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The anti-migraine 5-$HT_{1B/1D}$ agonist rizatriptan inhibits neurogenic dural vasodilation in anaesthetized guinea-pigs

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1 These studies investigated the pharmacology of neurogenic dural vasodilation in anaesthetized guinea-pigs. Following introduction of a closed cranial window the meningeal (dural) blood vessels were visualized using intravital microscopy and the diameter constantly measured using a video dimension analyser.

2 Dural blood vessels were constricted with endothelin-1 (3 μg kg^{-1}, i.v.) prior to dilation of the dural blood vessels with calcitonin gene-related peptide (CGRP; 1 μg kg^{-1}, i.v.) or local electrical stimulation (up to 300 μA) of the dura mater.

3 In guinea-pigs pre-treated with the CGRP receptor antagonist $CGRP_{(8\text{-}37)}$ (0.3 mg kg^{-1}, i.v.) the dilator response to electrical stimulation was inhibited by 85% indicating an important role of CGRP in neurogenic dural vasodilation in this species.

4 Neurogenic dural vasodilation was also blocked by the 5-$HT_{1B/1D}$ agonist rizatriptan (100 μg kg^{-1}) with estimated plasma levels commensurate with concentrations required for anti-migraine efficacy in patients. Rizatriptan did not reverse the dural dilation evoked by CGRP indicating an action on presynaptic receptors located on trigeminal sensory fibres innervating dural blood vessels.

5 In addition, neurogenic dural vasodilation was also blocked by the selective 5-HT_{1D} agonist PNU-142633 (100 μg kg^{-1}) but not by the 5-HT_{1F} agonist LY334370 (3 mg kg^{-1}) suggesting that rizatriptan blocks neurogenic vasodilation *via* an action on 5-HT_{1D} receptors located on perivascular trigeminal nerves to inhibit CGRP release.

6 This mechanism may underlie one of the anti-migraine actions of the triptan class exemplified by rizatriptan and suggests that the guinea-pig is an appropriate species in which to investigate the pharmacology of neurogenic dural vasodilation.

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Abbreviations: ET-1, endothelin-1; MABP, mean arterial blood pressure; MED, minimum effective dose

Introduction

Despite considerable research into the mechanisms that underlie migraine headache, the pathogenesis of this disorder remains poorly understood. One hypothesis is that fibres within the trigeminal nerve, which constitutes the sole sensory innervation of cranial blood vessels, become activated during migraine resulting in the release of vasoactive peptides and the subsequent development of a painful neurogenic dilation of meningeal blood vessels (Goadsby *et al*., 1990). This concept derives from studies which demonstrated that stretching of dural blood vessels in conscious patients evoked severe unilateral headache with nausea (Ray & Wolff, 1940) and the observation that levels of the potent vasodilator calcitonin gene-related peptide (CGRP) were elevated in jugular blood during the headache phase of migraine (Goadsby *et al*., 1990).

Initially it was thought that clinically effective anti-migraine agents with high 5-$HT_{1B/1D}$ affinity, such as the ergot alkaloids and the triptans, were effective in the treatment of migraine through actions at vascular 5-HT_{1-like} receptors to directly constrict the distended meningeal blood vessels (Humphrey & Feniuk, 1991). However, it is now clear that these agents can also act on receptors on both the peripheral and central terminals of trigeminal sensory fibres to inhibit the release of sensory neuropeptides such as CGRP. In humans, this concept is supported by observations that the elevated CGRP levels in jugular outflow during migraine were normalized by sumatriptan concomitant with headache relief (Goadsby & Edvinsson, 1993).

In rats, electrical stimulation of the dura mater has been shown to evoke a dilation of dural arteries mediated *via* the release of CGRP since these responses were almost completely abolished by the CGRP receptor antagonist $CGRP_{(8-37)}$ (Williamson *et al*., 1997b). Neurogenic dural vasodilation was also found to be blocked by the clinically effective 5-$HT_{1B/1D}$ receptor agonist rizatriptan, by the selective 5-HT_{1B} agonist CP-93,129 but not by the 5-HT_{1F} agonist LY334370, suggesting an important inhibitory role of 5-HT_{1B} receptors in this neurogenic dilator response in rats

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(Shepheard *et al*., 1997; 1999; Williamson *et al*., 1997c). In contrast, rizatriptan did not inhibit dural vasodilation evoked by intravenous CGRP, indicating that the 5-HT_{1B} receptors are located on the prejunctional terminals of trigeminal sensory fibres and activation of these receptors inhibits the release of CGRP (Williamson *et al*., 1997c).

In humans the 5-$HT_{1B/1D}$ receptor distribution within the trigeminovascular system differs from that in rats and it has been therefore difficult to extend observations with 5-$HT_{1B/1D}$ agonists in rats to humans. Razzaque *et al*. (1999) demonstrated that the potency of the triptans at constricting isolated human meningeal blood vessels correlated with their affinity at the 5-HT_{1B} receptor and the predominance of 5-HT_{1B} receptors on dural blood vessels has also been demonstrated immunohistochemically (Longmore *et al*., 1997). In contrast, the neuronal 5-HT_1 receptor subtypes expressed upon the trigeminal neurones in humans appear to be predominantly of the 5-HT_{1D} (Rebeck *et al*., 1994; Longmore *et al*., 1997) and the 5-HT_{1F} subtypes (Bouchelet *et al*., 1996), raising the possibility that selective agonists for these receptors may have anti-migraine actions without the side effect potential associated with direct vasoconstriction.

Studies in guinea-pigs suggest that the pharmacology and distribution of 5-HT_1 receptors within the trigeminovascular system is more similar to humans than rats. Bonaventure *et al*. (1998) demonstrated high levels of 5-HT_{1D}, but not 5-HT_{1B} mRNA and Johnson *et al*. (1997) found 5-HT_{1F} mRNA expressed in the guinea-pig trigeminal ganglion, suggesting that 5-HT_{1D} and 5-HT_{1F} receptors could have an important inhibitory role on both the central and peripheral terminals of trigeminal neurones. In support of this, both the selective 5-HT_{1D} and 5-HT_{1F} receptor agonists PNU109,291 and LY334370 have been shown to inhibit dural extravasation evoked by electrical stimulation of the trigeminal ganglion (Cutrer *et al*., 1999; Johnson *et al*., 1997). These observations suggest that if they are to be extrapolated to man, the guinea-pig may be a more appropriate species in which to study the role of 5-HT_{1D} and 5-HT_{1F} receptors in the trigeminovascular system.

The purpose of the present study in anaesthetized guinea-pigs was to investigate: (i) the role of CGRP in mediating neurogenic dural vasodilation using the CGRP receptor antagonist human-αCGRP$_{(8-37)}$, (ii) the effects of rizatriptan on neurogenic or rat-αCGRP-evoked dural vasodilation, and (iii) the effects of the selective 5-HT_{1D} agonist PNU142,633 and the selective 5-HT_{1F} agonist LY334370 on neurogenic dural vasodilation.

Methods

Male Dunkin Hartley guinea-pigs (300–450 g) were anaesthetized throughout experiments with pentobarbitone sodium (initially 50 mg kg^{-1}, i.p., then 18 mg kg^{-1}h^{-1}, i.v., constant infusion). The trachea, left carotid artery and jugular vein were cannulated for artificial ventilation (12 ml kg^{-1}, 50 strokes per min), measurement of mean arterial blood pressure (MABP) and intravenous injection of anaesthetic and drugs, respectively. All experiments were conducted under U.K. Home Office guidelines.

The methodology for dural blood vessel diameter measurement in rats has been described in detail previously (Williamson *et al*., 1997a,b). Briefly, guinea-pigs were placed in a stereotaxic frame, the skull exposed and the right parietal bone thinned by drilling with a saline-cooled drill, until the blood vessels of the dura were clearly visible through the intact skull. A branch of the middle meningeal artery was viewed using an intravital microscope and dural blood vessel diameter was continuously measured by a video dimension analyser. In neurogenic vasodilation studies a bipolar stimulating electrode was placed on the surface of the cranial window close to the vessel of interest.

In preliminary experiments it was found that, following introduction of the cranial window, the dural blood vessels typically were observed to be maximally dilated, so that electrical stimulation of the cranial window produced little if any increase in diameter. It was therefore necessary to preconstrict the dural vessels with intravenously administered endothelin-1 (ET-1, 3 μg kg^{-1}) which produced an approximate 50% reduction in dural blood vessel diameter (unpublished observations). Following administration of endothelin-1 (3 μg kg^{-1}, i.v.) dural vasodilation was reliably evoked approximately 3 min later by intravenous rat-αCGRP (1 μg kg^{-1}) or electrical stimulation of the cranial window (250–300 μA, 5-Hz, 1 ms for 10 s) and expressed as percentage increase in dural blood vessel diameter ± s.e.mean from baseline. Rizatriptan (0.01–1 mg kg^{-1}), PNU142,633 (0.01–1 mg kg^{-1}) or LY334370 (3 mg kg^{-1}) were administered intravenously 12 min before administration of ET-1 whereas human-αCGRP$_{(8-37)}$ (0.3 mg kg^{-1}) was given 2 min prior to ET-1. Statistical comparisons between drug and vehicle treated rats were made by *t*-tests (BMDP statistical software) and $P<0.05$ was considered significant.

Drugs

Rizatriptan (1H-Indole-3-ethanamine, N,N-dimethyl-5-(1H-1,2,4-triazol-1-ylmethyl)-,monobenzoate), PNU142,633 ((s)-3,4-dihydro-1-[2-[4-[4-(aminocarbonyl) phenyl]-1 piperazinyl] ethyl]-N-methyl-1H-2-benzopyan-6-carboximide and LY334370 (Benzamide, 4-fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]-, fumarate) (synthesised at Merck Sharp and Dohme Research Laboratories, U.K.) and human-αCGRP$_{(8-37)}$ (Peninsula, U.K.) were dissolved in 0.9% saline. Rat-αCGRP and ET-1 (Bachem, U.K.) 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^{-1} and doses refer to free base weight.

Results

Effects of vehicle or human-αCGRP$_{(8-37)}$ on dural blood vessel diameter, MABP and neurogenic dural vasodilation

Intravenous administration of saline had no effect on dural blood vessel diameter or MABP (data not shown). In this group of guinea-pigs, following administration of ET-1, electrical stimulation of the cranial window (approx. 275 μA, 1 ms, 5 Hz for 10 s) evoked a 122 ± 18% increase in dural blood vessel diameter ($n=11$). In separate animals, pre-treatment with 0.3 mg kg^{-1} i.v. human-αCGRP$_{(8-37)}$

evoked an immediate reduction in dural blood vessel diameter from 90±10 arbitrary units (AU) to 68±8 AU ($P<0.05$, $n=6$) and a small but significant increase in MABP (33±2 to 36±2 mmHg). Following subsequent ET-1 administration, electrical stimulation of the cranial window evoked a modest 18±7% increase in dural blood vessel diameter, which was significantly different from the response of the vehicle group (Figure 1a).

Effects of rizatriptan on neurogenic and CGRP-evoked dural vasodilation

Electrical stimulation of the cranial window in guinea-pigs pretreated with rizatriptan (0.01–1 mg kg^{-1}, $n=7-9$) prior to ET-1 preconstriction, produced a dose-related inhibition of neurogenic dural vasodilation compared to the vehicle group ($n=11$) with a minimum effective dose (MED) of 0.1 mg kg^{-1} and a maximum inhibition of 62% in the 1 mg kg^{-1} group (Figure 1b). In contrast, rizatriptan (1 mg kg^{-1}, i.v.) had no effect on vasodilation evoked by intravenous administration of rat-αCGRP (1 μg kg^{-1}, $n=6$, Figure 1b).

Effects of PNU142,633 and LY334370 on neurogenic dural vasodilation

PNU142,633 (0.01–1 mg kg^{-1}, i.v., $n=8$) administered prior to ET-1 also evoked a dose-related inhibition of neurogenic dural vasodilation with a MED of 0.1 mg kg^{-1} and a maximum inhibition of 53% in the 1 mg kg^{-1} group (Figure 2a). In contrast, in guinea-pigs treated with LY334370 (3 mg kg^{-1}, $n=7$) the neurogenic dural vasodilation response was no different from the vehicle group (Figure 2b, $n=11$).

Effects of rizatriptan, PNU142,633 and LY334370 on dural blood vessel diameter and MABP prior to ET-1

Intravenous administration of rizatriptan evoked a transient reduction in dural blood vessel diameter of 32% at 1 mg kg^{-1}, which returned to baseline within 10 min and a small but sustained increase in MABP after 1 mg kg^{-1} (41±2 to 44±2 mmHg, $P<0.05$). LY334370 (3 mg kg^{-1}, i.v.) also evoked a transient decrease in dural blood vessel diameter which returned to baseline within 3 min. Upon injection, LY334370 evoked a marked and immediate increase in MABP (36±2 to 46±3 mmHg, $P<0.05$) followed by a sustained decrease (30±2 mmHg, $P<0.05$). PNU142,633 had no consistent effects on dural blood vessel diameter or MABP.

Discussion

Effects of human-αCGRP$_{(8-37)}$ on dural blood vessel diameter and neurogenic dural vasodilation

The present studies have demonstrated that electrical stimulation of the cranial window evokes a neurogenic dural vasodilation of ET-1 preconstricted dural arteries in anaesthetized guinea-pigs. This vasodilation response is mediated by CGRP, presumably released from activated trigeminal fibres innervating the dural blood vessels, since the vasodilation was almost abolished by intravenous administration of the CGRP receptor antagonist human-αCGRP$_{(8-37)}$. These results are in agreement with previous studies in rats showing an important role of CGRP in neurogenic dural vasodilation

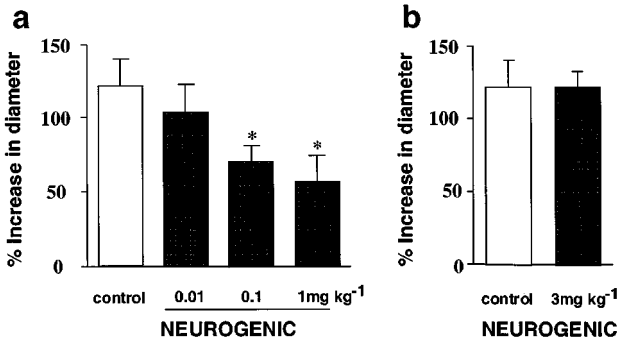

Figure 2 Effects of (a) PNU-142633 and (b) LY334370 on neurogenic dural vasodilation in anaesthetized guinea-pigs. Vasodilation was evoked by local electrical stimulation (approx. 275 μA, 1 ms, 5 Hz for 10 s) of the dura. Each column represents the mean percentage increase in diameter±s.e.mean of (a) 8-11 and (b) 7-11 guinea-pigs. *Significantly different from control ($P<0.05$).

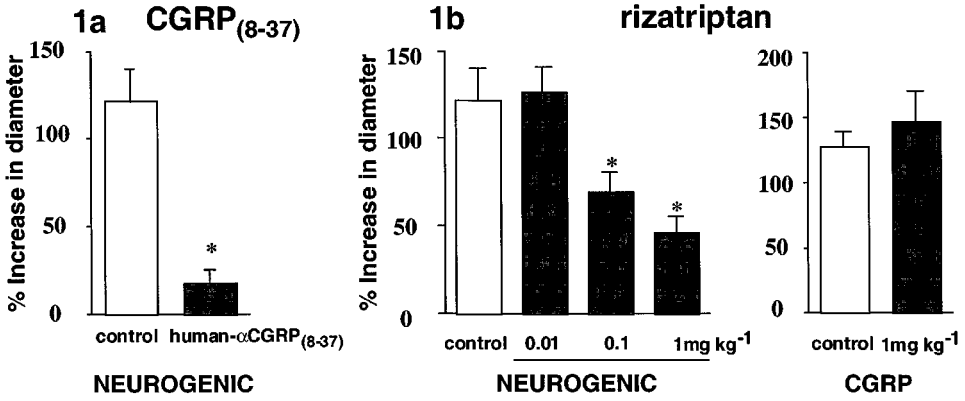

Figure 1 Effects of (a) 0.3 mg kg^{-1} i.v. human-αCGRP$_{(8-37)}$ on neurogenic dural vasodilation and (b) rizatriptan on neurogenic or rat-αCGRP-evoked dural vasodilation in anaesthetized guinea-pigs. Vasodilation was evoked by electrical stimulation (approx. 275 μA, 1 ms, 5 Hz for 10 s) of the dura mater or intravenous administration of rat-αCGRP (1 μg kg^{-1}). Each column represents the mean percentage increase in diameter±s.e.mean of (a) 6–11 and (b) 6–11 guinea-pigs. *Significantly different from control ($P<0.05$).

after electrical stimulation at similar intensities to those used in the present studies (Williamson *et al*., 1997b). One obvious difference between the guinea-pig and rat experiments was the need to constrict the dural blood vessels with ET-1 prior to the vasodilator challenge. In preliminary experiments following introduction of the cranial window the dural blood vessels were found to be dilated, possibly due to mechanical stimulation of trigeminal sensory fibres during the drilling process. In contrast to rats, the guinea-pig dural blood vessels did not constrict over time, so that subsequent electrical stimulation of cranial window only produced modest increases in dural blood vessel diameter. This sustained dilation of dural blood vessels may reflect the experimental conditions or physiological differences between guinea-pigs and rats. Intravenous administration of human-αCGRP$_{(8-37)}$ produced an immediate reduction in dural blood vessel diameter in the guinea-pig suggesting that under these experimental conditions there is a tonic release of CGRP within the meningeal circulation. This observation was somewhat surprising since it has been suggested that under normal physiological conditions the trigeminovascular system is not involved in the regulation of cranial blood flow in the cat (McCulloch *et al*., 1986). Although it is possible that CGRP plays an important role in the maintenance of cerebral blood flow in guinea-pigs under normal physiological conditions, several observations need to be considered. Firstly, under pentobarbitone anaesthesia the MABP in control animals was 38 ± 1 mmHg, whereas the resting MABP in conscious guinea-pigs is reported to be about 65 mmHg (Fossa *et al*., 1997). This marked reduction in blood pressure, could account for a tonic CGRP release since in rats a forced reduction in blood pressure of up to 40% results in a marked dilation of pial blood vessels mediated *via* CGRP release (Hong *et al*., 1994). Secondly, although Beattie & Connor (1994) reported that cannulation of the contralateral jugular vein and carotid artery had only transient effects on cerebral blood flow in guinea-pigs, it cannot be excluded that some interruption in cranial blood supply could account for the sustained vasodilation response following activation of a trigeminovascular reflex (Moskowitz, 1990) and subsequent release of CGRP.

Effects of rizatriptan on dural blood vessel diameter, neurogenic and CGRP-evoked dural dilation

The present experiments also demonstrated that neurogenic dural vasodilation, but not that evoked by intravenously administered rat-αCGRP, was inhibited by the 5-HT$_{1B/1D}$ receptor agonist rizatriptan indicating that rizatriptan prevents the release of CGRP *via* an action at receptors located on trigeminal sensory fibres. Intravenous administration of rizatriptan evoked a transient reduction in dural blood vessel diameter which recovered to baseline values within 10 min. Although it is likely that this observation reflects the direct constrictor action of rizatriptan on meningeal blood vessels (Razzaque *et al*., 1999), the reason for the transient nature of the vasoconstriction is not clear. In neurogenic vasodilation studies, rizatriptan significantly inhibited the response at a dose of 100 μg kg^{-1}, which would equate to a plasma level of approximately 40 nM (unpublished observations). The plasma concentration of rizatriptan active in this assay correlates well with plasma concentrations (30 – 70 nM) required for anti-migraine activity in the clinic (Longmore *et al*., 1998) and thus supports the guinea-pig as a relevant assay to investigate 5-HT$_1$ receptor pharmacology in animals.

Effects of PNU142,633 and LY334370 on neurogenic dural vasodilation

Neurogenic dural vasodilation in guinea-pigs was also inhibited by the selective 5-HT$_{1D}$ agonist PNU142,633 but not by the 5-HT$_{1F}$ agonist LY334370 suggesting that the inhibitory presynaptic receptors present on trigeminal fibres are 5-HT$_{1D}$, rather than 5-HT$_{1F}$ receptors in guinea-pigs. Further studies with a selective antagonist for 5-HT$_{1D}$ receptors, however, are required to unequivocally demonstrate that this receptor subtype mediates inhibition of neurogenic dural vasodilation.

We have previously reported that LY334370 is also inactive against neurogenic dural vasodilation in rats (Shepheard *et al*., 1999). suggesting that the neuronal population activated under these experimental conditions does not express functional 5-HT$_{1F}$ receptors in either species. It is unlikely that the lack of effect of LY334370 on neurogenic dural vasodilation can be explained in terms of dose used, since LY334370 has been shown to significantly inhibit activation of central trigeminal neurones in response to electrical stimulation of the dura mater in rats at the same dose that was tested in the present studies (Shepheard *et al*., 1999).

Perhaps the best explanation for the lack of effect of LY334370 against neurogenic dural vasodilation is that extravasation and vasodilation are mediated *via* different neuronal types. Neurogenic dural extravasation is produced by a high intensity (1 – 2.4 mA), sustained (5 min) electrical stimulation of the trigeminal ganglion and is mediated *via* neurokinins acting at NK$_1$ receptors on post capillary venules (Buzzi *et al*., 1992; Shepheard *et al*., 1993). In contrast, neurogenic vasodilation is evoked by a less intense (up to 300 μA), brief (10 s) stimulation of the skull and is mediated *via* the release of CGRP acting at CGRP receptors present on dural arteries. Immunocytochemical studies show that, whilst substance P is almost always co-localized with CGRP in trigeminal neurones, a significant population of neurones contain only CGRP (Lee *et al*., 1985; O'Connor & van der Kooy, 1988). Furthermore, substance P is predominantly contained within small diameter sensory C-fibres, whereas CGRP is contained in both small and larger diameter neurones (Lee *et al*., 1985). The larger diameter neurones contain only CGRP and may give rise to small, thinly myelinated Aδ-fibres, similar to the CGRP containing sensory Aδ-fibres that have been demonstrated in the rat spinal cord (McCarthy & Lawson, 1990). It is known that Aδ-fibres are activated at lower stimulus intensities than would be required to activate C-fibres, suggesting that in the present intravital studies the stimulation parameters used activate only the Aδ fibre population leading to the release of CGRP to evoke vasodilation. This observation may be of interest since jugular blood CGRP, but not substance P, levels are increased in migraine (Goadsby *et al*., 1990) and CGRP levels are normalized after successful sumatriptan treatment (Goadsby & Edvinsson, 1993). Furthermore, some agents shown to block experimental dural extravasation such

as NK_1 and the endothelin receptor antagonist bosentan are ineffective in the treatment of migraine (May *et al*., 1996; Goldstein *et al*., 1997). It is therefore possible that Aδ, rather than C-fibres may play a more important role in the pathogenesis of migraine and further supports the development of CGRP receptor antagonists as novel anti-migraine agents.

It is interesting to note that the present experiments do not mirror recent clinical trials with LY334370 and PNU142,633. LY334370 was reported to be effective in the treatment of migraine (Goldstein *et al*., 1999), whereas PNU142,633 failed to show efficacy in migraineurs (Cutler *et al*., 2000). If we propose that the guinea-pig is an appropriate species to extrapolate observations to man then discrepancies in the clinical trials must be explained. It is possible that the anti-migraine actions of LY334370 are mediated *via* a central mechanism rather than peripheral actions, since 5-HT_{1F} receptors have been detected in the trigeminal nucleus caudalis, a key central relay point for nociceptive neurotransmission (Waeber & Moskowitz, 1995). In support of this concept, 5-HT_{1F} agonists have been shown inhibit increases in trigeminal neuronal activity and c-*fos* expression (a marker for neuronal activation) within the trigeminal nucleus caudalis evoked by electrical or chemical stimulation of the dura mater in rats (Mitsikostas *et al*., 1999; Shepheard *et al*., 1999).

In contrast, the lack of effect of the 5-HT_{1D} agonist PNU142,633 in clinical trials is more difficult to explain. PNU142,633 was administered as a single 50 mg oral dose (Cutler *et al*., 2000) and it is possible, since plasma levels were not measured in this study, that this dose did not produce sufficient plasma concentrations in patients. Alternatively the lack of anti-migraine effects could also be explained by the observation that PNU142,633 is a weak agonist on cloned gorilla 5-HT_{1D} receptors *in vitro* compared to sumatriptan (Pregenzer *et al*., 1999). Although the efficacy of PNU142,633 on guinea-pig 5-HT_{1D} receptors is not known, the present observation that PNU142,633 blocked neurogenic dural vasodilation in guinea-pigs to a similar extent to the full agonist rizatriptan implies different efficacy across species.

Conclusions

The present studies have demonstrated that electrical stimulation of the dura mater evokes neurogenic vasodilation of preconstricted dural blood vessels in anaesthetized guinea-pigs and that the dilation is mediated by CGRP release from trigeminal fibres. In addition neurogenic, but not CGRP-evoked dural vasodilation, was also blocked by rizatriptan at clinically relevant doses *via* an action on presynaptic 5-HT_{1D} receptors, since neurogenic dural vasodilation was also blocked by the 5-HT_{1D} agonist PNU142,633 but not by the 5-HT_{1F} agonist LY334370. The present studies suggest that the guinea-pig may be an appropriate species in which to investigate the pharmacology of neurogenic dural vasodilation providing data that can be extrapolated to man.

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