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Blockade by oral or parenteral RPR 100893 (a non-peptide NK_1 receptor antagonist) of neurogenic plasma protein extravasation within guinea-pig dura mater and conjunctiva

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1 The ability of an NK_1 receptor antagonist, RPR 100893, and its enantiomer, RPR 103253 to block neurogenic plasma protein extravasation in guinea-pig dura mater and conjunctiva was assessed following ^{125}I-labelled bovine serum albumin ([^{125}I]-BSA, 50 μCi kg^{-1}, i.v.) and unilateral electrical stimulation of the trigeminal ganglion (0.6 mA, 5 ms, 5 Hz, 5 min) or capsaicin administration (150 μg kg^{-1}, i.v.).

2 When administered p.o. 60 min prior to electrical stimulation, RPR 100893 ($\geq$ 0.1 μg kg^{-1}) decreased plasma protein extravasation in dura mater in a dose-dependent manner, whereas the enantiomer (10 or 100 μg kg^{-1}, p.o.) was inactive.

3 When given i.v. 30 min prior to electrical stimulation, RPR 100893 ($\geq$ 0.5 ng kg^{-1}) significantly inhibited plasma protein extravasation in the dura mater evoked by electrical stimulation in a dose-dependent manner.

4 RPR 100893 (100 μg kg^{-1}, p.o.) also reduced the leakage when given 45 min before the guinea-pigs were killed and 10, 40 and 80 min after electrical trigeminal stimulation.

5 RPR 100893 given p.o. dose-dependently inhibited capsaicin-induced plasma protein extravasation with ID_{50}s of 7.4 μg kg^{-1} and 82 μg kg^{-1} for dura mater and conjunctiva, respectively.

6 These results are consistent with the contention that NK_1 receptors mediate neurogenic plasma protein leakage following trigeminal stimulation, and suggest that NK_1 receptor antagonists of the perhydroisoindolone series may be useful for treating migraine and cluster headaches.

Keywords: Migraine; neurogenic inflammation; tachykinins; trigeminal ganglion; NK_1 receptor antagonist

Introduction

Neurogenic inflammation (NI), a complex process characterized by vasodilatation, plasma extravasation, endothelium activation and mast cell degranulation develops in the dura mater following electrical or chemical stimulation of capsaicin-sensitive fibres projecting from neurones within trigeminal ganglia (Markowitz *et al.*, 1987; Dimitriadou *et al.*, 1992). There is considerable evidence that NI is mediated by the tachykinins, particularly substance P (SP), after release from perivascular trigeminovascular axons (Liu-Chen *et al.*, 1983; Moskowitz *et al.*, 1983; 1989; Dimitriadou *et al.*, 1991; Moussaoui *et al.*, 1993a,b; Shepheard *et al.*, 1993). SP binds preferentially to NK_1 receptors, whereas neurokinin A is a preferential ligand at NK_2 recognition sites (Quirion & Dam, 1985; Buck & Burcher, 1986; Regoli *et al.*, 1987). The selective antagonist, RP 67580 blocks neurogenic extravasation within dura mater after electrical or chemical trigeminal stimulation, thereby suggesting an NK_1 receptor-mediated response (Shepheard *et al.*, 1993; Moussaoui *et al.*, 1993a,b).

We have proposed that NI within dura mater may contribute to hyperalgesia and promote sensitization of dural trigeminovascular afferents to sustain headache pain (Moskowitz, 1984; 1991; 1992). Drugs useful for the treatment of acute migraine block NI within the dura mater. For example, sumatriptan or dihydroergotamine activate prejunctional 5-$HT_{1D/B}$ heteroreceptors and block the development of NI by inhibiting neuropeptide release from trigeminovascular fibres (Buzzi *et al.*, 1991).

RPR 100893, a novel, non-peptide, selective antagonist of human NK_1 receptors, is representative of the 7,7,4-triaryl-perhydroisoindol-4-ols, a new series of perhydroisoindolones of which RPR 67580 is a member (Tabart & Peyronel, 1994). We now demonstrate that low doses of orally or intravenously administered RPR 100893 potently block plasma protein extravasation in guinea-pig dura mater induced by electrical or chemical trigeminal stimulation, and that blockade can occur before as well as after nerve stimulation.

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Methods

Male Hartley guinea-pigs (200–250 g, Charles River Laboratories, Wilmington, MA, U.S.A. and Charles River Laboratories, France) were housed under diurnal lighting conditions and allowed food and water *ad libitum*.

Electrical trigeminal ganglion stimulation

Anaesthetized animals (pentobarbitone sodium, 40 mg kg^{-1}, i.p.) were placed in a stereotaxic frame (DKI 900, David Kopf Instruments, Tujunga, CA, U.S.A.) with the incisor bar set at − 4.5 mm from the horizontal, and the calvarium was exposed by a midsagittal incision. The right femoral vein was exposed and ^{125}I-labelled bovine serum albumin ([^{125}I]-BSA, 50 μCi kg^{-1}) was injected as a bolus. Symmetrical burr holes of 2 mm diameter were drilled 4.0 mm posterior to the bregma and 4.0 mm laterally on each side of the sagittal suture for electrode placement. Bipolar electrodes (50 mm shaft, Rhodes Medical Instruments, Woodland Hills, CA, U.S.A.) were lowered into the trigeminal ganglia to a depth of 10.5 mm from the dura mater overlying the dorsal surface of the brain. The right trigeminal ganglion was stimulated for 5 min (0.6 mA, 5 ms, 5 Hz) (Pulsemaster A300, Stimulus Isolator A365, World Precision Instruments, San Carlos, CA, U.S.A.; Oscilloscope V-134, Hitachi Densi, Tokyo, Japan), as previously described (Markowitz *et al.*, 1987; Matsubara *et al.*, 1991).

Pre-stimulation treatment

RPR 100893 or its enantiomer, RPR 103253, was administered via nasogastric tube in a volume of 2.5 ml kg^{-1} 60 min before electrical stimulation of trigeminal ganglion. Sixty min was selected as the interval between administration and stimulation because previous studies had demonstrated that plasma levels of RPR 100893 peak at this time (Moussaoui *et al.*, 1994). By i.v. route, RPR 100893 was administered 30 min before electrical stimulation. Control animals received the same volume of vehicle. [^{125}I]-BSA was injected via the right femoral vein 5 min before electrical stimulation. Immediately after stimulation, animals were perfused via the left cardiac ventricle with 0.9% saline for 2 min at a constant pressure of 100 mmHg. The dura mater was then dissected bilaterally, as previously described (Markowitz *et al.*, 1987).

Post-stimulation treatment

RPR 100893 was administered p.o. 10, 40 and 80 min after electrical trigeminal stimulation. Forty min later, [^{125}I]-BSA was injected. Five min later, animals were perfused and then killed. During these experiments, supplementary pentobarbitone (15 mg kg^{-1}, i.p.) was administered every 60 min to maintain anaesthesia.

Capsaicin administration

Capsaicin (150 μg kg^{-1}) was administered via the left jugular vein of the anaesthetized guinea-pig 5 min after [^{125}I]-BSA injection. RPR 100893 or sumatriptan was administered p.o. 60 min prior to capsaicin. Ten min after capsaicin treatment, animals were perfused with 0.9% saline transcardially at a constant pressure of 100 mmHg for 3 min. Immediately after transcardiac perfusion, the dura mater and conjunctiva were dissected.

Drugs

^{125}I-labelled bovine serum albumin ([^{125}I]-BSA; New England Nuclear, Boston, MA, U.S.A. and Du Pont de Nemours, France) was diluted in saline. Capsaicin (Sigma, France) was dissolved in ethanol/polysorbate 80/distilled water (1:1:8). RPR 100893 and RPR 103253 were synthesized in the Department of Chemistry of Rhone-Poulenc Rorer, France. RPR 100893 and RPR 103253 were dissolved in polyethyleneglycol 400. Sumatriptan (Glaxo, U.K.) was dissolved in saline. RPR 100893, [^{125}I]-BSA and capsaicin were injected i.v. as a bolus in a volume of 1.0 ml kg^{-1}. The same volume of vehicle was administered to the animals in the control groups in each experiment.

Data analysis

[^{125}I]-BSA extravasation is expressed as the ratio of c.p.m. mg^{-1} of wet wt. (stimulated side)/c.p.m. mg^{-1} of wet wt. (unstimulated side) for experiments involving electrical stimulation. Data are given as mean ± s.e.mean. Results with capsaicin are expressed as a percentage of c.p.m. mg^{-1} tissue in the capsaicin- versus vehicle-treated animals.

Each data point was calculated from at least two separate experiments. ID_{50} (the dose at which [^{125}I]-BSA extravasation was inhibited by 50%) was determined by regression analysis. Student's unpaired *t* test was used for statistical comparisons between vehicle- and drug-treated groups. Probability values (*P*) of less than 0.05 were considered significant.

Results

Unilateral electrical trigeminal ganglion stimulation increased the leakage of [^{125}I]-BSA within the dura mater of guinea-pigs treated with vehicle (p.o.) from 29.2 ± 3.0 to 48.2 ± 4.3 c.p.m. mg^{-1} wet wt. ($P<0.005$, $n=10$). The ratio between the stimulated and unstimulated sides was 1.69 ± 0.07 and was similar to previously reported values after saline-vehicle administration (Buzzi & Moskowitz, 1990).

When administered p.o. 60 min before electrical stimulation (Figure 1), RPR 100893 significantly decreased the ratio to 1.42 ± 0.05 at a threshold dose of 0.1 μg kg^{-1} ($P<0.05$, $n=6$). The ID_{50} was 0.5 μg kg^{-1}. RPR 100893 dose-dependently decreased plasma extravasation. The ratio decreased to 1.24 ± 0.03 at 1 μg kg^{-1} ($P<0.001$, $n=7$), 1.15 ± 0.05 at 10 μg kg^{-1} ($P<0.001$, $n=6$), and 1.04 ± 0.06 at 100 μg kg^{-1} ($P<0.001$, $n=5$). Extravasation on the unstimulated side did not differ between the treated and untreated groups.

When administered p.o. at 10 or 100 μg kg^{-1}, 60 min before electrical stimulation, RPR 103253, the enantiomer of RPR 100893, did not affect plasma protein extravasation within dura mater: 1.51 ± 0.03 ($n=5$) and 1.47 ± 0.04 ($n=7$), respectively; 1.56 ± 0.06 in vehicle group ($n=4$).

Pretreatment with RPR 100893 (0.05–50 ng kg^{-1}, i.v.) 30 min before electrical stimulation dose-dependently reduced plasma protein extravasation with an ID_{50} of 2.5 ng kg^{-1} (Figure 2). The threshold dose was 0.5 ng kg^{-1} (1.40 ± 0.03, $P<0.05$, $n=5$) and the maximum response was achieved with 50 ng kg^{-1} (1.08 ± 0.07, $P<0.001$, $n=5$) as compared to the vehicle-treated group (1.69 ± 0.07, $n=5$).

Table 1 shows the effect of RPR 100893 on the delayed plasma protein extravasation response that develops during the poststimulation period. At 100 μg kg^{-1}, p.o. administered 10, 40 or 80 min after electrical stimulation, RPR 100893 markedly attenuated the delayed extravasation response in those animals killed within 85 min poststimulation. There was little extravasation to block at 125 min after stimulation.

Capsaicin increased the leakage of [^{125}I]-BSA within dura mater (133.2 ± 4.8%, $P<0.01$, $n=24$) and conjunctiva (177.9 ± 9.4%, $P<0.01$, $n=20$) as compared to vehicle-treated animals. RPR 100893 (1–1,000 μg kg^{-1}, p.o.) blocked the response in a dose-dependent manner when administered 60 min prior to capsaicin injection [in dura mater; 124.0 ± 11.3% at 1 μg kg^{-1} ($n=6$), 117.8 ± 12.5% at 10 μg kg^{-1} ($n=6$), 102.5 ± 7.9% at 100 μg kg^{-1} ($P<0.01$, $n=9$),

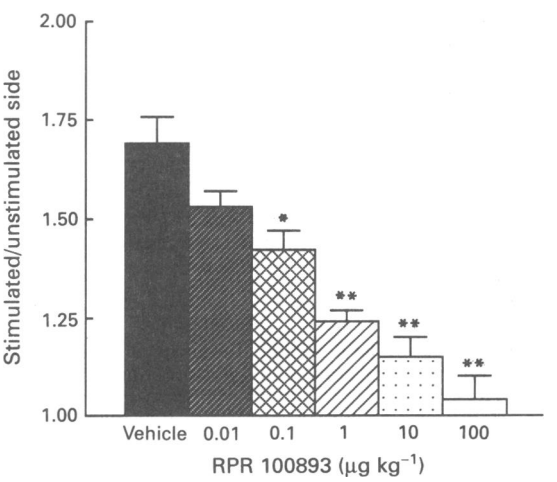

Figure 1 Oral RPR 100893 reduced plasma protein ([^{125}I]-BSA) extravasation within guinea-pig dura mater following electrical stimulation of trigeminal ganglion (0.6 mA, 5 ms, 5 Hz). Animals were treated with vehicle (solid column; $n=10$), 0.01 (heavily hatched column, $n=6$), 0.1 (cross-hatched column, $n=6$), 1 (hatched column, $n=7$), 10 (dotted column, $n=6$) or 100 (open column, $n=5$) μg kg^{-1} of RPR 100893 given 60 min before electrical stimulation and 55 min before [^{125}I]-BSA (50 μCi kg^{-1}) injection. Immediately after stimulation, animals were perfused with 0.9% saline and tissues harvested (see Methods). Results are expressed as the ratio of the c.p.m. mg^{-1} wet weight on the stimulated side to that on the unstimulated side (mean ± s.e.mean). *$P<0.05$, **$P<0.001$ as compared to vehicle-treated group.

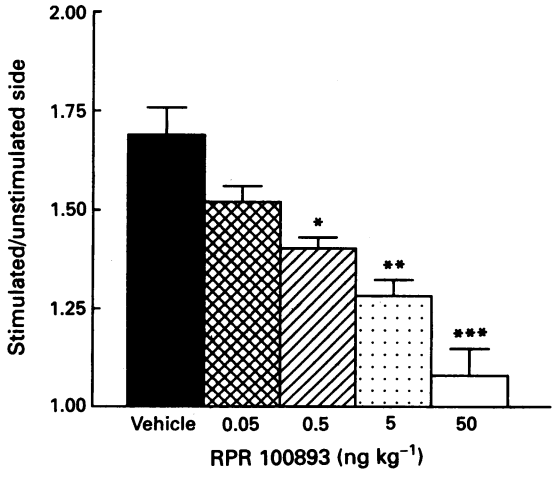

Figure 2 RPR 100893 administered i.v. decreased plasma protein extravasation within guinea-pig dura mater following electrical trigeminal ganglion stimulation. RPR 100893 was administered 0.05 (cross-hatched column), 0.5 (hatched column), 5 (dotted column) or 50 (open column) ng kg^{-1}, 30 min before electrical stimulation and 25 min before [^{125}I]-BSA injection. Immediately after stimulation, animals were perfused with 0.9% saline (see Methods). Data are expressed as described in legend to Figure 1. $*P<0.05$, $**P<0.01$; $***P<0.001$ as compared to vehicle-treated group ($n=5$ in each group).

101.3 ± 7.9% at 1,000 μg kg^{-1} ($P<0.01$, $n=8$): in conjunctiva; 161.3 ± 14.9% at 1 μg kg^{-1} ($n=6$), 138.5 ± 14.0% at 10 μg kg^{-1} ($P<0.05$, $n=6$), 130.6 ± 6.6% at 100 μg kg^{-1} ($P<0.01$, $n=8$), 117.7 ± 9.6% at 1,000 μg kg^{-1} ($P<0.01$, $n=6$)]. The ID_{50}s for dura mater and conjunctiva were 7.4 μg kg^{-1} and 82 μg kg^{-1}, respectively. Oral sumatriptan likewise administered 60 min prior to capsaicin injection was effective in dura mater only at a considerably higher dose [109.4 ± 10.4% at 10,000 μg kg^{-1} ($P<0.05$, $n=7$) vs. 155.6 ± 12.2% in vehicle group ($n=16$); ID_{50} of 1,000 μg kg^{-1}], but not in conjunctiva.

Discussion

The NK_1 receptor antagonist, RPR 100893 but not its stereoisomer RPR 103253, potently blocked NI within guinea-pig dura mater evoked by electrical trigeminal stimulation. RPR 100893 was effective at very low doses p.o. or i.v. when administered before, and also when given p.o. after electrical trigeminal stimulation. RPR 100893 was also effective against capsaicin-induced extravasation. When compared to RP 67580, pretreatment with RPR 100893 was about 240 times more potent. The ID_{50}s of RP 67580 (rat; Shepheard *et al.*, 1993) and RPR 100893 (guinea-pig) were 0.6 μg kg^{-1} and 2.5 ng kg^{-1}, i.v., respectively. Results of recent pilot experiments indicate that RPR 100893 does not alter basal plasma extravasation or vasodilatation in peripheral tissues (Moussaoui *et al.*, 1994).

RPR 100893

RPR 100893 binds with high affinity (nanomolar range) and selectively to the NK_1 recognition site on human IM9 cells and guinea-pig brain, but not to NK_2 or NK_3 binding sites (Fardin *et al.*, 1994). Its affinity for guinea-pig NK_1 receptors is 30 times greater than that of its enantiomer, RPR 103253 (Fardin *et al.*, 1994; Moussaoui *et al.*, 1994). RPR 100893 exhibits >1 μM affinity for a variety of other peptide receptors, receptors of classical neurotransmitters, calcium channels and the rat NK_1 receptor (Fardin *et al.*, unpublished data).

RPR 100893 inhibits nociceptive behaviours when administered to guinea-pigs, albeit at a lower potency than blockade of plasma extravasation within dura mater, and the response is not accompanied by a change in blood pressure. RPR 100893 but not its enantiomer blocked the licking response to formalin-injected guinea-pig paws (50 μl, 5%) with an ID_{50} of 3.1 mg kg^{-1}, s.c. (Moussaoui *et al.*, 1994). Although the mechanism may be complex and the specificity challenged (Rupniak *et al.*, 1993; Guard *et al.*, 1993), other NK_1 receptor antagonists, such as RP 67580 and CP-96,345 decreased pain-associated behaviours induced by chemical stimulation (formalin and phenylbenzoquinone tests in rat and mouse; Yamamoto & Yaksh, 1991; Nagahisa *et al.*, 1992; Carruette *et al.*, 1993). Moreover, RP 67580 blocked the nociception flexion reflex in an enantiomer-specific manner (Laird *et al.*, 1993). We recently found that relatively large doses of RPR 100893 (threshold, 1 μg kg^{-1}, i.v.) attenuated the expression of c-fos antigen within lamina I,II_o of guinea-pig trigeminal nucleus caudalis induced by intracisternal capsaicin (Cutrer *et al.*, unpublished data). The inhibition of c-fos antigen expression within this brain region was previously demonstrated after giving analgesics such as morphine, or after sumatriptan or dihydroergotamine following noxious meningeal stimulation (Presley *et al.*, 1990; Nozaki *et al.*, 1992; Moskowitz *et al.*, 1993).

Wang *et al.* (1994) noted certain structural similarities between non-peptide tachykinin receptor antagonists and local anaesthetics. They reported that RP 67580 and CP-96,345 blocked non-NK_1 related neurotransmission. RP 67580 and CP-96,345, like the anaesthetic bupivacaine, inhibited non-tachykinin-evoked neurotransmission in addition to tachykinin-evoked neurotransmission. Because RP

Table 1 Effect of RPR 100893 (100 μg kg^{-1}) administered p.o. 10, 40 or 80 min after electrical trigeminal stimulation on delayed plasma protein extravasation in guinea-pig dura mater

Poststimulation		*Stimulated side* (c.p.m. mg^{-1})	*Unstimulated side* (c.p.m. mg^{-1})	*Ratio*
55 min				
Vehicle	(8)	31.42 ± 0.47	21.90 ± 0.65	1.44 ± 0.03
RPR 100893	(8)	25.00 ± 1.05	23.11 ± 0.74	1.09 ± 0.07**
85 min				
Vehicle	(6)	32.54 ± 1.30	24.56 ± 1.09	1.33 ± 0.05
RPR 100893	(6)	26.98 ± 2.50	26.44 ± 2.73	1.03 ± 0.04*
125 min				
Vehicle	(6)	29.06 ± 1.97	26.40 ± 2.36	1.12 ± 0.06
RPR 100893	(6)	28.05 ± 3.27	27.93 ± 3.27	1.01 ± 0.05

RPR 100893 decreased plasma protein extravasation within the guinea-pig dura mater after 5 min of electrical trigeminal stimulation. RPR 100893 was administered p.o. 10, 40 or 80 min after electrical stimulation. Forty min later, [^{125}I]-BSA was injected and the animals perfused and killed after an additional 5 min. Numbers in parentheses represent the number of animals per group. $*P<0.01$, $**P<0.001$ as compared to corresponding vehicle-treated group.

67580 and RPR 100893 are structural analogues, the possibility that RPR 100893 possesses local anaesthetic-like actions in addition to actions at the NK_1 receptor cannot now be ruled out. However, the large concentrations of RP 67580 required to achieve non-specific blockade argues in favour of an NK_1 receptor-mediated mechanism in the studies reported herein. Additional work will be required to clarify this point.

NK_1 receptor and neurogenic inflammation in dura mater

The evidence supports NK_1 receptor involvement in the extravasation response. As noted above substance P (SP), the preferential NK_1 receptor endogenous ligand, is stored and released from trigeminal nerve terminals in the dura mater (Edvinsson *et al.*, 1983) where it produces vasodilatation and plasma protein extravasation (Moskowitz *et al.*, 1989). Exogenous SP and selective NK_1 receptor agonists, such as GR 73632 and SPOMe, but not NK_2 (GR 64349) or NK_3 (senktide) receptor agonists, enhance both plasma protein extravasation and vasodilatation in cranial tissues (Guard & Watson, 1991; Hagan *et al.*, 1991; Beattie *et al.*, 1993; O'Shaughnessy & Connor, 1993). Hence, the inhibition of NI by RPR 100893 is most consistent with selective blockade of NK_1 receptors.

RP 67580 (Moussaoui *et al.*, 1993c; Shepheard *et al.*, 1993) and RPR 100893 were more potent blockers of neurogenic extravasation within the dura mater than within extracranial tissues. When RP 67580 was administered prior to capsaicin treatment, its ID_{50} was as low as 35 $\mu g\,kg^{-1}$, i.v., whereas values of 109 and 309 $\mu g\,kg^{-1}$, i.v. were obtained in conjunctiva and bladder, respectively (Moussaoui *et al.*, 1993c). The explanation for this tissue-specific response is not yet known, but may suggest the existence of more than a single NK_1 receptor subtype.

The results after delayed RPR 100893 administration (Table 1) implicate sustained SP release as the mediator of plasma leakage developing beyond the period of electrical stimulation. Consistent with this formulation, endopeptidase 24.11 which degrades extracellular neuropeptides (Roques & Beaumont, 1990) blocks the delayed response as does 5-$HT_{1D/B}$ receptor agonists (Huang *et al.*, 1993). Together these findings are consistent with data showing that plasma extravasation in the skin can be blocked at the presynaptic level (by opioid agonists inhibiting SP release, by colchicine blocking axonal transport of neuromediators, or by neonatal capsaicin treatment destroying C-fibres; Moussaoui *et al.*, 1993a) as well as at the postsynaptic levels (by blocking NK_1 receptors or by dexamethasone which inhibits the mRNA expression of the NK_1 receptor; Ihara & Nakanishi, 1990). Our data showing that lignocaine (5 mg kg^{-1}, i.v.) blocked plasma protein extravasation in dura mater induced by SP (1 nmol kg^{-1}) or electrical trigeminal stimulation (Huang *et al.*, unpublished data) merit further study in view of one report showing that RP 67580 possesses local anaesthetic-like properties which are distinct from actions at the NK_1 receptor (Wang *et al.*, 1994).

In conclusion, the present work is the first to demonstrate the oral and parenteral effects of a potent human NK_1 receptor antagonist, RPR 100893, on neurogenic plasma extravasation, when administered either before or after chemical or electrical stimulation. If NI is important to the development of vascular headaches (Moskowitz *et al.*, 1989) and RPR 100893 exhibits antinociceptive activity (Moussaoui *et al.*, 1994), perhydroisoindoles such as RPR 100893 could be effective in the treatment of migraine and related headaches.

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