Suppression by the sumatriptan analogue, CP-122,288 of c-fos immunoreactivity in trigeminal nucleus caudalis induced by intracisternal capsaicin

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1 The effects of an intravenously administered sumatriptan analogue were examined on c-fos-like immunoreactivity (c-fos-LI), a marker of neuronal activation, evoked within trigeminal nucleus caudalis (TNC) and other brain stem regions 2 h after intracisternal injection of the irritant, capsaicin (0.1 ml, 0.1 mM), in pentobarbitone-anaesthetized Hartley guinea-pigs.

2 C-fos-LI was assessed in eighteen serial sections (50 µm) using a polyclonal antiserum. A weighted average, reflecting total expression within lamina I, II_o of TNC was obtained from three representative levels (i.e., at –0.225 mm, –2.475 mm and –6.975 mm.)

3 Capsaicin caused significant labelling within lamina I, II_o, a region containing axonal terminations of small unmyelinated C-fibres, as well as within the nucleus of the solitary tract, area postrema and medial reticular nucleus. A similar distribution of positive cells was reported previously after intracisternal injection of other chemical irritants such as autologous blood or carrageenin.

4 Pretreatment with a conformationally restricted sumatriptan analogue (with some selectivity for 5-HT_{1B} and 5-HT_{1D} receptor subtypes) CP-122,288, reduced the weighted average by approximately 50–60% ($P < 0.05$) in lamina I, II_o at ≥ 100 pmol kg^{–1}, i.v., but did not decrease cell number within area postrema, nucleus of the solitary tract or medial reticular nucleus. A similar pattern was reported previously following sumatriptan, dihydroergotamine or CP-93,129 administration after noxious meningeal stimulation.

5 We conclude that modifications at the amino-ethyl side chain of sumatriptan dramatically enhance the suppression of c-fos expression within TNC, a finding consistent with its remarkable potency against neurogenic plasma protein extravasation within dura mater. CP-122,288 and related analogues may serve as an important prototype for drug development in migraine and related headaches.

Keywords: CP-122,288; trigeminovascular system; capsaicin; c-fos expression; 5-HT_{1B/D} agonist; sumatriptan analogue; migraine; headache; intracisternal

Introduction

Very few animal models have been developed to evaluate compounds of potential importance for the treatment of migraine and related headaches. Moreover, traditional models do not assess the effects of drugs on nociception, but utilize measures of blood flow (Friberg, 1991; L'acombe *et al.*, 1993), blood velocity (Zanette *et al.*, 1992; Zwetsloot *et al.*, 1992) or assess the ability of compounds to constrict or dilate vascular smooth muscle (Feniuk & Humphrey, 1992).

We have utilized the expression of the transcription factor and immediate early gene c-fos to assess the response of postsynaptic cells within the trigeminal nucleus caudalis (TNC) to noxious chemical meningeal stimulation, and the effects of potential treatments on this response (Nozaki, 1992a,b). C-fos expression serves as a marker of functional activity in neurones and has been demonstrated within lamina I and II_o of the dorsal horn of the spinal cord (Hunt *et al.*, 1987; Menetrey *et al.*, 1989; Presley *et al.*, 1990).

We previously reported that the analgesic, morphine, the 5-HT_{1D} receptor agonists, dihydroergotamine or sumatriptan, (which are used as symptomatic migraine therapy), and the selective 5-HT_{1B} receptor agonist, CP-93,129, block c-fos antigen expression in the rat TNC (Nozaki, 1992b) after noxious meningeal stimulation. In this paper, we tested the effects of CP-122,288 on c-fos-LI after intracisternal injection

of the irritant, capsaicin. CP-122,288 is a conformationally restricted indole-containing analogue which belongs to a series of analogues which can be used to probe the receptor-ligand recognition elements at the 5-HT_{1D} binding site (Macor *et al.*, 1992a,b). CP-122,288 possesses a chemical structure similar to that of sumatriptan with the exception that a C3-(R)-pyrrolidin-2-ylmethyl group has been incorporated into the C3-aminoethyl side chain of sumatriptan (Macor *et al.*, 1992a,b). We now demonstrate that CP-122,288 when administered intravenously to guinea-pigs 30 min prior to capsaicin injection, potently decreases c-fos-like immunoreactivity (c-fos LI) within laminae I and II_o.

Methods

Animal preparation

Male Hartley guinea-pigs (200–300 g; Charles River Laboratories, Wilmington, MA, U.S.A.) were anaesthetized with sodium pentobarbitone (40 mg kg^{–1}, i.p.) and maintained with 10 mg kg^{–1}, i.p., every 1–2 h as needed to suppress the withdrawal response to hindpaw stimulation. A soft catheter (PE-10, 0.28 mm internal diameter; Intramedic, Clay Adams, Parsippany NJ, U.S.A.) was introduced into the cisterna magna after a midline skin incision was made from the occipital protuberance to the cervical area. The animals were then placed in lateral decubitus position for 5.5 h as

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previously described (Nozaki *et al.*, 1992a). The animals then received a femoral vein injection of either the vehicle or drug. Thirty minutes later (6 h after catheter placement), capsaicin solution (100 μ l of 0.1 μ mol ml⁻¹) was injected into the cisterna magna over 1 min with a tuberculin syringe. (In previously published experiments, it was shown that 100 μ l of $\leq$ 0.1 mM capsaicin produced brisk c-fos expression in TNC; 218 cells per section). Therefore, this dosage was used in experiments described here (Cutrer *et al.*, 1994).

To facilitate capsaicin distribution within the subarachnoid space, animals were placed in the reverse Trendelenberg position (-30 degrees) for 30 min and in lateral position for the next 90 min. Core temperature was maintained at $36-37^{\circ}\text{C}$ by a homeothermic blanket (Harvard Apparatus No. 551, South Natick MA, U.S.A.). An overdose of pentobarbitone (80 mg kg⁻¹, i.p.) was used for euthanasia 2 h after capsaicin installation.

Physiological monitoring was carried out in randomly selected animals. Arterial pH, PCO_2 , and PO_2 (Corning 178 Blood Gas Analyzer, Ciba Corning Diag. Corp. Medford MA, U.S.A.), arterial pressure (MAP), heart rate (HR) and respiratory rate (RR) (MAC Lab ADI Instruments Castle Hill, Australia) were measured.

Animals were perfused via the ascending aorta with saline (0.9% 200 ml), followed by 500 ml formaldehyde (4%) in 0.1 M phosphate buffer (PB, pH 7.3). Perfused brainstems with attached cervical cords were stored overnight in the same fixative and were then placed in a cryoprotectant solution (20% sucrose, 30% ethylene glycol in 0.1 M PB) for 48 h before sectioning. The brainstems and upper cervical spinal cords were sectioned serially with a freezing microtome (Reichert-Jung; 2000 Leica, U.S.A.) from rostral to obex (1 mm) to the C2 level (190 sections $\pm$ 10), and every third 50 μ m section or 18 selected sections (see below) were saved and processed for immunohistochemistry.

Protocol

Drug vehicle (normal saline; $n = 7$) or CP-122,288, 10 pmol kg⁻¹, ($n = 3$) 100 pmol kg⁻¹ ($n = 3$) or 1.0 nmol kg⁻¹ ($n = 8$) was injected via the femoral vein 5.5 h after catheter placement and 2.5 h before the animals were killed.

Immunohistochemistry

Tissue sections were processed as free floating sections with the avidin-biotin procedure using commercially available kits (Vectastain ABC; Vector Labs Burlingame, CA, U.S.A.) as described previously (Uemura *et al.*, 1991), with slight modifications (Cutrer *et al.*, 1994). Briefly, sections were incubated in 10% normal goat serum, 0.03% hydrogen peroxide in 0.1 M phosphate buffered saline (PBS; pH 7.3), primary c-fos antibody (kindly provided by Dr Dennis Slamon, the Department of Hematology and Oncology at the University of California, LA, U.S.A.) (1/5000 dilution in PBS with 0.3% Triton X-100) (PBS-T Sigma Labs St. Louis, MO, U.S.A.) (room temperature overnight), secondary antibody, a biotinylated anti-rabbit IgG antiserum (1/200 dilution in PBS-T, Vector Labs, Burlingame, CA, U.S.A.) (room temperature for 2 h), ABC-peroxidase complex (Vector Lab) (room temperature 2 h) and finally a solution of 3,3'-diaminobenzidine tetrahydrochloride (40 mg%; Sigma Labs) and 0.003% hydrogen peroxide in 50 mM Tris-HCl buffer (pH 7.6) (room temperature for 20 min).

After immunohistochemical processing, tissue sections were serially arranged on gelatin slides, air dried overnight and coverslipped.

Cell counting

C-fos positive cells (i.e., stained nuclei) were counted by an observer naive to the treatment group (F.M.C.) and confirmed (in randomly selected sections) by a second inves-

tigator (V.L.) also blind to the treatment groups, as described previously (Cutrer *et al.*, 1994).

Weighted average methods

Cell numbers were compared using a weighted average of cells per section. Based on the observation that c-fos expression was maximal at -2.25 to -2.55 mm and decreased linearly on either side of this level, a weighted average of the cell number per section was calculated as follows: Six 50 μ m sections (every third section) were counted at each of three levels: $+0.15$ to -0.60 (midpoint: -0.225); -2.10 to -2.85 (midpoint: -2.475); and -6.60 to -7.35 (midpoint: -6.975). The mean values from the three levels were designated x_1 , x_2 and x_3 respectively. (The use of six samples at each of three levels was based on established statistical methods; Efron, 1979.) The values x_1 , x_2 and x_3 correspond to the heights AB, DC and EF respectively (see Figure 1). The total number of labelled cells within the two trapezoids, ABCD and CDEF (see Figure 1), can be estimated by the formula $15(x_1 + x_2)/2$ for ABCD and $30(x_2 + x_3)/2$ for CDEF. The sum of the two values is then divided by the total number of sections (45) to obtain the weighted average.

To validate the weighted average method, brainstem sections from animals receiving capsaicin (i.c.) plus either vehicle ($n = 5$) or CP-122,288 1 nmol kg⁻¹ ($n = 5$), were analyzed by both the weighted average sampling method and the counting of positively stained cells in TNC in every 3rd section within the same sampling area (45 total sections).

Qualitative estimate of c-fos LI

An assessment of the extent of c-fos LI in nuclei other than TNC (present in 18 sections) was performed in: nucleus of the tractus solitarius (NTS, 12 sections), area postrema (AP, 2 sections), medullary reticular nucleus (MRN, 8 sections) and lateral reticular nucleus (LRN, 6 sections) by an observer naive to the treatment protocol. An estimate of c-fos staining in nuclei other than TNC was evaluated qualitatively by a three point scale: 3+ indicating intense expression comparable to the amount seen in TNC (> 30 cells); 2+ indicating moderate expression (11-30); 1+ (1-10 cells); and 0 indicating no cells.

Figure 1 Number of cells in a representative animal expressing c-fos antigen when sampled in every third 50 μ m section from just rostral to the obex through C₂ after intracisternal capsaicin injection (0.1 ml, 0.1 mM). Each dot represents the number of cells per section in lamina I, II_o of the entire TNC. For comparison, a weighted average method was used to estimate cell number based on mean values obtained at coordinates AB, CD and EF (6 sections at each of three levels -0.225 , -2.475 and -6.975). The weighted average per animal was determined by the total area of the 2 trapezoids (ABCD and CDEF, see Methods) divided by the number of sections.

Drugs

Capsaicin solution (Polyscience Inc. Warrington, PA, U.S.A.) was made fresh weekly. Capsaicin 3.05 mg was diluted in 1 ml of saline:ethanol:Tween 80 (8:1:1) and sonicated for 5 min. The solution was further diluted (1:100) in normal saline and stored at 4°C as a stock solution. Sodium pentobarbitone was obtained from Anthony Products Co. Arcadia, CA, U.S.A. Lignocaine HCl, 2% was from Astra Pharmaceutical Products Inc., Westborough MA, U.S.A. CP-122,288 (Pfizer Inc., Groton, CT, U.S.A.) was diluted in saline from frozen aliquots for each experiment.

Statistics

Data from the TNC are expressed as a weighted average $\pm$ standard error of the mean (s.e.mean). The weighted averages were compared by a one sided Williams test which controls for multiple comparisons in a dose-response experiment (Williams, 1972). Statistical comparisons of mean arterial blood pressure (MABP), heart rate (HR) and respiratory rate (RR) were made between vehicle and CP-122,288-treated groups using Analysis of Variance plus Bonferroni/Dunnett *post hoc* tests. Qualitative data were analysed by the Mann-Whitney unpaired *t* test.

Results

Data were obtained from 75 animals.

Physiological parameters after capsaicin

Baseline values did not differ between groups. Mean arterial blood pressure, heart rate, respiratory rate or end tidal CO_2 did not change after CP-122,288 alone (data not shown). Capsaicin caused transient changes in all of the above parameters but differences between groups reached statistical significance at 1 min only (HR and MABP $P < 0.05$ see Figure 2).

C-fos expression after capsaicin

Spatial distribution C-fos-like immunoreactivity was present bilaterally and most intensely within dorsal and ventral aspects of laminae I and II, at each sampled level of TNC. The magnitude of c-fos expression was greatest -2.475 from obex with a mean in the control animals ($n = 7$) of 357 ± 24 cells per section. At -0.225 and -6.975 there was a mean of 210 ± 24 and 69 ± 10 cells per section, respectively.

C-fos positive cells were also observed in association with arachnoid and pial blood vessels throughout, but were most prominent in that portion of meninges overlying the trigeminal nucleus caudalis or the dorsal horn of the spinal cord. Their identity was not determined. No evidence of subarachnoid haemorrhage was seen at the time of dissection.

Three plus c-fos-LI was observed in the area postrema (AP) at the level of -0.225 whereas 2+ was seen in the ventral portion of the medullary reticular nucleus (MRN) and NTS; 1+ positive cells were also seen in the lateral reticular nucleus (LRN). At -2.475 mm, 1+ c-fos-LI was present in the NTS and in the MRN. At -6.975 mm, 1+ staining was present in laminae III, IV, V and X. There was sparse labelling in lamina VII (see Figure 3).

Validation of the weighted average method

The numbers obtained by counting every third section (45 sections) were not statistically different from the weighted average values either in the control group ($P < 0.35$) or in the CP 122,288-treated group ($P < 0.52$) (see Table 1). The percentage reductions were likewise very similar for the two methods (all sections, 51.5% versus the weighted average method, 54.1%).

Figure 2 Time-dependent changes in mean arterial blood pressure (MABP), heart rate (HR), and respiratory rate (RR) following intracisternal injection of capsaicin (0.1 ml, 100 nm) in pentobarbitone anaesthetized guinea-pigs pretreated 30 min before with vehicle ($n = 4$) or CP-122,288 (1 nmol kg^{-1}) ($n = 3$). (See Methods). Baseline values for MABP, HR and RR were 56 ± 4 mmHg, 257 ± 17 beats per min or 60 ± 7 respirations per min respectively in the vehicle group and 69 ± 9 mmHg, 285 ± 29 beats per min or 48 ± 9 respirations per min respectively after CP-122,288 treatment. Data are expressed as percentage baseline $\pm$ s.e.mean.

Drug treatment

Two hundred and twenty eight ± 12 cells were present per $50 \mu\text{m}$ section after capsaicin ($n = 7$). CP-122,288 administration 30 min prior to capsaicin, dose-dependently reduced c-fos expression within TNC. Pretreatment with 10 pmol ($n = 3$), 100 pmol ($n = 3$) and 1 nmol ($n = 8$) kg^{-1} reduced c-fos-LI by 35%, 56% ($P < 0.01$) and 59% ($P < 0.01$), respectively (see Figure 4). A dose of 10 pmol kg^{-1} did not significantly decrease the weighted average ($P > 0.05$) but decreased the number of c-fos positive cells at -6.975 mm by 54% ($P < 0.05$). After administering 100 pmol kg^{-1} , cell counts were significantly decreased at -6.975 and -2.475 mm by 57% and 78% respectively ($P < 0.05$). At 1 nmol kg^{-1} , significant reductions were found at all three anatomical levels (see Figure 5). Treatment with CP 122,288 appeared to reduce c-fos LI in the ventral portion of TNC more than in the dorsal portion in a few animals; however, this was not a consistent finding. CP 122,288 did not change the estimate of positive cells in area postrema, nucleus of the tractus solitarius, lateral reticular nucleus and medullary reticular nucleus.

Figure 3 Camera lucida drawings showing the location of c-fos antigen immunostained cells (dots) in coronal brain stem sections taken from vehicle (a) and CP-122,288 (1 nmol kg^{-1} , i.v.) (b)-injected guinea-pigs given capsaicin intracisternally (0.1 ml, 100 nM) and killed 120 min later. The distance (mm) caudal or rostral to the obex is given to the right of each drawing. The findings from these 2 animals are representative of the results in 7 animals for each group. Not shown are the c-fos protein-like immunoreactive cells in pia mater, arachnoid and ependyma. TNC, trigeminal nucleus caudalis, NTS, nucleus of the solitary tract.

Figure 4 Pretreatment with CP-122,288 dose-dependently decreases the c-fos immunoreactive cells evoked by intracisternal capsaicin injection. Cell numbers are given per $50 \mu\text{m}$ section within the trigeminal nucleus caudalis (TNC) (lamina I, II_o) as determined by a weighted average method (see Methods). Vehicle ($n = 7$), CP-122,288 10 pmol kg^{-1} ($n = 3$), 100 pmol kg^{-1} ($n = 3$) or 1 nmol kg^{-1} ($n = 8$) was injected 30 min prior to capsaicin and the animals killed 120 min later. * $P < 0.01$ as compared to vehicle-treated group.

Table 1 Comparison of average cells per section counted in every third section with the weighted average method obtained from eighteen selected sections in the same 10 guinea-pigs (5 NS vehicle and 5 CP-122,288 1 nmol kg^{-1} , i.v.)

Group	Animal	Method I	Method II		
NS	302931	153	193		
NS	302932	284	307		
NS	302935	262	263		
NS	311931	177	262		
NS	316932	152	184		
CP-122 288	302933	64	80		
CP-122 288	302934	102	103		
CP-122 288	311934	97	129		
CP-122 288	316931	94	102		
CP-122 288	316934	143	140		
	NS all cells	CP all cells	NS select	CP select	
Mean	206	100	242	111	
s.d.	63	29	52	24	
s.e.	28	13	23	11	
% Reduct.	All sections	Selected sections			
	51.5%	54.1%			

NS = normal saline; Method I, every third section counted; Method II, weighted average method in which 18 selected sections (6 each from obex, -2.25 and -6.75) were counted.

Figure 5 Distribution of c-fos protein-like immunoreactive cells at three different levels within TNC (lamina I, II_o) in animals that received capsaicin injection (0.1 ml, 100 nm) into the cisterna magna 30 min prior to vehicle ($n = 7$) or CP-122,288 (1 nmol kg^{-1} , $n = 8$), and killed 120 min later. Cells were counted in six $50 \mu\text{m}$ sections sampled every $150 \mu\text{m}$ at -0.225 (6 sections), -2.475 mm (6 sections) and -6.975 mm (6 sections). * $P < 0.05$ compared with vehicle-treated animals. Top figure shows a midsagittal section through brain stem and cerebellum. The arrows refer to the brain stem levels from which the analysis was performed.

Discussion

Intracisternal injection of the irritant, capsaicin, evoked c-fos-LI within TNC, particularly lamina I, II_o. The magnitude of expression was greatest -2.475 mm from obex. At more caudal levels, the number of positive cells gradually decreased to amounts comparable to vehicle-injected animals. The rostro-caudal distribution is similar to that previously reported by Nozaki *et al.* (1992a) after intracisternal instillation of autologous blood in the rat. Pretreatment with CP-122,288 ($\geq 100 \text{ pmol kg}^{-1}$, i.v.) significantly and dose-dependently attenuated the number of positive cells in TNC. The number of stained cells was reduced by a maximum of 59% at 1 nmol kg^{-1} . The reduction was not apparent in AP, MRN, LRN or NTS.

These findings are also consistent with the study of Nozaki *et al.* (1992b) showing that pretreatment with 5-HT_{1B} receptor agonist such as sumatriptan, dihydroergotamine or CP-93,129 significantly attenuated the c-fos response in TNC but not within AP or NTS after intracisternal injection of autologous blood. The 59% reduction after CP-122,288 (1 nmol kg^{-1} , i.v.) is larger than the maximal reduction after pretreatment with sumatriptan (720 nmol kg^{-1} , i.v. $\times 2$) or dihydroergotamine (86 nmol kg^{-1} , i.v. $\times 2$) [31% or 33% respectively], but is smaller than after pretreatment with morphine ($15 \mu\text{mol kg}^{-1}$, s.c.) [63%]. These reductions approximate the maximum decrease (62% to 67%) reported by others in lamina I, II_o following morphine 10 mg kg^{-1} administration (Presley *et al.*, 1990; Toelle *et al.*, 1990). Although the models used to test sumatriptan and dihydroergotamine are not identical to the capsaicin model described here, the potency of CP-122,288 is remarkably similar.

Cell counting methods

We developed a relatively simple method for estimating the extent of c-fos expression within trigeminal nucleus caudalis. The use of this estimation strategy has several advantages over counting every third section. It allows one to increase

the number of animals per treatment group, while also conserving antibody. We sampled six sections at each site because six gave the least variance.

C-fos expression and nociception

It is likely that activation of meningeal afferents is the major source of c-fos LI in TNC. Following the intracisternal instillation of autologous blood or carageenin, c-fos positive cells appear within 1 h, peak at 2 h and decline thereafter (Nozaki *et al.*, 1992a). As in the present study, labelling was found in both the ventral and dorsal aspects of TNC within lamina I, II_o which reflects the termination sites for small unmyelinated C-fibres from all three trigeminal divisions. The destruction of unmyelinated fibres by neonatal capsaicin treatment significantly decreased the number of positive cells as did surgical transection of trigeminal meningeal afferents (Nozaki *et al.*, 1992a). We presume that similar mechanisms are operating after capsaicin instillation.

Capsaicin as a nociceptive stimulus

Capsaicin has been extensively employed as an activating stimulus for c-fos expression (Ceccatelli *et al.*, 1989; Peltto-Huikko *et al.*, 1991). Strassman & Vos showed that injection of capsaicin into the supraorbital region of rats evoked c-fos expression in laminae I, II of the medullary trigeminal nuclear complex (Strassman & Vos, 1993). Capsaicin proved to be a superior irritant to autologous blood when injected into the subarachnoid space. The average number of cells within the TNC after subarachnoid haemorrhage was 37 ± 4 (Nozaki *et al.*, 1992a); whereas in the present study the number of labelled cells after capsaicin was 228 ± 12 . Capsaicin specifically activates the predominant population of meningeal nerve fibres which are the small unmyelinated C fibres. The evoked c-fos expression is 20 times that of animals receiving intracisternal vehicle injection (Cutrer *et al.*, 1994). Capsaicin solutions also proved less cumbersome to prepare and inject and unlike blood, its precise composition is known.

CP-122,288

The remarkable potency of CP-122,288 parallels its effect on neurogenic inflammation. CP-122,288 blocks neurogenic plasma protein extravasation within dura mater of guinea-pig with an 1800 fold greater potency than sumatriptan (EC_{50} of $1.66 \text{ pmol kg}^{-1}$ versus 3.0 nmol kg^{-1} for CP-122,288 and sumatriptan respectively) following electrical stimulation of the trigeminal ganglion (Lee & Moskowitz, 1993). CP-122,288, like sumatriptan, appears to block neurogenic inflammation via a prejunctional receptor and its effect is reversed partially by metergoline, a non-selective 5-HT_{1D} antagonist. However, the mechanism for its high potency in both the c-fos and plasma extravasation assays is not yet fully understood and is not likely to be accounted for by differences in affinities for the 5-HT_{1D} receptor as determined by autoradiographic ligand binding studies (sumatriptan: $252 \pm 40 \text{ nM}$ vs CP-122,288: $155 \pm 13 \text{ nM}$; EC_{50} to displace 1 nM [^3H]-5-CT in guinea-pig substantia nigra; C. Waeber unpublished data). Nevertheless, the existence of the mRNA encoding the 5-HT_{1D α} receptor subtype has recently been identified in trigeminal ganglia using the reverse transcriptase polymerase chain reaction (Rebeck *et al.*, 1993). More than a single receptor subtype or mechanism (perhaps non-5-HT-mediated) may be involved (Lee & Moskowitz, 1993). The development of a potent and selective receptor antagonist would help to clarify the role of the 5-HT_{1D} receptor subtype, as would experiments to determine the affinity of CP-122,288 for the 5-HT_{1F} receptor subtype, which also binds sumatriptan with nanomolar affinity. The high potency of CP 122,288 in this model might also be explained by increased brain penetration. However, information about its lipid partition

coefficient compared to sumatriptan is not at present available. It should be noted that CP 122,288 more potently reduces neurogenic plasma extravasation within the dura mater than sumatriptan (Lee & Moskowitz, 1993), an effect that does not require penetration of the blood brain barrier. We remain uncertain as to the relative importance of central and/or peripheral trigeminovascular receptors as to the effects of CP-122,288 or sumatriptan on the c-fos response following noxious meningeal stimulation. The results are consistent with both or either.

CP-122,288 constricts cat pial vessels with no greater potency than sumatriptan (Wei et al., unpublished data). In fact, topical application of CP-122,288 ($\geq 1 \mu\text{M}$) dilated pial vessels (5–10% above baseline). Barring significant species differences or differences due to intravenous versus topical

administration, these data strongly suggest that CP-122,288 does not block neurogenic inflammation or c-fos expression within TNC by a mechanism dependent upon vasoconstriction. Modifications at the amino ethyl side chain of sumatriptan may be a useful strategy to enhance the antimigraine properties of 5-HT_{1D} agonists without increasing vasoconstriction.

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