}). Paired, non-concentric bipolar electrodes were then lowered into the trigeminal ganglia to a depth of 10.5 mm from the dura mater and the right or left ganglion arbitrarily assigned for stimulation (1.2 mA, 5 Hz, 5 ms duration for 5 min). Immediately after the stimulation period, animals were perfused for 3 min with saline at 100 mmHg via the left cardiac ventricle. The dura mater was dissected free, bisected, the blotted wet weight recorded and the ^{125}I in each side counted for 20 min in a Wallac Gamma Counter. Extravasation of [^{125}I]-albumin was calculated as the ratio of c.p.m. mg^{-1} wet weight for the stimulated over the unstimulated side. On each experimental day, at least one animal was injected with drug vehicle to determine day-to-day variation in stimulation-evoked albumin leakage and to serve as time-matched controls.

Blood flow studies Laser doppler flowmetry (MBF3 Laser Doppler Monitor, Moore Instruments, U.K.) was used to monitor blood flow continuously in the right ear of animals used in the extravasation experiments. The flow probe and monitor were set to a frequency of 10 Hz and a time constant of 1 s. Blood flow was recorded from small, marginal ear arteries as red cell flux determined as a percentage of a standardised signal obtained in a particular control medium. Changes in vascular conductance were calculated by dividing the flow signal by mean blood pressure at the peak of drug-induced changes in flow.

Data analysis

Isolated intact tissue studies Either single or paired agonist E/[A] curves were obtained, from which computed estimates of agonist potency ($[A_{50}]$: actually estimated as $p[A_{50}]$), intrinsic activity (α) and the Hill coefficient (n_H) were obtained by fitting the data to a logistic function of the following form;

$$E = \frac{\alpha.[A]^{n_H}}{[A_{50}]^m + [A]^m} \quad (1)$$

Parameter estimates obtained in paired curve experiments were compared by use of a paired t test. When this design was used to test antagonist effects, mid-point i.e. $[A_{50}]$ concentration-ratios (CR) were calculated and presented as geometric means with 95% confidence limits, or they were used to calculate apparent pK_B values by use of the Schild equation:

$$pK_B = \log_{10}CR - 1 + n \log_{10}[B] \quad (2)$$

in which B refers to the antagonist concentration and n is equivalent to the Schild plot slope. Estimates of agonist affinity (determined a log K_A) and efficacy (determined as log τ) were obtained by use of operational model-fitting methods. In most cases, curves to 311C90 and sumatriptan were 'partial' with respect to 5-HT, hence curve pairs were constructed, the first of which was always to 5-HT. Since K_A for 5-HT is known from previous receptor inactivation experiments ($pK_A = 7.12$; Martin & MacLennan, 1990), 5-HT/test agonist curve pairs were fitted directly to the operational model of agonism (Eq.3; Black & Leff, 1983) with K_A for 5-HT constrained to 7.12. This provided model estimates of K_A and τ for the test agonist, and a value of τ for 5-HT (see Leff *et al.*, 1990). When the test agonist curve maximum was indistinguishable from that for 5-HT (i.e. the test agonist behaved as a full agonist relative to 5-HT), a third curve to the test agonist was constructed after fractional inactivation of the receptors with benextramine tetrahydrochloride (1 μM for 30 min: see Martin & MacLennan, 1990). Direct fitting of the second and third curves (test agonist before and after receptor inactivation) provided estimates of affinity and efficacy in the usual way. In both cases, the form of the operational model was the same:

$$E = \frac{E_{max}.\tau^n.[A]^n}{(K_A + \tau)^n + \tau^n.[A]^n} \quad (3)$$

where K_A is the agonist dissociation equilibrium constant, τ is the efficacy of the agonist in a particular tissue, E_{max} denotes the maximum possible effect of an agonist in the system and n defines the slope of the occupancy-effect relation.

Radioligand displacement and cyclic AMP accumulation studies Competition displacement curves and cyclic AMP accumulation E/[A] curves were constructed in each experiment with duplicates at each ligand concentration. The resulting curves were then fitted to a four parameter logistic function to provide potency estimates (pIC_{50} or $p[A_{50}]$, respectively). When the Hill slope estimates for competition displacement curves were not different from unity, pIC_{50}s were converted to pK_i values by use of the Cheng-Prusoff equation (Cheng & Prusoff, 1973).

Drugs

BW311C90WH (311C90, zolmitriptan: (*S*)-4[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]-2-oxazolidinone); sumatriptan (synthesized by Dr D. Selwood, Medicinal Chemistry Department, Wellcome Research Laboratories, Kent, U.K.); methysergide bimaleate (Sandoz, Basle, Switzerland); methiothepin mesylate (Pharmaceutical and Biochemical Research Institute, Prague, Czechoslovakia); MDL 72222 (1 αH, 3α, 5αH-tropan-3-yl-3,5-dichlorobenzoate methanesulphonate, Marrion-Merrell-Dow, Strasbourg, France); 5-hydroxytryptamine creatinine sulphate; benextramine tetrahydrochloride; pargyline hydrochloride; ketanserin tartrate (all from Sigma, St. Louis, M.O., U.S.A.); 311C90 and sumatriptan were prepared as aqueous solutions with equimolar concentrations of HCl. All drugs were dissolved and diluted in

distilled water.

Results

Actions at vascular '5-HT$_{1B}$-like' receptors

Rabbit saphenous vein In rings of rabbit saphenous vein 5-HT produced a monophasic concentration-contraction curve with a mid-point (p[A_{50}]) that ranged between 7.3–7.6. Paired curves to 5-HT obtained in the same vascular ring were not different ($\Delta p[A_{50}] = 0.18 \pm 0.09$; $\Delta max = -0.04 \pm 0.05$; $n = 6$). Figure 1 illustrates average E/[A] curve pairs obtained for 5-HT followed by either 311C90 (a) or sumatriptan (b) and shows that, relative to 5-HT, 311C90 behaved as a potent partial agonist, with a p[A_{50}] of 6.79 ± 0.06 and a maximum response $77 \pm 5\%$ of that obtained with 5-HT. Sumatriptan (p[A_{50}] = 6.48 ± 0.04) was half as potent as 311C90 and achieved $97 \pm 2\%$ of the 5-HT maximum.

Affinity and efficacy estimates for 311C90 and sumatriptan were obtained from a subsequent series of consecutive-curve experiments, in which the first curve was always to 5-HT (see Method). Average parameter estimates are summarised in Table 2. The results confirm that 311C90 displays a high affinity at the '5-HT$_{1B}$-like' receptor mediating vascular contraction ($pK_A = 6.63 \pm 0.04$ cf 7.12 for 5-HT) and is a partial agonist with respect to 5-HT ($\tau_{rel} = 0.61 \pm 0.03$). For sumatriptan, estimates of pK_A and τ_{rel} were 6.16 ± 0.03 and 0.63 ± 0.10, respectively, demonstrating that the lower potency of this drug results from a lower affinity, since it displays similar efficacy to 311C90.

Responses to 311C90 were unaffected by the selective 5-HT$_3$ receptor antagonist MDL72222 (1 μM), but were surmountably antagonised by the 5-HT$_{2A}$ selective antagonist ketanserin (0.3 μM) and methiothepin (10 nM) in a manner consistent with '5-HT$_{1B}$-like' receptor effects in rabbit saphenous vein (Martin & MacLennan, 1990). 5-HT was equally sensitive to these antagonists, providing confidence that these agonists act at a single receptor type in this tissue (Table 3).

Primate basilar artery Consistent with its action in rabbit saphenous vein, 311C90 also contracted basilar artery rings from cynomolgus monkey. Preliminary experiments showed that two successive E/[A] curves to 5-HT were highly reproducible, with no differences ($P > 0.05$) in maximum, mid-point slope parameter or location. E/[A] curves to 311C90 and sumatriptan were therefore compared to a 5-HT curve constructed in the same tissue. Figure 2 shows that 311C90 was a potent agonist (p[A_{50}] = 6.92 ± 0.07 cf 7.03 ± 0.12 for 5-HT), but attained only $56 \pm 9\%$ of the maximum to 5-HT in the same tissue. Sumatriptan produced a similar magnitude of contraction ($48 \pm 12\%$), but was less potent (p[A_{50}] = 6.46 ± 0.03). Although direct evidence for an action at the '5-HT$_{1B}$-like' receptor was not obtained in this limited study, the results agree well with those obtained for activation of this receptor in rabbit saphenous vein. However, the lower intrinsic activities of 311C90 and sumatriptan relative to 5-HT in this vessel cannot be taken as evidence for partial agonist behaviour, since Connor *et al.* (1989) have shown that 5-HT contractions involve both '5-HT$_{1B}$-like' and 5-HT$_{2A}$ receptors, which, as

Table 2 Estimates of affinity (pK_A) and efficacy relative to 5-HT (τ_{rel}) for 311C90 and sumatriptan obtained by direct operational model-fitting

Agonist	pK_A	τ_{rel}
5-HT*	7.12	1.00
311C90	6.63 ± 0.04	0.61 ± 0.03
Sumatriptan	6.16 ± 0.03	0.63 ± 0.10

Values are the averages of 14 estimates in the case of 311C90 and 4 in the case of sumatriptan.
*Data from Martin & MacLennan (1990).

Table 3 Antagonism of responses to 311C90 and 5-HT by some standard 5-HT receptor ligands

Agonist	*MDL 72222* (1 μM)	*Methiothepin* (10 nM)	*Ketanserin** (0.3 μM)
5-HT	1.0 (0.2–3.9)	19.2 (8.5–42.6)	2.8 (1.8–4.3)
311C90	0.8 (0.7–1.3)	14.7 (5.4–40.7)	4.7 (2.6–6.8)

Values are mid-point concentration-ratios determined from the surmountable right-shift of agonist E/[A] curves obtained at the antagonist concentration shown (geometric mean and 95% confidence interval; $n = 3–6$). In each case, agonist maximum responses and mid-point slopes were unchanged ($P > 0.05$) by the antagonist.
*Ketanserin is non-competitive, producing saturable, parallel right-shifts of agonist curves under the present experimental conditions (Martin & MacLennan, 1990).

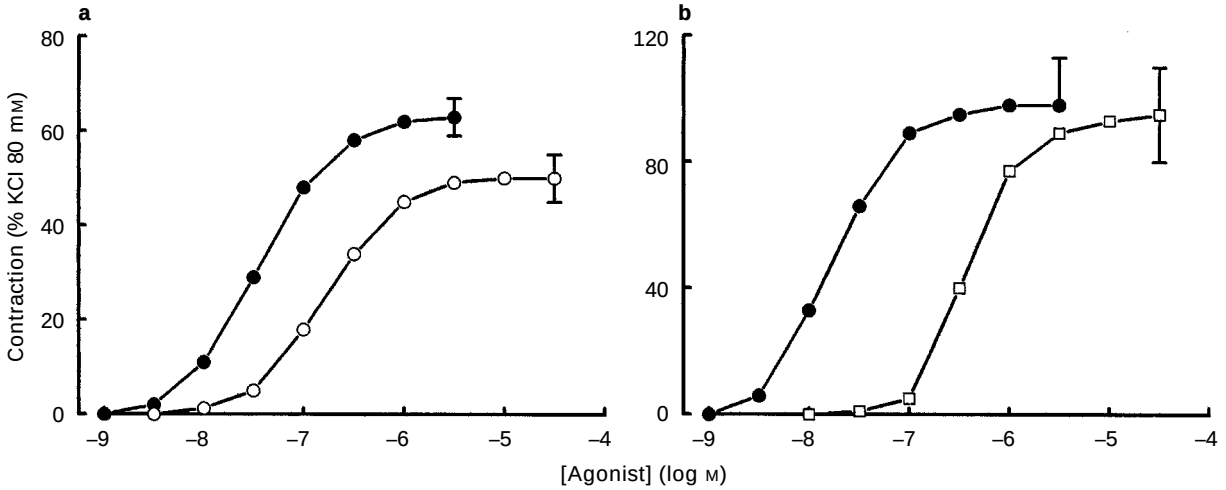

Figure 1 Paired average contraction-response curves to 5-HT (●) and either 311C90 (○) or sumatriptan (□) obtained in rings of rabbit saphenous vein. (a) Replicates from 14 paired curve experiments; (b) replicates from 4 similar experiments. Note the difference in ordinate scales. Vertical lines show s.e.mean.

judged by sensitivity to selective 5-HT_{2A} receptor block with ketanserin, contribute 60% and 40%, respectively, to the total 5-HT response in this tissue.

Human coronary artery Figure 3 shows that in coronary artery rings from 5 patients, 311C90 produced concentration-related contractions ($p[A_{50}] = 7.3 \pm 0.1$) achieving an average maximum effect which was $37 \pm 8\%$ of the 5-HT maximum. In parallel experiments sumatriptan produced the same magnitude of contraction ($35 \pm 7\%$ cf 5-HT), but, as in all other vessels tested, was $\sim$4 fold less potent ($p[A_{50}] = 6.7 \pm 0.1$). Like the primate basilar artery, human coronary artery possesses 5-HT_{2A} receptors which also contribute to 5-HT-induced contractions and account for the greater maximum effect produced by the natural agonist.

Actions at human recombinant 5-HT_{1B} and 5-HT_{1D} receptors

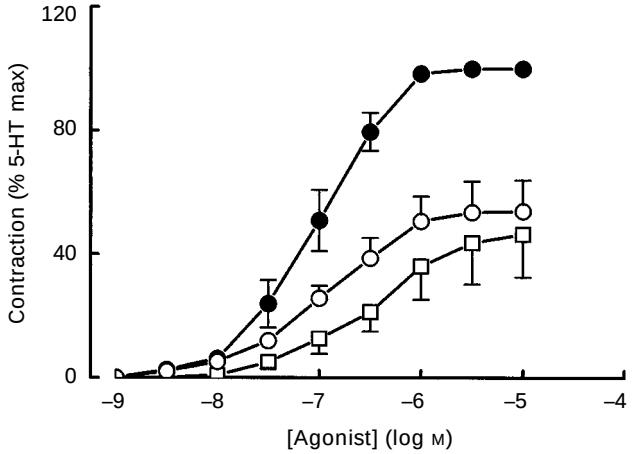

Figure 2 Comparative contractile effects of 311C90 (○), sumatriptan (□) and 5-HT (●) in rings preparations of primate basilar artery. Data are from second curves constructed after a first curve to 5-HT in each case. The results are expressed as a percentage of the 5-HT first curve maximum. Shown are the averages of 3–4 E/[A] curves obtained in vessels from two animals. Vertical lines show s.e.mean.

In addition to the '5-HT_{1B}-like' receptor in vasculature, 311C90 displayed high affinity and agonist potency at human recombinant 5-HT_{1B} and 5-HT_{1D} receptors stably transfected into CHO-K1 cells (Table 4). With membranes prepared from these cells, 311C90 displaced specifically bound [^{3}H]-5-HT with pIC_{50} values of 8.32 ± 0.09 (5-HT_{1B}) and 9.16 ± 0.12 (5-HT_{1D}). In both cases, monophasic displacement curves were produced, but Hill slope estimates were significantly less than unity (0.84 ± 0.02 and 0.92 ± 0.08, respectively). Since this is a typical feature of these assays in our hands, conversion to affinity (pK_i) estimates was not performed.

In intact cells, 311C90 produced a concentration-dependent, monophasic inhibition of forskolin-stimulated cyclic AMP accumulation in cells stably transfected with either 5-HT_{1B} or 5-HT_{1D} receptors ($p[A_{50}] = 9.5 \pm 0.1$ and 9.9 ± 0.1, respectively) and in both cases achieved the same maximum inhibition as 5-HT.

Actions at other receptor types

As summarized in Table 4, 311C90 displayed modest affinity at

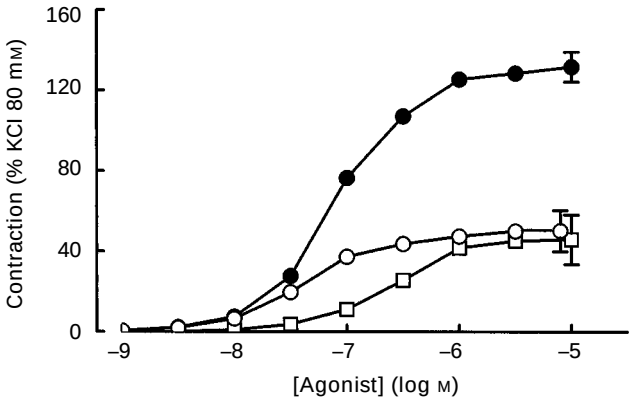

Figure 3 Contractile effects of 311C90 (○), sumatriptan (□) and 5-HT (●) in ring preparations of human epicardial coronary artery. Data are the averages of 7–10 E/[A] curves obtained in vessels from five transplant recipients. Vertical lines show s.e.mean.

Table 4 Receptor profile of 311C90 obtained with functional receptor assays and radioligand binding assays

Receptor	*Functional assay*	*Radioligand binding assay*
	*$p[A_{50}]$/max or pK_B**	*pK_i (pIC_{50})*
'5-HT_{1B}-like'	6.8/0.77	
5-HT_{1A}		6.5
5-HT_{1D}		9.2
5-HT_{1B}		8.3
5-ht_{1F}		7.2
5-HT_{2A}	< 4.5/–	
5-HT_{2B} (endothelial 5-HT receptor)	< 4.5/–	
5-HT_{2C}		4.1
5-HT_3	< 4.5/–	
5-HT_4	< 4/5/–	
5-HT_7 (smooth muscle relaxation)	5.3/–	
α_1-Adrenoceptor	< 4.5/–	
α_2-Adrenoceptor		4.1
β_1-Adrenoceptor	4.8/–	
Histamine H_1	5.5/–	
Histamine H_2	< 4.5/–	
Muscarinic M_2	5.0/–	
Muscarinic M_3	< 4.5/–	
Dopamine D_1		< 4.0
Dopamine D_2		< 4.0

Each value is the mean of at least 3 estimates (in the case of binding studies, each obtained in duplicate).
*Where 311C90 behaved as an agonist, the result is expressed as $p[A_{50}]$/maximum cf 5-HT (= 1). When the compound failed to display agonism (indicated by –), it was evaluated as an antagonist and the result expressed as a pK_B value.

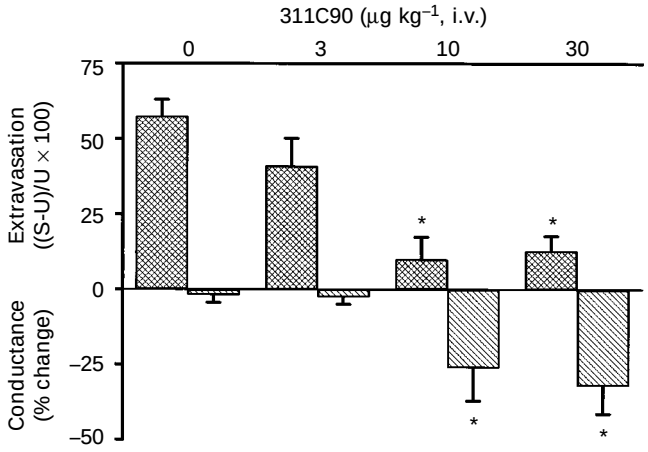

Figure 4 Effect of 311C90 on neurogenic plasma protein extravasation (ratio stimulated: unstimulated, cross-hatched columns) into the dura and on ear blood flow (conductance, hatched columns) in anaesthetized guinea-pigs. Data are the means ± s.e.mean of values obtained from 5–8 animals in each treatment group. Effects on ear blood flow are the peak changes observed after intravenous bolus injection. Extravasation was measured following electrical stimulation of a trigeminal ganglion (1.2 mA, 5 Hz, 5 ms duration for 5 min) 10 min after drug administration.

human recombinant 5-HT_{1A} receptors ($pIC_{50} = 6.45 \pm 0.11$) and slightly higher affinity at recombinant 5-ht_{1F} receptors ($pIC_{50} = 7.22 \pm 0.12$). When inhibition of forskolin-stimulated cyclic AMP formation was measured in intact cells, the drug behaved as a full, or near-full agonist at both receptor types (5-HT_{1A}: $p[A_{50}] = 6.4 \pm 0.1$; 5-$ht_{1F} = 8.2 \pm 0.1$). 311C90 had low or negligible affinity ($pIC_{50}/pK_B < 5.0$) at a number of other monoamine receptors tested, including other 5-HT subtypes.

Effect on neurogenically-evoked [^{125}I]-albumin extravasation into guinea-pig dura mater and on ear vascular conductance

Mean resting haemodynamic variables (BP – blood pressure or HR – heart rate) were not different between animals treated intravenously with vehicle ($n = 8$) or 311C90 (3 μg kg^{-1} ($n = 5$), 10 μg kg^{-1} ($n = 6$) or 30 μg kg^{-1} ($n = 6$)). In order, the measures of mean blood pressure were 37.6 ± 4.3, 42.9 ± 2.8, 39.7 ± 3.7 and 36.6 ± 3.7 mmHg, whilst heart rate was 251 ± 17, 248 ± 24, 288 ± 17 and 253 ± 14 beats min^{-1}. In vehicle-treated animals, unilateral electrical stimulation of the trigeminal ganglion increased extravasation of [^{125}I]-albumin into the dura on the side ipsilateral to stimulation producing a stimulated/unstimulated ratio of 1.57 ± 0.07 ($n = 8$, $P < 0.05$). 311C90 decreased this ratio in a dose-dependent fashion. After injection of 3, 10 or 30 μg kg^{-1}, i.v., the ratios were (comparator vehicle controls in parentheses): 1.42 ± 0.10 (1.51 ± 0.08), NS; 1.10 ± 0.08 (1.60 ± 0.07), $P < 0.05$ and 1.13 ± 0.05 (1.58 ± 0.08), $P < 0.05$. In the same animals, the drug produced concomitant, dose-related falls in ear conductance (Figure 4).

Discussion

5-HT_{1B} and/or 5-HT_{1D} receptors are recognised as an important target for drugs effective in the acute treatment of migraine (Humphrey *et al*., 1990; Visser *et al*., 1994). Within the cranial circulation, '5-HT_{1B}-like' receptors are present on the smooth muscle of meningeal (Humphrey *et al*., 1991) and conduit blood vessels such as the basilar and middle cerebral arteries (Parsons *et al*., 1989; Friberg *et al*., 1991; Hamel *et al*., 1993; Deckert *et al*., 1994). They appear to be particularly prominent within cranial arterio-venous anastomoses (AVAs) where their activation produces a flow-limiting vasoconstriction, resulting in a decrease in total carotid arterial blood flow (Saxena, 1978; Perren *et al*., 1989; Den Boer *et al*., 1991). The same, or similar receptors are also claimed to mediate inhibition of neurogenically evoked plasma-protein extravasation in the dura mater (Buzzi *et al*., 1991). These effects are considered to be the most pertinent actions of 5-$HT_{1B/1D}$ agonist drugs which have been shown to be effective in the acute treatment of migraine (Humphrey & Feniuk, 1991; Moskowitz, 1992).

In the studies described here, we have used the rabbit saphenous vein as a primary bioassay for '5-HT_{1B}-like' receptors mediating vasoconstriction. This vessel offers an advantage over, for example, the dog saphenous vein, in that 5-HT effects are mediated by a homogenous population of the target receptor with no contamination by 5-HT_{2A} receptors (Martin & Maclennan, 1990). Comparative studies showed that 311C90 behaved as a potent partial agonist in this vessel, achieving 77% of the maximum response to 5-HT. Parallel experiments with sumatriptan showed that it was 2–3 times less potent but achieved a greater maximum (97%), comparable to that obtained with 5-HT. Whilst these data indicate that sumatriptan behaves as a full agonist in the saphenous vein, subsequent estimation of affinity and intrinsic efficacy with the alkylating agent benextramine showed that both sumatriptan and 311C90 are in fact '5-HT_{1B}-like' receptor partial agonists with a similar efficacy relative to 5-HT (~ 0.6). This means that the higher agonist potency of 311C90 is due solely to its higher receptor affinity. These results once again reinforce the need to eliminate the tissue component of biological variability when making quantitative comparisons of agonist effects. Indeed, our experience with a number of smooth muscle preparations suggests that there can be considerable variation in the coupling efficiency of '5-HT_{1B}-like' receptors mediating contraction.

As expected from its '5-HT_{1B}-like' receptor actions in saphenous vein, 311C90 also produced concentration-dependent contraction of primate basilar and human epicardial coronary artery. In both of these vessels, the potency ratio with sumatriptan was similar, although, interestingly, the absolute potency of both drugs appeared higher in the human tissue. The simplest explanation is a more efficient receptor-effector coupling in human vessels, but the possibility that structural differences in the human receptor result in a higher affinity for these indole analogues cannot be ruled out. Maximum responses to 311C90 were also the same as those obtained with sumatriptan in these two vessels, although the additional presence of 5-HT_{2A} receptors meant that 5-HT achieved a higher maximum response. The co-existence of '5-HT_{1B}-like' and 5-HT_{2A} receptors mediating contraction has been found in a variety of blood vessels including human coronary and primate basilar artery (see Martin, 1994). Moreover, the relative contributions made by each receptor type varies considerably between animals/patients as well as between different rings prepared from the same vessel segment (e.g. Kaumann *et al*., 1994). This almost certainly accounts for the lower average maximum contraction obtained here for sumatriptan and 311C90 in primate basilar artery ($\sim 50\%$ cf 5-HT) compared to previously published results (78%: Connor *et al*., 1989). By contrast, the results obtained in isolated human coronary artery ($\sim 35\%$ cf 5-HT) are within the range described elsewhere (12–35%: Chester *et al*., 1990; Connor *et al*., 1989; Kaumann *et al*., 1993; Cocks *et al*., 1993). These results provide reassurance that, on average, in man, '5-HT_{1B}-like' receptor-mediated contraction of both cerebral and coronary vessels accounts for only about one third to one half of the maximum produced by the endogenous agonist 5-HT. On the basis of extensive clinical studies with sumatriptan, this effect appears to be of no clinical consequence (Brown *et al*., 1991).

In addition to its actions on vascular smooth muscle *in vitro*, intravenously administered 311C90 potently inhibited neurogenic plasma protein extravasation into guinea-pig dura provoked by stimulation of the trigeminal ganglion. At the same doses the drug produced a flow-limiting vasoconstriction in the ear, as judged by falls in auricular arterial conductance, but was without significant effect on either blood pressure or heart

rate. Unpublished results from our laboratory (A. Honey) have shown that the vascular response in the ear displays the typical pharmacology of a '5-HT_{1B}-like' receptor-mediated effect, and since these receptors are also present in guinea-pig basilar artery (Chang & Owman, 1989), it might be reasoned that some intracranial vessels could also be tonically activated in these experiments. Whilst it is therefore conceivable that this vascular action of 5-$HT_{1B/1D}$ agonists accounts for the inhibition of dural plasma protein extravasation, as advocated by Humphrey and colleagues, there is increasing evidence to support a direct prejunctional inhibitory action (Moskowitz, 1992; Kaube *et al*., 1992; see Humphrey & Goadsby, 1994). Perhaps the most convincing evidence is the demonstration that sumatriptan and dihydroergotamine block the expression of fos-like immunoreactivity in rat trigeminal nucleus caudalis provoked by injection of autologous blood into the subarachnoid space (Nozaki *et al*., 1992). Since this manoeuvre is accompanied by intense cerebroarterial constriction, the inhibitory action of 5-$HT_{1B/1D}$ agonists would seem to be independent of their vasoconstrictor effects.

Receptor specificity studies confirmed that 311C90 exhibits a high degree of selectivity for the human 5-HT_{1B} and 5-HT_{1D} receptor types, with low, or no detectable affinity (pK_i or $pK_B \leqslant 5.5$) at numerous other monoamine receptors. Only at recombinant human 5-HT_{1A} and 5-ht_{1F} receptors did the drug display moderate affinity ($pIC_{50} = 6.45 \pm 0.11$ and 7.22 ± 0.12, respectively) and agonist potency ($p[A_{50}] = 6.4 \pm 0.1$ and 8.2 ± 0.1, respectively), but the relevance of these non-5-$HT_{1B/1D}$ receptor actions remains unknown. Whilst it is generally accepted that the trigemino-vascular effects of 311C90, sumatriptan and the ergots are mediated by a '5-HT_{1B}-like' receptor, the precise identity of the receptor(s) involved remains uncertain. Available data favour the view that vasoconstriction is mediated primarily by 5-HT_{1B} receptors, whilst neuro-inhibition involves predominantly the 5-HT_{1D} subtype. This is based upon data from studies describing mRNA transcripts for the 5-HT_{1B} but not the 5-HT_{1D} receptor in human and bovine cerebral arteries (Hamel *et al*., 1993), coupled with an apparently closer operational correlation between cerebral vessel constriction and 5-HT_{1B} receptor pharmacology (Kaumann *et al*., 1993; Hamel *et al*., 1993; Connor *et al*., 1995). Conversely, 5-HT_{1D} but not 5-HT_{1B} message has been found in human and guinea-pig trigeminal ganglia (Rebeck *et al*., 1994). However, this apparently selective distribution of the two receptor subtypes within the trigemino-vascular system appears to be species-dependent. For example, gene-deletion studies have shown that in mice lacking the 5-HT_{1B} receptor, sumatriptan no longer blocks neurogenic plasma protein extravasation, implying little or no role for 5-HT_{1D} receptors in these animals (Yu *et al*., 1995). Nevertheless, it remains possible that the actions of available drugs includes an action at 5-ht_{1F} receptors, since ergotamine, methylergometrine, sumatriptan and 311C90 display modest to high affinity at this subtype (Adham *et al*., 1993b; present study) and, in the case of methylergometrine and sumatriptan, behave as full agonists in transfected NIH-3T3 cells (Adham *et al*., 1993a).

The studies summarised here show that 311C90 is a selective and potent partial agonist at '5-HT_{1B}-like' receptors mediating vascular contraction and is a highly potent agonist at recombinant human 5-HT_{1B} and 5-HT_{1D} receptor subtypes, with about ten fold selectivity for the latter. These subtypes, either independently or as a mixed population, are presumed to mediate the cranial vascular actions of the drug as well as its ability to inhibit plasma protein extravasation in the dura mater following trigeminal stimulation. In these respects, 311C90 displays a similar pharmacological profile to sumatriptan (Humphrey *et al*., 1988). However, combined with good oral bioavailability in man (48%: Seaber *et al*., 1995), the ability of 311C90 to inhibit trigemino-vascular activation centrally as well as peripherally (Goadsby & Edvinsson, 1994) might be expected to confer additional therapeutic benefit over existing acute treatments for migraine. The extent to which this is the case will become evident from continuing comparative clinical studies.

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