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Short communication

The novel anti-migraine agent rizatriptan inhibits neurogenic dural vasodilation and extravasation

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Abstract

These studies in anaesthetised rats showed, using intravital microscopy, that the novel anti-migraine agent, rizatriptan, significantly reduced electrically stimulated dural vasodilation but had no effect on increases in dural vessel diameter produced by exogenous substance P or calcitonin gene-related peptide (CGRP). Rizatriptan also significantly inhibited dural plasma protein extravasation produced by high intensity electrical stimulation of the trigeminal ganglion. We suggest that rizatriptan inhibits the release of sensory neuropeptides from perivascular trigeminal nerves to prevent neurogenic vasodilation and extravasation in the dura mater. These prejunctional inhibitory effects may be involved in the anti-migraine action of rizatriptan.

Keywords: Rizatriptan; Microscopy, intravital; Meningeal artery, middle; CGRP (calcitonin gene-related peptide); Migraine

1. Introduction

Migraine headache pain is thought to arise from activation of sensory trigeminal nerves surrounding blood vessels of the dura mater and large cerebral arteries (Ray and Wolff, 1940). Perivascular trigeminal sensory nerves, when activated, will release the pro-inflammatory neuropeptides calcitonin gene-related peptide (CGRP), substance P and neurokinin A (Edvinsson and Goadsby, 1994). High intensity electrical stimulation of the trigeminal ganglion produces plasma protein extravasation in the dura mater by the actions of neurokinins released from the perivascular sensory nerves. This neurogenic extravasation can be inhibited by a prejunctional action of some serotonergic antimigraine drugs and, since the consequent activation of meningeal sensory nerves would thus be reduced, it was proposed that this may contribute to their anti-migraine effect (Moskowitz, 1992). Alternatively, it has been proposed that activation of the trigeminal system by blood vessel vasodilation is the primary factor in the pathogenesis of migraine headache pain and that normalisation of vessel calibre is the most important mechanism for pain relief (Humphrey and Feniuk, 1991). Studies in animals have shown that stimulation of meningeal trigeminal nerves, such as might be expected to occur when blood vessels become painfully distended, increases blood flux and vessel diameter within dural vascular beds predominantly via the release of the potent vasoactive neuropeptide CGRP and this would perpetuate any ongoing vasodilation response (Kurosawa et al., 1995; Williamson et al., 1996). Serotonergic anti-migraine agents have direct vasoconstrictor effects on large intracranial, extracerebral arteries and it has been suggested that this mechanism underlies their therapeutic action. However, it is possible that vessel diameter could be normalised without frank vasoconstriction through an inhibition of CGRP release, and this too could contribute to headache pain relief. The present studies investigated the effects of the novel anti-migraine 5-$HT_{1B/1D}$ receptor agonist rizatriptan (Visser et al., 1996) on (1) the vasodilation responses of dural blood vessels evoked by substance P and CGRP or by electrical stimulation and (2) the neurogenic dural plasma extravasation evoked by electrical trigeminal simulation.

2. Materials and methods

2.1. Vasodilation experiments

Male Sprague-Dawley rats (300–400 g) were anaesthetised with pentobarbitone sodium (initially 60 mg/kg,

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i.p., then 18 mg/kg per h, i.v. infusion). A femoral artery and both femoral veins were cannulated for blood pressure recording, intravenous injection of drugs and infusion of anaesthetic, respectively. Rats were placed in a stereotaxic frame, the skull exposed and thinned by drilling to reveal a branch of the middle meningeal artery whose diameter was measured continuously (in arbitrary units) through the intact skull using intravital microscopy (Microvision MV2100, UK) and a video dimension analyser (Living Systems Instrumentation, USA). Vasodilation was evoked by substance P (100 ng/kg, i.v.), rat-α-CGRP (300 ng/kg, i.v.) or neurogenically using a bipolar stimulating electrode (NE 200X, Clark Electromedical) placed on the surface of the cranial window approximately 200 μm from the vessel of interest (see Fig. 1). The stimulus intensity required to produce a maximal vasodilation was established and was 5 Hz, 1 ms, 50–300 μA for 10 s. In the first series of experiments, a control response to substance P (100 ng/kg, i.v.) and electrical stimulation were produced and 5 min later, rizatriptan (1 mg/kg, i.v.) or vehicle was given and the vasodilator challenges repeated after 15 min. Using the same protocol, a second dose of rizatriptan (10 mg/kg, i.v.) or vehicle was given followed again by the vasodilator challenges. In the second series, a control response to rat-α-CGRP (300 ng/kg, i.v.) and electrical stimulation was obtained and then 5 min later, rizatriptan (3 mg/kg, i.v.) or vehicle was administered and the vasodilator challenges repeated after 15 min. The mean maximum percentage increase in dural vessel diameter relative to pre-stimulus baseline was calculated for each vasodilator challenge. Comparisons of vasodilation responses evoked in the presence or absence of rizatriptan were made by analysis of variance (ANOVA) followed by paired *t*-tests.

2.2. Plasma extravasation studies

In male Sprague-Dawley rats (180–230 g) anaesthetised with pentobarbitone (60 mg/kg, i.p.), dural plasma extravasation produced by electrical stimulation (1.2 mA given as a ± 0.6 mA square wave pulse, 5 Hz, 5 ms, for 5 min) of the right trigeminal ganglion was measured using ^{125}I-bovine serum albumin as a plasma marker as described by Shepheard et al. (1995). The effects of intravenous vehicle or rizatriptan (1–1000 μg/kg) on this dural extravasation were examined. The amount of extravasation was calculated as an extravasation ratio i.e. extravasation in stimulated/unstimulated sides. Statistical comparisons between drug and vehicle treated rats were made by ANOVA followed by unpaired *t*-tests.

2.3. Drugs

Rizatriptan (Merck Sharp and Dohme Research Laboratories, Terlings Park, UK) was dissolved in 0.9% saline. Rat-α-CGRP (Cambridge Research Biochemicals, UK) and substance P (Bachem, UK) were initially dissolved in distilled water, aliquotted and frozen. Subsequent dilutions were made in 0.9% saline. All drugs were administered at a volume of 1 ml/kg and doses refer to free base weight.

3. Results

3.1. Effects of rizatriptan on baseline dural blood vessel diameter and vasodilation

Rizatriptan had no intrinsic vasoconstrictor activity in meningeal blood vessels (mean dural vessel diameter was 38 ± 3 (arbitrary units) prior to rizatriptan (10 mg/kg, i.v.) and was 39 ± 2 15 min later. Fig. 2A shows that rizatriptan had no significant effects on substance P or rat-α-CGRP-induced vasodilation but at 3 and 10 mg/kg (i.v.) inhibited electrically evoked neurogenic vasodilation (from $103 \pm 12\%$ to $62 \pm 9\%$ and $100 \pm 12\%$ to $49 \pm 7\%$, respectively). There was no significant difference in the size of the response evoked by injection of substance P,

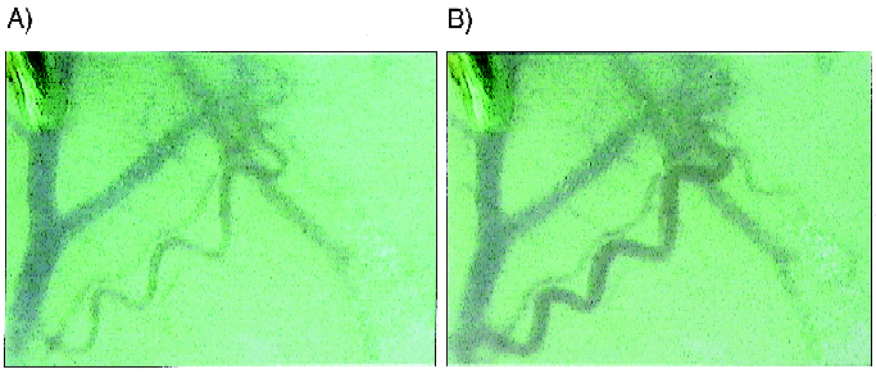

Fig. 1. Videomicroscope image of a branch (30 μm diameter) of the middle meningeal artery. (A) control and (B) 30 s after electrical stimulation of the cranial window (100 μA, 1 ms, 5 Hz for 10 s).

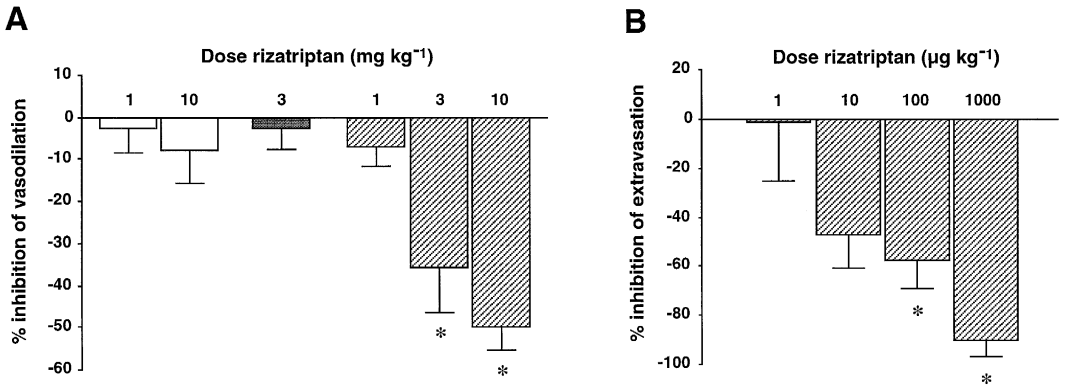

Fig. 2. (A) Effects of rizatriptan (1, 3 or 10 mg/kg, i.v.) on vasodilation responses evoked by substance P (100 ng/kg, i.v.; open bars), rat-α-CGRP (300 ng/kg, i.v.; filled bar) or electrical stimulation of the surface of the cranial window (50–300 μA, 1 ms, 5 Hz for 10 s; hatched bars). Data are presented as the mean percent inhibition of vasodilation ± S.E.M. compared to control, statistical analysis was performed by ANOVA and paired *t*-test on the actual changes, $n = 5–8$, * $P < 0.05$ from control. (B) Effects of rizatriptan (1–1000 μg/kg) on dural plasma extravasation produced by electrical stimulation (±0.6 mA square wave pulse, 5 Hz, 5 ms, 5 min) of the trigeminal ganglion. Data are expressed as the mean percent inhibition of extravasation ± S.E.M. compared to vehicle, statistical analysis was performed by ANOVA followed by unpaired *t*-tests, $n = 10–19$ per group, * $P < 0.05$ from vehicle.

rat-α-CGRP or repeated electrical stimulation when the animals were treated with vehicle using the same experimental protocols.

3.2. Effect of rizatriptan on neurogenic plasma extravasation

After electrical stimulation of trigeminal ganglion the extravasation ratio in vehicle treated animals was 2.06 ± 0.22 ($n = 19$). Pretreatment with rizatriptan (1–1000 μg/kg, $n = 10$ per group) produced a significant dose related inhibition of dural extravasation in comparison to control (Fig. 2B). A two-parameter inhibition curve was fitted to the data using Grafit software (Ericathus) and the dose producing half-maximal inhibition (ID_{50}) was 31 μg/kg, i.v.

4. Discussion

These results show that the 5-$HT_{1B/1D}$ receptor agonist rizatriptan inhibits dural vasodilation evoked by electrical stimulation of the surface of a closed cranial window in anaesthetised rats. This novel preparation avoids the complications of brain swelling and altered blood vessel reactivity that are often associated with open cranial window techniques. Moreover, the technique measures vessel diameter directly rather than relying on blood flux measurements that can be affected by changes in tissue perfusion without changes in vessel calibre. Rizatriptan had no direct constrictor effects per se, so it is unlikely that its inhibition of dural neurogenic vasodilation is by a physiological antagonism. We have previously shown that this dural vasodilation is mediated predominantly by CGRP since it is unaffected by the rat tachykinin NK_1 receptor antagonist RP 67580 but almost completely abolished by the CGRP receptor antagonist human-α-CGRP-(8–37) (Williamson et al., 1996). Rizatriptan had no effect on the vasodilation produced by exogenous rat-α-CGRP. Thus, the mechanism through which rizatriptan inhibits neurogenic vasodilation in this preparation is probably by an inhibition of the release of CGRP via activation of prejunctional receptors located on the terminals of trigeminal sensory nerves.

The lack of direct constrictor effects of rizatriptan on the dural vessels in this anaesthetised rat preparation was surprising since rizatriptan produces potent constriction of human middle meningeal arteries in vitro (J. Longmore, personal communication). The reason for this apparent contradiction may relate to differences in the anatomical localisation or density of receptors between the large cranial conduit arteries and the smaller arterial branches within the dura mater itself.

This study also demonstrates that rizatriptan inhibits dural plasma extravasation produced by electrical stimulation of the trigeminal ganglion. In situ hybridisation studies (Bruinvels et al., 1993) have detected 5-HT_{1B} and 5-HT_{1D} mRNA in rat trigeminal ganglia, suggesting both receptor subtypes could be expressed on the terminals of trigeminal sensory fibres. The ID_{50} of rizatriptan in blocking dural plasma extravasation was 30 μg/kg after which the plasma concentration was predicted to be approximately 19 nM. Comparison of this plasma level with the potency and efficacy of rizatriptan measured using agonist induced [^{35}S]GTPγS binding (M. Beer, personal communication) at rat cloned 5-HT_{1D} (11 nM, 105% of 5-HT response) and rat cloned 5-HT_{1B} (1700 nM, 101% of 5-HT response) receptors suggests that blockade of dural plasma extravasation by rizatriptan in rats is consistent with an action at prejunctional 5-HT_{1D} receptors to inhibit release of neuropeptides from trigeminal fibres. However, data from similar trigeminal extravasation studies with CP-122,288 (Gupta et al., 1995) indicate that another, as yet unidentified, extravasation receptor subtype may also be involved in this inhibitory response.

In neurogenic vasodilation studies, the plasma concentration of rizatriptan at 15 min after the minimum effective intravenous dose of 3 mg/kg was predicted to be approximately 1560 nM. Comparison of this plasma level with the potency and efficacy of rizatriptan at rat cloned 5-HT_{1D} (11 nM) and rat cloned 5-HT_{1B} (1700 nM) receptors (see above) suggests that rizatriptan inhibits neurogenic dural vasodilation in rats by an action at 5-HT_{1B} receptors. The affinity of rizatriptan for human cloned 5-HT_{1D} receptors is 20 nM and for human cloned 5-HT_{1B} receptors is 50 nM (Connor and Beattie, 1996). In humans, the plasma concentrations of rizatriptan achieved after clinically active doses are approximately 40–100 nM. Thus, rizatriptan could act at both subtypes of receptor after therapeutic dosing and it is therefore of interest to consider whether inhibition of substance P-mediated neurogenic extravasation or CGRP-mediated neurogenic vasodilation is its most important inhibitory anti-migraine mechanism in the meninges.

The prejunctional inhibition of neuropeptide release from trigeminovascular fibres in the dura mater has been suggested to be one mechanism by which anti-migraine drugs of the 5-$HT_{1B/1D}$ class produce their therapeutic effect. It is not yet known how the stimulus intensities used in these experimental studies relate to the level of trigeminal activation during migraine. Peripherally acting tachykinin NK_1 receptor antagonists are highly active in the dural extravasation assay; however, recent negative clinical results with RPR 100893 (communicated by C. Diener, 6th International Headache Research Seminar, Copenhagen, 1995) and LY 303870 (communicated by D. Goldstein, Conference on Tachykinins and their Antagonists, London, 1996) suggest that inhibition of meningeal extravasation alone may be insufficient to give acute migraine headache relief. It could be questioned whether the high stimulus intensity conditions required to evoke substance P release from trigeminal fibres and thus dural plasma extravasation actually occur during a migraine attack. In contrast, it has been shown that levels of the potent vasodilator neuropeptide CGRP are increased in cranial venous effluent blood during a migraine attack and that the clinically effective anti-migraine drug sumatriptan normalises these elevated levels concomitantly with giving migraine headache relief (Goadsby and Edvinsson, 1993). The findings of the current intravital microscope studies concur with these clinical findings and suggest a role for CGRP in painful meningeal vasodilation.

The present studies show that rizatriptan blocks neurogenic dural vasodilation and extravasation by inhibiting the release of inflammatory neuropeptides such as CGRP and substance P. If these mechanisms are involved in the pathogenesis of migraine headache pain then their inhibition by rizatriptan may contribute to its anti-migraine action.

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