SPECIAL REPORT

Effect of a 5-HT₁ receptor agonist, CP-122,288, on oedema formation induced by stimulation of the rat saphenous nerve

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Neurogenic oedema formation in the rat hind paw skin induced by electrical stimulation of the saphenous nerve and measured by extravasation of [¹²⁵I]-albumin, was inhibited by the 5-HT_{1B} receptor agonist, CP-93,129, and the novel tryptamine analogue, CP-122,288. Significant inhibition of up to 66% of control was observed with CP-122,288 (2×10^{-14} – 2×10^{-7} mol kg⁻¹) and CP-93,129 (5×10^{-7} – 5×10^{-6} mol kg⁻¹), with the minimum effective dose for CP-122,288 being about 10⁷ fold less than that for CP-93,129. Oedema formation induced by the intradermal administration of exogenous mediators (substance P and histamine) in rat dorsal skin was not inhibited by CP-122,288 (2×10^{-10} mol kg⁻¹). These results suggest that CP-122,288 is a potent inhibitor of neurogenic inflammation in rat skin and that the effect may be due to a prejunctional inhibition of neuropeptide release.

Keywords: Neurogenic inflammation; 5-hydroxytryptamine; 5-HT_{1B}; CP-122,288; substance P; CGRP; sensory nerves

Introduction Electrical stimulation of the rat saphenous nerve leads to increased blood flow and oedema formation in skin of the hind paw (Lembeck & Holzer, 1979). Studies, using receptor antagonists, suggest that substance P and calcitonin gene-related peptide (CGRP) are major mediators of plasma protein extravasation and vasodilatation in this model (Garret *et al.*, 1991; Escott & Brain, 1993). Thus, attenuation of neurogenic inflammation may be achieved with selective receptor antagonists; however, this approach is limited to inhibiting postjunctional responses mediated *via* specific receptor types. An alternative approach is to inhibit neuropeptide release *via* a prejunctional mechanism. Sumatriptan, a 5-HT_{1D} receptor agonist and CP-93,129, a selective 5-HT_{1B} receptor agonist inhibit neurogenic plasma extravasation in the dura mater of rat and/or guinea-pig by inhibiting neuropeptide release (Buzzi & Moskowitz, 1990; Matsubara *et al.*, 1991). Furthermore, it has been suggested that the therapeutic efficacy of sumatriptan in migraine is related to this activity and that the inhibitory effects of sumatriptan-like agents have, to date, only been reported in selected tissues innervated by the trigeminal nerve. Recently, a C-3 conformationally restricted tryptamine analogue, CP-122,288 (Macor *et al.*, 1992), has been shown to be considerably more potent than sumatriptan in the guinea-pig dura mater (Lee & Moskowitz, 1993). In this study we provide evidence, for the first time, that CP-122,288 and CP-93,129 can also act to inhibit neurogenic inflammation induced after stimulation of non-trigeminal sensory nerves.

Methods Male Wistar rats (200–250 g), anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.p.), were prepared to enable electrical stimulation (10 V, 1 ms, 2 Hz for 5 min) of the saphenous nerve of the test leg, while the contralateral leg served as a sham control (Escott & Brain, 1993). [¹²⁵I]-albumin (50 kBq), mixed with Evans Blue dye (25 mg kg⁻¹) and test ligand or saline (control) were given i.v. 5 min prior to stimulation. After stimulation, blood was taken and the rat killed. [¹²⁵I]-albumin was measured in a plasma sample and in the skin from the paws. Results are calculated as oedema (µl 100 mg⁻¹ tissue) and expressed as a ratio of

stimulated to unstimulated skin. The effect of test agents is shown as percentage (%) inhibition of the control response in saline-treated animals. Oedema responses (µl plasma per site) produced to intradermally-injected (i.d., 0.1 ml) agents were measured in rat dorsal skin (Brain & Williams, 1985). In separate experiments the haemodynamic effect of compounds following i.v. administration was studied in anaesthetized rats by measuring blood pressure *via* a carotid artery cannula.

Materials CP-122,288 ((R)-N-methyl-[3-(1-methyl-2-pyrroli-dinylmethyl)-1H-indol-5-yl] methanesulphonamide) (Pfizer Central Research, Sandwich, U.K.), CP-93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one) (Central Research Division, Pfizer Inc., Groton, U.S.A.), human α-CGRP (a gift from Dr U. Ney, Celltech, U.K.), substance P, histamine diphosphate salt and Evans Blue dye (Sigma Chemical Co., Dorset, U.K.), [¹²⁵I]-human serum albumin (Amersham International, U.K.) and sodium pentobarbitone (Sagatal, May and Baker, Essex, U.K.) were used.

Statistical analysis ANOVA followed by multiple comparisons test (Dunnnett, Figure 1a and Bonferroni, Figure 1b).

Results In control studies (saline, 1 ml kg⁻¹, i.v.) plasma extravasation in the rat hind paw was 14.07 ± 0.96 µl in the stimulated, and 1.68 ± 0.08 µl in the sham paw, mean $\pm$ s.e.mean, $n = 29$. CP-93,129 and CP-122,288 inhibited oedema formation in the stimulated paw (Figure 1a), and the minimum effective doses for CP-93,129 and CP-122,288 were 5×10^{-7} mol kg⁻¹ and 2×10^{-14} mol kg⁻¹, respectively. At the highest doses tested, both compounds produced a similar inhibitory effect (upto 66%). Thus, CP-122,288 was about 10⁷ fold more potent than CP-93,129. Neither CP-122,288 nor CP-93,129 affected oedema formation in the sham paw (plasma extravasation in control 1.9 ± 0.3 µl, CP-122,288 (2×10^{-10} mol kg⁻¹) 2.07 ± 0.2 µl; CP-93,129 (5×10^{-6} mol kg⁻¹) 1.6 ± 0.1 µl; $n = 6$ for each group). Furthermore, no change in mean arterial pressure (MAP) was observed following i.v. administration of CP-122,288 (MAP: before CP-122,288 (2×10^{-10} mol kg⁻¹, i.v.), 82 ± 3 , and 10 min after, 84 ± 3 mmHg; before saline (i.v.) 93 ± 12 , and 10 min after, 98 ± 14 mmHg; $n = 5$ for both

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Figure 1 (a) Effect of CP-122,288 (■) and CP-93,129 (○) on plasma extravasation in rat skin after electrical stimulation (10 V, 1 ms, 2 Hz for 5 min) of the saphenous nerve. Results are expressed as % inhibition of oedema in test rats compared to control rats, mean $\pm$ s.e.mean, $n = 6$ in each group. ** $P < 0.01$, Dunnett multiple comparisons test. (b) Effect of i.v. CP-122,288 on oedema induced by substance P and histamine in rat skin. Mediators were injected i.d. 5 min after i.v. agent and oedema formation measured over 30 min. Responses to substance P (SP) and histamine (HA) $\pm$ calcitonin gene-related peptide (CGRP) are shown. Open columns represent control (saline) animals and solid columns represent CP-122,288 (2×10^{-10} mol kg⁻¹)-treated animals. Results are expressed as the mean $\pm$ s.e.mean, $n = 6$. No significant difference of treatments was observed in CP-122,288 and saline-treated animals.

groups, $P > 0.05$). CP-122,288 (2×10^{-10} mol kg⁻¹, i.v.), as shown in Figure 1b, did not significantly inhibit oedema induced by i.d. administered substance P or histamine in either the presence or absence of a potentiating dose of the vasodilator CGRP (see Brain & Williams, 1985).

Discussion These results demonstrate that neurogenically-induced oedema formation in the rat paw is attenuated by CP-122,288 and CP-93,129. The inability of CP-122,288 to alter oedema formation induced by exogenously administered mediators of inflammation indicates that it does not act postjunctionally as a receptor antagonist. These findings lead us to suggest that CP-122,288 may inhibit neuropeptide release by acting *via* prejunctional receptors. It has been proposed that sumatriptan and CP-93,129 act selectively to inhibit neurogenic inflammation in the dura mater (Mos-

kowitz, 1992) and our results indicate that CP-93,129 and sumatriptan (Escott & Brain, unpublished) possess only a weak activity in the rat paw. However, the inhibitory threshold level for CP-122,288 in the rat paw is comparable to that observed in the guinea-pig dura mater (Lee & Moskowitz, 1993). The receptor subtype at which CP-122,288 acts is not yet determined and the fact that a total inhibition of oedema formation was not observed will be the subject of further study. Nevertheless, the potent ability of CP-122,288 to inhibit neurogenic oedema in non-trigeminal sensory nerve-innervated tissue is clearly demonstrated. These results indicate that a drug such as CP-122,288 could be of use for the treatment of inflammatory disease states, in which neurogenic plasma extravasation occurs.

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