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The *in vivo* pharmacological profile of a 5-HT_1 receptor agonist, CP-122,288, a selective inhibitor of neurogenic inflammation

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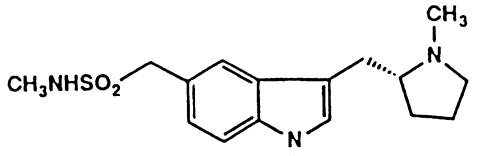
1 The aim of the present study was to investigate the *in vivo* pharmacological profile of CP-122,288, an indole-derivative with a conformationally restricted N-methylpyrrolidinyl basic side chain in the C-3 position. This C-3 substituent structurally differentiates CP-122,288 from the 5-HT_{1D} receptor agonist sumatriptan, which possesses an N,N-dimethylaminoethyl group.



CP-122,288

2 When administered prior to electrical stimulation of the trigeminal ganglion, CP-122,288 (0.3-300 ng kg^{-1}, i.v.) produced a dose-related inhibition of plasma protein extravasation in rat dura mater (minimum effective dose, MED, 3 ng kg^{-1} i.v., $P<0.05$; maximal inhibition of plasma extravasation at 30 ng kg^{-1} i.v., $P<0.01$). Sumatriptan produced a similar inhibition of plasma leakage in the dura, but at much higher dose levels (MED, 100 μg kg^{-1} i.v., $P<0.05$). Thus, CP-122,288 is of the order of 10^4 fold more potent than sumatripan.

3 At all doses tested, CP-122,288 did not inhibit plasma protein extravasation measured in extracranial tissues such as the lower lip, eyelid, and conjunctiva.

4 In a separate series of studies in the anaesthetized rat, CP-122,288 (0.003-3 μg kg^{-1} i.v.) produced no change in either heart rate or mean arterial blood pressure, thus demonstrating that doses of CP-122,288 which inhibit plasma protein leakage in rat dura, are devoid of haemodynamic effects.

5 Following a 5 min period of electrical stimulation of the trigeminal ganglion, a 20 min period of sustained neurogenically-driven plasma extravasation, occurring in the absence of electrical stimulation, was initiated. By administration of the compound 5 min after completing the phase of electrical stimulation, this protocol permitted the evaluation of the activity of CP-122,288 on an ongoing and established inflammatory event. CP-122,288 (30 and 300 ng kg^{-1}, i.v., $P<0.01$ and $P<0.05$, respectively) produced a complete inhibition of plasma protein leakage which was consistent with its effects when administered prior to trigeminal ganglion stimulation.

6 In the anaesthetized dog, CP-122,288 and sumatriptan, at 1-300 μg kg^{-1}, i.v., produced a dose-dependent reduction in carotid arterial blood flow and coronary arterial diameter. These data demonstrate that sumatriptan inhibits neurogenic inflammation in the rat (MED, 100 μg kg^{-1}, i.v.), and produces vasoconstriction in the dog, over a similar dose-range. Interestingly, doses of CP-122,288 that inhibit neurogenic inflammation in rat dura mater (0.3-300 ng kg^{-1}) were demonstrated to be devoid of vasoconstrictor activity in either the carotid or coronary vascular beds of dog.

7 These data demonstrate that in the rat, CP-122,288 is a highly potent and selective inhibitor of neurogenic inflammation in intracranial tissues, at doses which are devoid of vasoconstrictor activity in dog. Potentially, CP-122,288 may be of use for the acute treatment of migraine, without the risk of cardiovascular side-effects.

Keywords: CP-122,288; sumatriptan; dura mater; neurogenic inflammation; rat; regional haemodynamics; dog

Introduction

It is claimed that clinical efficacy of acute anti-migraine therapies, such as sumatriptan and other 5-HT_{1D} receptor agonists, may be due to either an inhibition of neurogenic inflammation *via* a prejunctional inhibition of neuropeptide release from intracranial perivascular trigeminal afferents (Moskowitz, 1992), and/or due to the activation of cranial vascular '5-HT_{1D}-like' receptors which mediate constriction (Humphrey & Feniuk, 1991). The relative importance of the ability of sumatriptan to modulate sensory neurotransmission and constrict cranial blood vessels remains an important debate in migraine research, and will be resolved when agents which are able to discriminate between these two actions are identified and tested in the clinic.

CP-122,288, an indole-derivative with a conformationally restricted N-methylpyrrolidinylmethyl basic side-chain in the C-3 position and the C-5 sulphonamide group found in sumatripan, has been demonstrated to be a potent inhibitor of neurogenic inflammation in guinea-pig dura mater (minimum

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effective dose 300 pg kg^{-1}, i.v., Lee & Moskowitz, 1993). It is likely that CP-122,288 inhibits neurogenic inflammation by preventing neuropeptide release *via* a prejunctional mechanism, since plasma extravasation produced following the intravenous administration of substance P was unaltered by pretreatment with CP-122,288 (Lee & Moskowitz, 1993). Thus, CP-122,288 could represent the first of a new class of agents of use for the treatment of migraine, and could singularly resolve the debate regarding the relative clinical importance of the pharmacological actions of sumatripan. Furthermore, it is evident from clinical studies with sumatriptan that its vasoconstrictor effects are not restricted exclusively to the cranial vasculature, and marked changes in peripheral haemodynamics have been reported over the therapeutic dose-range (MacIntyre *et al.*, 1993). These data are consistent with the ability of sumatripan to contract preparations of human isolated saphenous vein (Bax *et al.*, 1992) and coronary artery (Connor *et al.*, 1989; Cocks *et al.*, 1993; Kaumann *et al.*, 1994). In fact, cardiovascular side-effects have been reported in response to sumatripan (Willett *et al.*, 1992; Ottervanger *et al.*, 1993) and the compound is contraindicated in patients with, or at risk of cardiovascular disease. Thus, the development of an anti-migraine agent that is clinically efficacious through an ability to inhibit neurogenically-evoked inflammation and vasodilatation, whilst lacking the direct vasoconstrictor properties of sumatriptan, would be an atttractive clinical alternative.

However, one caveat of an approach to inhibit selectively neurogenic inflammation *via* an inhibition of peptide release is whether such an effect would translate to a rapid onset of relief of migraine symptoms in the clinic. It has been argued that only a vasoconstrictor mechanism would be able to reverse rapidly a pathophysiological event of vascular origin. Interestingly, in a recent study by Moskowitz and co-workers, it was demonstrated that after the completion of a 5 min period of electrical stimulation of trigeminal ganglion in the guinea-pig, the trigeminal fibres which innervate the dura mater continued to fire, in the absence of electrical stimulation, for up to 45 min (Huang *et al.*, 1993). The processes which are responsible for this continued release of neuropeptides have not been examined; however, this experimental protocol provided a simple and convenient method of demonstrating that sumatriptan and endopeptidase 24,11 could reverse an established and ongoing neurogenically-driven sterile inflammation within minutes when administered during this period of continual neuronal firing (Huang *et al.*, 1993). Thus, if the inflammatory environment that develops using this experimental protocol mimics those events which occur pathophysiologically during migraine, then it appears that the inflammatory process could be inhibited by a prejunctional inhibition of peptide release within a clinically relevant timescale.

The aims of the work described in this paper were as follows:- (i) since differences in pharmacology are frequently observed between different animal species, the ability of CP-122,288 to inhibit intracranial neurogenic inflammation in a second animal species, rat, was examined; (ii) to investigate the ability of CP-122,288 to reverse an established and ongoing neurogenic inflammation using the method of Huang *et al.* (1993), and (iii) to define the dose-range over which CP-122,288 produced haemodynamic and vasoconstrictor effects in the anaesthetized dog, a species which has been of use, unlike rat (Spokes & Middlefell, 1993), for investigating the contractile activity of compounds mediated *via* activation of vascular '5-HT_{1D}-like' receptors (Feniuk *et al.*, 1989).

Methods

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

Male Sprague Dawley rats (380–450 g, Charles River, Manston, U.K.) were kept under diurnal lighting conditions and allowed water *ad libitum*. Animals were anaesthetized with pentobarbitone (60 mg kg^{-1}, i.p.) and a femoral vein was cannulated for intravenous injections. Animals were placed in a stereotaxic frame (Kopf 900 Instruments) with the incisor bar set at −1.5 mm. Symmetrical burr holes were drilled at 4.0 mm laterally and 4.0 mm anteriorly from bregma for a 400 g rat, and adjusted proportionally according to the rat weight. Paired non-concentric bipolar electrodes (5 cm shaft, Clark Electro-medical, Pangbourne) were lowered 9.5 mm from the dura mater bilaterally into the trigeminal ganglia. ^{125}I-radiolabelled human serum albumin (50 μCi kg^{-1}) and Evans blue (20 mg kg^{-1}) were injected *via* the femoral vein, 5 min later either drug or vehicle were administered, and after a further 10 min, the trigeminal ganglion was electrically stimulated (3 min, 2.2 mA, 5 Hz, 2 ms duration; Isostim Stimulus Isolator A320, World Precision Instruments, Florida, U.S.A.). The left or right ganglion was arbitrarily designated for stimulation. Immediately after stimulation, animals were perfused with 0.9% saline *via* the left cardiac ventricle for a 5 min period at a constant pressure of 120 mmHg to wash the blood from the head region. Following removal of the brain, the dura mater lining the anterior fossae was removed and dissected bilaterally. Samples of extracranial tissues innervated by the trigeminal nerve (eyelid, conjunctiva and lower lip) were also removed, weighed and counted for radioactivity (1277 Gammamaster, LKB, Finland). The recorded counts mg^{-1} wet weight of tissue were used to calculate an extravasation ratio between the tissues from the stimulated and unstimulated sides.

In some experiments, the protocol described above was modified according to the method of Huang *et al.* (1993). These workers demonstrated that following the completion of a 5 min period of electrical stimulation of the trigeminal ganglion, plasma extravasation continued to develop for up to 45 min by a process which was dependent upon neuropeptide release, since it could be inhibited by both endopeptidase 24.11 and sumatriptan. Thus, by administering compound post electrical stimulation, this protocol provides a simple method of evaluating the effect of compounds on an established and ongoing neurogenically-driven inflammatory response. In the present study, compound or vehicle were adminstered 5 min after the completion of a 5 min period of electrical stimulation of the trigeminal ganglion and ^{125}I-radiolabelled human albumin (50 μCi kg^{-1}) and Evans blue (20 mg kg^{-1}) were administered after a further 5 min. Finally, 10 min after the administration of the radiolabelled albumin, animals were perfused with 0.9% saline (see above for detail), and tissues were removed as before.

Measurement of cardiovascular parameters in the anaesthetized rat

In a separate series of experiments using rats anaesthetized with Inactin (120 mg ml^{-1} solution in 0.9% saline; 0.1 ml 100 g^{-1} body weight i.p.), arterial blood pressure was recorded *via* the femoral artery with a Gould pressure transducer (model P231D). Heart rate was subsequently derived from the blood pressure signal. Drug or vehicle were administered *via* the femoral vein. Animals were artificially ventilated to maintain blood gases (pH 7.36–7.51; PCO_2 29–44 mmHg; PO_2 87–106 mmHg). After surgery, rats were allowed to stabilize and doses of compound (CP-122,288, 3 ng kg^{-1}–3 μg kg^{-1}) or vehicle (0.9% saline) were given at 5 min intervals. Data were collected using an in-house on-line data acquisition system, based on the Motorola 68,000 family computer.

Measurement of cardiovascular parameters in the anaesthetized dog

Female Beagle dogs (Pfizer colony, 11–14.5 kg) were premedicated with piritramide (5.0 ml, s.c.) and anaesthetic was administered *via* the right cephalic vein (mixture of 1:11 volumes of 10% α-chloralose in PEG300 and 11% urethane in 0.9% saline). All dogs were intubated with an endotracheal

tube and artificially ventilated with a Bird respirator MK7A (Viamed, Keighley, W. Yorks) to maintain arterial pH, $PaCO_2$ and PaO_2 within normal limits (pH 7.35–7.45; $PaCO_2$ 35–45 mmHg; PaO_2 85–120 mmHg). Body temperature was maintained at 37–39°C. Arterial blood pressure was recorded from a cannulated femoral artery with a Gould pressure transducer (model P231D). Blood samples for blood gas analysis were also taken from the arterial line. Lead II of the electrocardiogram was recorded from sub-epidermal needles to allow constant monitoring of cardiac rhythm and to derive heart rate. Blood flow in the left common carotid artery was measured using an electromagnetic flow probe (Skalar Medical bv, Delft, The Netherlands, 2.5 mm diameter).

A left thoracotomy was performed by removing part of the 6th rib and the heart was suspended in a pericardial cradle. The external diameter of the left circumflex coronary artery was measured with a sonomicrometer (ultrasonic transit-time dimension gauge, model 120.2, Triton Technology Inc., San Diego, U.S.A.). A pair of 5 MHz piezoelectric crystals attached to backing material were sutured to opposing surfaces of the left circumflex coronary artery, 3–5 cm from its origin. Correct alignment of the crystals was verified by on-line sonomicrometer and oscilloscope monitoring. Left circumflex coronary artery blood flow was measured with an electromagnetic flow probe (Skalar Medical bv, Delft, The Netherlands, 2.0 mm diameter). Care was taken during instrumentation to limit dissection and damage of visible nerves. All primary parameters (ECG, blood pressure, coronary diameter and blood flows) were displayed continuously on a Grass 79D polygraph and simultaneously transferred to the on-line data acquisition system described above.

After a 60 min stabilization period, a set of control readings were taken. Cumulative doses of compound (0.001–300 $\mu g\ kg^{-1}$) were administered by bolus injection at 5 min intervals. For each dose of compound, the response to compound for the measured parameters was reported at the peak change in carotid flow, with the exception of coronary diameter, where maximal reductions generally occurred after the maximal changes in carotid flow.

Drugs and solutions

The following compounds were purchased: ^{125}I-human serum albumin (Amersham International, U.K.); Evans Blue dye and α-chlorolose (Sigma Chemical Co., Dorset, U.K.), sodium pentobarbitone (Sagatal, May and Baker, Essex, U.K.), piritramide (Dipidolor, Jansen Pharmaceuticals, Beerse, Belgium) and urethane (Aldrich Chemicals Ltd., Steinheim, Germany).

The following compounds were synthesized at Pfizer Central Research (U.K.): CP-122,288 ((**R**)-N-methyl-[3-(1-methyl-2-pyrrolidinylmethyl)-1*H*-indol-5-yl]methanesulphonamide) and sumatriptan succinate. CP-122,288 was dissolved in 0.1 M citric acid and further diluted in 0.9% saline. Sumatriptan was dissolved in 0.9% saline.

Statistical analysis and calculations

In plasma extravasation studies, the level of plasma leakage produced in the presence of each dose of test compound was compared with that produced in a separate vehicle-treated control group. Differences between means were determined by Student's unpaired *t* test, and *P*-values of less than 0.05 were considered to indicate a significant difference between the responses being compared. In studies to measure the vasoconstrictor properties of test compounds in the anaesthetized dog, contractile potency is expressed as the ED_{50} value, which represents the dose of agonist required to produce 50% of the maximum response attainable for that particular agonist. ED_{50} values were derived by an in-house logistic curve-fitting programme based on ALLFIT (DeLean *et al.*, 1978), and are expressed as the geometric mean with 95% confidence limits in parentheses.

Results

Neurogenic inflammation studies

Electrical stimulation of the trigeminal ganglion in rat produced a two fold increase in the extravasation of ^{125}I-radiolabelled human albumin within the dura mater from the side ipsilateral to stimulation compared with the contralateral side (mean d.p.m. mg^{-1} dura mater, ±s.e.mean: unstimulated 35±3, stimulated 69±8, corresponding to an extravasation ratio of 2.0±0.1, $n=43$).

When administered prior to electrical stimulation of the trigeminal ganglion, CP-122,288 (0.3–300 ng kg^{-1}) produced a dose-dependent reduction in plasma protein leakage measured in the dura mater, when compared with control (Figure 1a). The minimum effective dose (MED) of CP-122,288 that produced a significant inhibition of plasma protein leakage, when compared to control, was 3 ng kg^{-1}, and a maximal and complete inhibition of the extravasation ratio was observed from 30 ng kg^{-1} (mean extravasation ratio ± s.e.mean: control 2.0±0.3, $n=10$; CP-122,288 3 ng kg^{-1}, 1.4±0.2, $n=8$, $P<0.05$; control 1.7±0.3, $n=10$, CP-122,288 30 ng kg^{-1}, 1.0±0.1, $n=7$, $P<0.01$). When compared to CP-122,288, sumatriptan produced a similar level of inhibition of plasma

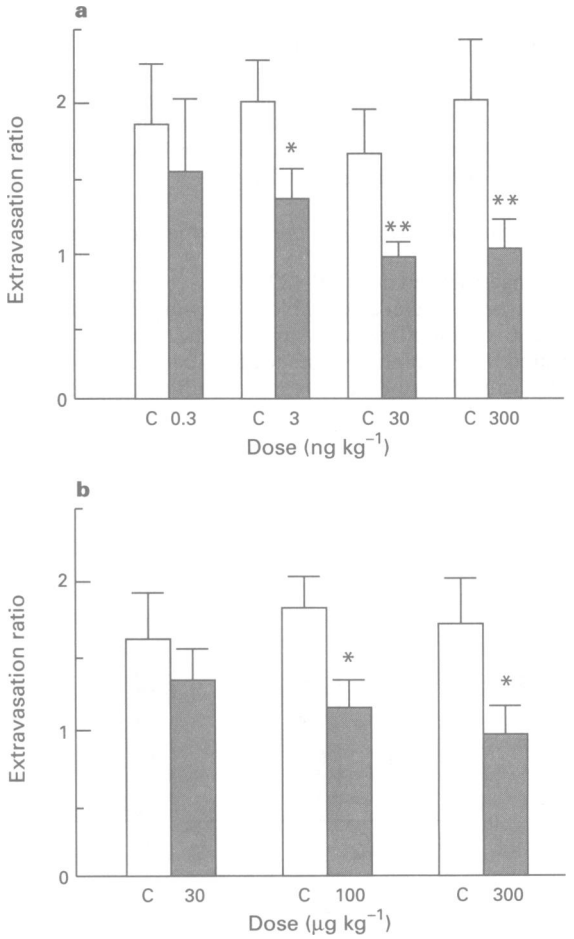

Figure 1 Effect of (a) CP-122,288 (0.3–300 ng kg^{-1}, i.v.) and (b) sumatriptan (30–300 $\mu g\ kg^{-1}$, i.v.) on plasma protein extravasation produced in dura mater following electrical stimulation of the trigeminal ganglion in rat. Data are expressed on the ordinate scale as a mean extravasation ratio in stimulated/unstimulated sides, and on the abscissa scale, as drug dose (ng kg^{-1} or $\mu g\ kg^{-1}$). Open columns (C) = vehicle control (0.9% saline); shaded columns = compound-treated. Mean with s.e.mean of $n=7-10$ separate experiments are shown. Statistical analysis was performed using Student's unpaired *t* test, $^*P<0.05$ and $^{**}P<0.01$ when compared to each respective control.

protein leakage in the dura mater, but these effects were observed only at much higher dose-levels, 30–300 $\mu g\ kg^{-1}$. The MED for sumatriptan that produced a significant inhibition of the extravasation ratio was 100 $\mu g\ kg^{-1}$ (mean extravasation ratio ± s.e.mean: control 1.8 ± 0.2, $n=10$; sumatriptan 100 $\mu g\ kg^{-1}$, 1.1 ± 0.2, $n=8$, $P<0.05$; see Figure 1b). When the MED levels of both compounds are compared, CP-122,288 is in the order of 10^4 fold more potent than sumatriptan as an inhibitor of neurogenic inflammation in rat dura mater. In contrast, neither CP-122,288 nor sumatriptan inhibited the extravasation of plasma protein in extracranial tissues such as the lower lip, eyelid and conjunctiva, even at the highest doses tested, 300 ng kg^{-1} and 300 $\mu g\ kg^{-1}$, respectively.

In further studies using a modified experimental protocol, CP-122,288 was administered during a period of continual firing of the trigeminal nerve. Under these conditions, CP-122,288 (30–300 ng kg^{-1}) produced an immediate and effective inhibition of plasma protein leakage in the dura mater (mean extravasation ratio ± s.e.mean: control 1.6 ± 0.2, $n=10$; CP-122,288 30 ng kg^{-1}, 0.9 ± 0.1, $n=7$, $P<0.01$; see Figure 2). Since a full dose-response curve was not prepared, a determination of the MED of CP-122,288 in the modified model cannot be made. However, these data demonstrate that at 30 ng kg^{-1}, there is no difference in the level of efficacy of CP-122,288 when administered prior to, or during, activation of trigeminal neurones.

In a separate series of studies in the anaesthetized rat, CP-122,288 (0.003–3 $\mu g\ kg^{-1}$, i.v.) produced no change in either heart rate (beats min^{-1}: vehicle control, 401 ± 6, $n=4$; CP-122,288, 3 $\mu g\ kg^{-1}$, 390 ± 9, $n=4$) or mean arterial blood pressure (mmHg: vehicle control, 121 ± 5, $n=4$; CP-122,288, 3 $\mu g\ kg^{-1}$, 116 ± 8, $n=4$), thus demonstrating that doses of CP-122,288 which inhibit plasma protein leakage in rat dura, are devoid of haemodynamic effects.

Haemodynamic profile in the anaesthetized dog

In the anaesthetized dog, CP-122,288 (1–100 ng kg^{-1}) did not alter heart rate, mean arterial blood pressure, coronary arterial diameter, or blood flow in any of the vascular territories measured. At the highest doses tested (100–300 $\mu g\ kg^{-1}$), CP-122,288 caused a modest increase in both mean arterial blood pressure (4 mmHg) and heart rate (10 beats min^{-1}) when compared to the vehicle-treated control.

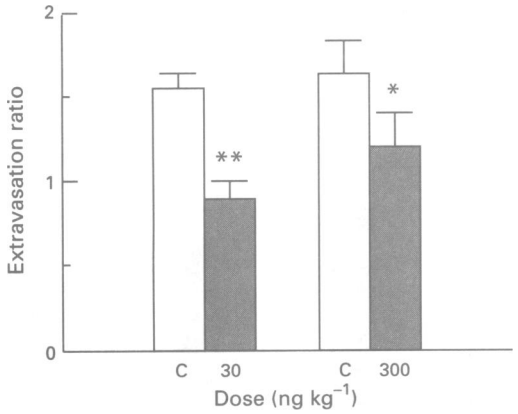

Figure 2 Effect of CP-122,288 (30–300 ng kg^{-1}, i.v.) on an established and ongoing neurogenically-driven plasma protein extravasation produced in dura mater. CP-122,288 or vehicle were administered 5 min after the completion of a 5 min period of electrical stimulation of the trigeminal ganglion in rat. ^{125}I-labelled albumin leakage in the dura mater was measured after a further 10 min period. Data are expressed on the ordinate scale, as a mean extravasation ratio in stimulated/unstimulated sides, and on the abscissa scale as drug dose (ng kg^{-1}). Open columns (C) = vehicle control (0.9% saline), shaded columns = compound-treated. Mean with s.e.mean of $n=7-11$ separate experiments are shown. Statistical analysis was performed using Student's unpaired t test, $^*P<0.05$ and $^{**}P<0.01$ when compared to each respective control.

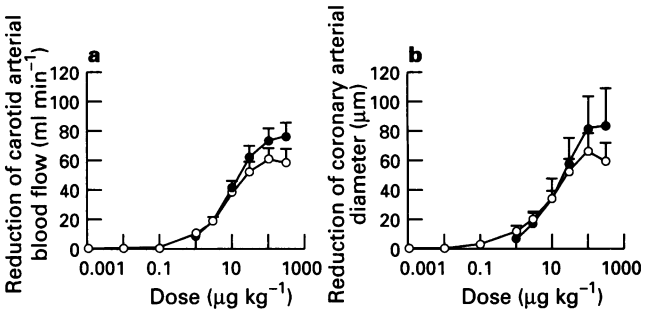

Figure 3 Comparison of the effects of CP-122,288 (○) and sumatripan (●) on (a) carotid arterial blood flow and (b) coronary artery diameter in the anaesthetized dog (for detailed methodology, see Methods). In (a) and (b), data are expressed on the ordinate scale as reduction of carotid arterial blood flow (ml min^{-1}), and reduction of coronary arterial diameter (μm), respectively; data on the abscissa are expressed as drug dose ($\mu g\ kg^{-1}$). Each point represents the mean response of between 8–10 separate experiments, with s.e.mean. Baseline carotid blood flow (ml min^{-1}) was 129 ± 10 ($n=10$) and 154 ± 12 ($n=10$) for CP-122,288 and sumatriptan, respectively; baseline coronary artery diameter (μm) was 3048 ± 231 ($n=8$) and 3351 ± 133 ($n=10$) for CP-122,288 and sumatriptan, respectively.

Over a dose-range of 1–300 $\mu g\ kg^{-1}$, CP-122,288 caused a dose-dependent reduction in carotid blood flow with a potency similar to that of sumatriptan (mean ED_{50} value [95% confidence limits]: CP-122,288 7 $\mu g\ kg^{-1}$ [5–11], $n=10$; sumatriptan 9 $\mu g\ kg^{-1}$ [7–11], $n=10$; Figure 3a). Neither compound produced a significant effect on coronary arterial blood flow at any of the doses tested. However, CP-122,288 and sumatriptan produced similar dose-related reductions in coronary artery diameter (mean ED_{50} value [95% confidence limits]: CP-122,288 18 $\mu g\ kg^{-1}$ [8–37], $n=8$; sumatriptan 19 $\mu g\ kg^{-1}$ [11–31], $n=10$; Figure 3b). Thus, both CP-122,288 and sumatriptan reduce carotid blood flow, and coronary arterial dimensions, at similar doses (1–300 $\mu g\ kg^{-1}$), but at the low doses of CP-122,288 which inhibit neurogenic inflammation in rat dura (<1 $\mu g\ kg^{-1}$), CP-122,288 is devoid of vasoconstrictor activity.

Discussion

In this paper, we have described the *in vivo* pharmacological profile of the novel indole-derivative, CP-122,288. Previously, this compound has been demonstrated to be an extremely potent inhibitor of a sterile neurogenic inflammation that occurs in guinea-pig dura mater following electrical stimulation of the trigeminal ganglion (Lee & Moskowitz, 1993). In the present study, experiments have been performed to evaluate this activity in a second animal species, rat, and to compare the dose-levels of CP-122,288 which inhibit neurogenic inflammation with those which alter cardiovascular parameters in the anaesthetized dog. In addition, we have considered whether CP-122,288 can inhibit an established and ongoing neurogenically-mediated inflammation within a timecourse that could be expected to translate to a rapid onset of relief of migraine symptoms in the clinic.

Thus, CP-122,288 has been shown to be an extremely potent (MED 3 ng kg^{-1}) and effective inhibitor of neurogenic inflammation in rat dura mater, being approximately 10^4 fold more potent than sumatriptan (MED 100 $\mu g\ kg^{-1}$). This profile is consistent with the effect of CP-122,288 described in other studies for example, in an equivalent guinea-pig model of neurogenic inflammation (MED 300 pg kg^{-1}, Lee & Moskowitz, 1993), at suppressing c-fos expression within guinea-pig trigeminal nucleus caudalis (MED 30 ng kg^{-1}, Cutrer *et al.*, 1995), and at inhibiting neurogenic inflammation induced after

stimulation of non-trigeminal sensory nerves in rat skin (MED 6 pg kg^{-1}, Kajekar *et al.*, 1995). From separate studies in the anaesthetized rat, it is unlikely that the high potency of CP-122,288 could be explained by an effect on haemodynamic parameters, since no significant changes in mean arterial blood pressure, or heart rate, were observed over the dose-range that inhibits neurogenic inflammation. Like sumatriptan, CP-122,288 had no effect on plasma protein extravasation in extracranial tissues which receive inputs from the second and third divisions of the trigeminal ganglion. Whilst the identity of the receptor, or receptors (see Buzzi *et al.*, 1991), which mediates the inhibition of neurogenic inflammation to CP-122,288, sumatriptan and the ergot alkaloids (Saito *et al.*, 1988) remains to be determined, it is emerging that its cranial distribution seems to be restricted to intracranial, rather than extracranial, perivascular trigeminal afferents. By use of the polymerase chain reaction, only the mRNA for the 5-HT_{1B} and 5-$HT_{1D\alpha}$ receptors have, so far, been shown to be expressed in trigeminal ganglia of rat (Bruinvels *et al.*, 1993) and man (5-$HT_{1D\alpha}$ only, Rebeck *et al.*, 1994). Since the 5-$HT_{1D\alpha}$ receptor is common to both rat and guinea-pig unlike the 5-HT_{1B} subtype, it is possible that CP-122,288 might inhibit neurogenic inflammation *via* activation of a prejunctional 5-$HT_{1D\alpha}$ heteroreceptor. However, from radioligand binding studies, we have demonstrated that CP-122,288 and sumatriptan share a similar nM affinity at the human recombinant 5-$HT_{1D\alpha}$ receptor (and also the 5-$HT_{1D\beta}$-subtype) which suggests that an action at the 5-HT_{1D} receptor may not account for the activity of CP-122,288 (p*Ki* values at 5-$HT_{1D\alpha}$ subtypes, respectively: CP-122,288 8.2 and 7.5, sumatriptan 8.0 and 7.6, unpublished observations). Therefore, the identity of the receptor which recognises CP-122,288, and perhaps sumatriptan, in models of neurogenic inflammation remains to be determined.

Studies described in the anaesthetized dog illustrate fully the potential significance of the high potency exhibited by CP-122,288 in models of neurogenic inflammation. In dog, both CP-122,288 and sumatriptan produced a dose-dependent reduction of carotid arterial blood flow over an identical dose-range (1–300 $\mu g\ kg^{-1}$). Notably, over the ng kg^{-1} dose-range at which CP-122,288 inhibits neurogenic inflammation, no reduction in carotid arterial blood flow was observed. When coronary arterial blood flow was measured, no effect was observed with either compound, even at the highest dose tested, 300 $\mu g\ kg^{-1}$. Although contractile '5-HT_{1D}-like' receptors have been described in canine (Parsons *et al.*, 1992) and human isolated coronary artery (Connor *et al.*, 1989; Cocks *et al.*, 1993), these are conductance rather than resistance vessels, and therefore it is perhaps not surprising that blood flow remained unaffected. However, when coronary arterial diameter was measured, both compounds produced a dose-dependent reduction in vessel diameter with equivalent potency. Importantly, dose levels of CP-122,288 which inhibited neurogenic inflammation ($<1\ \mu g\ kg^{-1}$) did not reduce coronary arterial dimensions. In contrast, sumatriptan produced an inhibition of neurogenic inflammation and a reduction of carotid blood flow and coronary dimensions over the same dose-range. These observations suggest that, potentially, CP-122,288 could be a useful acute treatment of migraine, without risk of cardiovascular side-effects.

The neurogenic inflammation hypothesis of migraine has not yet been accepted universally. Given that sumatriptan (6 mg) administered subcutaneously can produce a rapid relief of migraine symptoms (Pilgrim & Blakeborough, 1994), it could be argued that only a vasoconstrictor mechanism would be able to rapidly reverse a pathophysiological event(s) of vascular origin. By use of the original protocol to evaluate inhibitors of neurogenic inflammation, when compound is administered prior to stimulation of the trigeminal ganglion, it is not possible to deduce whether an established and ongoing inflammation could be inhibited rapidly by the prejunctional inhibition of peptide release. However, following a brief period of electrical stimulation of the trigeminal ganglion (5 min), Moskowitz and co-workers have demonstrated that a period of neurogenically-driven plasma protein extravasation from the dural vasculature remains for up to 45 min (Huang *et al.*, 1993). Thus, under these modified experimental conditions, CP-122,288 (present study) and sumatriptan (Huang *et al.*, 1993) produced an immediate and effective inhibition of plasma protein leakage. Importantly, the administration of compound during a period of continual firing of the trigeminal nerve did not reduce the ability of low ng kg^{-1} doses of CP-122,288 to inhibit plasma protein leakage. Therefore, if the inflammatory environment that develops in this modified experimental model mimics those events which occur pathophysiologically during migraine headache, it is possible that the ongoing inflammatory process could be inhibited by prejunctional inhibitors of peptide release within a timescale that could translate into a rapid onset of symptomatic relief in the clinic.

In conclusion, the work described in the present study demonstrates that CP-122,288 inhibits intracranial neurogenic inflammation at doses that are devoid of vasoconstrictor actions. Thus, these data demonstrate that CP-122,288 has the potential to be an effective acute treatment of migraine, without the risk of cardiovascular side-effects. Furthermore, CP-122,288 may prove to be a useful agent in delineating the relative importance of the pharmacological actions of current acute anti-migraine therapies.

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