The non-peptide neurokinin₁ receptor antagonist, RP 67580, blocks neurogenic plasma extravasation in the dura mater of rats

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A non-peptide neurokinin₁ (NK₁) receptor antagonist, RP 67580, that is selective for the rodent subtype of the NK₁ receptor, dose-dependently reduced plasma extravasation in the dura mater produced by electrical stimulation of the trigeminal ganglion in rats, with an ID₅₀ of 0.6 µg kg⁻¹. Its enantiomer RP 68651 was some 400 fold less active. The results indicate that neurogenic plasma extravasation within the dura mater is NK₁ receptor-mediated and suggest that NK₁ receptor antagonists may have a role as antimigraine agents.

Keywords: Neurokinin, receptor extravasation; trigeminal ganglion; migraine

Introduction Neurogenic inflammation within cranial tissues has been proposed as a mechanism in the pathogenesis of migraine (Moskowitz, 1992). The dura mater, a pain-producing cranial structure, and its blood vessels are innervated by the trigeminal and upper cervical sensory nerves. Neurogenic plasma extravasation mediated presumably through the release of sensory neuropeptides e.g. substance P and calcitonin gene-related peptide, has been shown to develop within the dura and other receptive fields (lip, conjunctiva, eyelid) of trigeminal sensory nerve fibres upon electrical stimulation of the trigeminal ganglion. It has previously been shown that ergot alkaloids, with clinical effectiveness against migraine, block neurogenic extravasation within the dura evoked by electrical stimulation of the trigeminal ganglion (Saito *et al.*, 1988). Recently sumatriptan, a 5-HT_{1D} receptor agonist with proven anti-migraine activity, has been shown to have similar effects in the trigeminal stimulation model (Buzzi & Moskowitz, 1990) and it was suggested that this was due to inhibition of sensory neuropeptide release. Fardin *et al.* (1992) have described a non-peptide NK₁ receptor antagonist, RP 67580 ((3aR, 7aR)-7, 7-diphenyl-2-(1-imino-2-(2-methoxyphenyl)ethyl)perhydroindole) which is selective for rodent NK₁ receptors whilst its (3aS, 7aS) enantiomer, RP 68651, is much less active. These enantiomers, synthesized in our laboratories, had IC₅₀s against [¹²⁵I]-Tyr⁸-substance P on cloned rat NK₁ receptors expressed in CHO cells of 4.5 nM for RP 67580 and > 3 µM for RP 68651 (Cascieri; personal communication). Using RP 67580 and RP 68651 we have investigated the role of NK₁ receptors in mediating neurogenic plasma extravasation in the dura and extracranial tissues of rats following trigeminal ganglion stimulation.

Methods Male Sprague-Dawley rats (approx 200 g) were anaesthetized with pentobarbitone sodium (60 mg kg⁻¹, i.p.) and a femoral vein and artery were cannulated for intravenous injections and the monitoring of arterial blood pressure respectively. Animals were placed in a stereotaxic frame and burr holes drilled in the skull bilaterally 3.2 mm posterior and 2.5 mm lateral from bregma to allow for placement of stimulating electrodes. After intravenous administration of either RP 67580 (0.1–1000 µg kg⁻¹), RP 68651 (300 µg kg⁻¹) or distilled water vehicle (*n* = 8–10 per dose group), concentric bipolar stimulating electrodes were lowered 9 mm below the surface of the dura bilaterally into the trigeminal ganglia. [¹²⁵I]-radiolabelled bovine serum albumin (70 µCi kg⁻¹) was then given intravenously as a plasma marker. At

10 min after the drug or vehicle injection, the right trigeminal ganglion only was electrically stimulated (5 Hz, 5 ms, 25 V for 5 min, giving a maintained stimulus current in the range of 1 to 2.5 mA measured by voltage drop across a resistance). At the end of the stimulation period the animals were left for 5 min before being exsanguinated prior to dissection of the dura from both sides of the skull. The area of dura immediately surrounding the sites of electrode penetration was discarded. Samples of extracranial tissues (conjunctiva, eyelid and lip) innervated by the trigeminal nerves were also taken. Tissues were rinsed, dried overnight, weighed, then counted for radioactivity. The counts mg⁻¹ dry weight of tissue in the stimulated and unstimulated sides were calculated and results are expressed as an extravasation ratio, i.e. the ratio of the stimulated to unstimulated sides. For example, the mean c.p.m. mg⁻¹ in the dura of stimulated and unstimulated sides were 455 and 220 respectively giving a ratio of 2.1. Differences were assessed by ANOVA and a Dunnett's *t* test.

Results Following electrical stimulation of the trigeminal ganglion in vehicle-treated animals, the extravasation ratio in dura, conjunctiva, eyelid and lip was 2.1 ± 0.1, 1.6 ± 0.2, 7.6 ± 1.5 and 7.7 ± 1.2 respectively (*n* = 10, values are mean ± s.e.mean). Pretreatment with RP 67580 produced dose-related reductions in the ratio of extravasation within the dura that were significant (*P* < 0.05) at doses of 10 µg kg⁻¹ and greater when compared to vehicle pretreatment (Figure 1). An inhibition curve fitted to these data using non-linear least squares analysis (RS1) gave an ID₅₀ (i.e. dose of RP 67580 producing half maximal inhibition of extravasation) of 0.6 µg kg⁻¹. RP 67580 reduced extravasation in the facial tissues (conjunctiva, eyelid and lip) only at doses of 100 µg kg⁻¹ or more. A comparison of the effects of RP 67580 at a dose of 10 µg kg⁻¹ on the dura and the facial tissues (Figure 1) shows that the extravasation in the dura is almost completely blocked by this dose, but there is little effect on the extravasation in the facial tissues. Thus at low doses (< 100 µg kg⁻¹) RP 67580 appears relatively selective in reducing extravasation in the dura over the facial tissues, but at higher doses it loses this apparent tissue selectivity. Pretreatment with RP 68651, the less active (3aS, 7aS) enantiomer of RP 67580, at a dose of 300 µg kg⁻¹, i.v. gave an extravasation ratio of 1.53 ± 0.1 (*n* = 7) in the dura and had no effect in the facial tissues. In all experiments the mean arterial blood pressure after dosing with vehicle or antagonist was no different at the point of trigeminal ganglion stimulation from that before dosing.

Discussion In the present experiments the NK₁ receptor-selective antagonist, RP 67580, was some 400 fold more

Figure 1 Histograms illustrating the effects of RP 67580 on plasma extravasation produced in dura (a) and facial tissues (conjunctiva (b), eyelid (c), lip (d)) by electrical stimulation of the trigeminal ganglion in the rat. Data are expressed as an extravasation ratio, i.e. ratio of extravasation in stimulated/unstimulated sides. Open column = vehicle (distilled H₂O); hatched column = RP 67580, (0.1, 1.0, 10, 100, or 1000 $\mu\text{g kg}^{-1}$ i.v., $n = 8-10$). Statistical analysis was performed by ANOVA and a Dunnett's test; * $P < 0.05$, vertical bars = s.e.mean.

active than RP 68651, demonstrating a highly effective ($\text{ID}_{50} = 0.6 \mu\text{g kg}^{-1}$) and enantiomerically selective inhibition of plasma extravasation in the dura of the rat. This indicates that plasma extravasation in the dura after electrical stimulation of trigeminal sensory afferent fibres is predominantly NK₁ receptor-mediated and is likely to be due to substance P release, as this peptide is the endogenous ligand that acts mainly at the NK₁ receptor. The present result contrasts with that of Buzzi and colleagues (cited as unpublished observations, Buzzi *et al.*, 1992) who suggested that NK₁ antagonists have poor activity in this rodent model, although the NK₁ antagonist, CP-96,345 used in their experiments has selectivity for the human NK₁ receptor subtype over the rodent subtype (Beresford *et al.*, 1991; Fardin *et al.*, 1992).

RP 67580 shows significant inhibition of dural extravasation at doses that have no effect on cardiovascular parameters and thus it is unlikely that the reduction in extravasation seen with this compound is due to any reduction in tissue perfusion pressure. The apparent dural selectivity of RP 67580 over the facial tissues has been observed previously by Buzzi & Moskowitz (1990) with the 5-HT_{1D} agonist, sumatriptan, which markedly inhibited the extravasation in the dura at a dose of 100 $\mu\text{g kg}^{-1}$ but had no effect on extravasation in extracranial tissues at a dose of 300 $\mu\text{g kg}^{-1}$. The reasons for this selectivity are as yet unknown, but may reflect differences in the physical limits imposed on extravasation in the different tissue types, or differences in the receptor mediated stimulus-response mechanisms.

This study has shown that RP 67580 is more active (maximal inhibition at 10 $\mu\text{g kg}^{-1}$) at this rodent trigeminal model of migraine than ergotamine tartrate and dihydroergotamine (100 and 50 $\mu\text{g kg}^{-1}$ respectively, Saito *et al.*, 1988) or sumatriptan (100 $\mu\text{g kg}^{-1}$, Buzzi & Moskowitz, 1990). Clinical studies have shown that the ergot alkaloids and the 5HT_{1D} agonist, sumatriptan, are effective anti-migraine agents. The present studies therefore indicate that NK₁ receptor antagonists could also prove effective in the treatment of migraine headache.

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