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Short communication

The pre- and postjunctional activity of CP-122,288, a conformationally restricted analogue of sumatriptan

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Abstract

The present study investigated the pre- and postjunctional activity of CP-122,288 (5-methyl-aminosulphonylmethyl-3-(*N*-methylpyrrolidin-2*R*-yl-methyl)-1*H*-indole), an analogue of the vascular 5-HT_1 receptor agonist, sumatriptan. CP-122,288 inhibited neurogenic plasma protein extravasation in rat dura with a potency approximately 40 000-fold greater than sumatriptan (ID_{50} values of 0.3 pmol/kg and 13.9 nmol/kg i.v. respectively). However, CP-122,288 was only approximately 2-fold more potent than sumatriptan at inhibiting neurogenically mediated contractions of the dog saphenous vein. CP-122,288 contracted the dog saphenous vein and basilar artery with a potency approximately 2-fold greater than that of sumatriptan. Both compounds possessed similar affinities at either human 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptors. It is concluded that CP-122,288 exhibits a prejunctional selectivity in the meninges to inhibit dural plasma protein extravasation independent of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor activation.

Keywords: CP-122,288; Sumatriptan; 5-HT receptor; Vasoconstriction

1. Introduction

The efficacy of the vascular 5-HT_1 receptor agonist, sumatriptan, in migraine has been attributed to constriction of large cranial blood vessels, which are distended and inflamed during migraine headache (Friberg et al., 1991). Vasoconstriction is thought to result in a reduction of trigeminal sensory nerve activity and thus, attenuation of nociceptive transmission to the central nervous system. However, it is evident that sumatriptan can also act prejunctionally on trigeminal nerve terminals to inhibit the release of vasodilatory peptides such as substance P and calcitonin gene-related peptide (CGRP) and this has been suggested to be the principal mechanism by which sumatriptan aborts the pain of migraine (Buzzi and Moskowitz, 1990). Consistent with this proposal, sumatriptan has been shown to attenuate the increased plasma levels of CGRP which occur during a migraine attack (Goadsby and Edvinsson, 1993).

Recently, the pharmacology of CP-122,288 (5-methyl-aminosulphonylmethyl-3-(*N*-methylpyrrolidin-2*R*-yl-methyl)-1*H*-indole), a conformationally restricted analogue of sumatriptan, was described (Lee and Moskowitz, 1993). CP-122,288 inhibited plasma protein extravasation in the dura mater of anaesthetised guinea-pigs approximately 2000 times more potently than sumatriptan. The present study has investigated further the activity of CP-122,288, and for comparison, sumatriptan, to determine their relative neuronal inhibitory and vasoconstrictor activities and the mechanisms involved. The potencies of CP-122,288 and sumatriptan were compared in a number of in vitro preparations, each of which is known to contain sumatriptan-sensitive vascular 5-HT_1 receptors. Postjunctional constrictor activity was determined in the dog isolated saphenous vein and basilar artery and prejunctional activity studied in the dog isolated saphenous vein. Prejunctional activity was also determined in vivo in the rat dural plasma protein extravasation model. As the 5-$HT_{1D\alpha}$ and/or 5-$HT_{1D\beta}$ receptors are considered to be the most likely subtypes via which sumatriptan acts pre- and postjunctionally (Hamel et al., 1993; Rebeck et al., 1994), the binding affinity of CP-122,288 and sumatriptan was assessed at both these human 5-HT_{1D} receptor subtypes.

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2. Materials and methods

2.1. Radioligand binding experiments

Monkey kidney epithelial (COSM6) cells were transiently transfected (by the DEAE Dextran method) with a plasmid containing the human 5-$HT_{1D\alpha}$ receptor gene (purchased from Dr M. Hamblin, Seattle Veterans Affairs Medical Centre). Cells were cultured in Dulbecco minimum Eagle's medium (37° C, in 5% CO_2 and 92% humidity), supplemented with 10% foetal calf serum, 1% glutamine and 1% non-essential amino acids. HeLa cells, stably transfected with a plasmid containing the human 5-$HT_{1D\beta}$ receptor gene (Hamblin and Metcalf, 1991), were grown in minimum essential medium (Earle's salt) supplemented with 1% glutamine and 1% non-essential amino acids. Cells were centrifuged (4500 × *g* for 35 min) and the pellet homogenised in binding incubation buffer (100 mM Tris HCl, 20 mM Mg SO_4 and 1 mM EDTA at pH 7.7). For the binding assay, homogenates were diluted in buffer (250 μg protein/ml) and [^{3}H]5-carboxamidotryptamine ([^{3}H]5-CT; 1.0 nM), was used as the radioligand. Incubations were terminated by rapid filtration, followed by washing with Tris HCl buffer (4° C). Specific [^{3}H]5-CT binding at 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors, defined by inclusion of cold ligand (5-CT; 10 μM), exceeded 90% of total binding.

2.2. Postjunctional activity in the dog isolated basilar artery and saphenous vein

Beagle dogs (7–10 kg, either sex) were killed with pentobarbitone (100 mg/kg i.v.) and the basilar artery removed and stored overnight in modified Krebs solution (Apperley et al., 1976) at 4° C. The artery was perfused (0.5 ml/min for 30 s) with Triton X-100 (0.1%) to remove the endothelium. Ring segments (3–4 mm) of the basilar artery were placed in separate 10 ml organ baths containing the modified Krebs solution at 37° C, bubbled with 95% O_2, 5% CO_2, to record changes in isometric tension. Preparations were maintained at an initial resting tension of 0.3 g for 1 h and then tension was increased to 1 g prior to construction of agonist concentration-effect curves. Saphenous veins were removed from beagle dogs (7–10 kg, either sex), anaesthetized with thiopentone sodium (25 mg/kg i.v.) followed by barbitone (300 mg/kg i.p.). Tissues were used immediately after dissection. Isometric contractions were recorded from spirally cut strips of the saphenous vein, suspended in modified Krebs solution (Apperley et al., 1976) bubbled with 95% O_2 and 5% CO_2, under a resting tension of 0.5 g. Tissues were allowed to equilibrate for at least 1 h prior to construction of agonist concentration-effect curves.

2.3. Prejunctional activity in the dog isolated saphenous vein

Freshly dissected, isolated spiral strips of the saphenous vein from beagle dogs (7–10 kg, either sex), anaesthetized with thiopentone sodium (25 mg/kg i.v.) followed by barbitone (300 mg/kg i.p.), were suspended in Krebs solution between platinum electrodes. Tissues, under a resting tension of 0.5 g, were allowed to equilibrate for 1 h before commencing electrical stimulation (2 Hz for 10 s every 180 s, 0.1 ms pulse width, supramaximal voltage). The contraction produced under these conditions is due to stimulation of noradrenergic nerves (Feniuk et al., 1979). Inhibitory-effect curves to drugs were commenced when constant responses were obtained to electrical stimulation. Tension was reset to 0.5 g between each concentration-response curve. Experiments were performed in the continuous presence of indomethacin (2.8 μM); cocaine (30 μM), atropine, mepyramine and cyproheptadine (all at 1 μM) which were included to optimize the experimental conditions and to exclude possible effects of sumatriptan and CP-122,288 at a variety of other receptor sites.

2.4. Measurement of in vitro agonist potencies in dog saphenous vein and basilar artery

Cumulative concentration-effect curves to sumatriptan were obtained in all tissues and the sumatriptan was then washed from the bath. Sixty minutes later, a cumulative concentration-effect curve to CP-122,288 was constructed in some tissues, whilst other preparations were again dosed with sumatriptan as a control, to monitor any spontaneous changes in sensitivity to sumatriptan. The equiactive molar ratio was determined by dividing the EC_{50} value (molar concentration producing 50% of the maximum effect) for CP-122,288 by the EC_{50} for sumatriptan in the same preparation. This value was then corrected for any spontaneous change in sensitivity by dividing it by the ratio of the EC_{50} values for sumatriptan in the control strips. This ratio varied by less than two. Results were expressed as the geometric means (with 95% confidence limits).

2.5. Measurement of dural plasma protein extravasation in rat dura mater

Plasma protein extravasation was measured essentially as described by Buzzi and Moskowitz (1990). Male Sprague-Dawley rats (190–220 g) were anaesthetised with pentobarbitone (60 mg/kg i.p.) and the left femoral vein cannulated for drug administration. Animals were placed in a stereotaxic frame (Lab Standard S1600) and the skull was exposed by a mid-sagittal incision. A 2 mm hole was then drilled on either side of

the sagittal suture (3 mm posterior to bregma and 3 mm lateral to the sagittal suture). [^{125}I]Human serum albumin was injected (370 kBq/animal i.v.) together with Evans blue (50 mg/kg i.v.), which aided verification of electrode placements post mortem. Bipolar electrodes (NEX-200, Rhodes) were lowered bilaterally into each trigeminal ganglion and, 5 min after [^{125}I]human serum albumin administration, biphasic electrical stimulation (5 ms, 5 Hz, 1.2 mA for 5 min) was delivered to one ganglion. Sumatriptan, CP-122,288 or vehicle was administered (i.v.) 15 min before electrical stimulation.

Immediately after electrical stimulation, animals were perfused (0.9% saline for 3 min) via the left cardiac ventricle at mean arterial blood pressure (100 mm Hg). The right atrium was incised to allow outflow of perfusate. Conjunctiva, eyelid and lip were dissected out, the skull opened and the dura mater dissected from the cranium. Tissues from the stimulated and unstimulated sides were weighed and counted for radioactivity. Results were expressed as cpm/mg tissue and the difference between unstimulated and stimulated sides was assessed using either Student's paired *t*-test or analysis of variance (ANOVA) followed by a Dunnett's *t*-test with significance levels set at $P < 0.05$. Data were then expressed for each animal as a ratio of cpm/mg tissue between stimulated and unstimulated sides.

2.6. Drugs used

The following drugs were used: atropine sulphate (Sigma), cocaine hydrochloride (Sigma), cyproheptadine hydrochloride (Merck, Sharp and Dohme), indomethacin (Sigma), mepyramine maleate (May and Baker), sumatriptan and CP-122,288 (both synthesised in the Medicinal Chemistry department, Glaxo Research and Development, Ware, UK). [^{3}H]5-CT (9.25 Bq/mmol) and [^{125}I]human serum albumin (1.85 MBq/ml) (NEN-DuPont and Amersham, UK, respectively) were stored at 4° C until used. Drugs were dissolved in distilled water with the exception of CP-122,288, which was dissolved initially in 10% HCl (1 M).

3. Results

3.1. Binding affinity of CP-122,288 and sumatriptan at 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors

Membranes from transiently transfected COSM6 (5-HT$_{1D\alpha}$) and stably transfected HeLa cells (5-HT$_{1D\beta}$) displayed high affinity (K_d values of 0.7 and 5.2 nM respectively) saturable [^{3}H]5-CT binding. CP-122,288 and sumatriptan (0.03 nM–10 μM) displaced, in a concentration-dependent manner, [^{3}H]5-CT binding to 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ receptors. The pK_i values (means ± S.E.M.) for CP-122,288 and sumatriptan were 8.1 ± 0.1 (slope of 1.0 ± 0.1; $n = 3$) and 7.9 ± 0.2 (slope of 1.1 ± 0.2; $n = 6$), respectively, at the 5-HT$_{1D\alpha}$ receptor and 7.5 ± 0.1 (slope of 0.8 ± 0.1; $n = 3$) and 7.1 ± 0.1 (slope of 0.9 ± 0.1; $n = 6$) at the 5-HT$_{1D\beta}$.

3.2. Postjunctional activity in the dog isolated basilar artery and saphenous vein

In the dog isolated basilar artery, CP-122,288 and sumatriptan (each 0.001–5 μM) produced concentra-

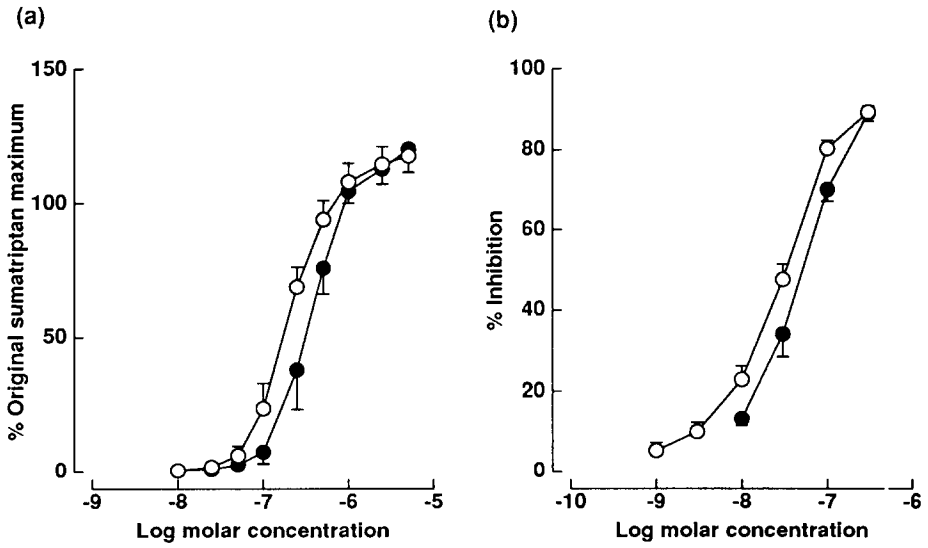

Fig. 1. The activity of sumatriptan (filled circles) and CP-122,288 (open circles) in the dog isolated saphenous vein. (a) Contractile responses are expressed as a mean (±S.E.M.) percentage of the original maximum to sumatriptan ($n = 4$). (b) The influence on sympathetic nerve-mediated contractions is expressed as the mean (±S.E.M.) percentage inhibition of the twitch response ($n = 4$).

tion-dependent contractions (EC_{50} values (with 95% confidence limits) of 0.06 μM (0.005–0.77) and 0.14 μM (0.02–1.01) respectively ($n = 3$)). CP-122,288 was 2-fold more potent than sumatriptan (equiactive molar ratio, relative to sumatriptan, of 0.47 (0.28–0.8; $n = 3$)). Both compounds produced a similar maximum response. CP-122,288 and sumatriptan (each 0.01–5 μM) produced concentration-dependent contractile responses of the dog isolated saphenous vein (EC_{50} values were 0.2 μM (0.14–0.31) and 0.35 μM (0.19–0.66) respectively; $n = 4$). The equiactive molar ratio for CP-122,288, relative to sumatriptan, was 0.68 (0.4–1.16; $n = 4$) and both compounds produced similar maximum responses (Fig. 1).

3.3. Prejunctional activity in the dog isolated saphenous vein

Electrical stimulation (2 Hz for 10 s every 180 s, 0.1 ms pulse width, supramaximal voltage) produced reproducible contractions of the dog isolated saphenous vein. CP-122,288 and sumatriptan (each 0.001–0.3 μM) inhibited the electrically evoked responses in a concentration-dependent manner (Fig. 1). The mean IC_{50} values (with 95% confidence limits) for CP-122,288 and sumatriptan were 32.4 nM (20.5–51.0) and 49.0 nM (28.2–85.3) respectively ($n = 4$). CP-122,288 was approximately 2-fold more potent than sumatriptan (equiactive molar ratio = 0.43 (0.25–0.73; $n = 4$), relative to sumatriptan).

3.4. Plasma protein extravasation in rat dura mater

Electrical stimulation of the trigeminal ganglion induced a leakage of [^{125}I]human serum albumin in rat dura (29.6 ± 2.1 and 20.2 ± 1.7 cpm/mg tissue on ipsilateral and contralateral sides to stimulation, $P < 0.05$, $n = 18$). Extravasation also occurred in extracranial tissues. The extravasation ratios, calculated from the cpm/mg tissue in the stimulated and unstimulated sides, were 1.5 ± 0.1 (dura), 3.8 ± 0.4 (conjunctiva), 3.4 ± 0.3 (eyelid) and 6.6 ± 0.6 (lip) ($n = 18$).

Pretreatment of rats with either sumatriptan (3.4, 34 and 340 nmol/kg i.v.; $n = 4$, 5 and 4 respectively) or CP-122,288 (0.01, 0.1, 0.9, 9.3 and 93 pmol/kg i.v.; $n = 4$, 7, 5, 4 and 6 respectively) produced a dose-dependent inhibition of plasma protein extravasation in the dura mater (Fig. 2). CP-122,288 was approximately 40 000-fold more potent than sumatriptan; the ID_{50} values (i.e. the doses producing 50% inhibition) were 0.3 pmol/kg and 13.9 nmol/kg respectively. Sumatriptan (3.4–340 nmol/kg i.v.) and CP-122,288 (0.01–93 pmol/kg i.v.) had no effect on extravasation in conjunctiva, eyelid and lip (data not shown).

4. Discussion

Neurogenic plasma protein extravasation in the dura mater is considered to be a model of the sterile neurogenic inflammation which is thought to occur, following trigeminal nerve activation, in migraine headache (Buzzi and Moskowitz, 1990). It is likely that the inhibition of plasma protein extravasation produced by sumatriptan is due to a reduction of substance P release from trigeminal nerve terminals and this mechanism has been suggested to account for the efficacy of sumatriptan in migraine (Buzzi and Moskowitz, 1990). Recently, a conformationally restricted analogue of sumatriptan, CP-122,288, was shown to inhibit neuro-

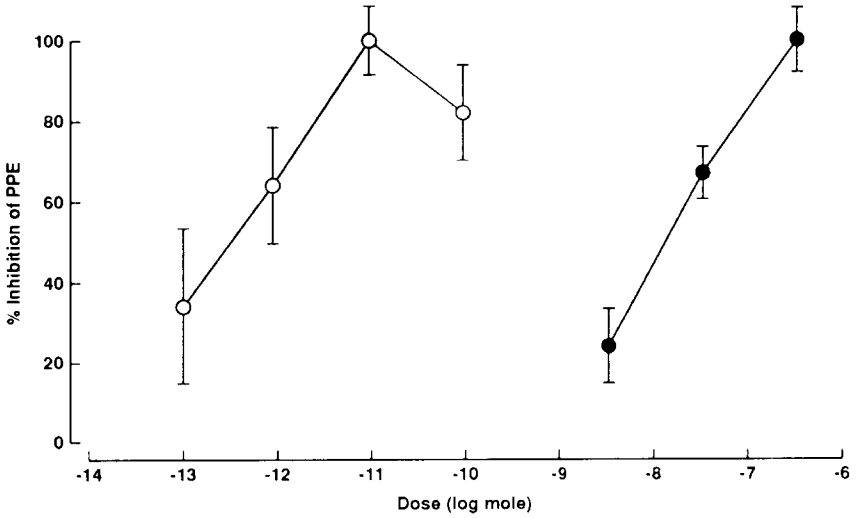

Fig. 2. The influence of sumatriptan (3.4, 34 and 340 nmol/kg i.v. ($n = 4$, 5 and 4 respectively); filled circles) and CP-122,288 (0.1, 0.9, 9.3 and 93 pmol/kg i.v. ($n = 7$, 5, 4 and 6 respectively); open circles) on plasma protein extravasation in rat dura evoked by trigeminal ganglion electrical stimulation. Results are expressed as a mean (± S.E.M.) percentage inhibition of plasma protein extravasation.

genic plasma protein extravasation in guinea-pig dura with a potency approximately 2000-fold greater than sumatriptan (Lee and Moskowitz, 1993). CP-122,288 appeared to be acting prejunctionally as plasma protein extravasation evoked by substance P administration was unaffected (Lee and Moskowitz, 1993). The present study has confirmed, in another species (i.e. the rat), that CP-122,288 is a potent inhibitor of dural plasma protein extravasation. Indeed, CP-122,288 exhibited even greater potency than sumatriptan (approximately 40000-fold higher) in the rat than that reported for the guinea-pig. This may not reflect a true species difference as the lower potency of sumatriptan in the present study compared to that obtained by Lee and Moskowitz (1993) (ID_{50} values of 13.9 and 4.1 nmol/kg respectively) may have exaggerated any genuine difference in the relative potencies of CP-122,288 and sumatriptan. Species differences are evident for many of the 5-HT receptor subtypes. The 5-HT_{1B} receptor, for example, is the rat equivalent of the human 5-$HT_{1D\beta}$ receptor subtype (Hoyer and Middlemiss, 1989) and has been identified in the rat trigeminal ganglion (Bruinvels et al., 1992). The ability of sumatriptan to inhibit plasma protein extravasation in rat dura has been attributed to activation of a receptor similar to, although not identical with, the 5-HT_{1B} receptor (Buzzi et al., 1991).

The increased prejunctional potency of CP-122,288, relative to sumatriptan, in the plasma protein extravasation experiments may be restricted to the cranial vasculature. In the dog saphenous vein, CP-122,288 was only 2-fold more potent than sumatriptan at inhibiting, via a prejunctional action, contractions mediated by stimulation of sympathetic noradrenergic nerves. This suggests that the receptors which are activated selectively by CP-122,288 on the trigeminal nerve terminals are absent on the sympathetic innervation of the dog saphenous vein. Moreover, the inhibitory activity of CP-122,288 was restricted to the dura; extravasation in extracranial tissues (i.e. conjunctiva, eyelid and lip) was unaffected ruling out a non-specific (e.g. local anaesthetic-like) action.

The increased potency of CP-122,288 in the plasma protein extravasation model, compared to sumatriptan, is not reflected postjunctionally in terms of its ability to contract the dog isolated saphenous vein and basilar artery. CP-122,288 contracted the saphenous vein and basilar artery with a potency approximately 2-fold greater than sumatriptan. As these responses are likely to be mediated via 5-$HT_{1D\beta}$ receptor activation (Hamel et al., 1993), the results suggest that the prejunctional receptors activated by CP-122,288 to inhibit plasma protein extravasation differ from the 5-$HT_{1D\beta}$ receptor subtype mediating vasoconstriction. Consistent with this view, 5-CT is more potent (threshold dose of 3.1 pmol/kg i.v.) at inhibiting dural plasma protein extravasation than would be predicted from its 5-HT_{1D} receptor binding affinities (Buzzi et al., 1991). Furthermore, the non-selective 5-HT_{1D} receptor antagonist, methiothepin, fails to influence the sumatriptan-mediated inhibition of plasma protein extravasation in dura, but is able to block its vasoconstrictor activity in the cerebral vasculature (Buzzi et al., 1991; Connor et al., 1989).

The binding affinities of CP-122,288 and sumatriptan at the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors were measured to determine whether the relative pre- and postjunctional potencies of the compounds could be explained in terms of differences in their affinity at either subtype. The presence of mRNA for the 5-$HT_{1D\beta}$, but not 5-$HT_{1D\alpha}$, receptor subtype has been demonstrated in cerebral blood vessels while the opposite may be true for the human trigeminal ganglion (Hamel et al., 1993; Rebeck et al., 1994). However, in the present study, CP-122,288 and sumatriptan had similar affinities at each subtype. It remains to be determined whether this is also true at the other recently identified 5-HT receptor subtypes. However, data with sumatriptan and 5-CT appear to rule out a role for the 5-HT_{1E}, 5-HT_{1F} or 5-HT_7 receptor subtypes in mediating inhibition of dural plasma protein extravasation. 5-CT has low affinity at the human 5-HT_{1E} and 5-HT_{1F} receptor subtypes (pK_i value of 5.5 and 6.1 respectively; McAllister et al., 1992; Adham et al., 1993) while the same is true for sumatriptan at the rat 5-HT_7 receptor (pK_i value of 6.6; Shen et al., 1993). However, this assumes that CP-122,288 activates the same receptor as sumatriptan and 5-CT in dura. It is possible that non-5-HT receptors may be involved in the inhibition of dural plasma protein extravasation by CP-122,288, although the compound has little affinity ($pIC_{50} < 6.0$) at, for example, noradrenergic, muscarinic, histaminergic, neurokinin and CGRP receptors; Glaxo, unpublished observations). Alternatively, an, as yet uncharacterised 5-HT receptor type, may be involved.

The ability of CP-122,288 to block plasma protein extravasation in the dura mater of anaesthetised guinea-pigs and rats presumably reflects its inhibitory action on the release of substance P from trigeminal nerve terminals, and may imply that the compound will possess anti-migraine activity. The question of whether a prejunctional action alone is sufficient to confer anti-migraine activity remains unanswered. The clinical efficacy of sumatriptan in migraine has been ascribed to both its postjunctional vasoconstrictor activity in the cranial vasculature (Friberg et al., 1991) and to its prejunctional inhibitory effects on the trigeminal nerve (Buzzi and Moskowitz, 1990). The results from experiments with CP-122,288 demonstrate that selectivity for prejunctional sites in the cranial circulation is possible. It would be interesting to compare the clinical efficacy

of a compound such as CP-122,288, at a dose devoid of any vasoconstrictor activity, with that of sumatriptan. If CP-122,288, like sumatriptan, is poorly brain penetrant, and therefore unlikely to act directly on the central trigeminal nerve terminals in the trigeminal nucleus caudalis, such a comparison should determine the importance of prejunctional trigeminal nerve inhibition in the cranial vasculature as a mechanism for anti-migraine activity in man.

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References

Adham, N., H.-T. Kao, L.E. Schechter, J. Bard, M. Olsen, D. Urquhart, M. Durkin, P.R. Hartig, R.L. Weinshank and T.A. Branchek, 1993, Cloning of another human serotonin receptor (5-HT_{1F}): a fifth 5-HT_1 receptor subtype coupled to the inhibition of adenylate cyclase, Proc. Natl. Acad. Sci. USA 90, 408.

Apperley, E., P.P.A. Humphrey and Y.S. Bakhle, 1976, Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta, Br. J. Pharmacol. 58, 211.

Bruinvels, A.T., B. Landwehrmeyer, M.A. Moskowitz and D. Hoyer, 1992, Evidence for the presence of 5-HT_{1B} receptor messenger RNA in neurons of the rat trigeminal ganglia, Eur. J. Pharmacol. 227, 357.

Buzzi, M.G. and M.A. Moskowitz, 1990, The anti-migraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater, Br. J. Pharmacol. 99, 202.

Buzzi, M.G., M.A. Moskowitz, S.J. Peroutka and B. Byun, 1991, Further characterization of the putative 5-HT receptor which mediates blockade of neurogenic plasma protein extravasation in rat dura mater, Br. J. Pharmacol. 103, 1421.

Connor, H.E., W. Feniuk and P.P.A. Humphrey, 1989, Characterization of 5-HT receptors mediating contraction of canine and primate basilar artery by use of GR43175, a selective 5-HT_1-like receptor agonist, Br. J. Pharmacol. 96, 379.

Feniuk, W., P.P.A. Humphrey and A.D. Watts, 1979, Presynaptic inhibitory action of 5-hydroxytryptamine in dog isolated saphenous vein, Br. J. Pharmacol. 67, 247.

Friberg, L., J. Olesen, H.K. Iversen and B. Sperling, 1991, Migraine pain associated with middle cerebral artery dilatation: reversal by sumatriptan, Lancet 338, 13.

Goadsby, P.J. and L. Edvinsson, 1993, The trigeminovascular system and migraine: studies characterising cerebrovascular and neuropeptide changes seen in humans and cats, Ann. Neurol. 33, 48.

Hamblin, M.W. and M.A. Metcalf, 1991, Primary structure and functional characterisation of a human 5-HT_{1D}-type receptor, Mol. Pharmacol. 40, 143.

Hamel, E., L. Gregoire and B. Lau, 1993, 5-HT_1 receptors mediating contraction in bovine cerebral arteries: a model for human cerebrovascular '5-$HT_{1D\beta}$' receptors, Eur. J. Pharmacol. 242, 75.

Hoyer, D. and D.N. Middlemiss, 1989, Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain, Trends Pharmacol. Sci. 10, 130.

Lee, W.S. and M.A. Moskowitz, 1993, Conformationally restricted sumatriptan analogues, CP-122,288 and CP-122,638 exhibit enhanced potency against neurogenic inflammation in dura mater, Brain Res. 626, 303.

McAllister, G., A. Charlesworth, C. Snodin, M.S. Beer, A.J. Noble, D.N. Middlemiss, L.L. Iversen and P. Whiting, 1992, Molecular cloning of a serotonin receptor from human brain (5HT_{1E}): a fifth 5HT_1-like subtype, Proc. Natl. Acad. Sci. USA 89, 5517.

Rebeck, G.W., K.I., Maynard, B.T. Hyman and M.A. Moskowitz, 1994, Selective 5-$HT_{1D\alpha}$ serotonin receptor gene expression in trigeminal ganglia: implications for antimigraine drug development, Proc. Natl. Acad. Sci. USA 91, 3666.

Shen, Y., F.J. Monsma, M.A. Metcalf, P.A. Jose, M.W. Hamblin and D.W. Sibley, 1993, Molecular cloning and expression of a 5-hydroxytyramine$_7$ serotonin receptor subtype, J. Biol. Chem. 268, 1820.