Br. J. Pharmacol. (1991), **104**, 3–4

SPECIAL REPORT

CP-93,129, a potent and selective 5-HT_{1B} receptor agonist blocks neurogenic plasma extravasation within rat but not guinea-pig dura mater

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Pretreatment with CP-93,129 blocked plasma extravasation in rat dura mater induced by electrical trigeminal ganglion stimulation when administered at $\geqslant 140\,nmol\,kg^{-1}$, i.v. but did not affect plasma leakage in guinea-pig at 460 or $1400\,nmol\,kg^{-1}$. Sumatriptan, a 5-HT_{1D}-like receptor agonist, blocked plasma extravasation in the guinea-pig model when administered at $7\,nmol\,kg^{-1}$. In as much as CP-93,129 binds with micromolar affinities to 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D}, and 5-HT_2 recognition sites, and with nanomolar affinity to the 5-HT_{1B} receptor subtype, blockade of plasma extravasation in the rat dura mater may be mediated by 5-HT_{1B} receptors whereas the 5-HT_{1D} receptor may be more relevant to the guinea-pig.

Keywords: 5-HT_{1B} receptors; 5-HT_{1D} receptors; migraine; dura mater; neurogenic inflammation

Introduction 5-HT_{1B} and 5-HT_{1D} are pharmacologically distinct but analogous subtypes of 5-hydroxytryptamine (5-HT) receptors which mediate similar functions in different species. 5-HT_{1B} binding sites are present in rat and mouse brain but are not found in membranes prepared from guinea-pig or human brain (Hoyer & Middlemiss, 1989). CP-93,129, the tautomer of 5-hydroxy-3(4-1,2,5,6-tetrahydropyridyl)-4-azaindole, exhibits marked affinity for 5-HT_{1B} binding sites in rat brain membranes (IC_{50} 15 nM with [^{3}H]-5-HT) and selectively inhibits adenylate cyclase activity in brain areas possessing a high density of 5-HT_{1B} (rat substantia nigra) but not 5-HT_{1A} (guinea-pig hippocampus) or 5-HT_{1D} (guinea-pig substantia nigra) receptors (Macor *et al.*, 1990).

5-HT_1 receptors mediate inhibition of plasma leakage within rat dura mater following trigeminal electrical stimulation (Buzzi *et al.*, 1991b). Leakage is attenuated or blocked in the rat by pretreatment with 5-carboxamidotryptamine $\gg$ 5-benzyloxytryptamine > dihydroergotamine > sumatriptan > 8-hydroxydipropylaminotetralin in descending order of potency. This potency order is most consistent with a 5-HT_{1B} or 5-HT_{1D} response among the known 5-HT_1 family of receptors although methiothepin did not block the effect of sumatriptan nor metergoline the effects of 5-CT.

Studies were therefore undertaken in order to clarify the receptor subtype which mediates inhibition of neurogenic plasma extravasation, and to examine one possible functional correlate of the marked ligand binding selectivity.

Methods *Electrical trigeminal stimulation* Male Sprague-Dawley rats (150–200 g) and male Hartley guinea-pigs (200–250 g) (Charles River Laboratories, Wilmington, MA, U.S.A.) were anaesthetized with pentobarbitone (50 or $40\,mg\,kg^{-1}$, i.p., rats or guinea-pigs, respectively), placed in a stereotaxic frame with the incisor bar set at −1.5 mm (rats) or −4.0 mm (guinea-pigs). Symmetrical burr holes were drilled 3.0 mm laterally and 3.7 mm posteriorly from bregma (rats) or 4.0 mm and 4.0 mm, respectively in guinea-pigs. [^{125}I]-BSA $50\,\mu Ci\,kg^{-1}$ was then injected. After 5 min, electrodes were lowered 9.5 mm (rats) or 10.5 mm (guinea-pigs) from dura mater. The right ganglion was stimulated (5 min, 1.2 mA, 5 Hz, 5 ms duration). Ten minutes before stimulation and 5 min before [^{125}I]-albumin administration, rats were injected with CP-93,129 (46, 140 or $460\,nmol\,kg^{-1}$; $n = 5$, 9 or 6, respectively). Guinea-pigs were injected with CP-93,129 (460 or $1400\,nmol\,kg^{-1}$; $n = 7$, 8, respectively) or sumatriptan (2, 7 $nmol\,kg^{-1}$; $n = 5$, 6, respectively). Each dose was tested in at least two separate experiments. Animals were perfused with saline via the left cardiac ventricle for 2 (rats) or 3 min (guinea-pigs) at constant pressure (100 mmHg) to remove intravascular [^{125}I]-BSA. The dura mater was dissected as previously described (Markowitz *et al.*, 1987; Buzzi & Moskowitz, 1990) and radioactivity determined on the two sides.

Capsaicin administration The left femoral vein was exposed in pentobarbitone-anaesthetized guinea-pigs, and CP-93,129 injected. Five minutes later, [^{125}I]-BSA ($50\,\mu Ci\,kg^{-1}$) was injected as a bolus. After an additional 5 min, capsaicin ($0.5\,\mu mol\,kg^{-1}$) or vehicle was infused over 3 min. Ten minutes later, animals were perfused with saline as described above.

Data analysis [^{125}I]-BSA extravasation is expressed as the ratio: c.p.m. mg^{-1} (stimulated side)/c.p.m. mg^{-1} (unstimulated side). In capsaicin experiments, data are expressed as % c.p.m. mg^{-1} between vehicle- and drug-treated animals. Results are expressed as mean ± s.e.mean. Unpaired Student's t test was used for statistical analysis. Probability values (P) of less than 0.05 were considered significant.

Drugs [^{125}I]-bovine serum albumin (BSA; New England Nuclear, Boston, MA) and sumatriptan (Glaxo Ltd, Hertfordshire, England) were diluted in saline; capsaicin (Polyscience Inc., Wilmington, Pennsylvania) was solubilized in saline:ethanol:Tween 80 8:1:1; CP-93,129 (Pfizer, Inc, Groton, CT, U.S.A.) was dissolved in dimethylsulphoxide:saline 1:9. All drugs were injected intravenously ($1\,ml\,kg^{-1}$). The same volume of vehicle was administered.

Results There was no mortality after electrical stimulation or drug administration. CP-93,129 ($140\,nmol\,kg^{-1}$) did not change arterial blood pressure when intra-arterial monitoring (20 min) was performed in selected animals ($n = 3$ rats).

Albumin (c.p.m. mg^{-1} wet wt.) was 16.0 ± 1.6 (rats) and 17.3 ± 1.2 (guinea-pigs) on the side contralateral to stimu-

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lation. Leakage ipsilateral to the stimulation was 28.8 ± 2.8 (rats) and 28.3 ± 2.1 (guinea-pigs). The ratio ranged from 1.6–2.1 (rats) or from 1.3–2.0 (guinea-pigs) in 5 (rats) or 6 (guinea-pigs) separate experiments.

The amount of protein leakage on the unstimulated side did not differ between vehicle- or CP-93,129-treated rats or guinea-pigs. The data (c.p.m. mg^{-1} wet wt.) contralateral to stimulation in rat was 20.1 ± 2.2 (vehicle, $n = 5$) versus 21.5 ± 3.4 ($n = 5$) after 46 nmol kg^{-1}, 16.2 ± 1.9 (vehicle, $n = 8$) versus 14.7 ± 1.5 ($n = 9$) after 140 nmol kg^{-1}, and 15.3 ± 1.7 (vehicle, $n = 4$) versus 17.2 ± 3.2 ($n = 6$) after 460 nmol kg^{-1}.

Pretreatment with CP-93,129 (140 or 460 nmol kg^{-1}) decreased the ratio in rats but not guinea-pigs (460, 1400 nmol kg^{-1}). Sumatriptan (7 nmol kg^{-1}) decreased the ratio in the guinea-pigs from 1.60 ± 0.12 to 1.15 ± 0.06 (Figure 1).

Capsaicin increased [^{125}I]-BSA leakage in guinea-pig dura mater but CP-93,129 (1400 nmol kg^{-1}) did not block the extravasation response [149 ± 7% in capsaicin-treated group ($n = 9$) versus 139 ± 5% in CP-93,129-treated animals ($n = 8$)].

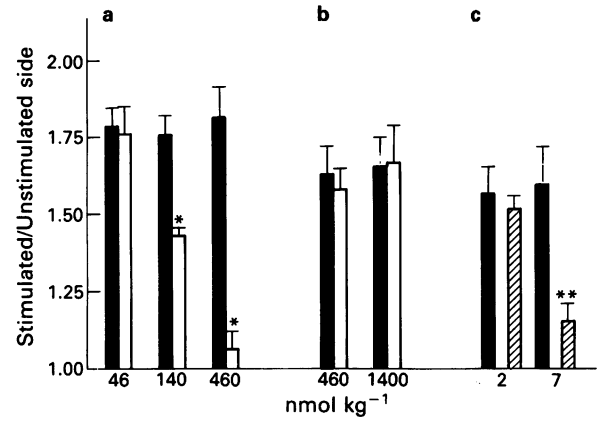

Figure 1 CP-93,129, a potent and selective 5-HT_{1B} receptor agonist, blocks neurogenic plasma extravasation within rat (a) but not guinea-pig (b) dura mater whereas sumatriptan, a potent 5-HT_{1D}-like agonist, blocks the response in the guinea-pig dura mater (c). CP-93,129 (46, 140, 460 nmol kg^{-1}, i.v. to rats; 460, 1400 nmol kg^{-1}, i.v. to guinea-pigs) or sumatriptan (2, 7 nmol kg^{-1}, i.v. to guinea-pigs) was administered to pentobarbitone-anaesthetized animals at the indicated doses 10 min prior to electrical trigeminal stimulation (1.2 mA, 5 Hz, 5 ms, 5 min) and 5 min prior to [^{125}I]-albumin (i.v., 50 μCi kg^{-1}) (see Methods). Animals were perfused with saline, and the tissues removed and counted for radioactivity. Data are expressed as the ratio of c.p.m. mg^{-1} on the stimulated and unstimulated sides. The solid columns represent the vehicle-treated animals. The open columns represent the CP-93,129-treated animals, the hatched columns the sumatriptan-treated animals; vertical bars show s.e.mean. * $P < 0.005$ or ** $P < 0.01$ as compared to vehicle-treated animals.

Discussion The importance of the 5-HT_{1B} receptor in the rat plasma extravasation model is documented in this report. The virtual selectivity of CP-93,129 for the 5-HT_{1B} recognition site provides strong evidence in support of this conclusion. Ligand binding experiments indicate that CP-93,129 exhibits 200 fold greater affinity for 5-HT_{1B} than 5-HT_{1A} (rat cortex), 150 fold greater affinity for 5-HT_{1B} than 5-HT_{1D} (bovine striatum), 400 fold greater affinity for 5-HT_{1B} than 5-HT_{1C} (pig choroid plexus), 2,400 fold greater affinity for 5-HT_{1B} than 5-HT_2 binding sites (Macor *et al.*, 1990). The compound does not bind to dopamine, noradrenaline or adenosine recognition sites.

CP-93,129 was inactive when tested in the guinea-pig model at 1,400 nmol kg^{-1} whereas sumatriptan was quite potent. The threshold dose of sumatriptan in the guinea-pig electrical stimulation model was 30 fold lower than in the rat model. This correlates with the apparent greater affinity (26 fold) of sumatriptan at the 5-HT_{1D} than 5-HT_{1B} recognition sites (Hoyer *et al.*, 1989).

Inhibition of neurogenic plasma extravasation in rat dura mater is mediated by prejunctional 5-HT_1 heteroreceptors (Buzzi *et al.*, 1991b). Receptor activation attenuates mast cell secretion and degranulation, platelet aggregation, endothelial activation which develops within postcapillary venules during electrical trigeminal ganglion stimulation (Buzzi *et al.*, 1990; Dimitriadou *et al.*, 1990). Dihydroergotamine or sumatriptan does not block the substance P- or neurokinin A-induced extravasation (Saito *et al.*, 1988; Buzzi & Moskowitz, 1990) but attenuates stimulation-induced elevations in plasma immunoreactive calcitonin gene-related peptide levels within sagittal sinus blood (Buzzi *et al.*, 1991a).

5-HT_{1B} receptor-mediated inhibition of neurotransmitter release is well known within rat brain (Macor *et al.*, 1990). Taken together with the data reported here, an important inhibitory role for the 5-HT_{1B} receptor in both the peripheral and central nervous system of rodents such as the rat seems likely.

Supported by NIH grant NS 21558.

References

BUZZI, M.G., CARTER, W.B., SHIMIZU, T., HEATH, H. & MOSKOWITZ, M.A. (1991a). Dihydroergotamine and sumatriptan attenuate the increase in plasma CGRP levels within rat superior sagittal sinus during electrical trigeminal stimulation. *Neuropharmacol.*, (in press).

BUZZI, M.G., DIMITRIADOU, V., THEOHARIDES, T.C. & MOSKOWITZ, M.A. (1990). Morphological effects of electrical trigeminal ganglion stimulation (ETG) on intra- and extracanial vessels. *Soc. Neurosci.*, **16**, 160.

BUZZI, M.G. & MOSKOWITZ, M.A. (1990). The antimigraine drug, sumatriptan (GR43175), selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. *Br. J. Pharmacol.*, **90**, 202–206.

BUZZI, M.G., MOSKOWITZ, MA., PEROUTKA, S.J. & BYUN, B. (1991b). Further characterization of the putative 5-HT receptor which mediates blockade of neurogenic plasma extravasation in rat dura mater. *Br. J. Pharmacol.*, (in press).

DIMITRIADOU, V., BUZZI, M.G., LAMBRACHT-HALL, M., MOSKOWITZ, M.A. & THEOHARIDES, T.C. (1990). *In vivo* and *in vitro* ultrastructural evidence for stimulation of dura mast cells by neuropeptides. *Soc. Neurosci.*, **16**, 161.

HOYER, D. & MIDDLEMISS, D.N. (1989). Species difference in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol. Sci.*, **10**, 130–132.

HOYER, D., SCHOEFFTER, P. & GRAY, J.A. (1989). A comparison of the interactions of dihydroergotamine, ergotamine and GR 43175 with 5-HT_1 receptor subtypes. *Cephalalgia*, **9**(Suppl 10), 340–341.

MACOR, J.E., BURKHART, J.H., HEYM, J.H., IVES, J.L., LEBEL, L.A., NEWMAN, M.E., NIELSEN, J.A., RYAN, K., SCHULZ, D.W., TORGERSEN, L.K. & KOE, B.K. (1990). 3-(1,2,5,6-Tetrahydropyrid-4-yl) pyrrolo[3,2-b]pyrid-5-one: a potent and selective serotonin (5-HT_{1B}) agonist and rotationally restricted phenolic analogue of 5-methyl-3-(1,2,5,6-tetrahydropyrid-4-yl)indole. *J. Med. Chem.*, **33**, 2087–2093.

MARKOWITZ, S., SAITO, K. & MOSKOWITZ, M.A. (1987). Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain. *J. Neurosci.*, **7**, 4129–4136.

SAITO, K., MARKOWITZ, S. & MOSKOWITZ, M.A. (1988). Ergot alkaloids block neurogenic extravasation in dura mater: Proposed action in vascular headache. *Ann. Neurol.*, **24**, 732–737.

(Received March 11, 1991
Accepted May 9, 1991)