Vanilloid type 1 receptors (VR1) on trigeminal sensory nerve fibres play a minor role in neurogenic dural vasodilatation, and are involved in capsaicin-induced dural dilation

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1 Capsaicin, the active substance in chilli peppers, activates the vanilloid type 1 receptor (VR1) rather than the vanilloid-like receptor (VRL1) in the trigeminal ganglion and nucleus of small and medium C- and Aδ-fibres.

2 Capsaicin induces calcitonin gene-related peptide (CGRP) release when VR1 receptors are activated, and this can be reversed by both the VR1 receptor antagonist capsazepine and the CGRP blocker αCGRP$_{8-37}$ in vitro.

3 In this study we used intravital microscopy to look at the possible role of the VR1 receptor in the trigeminovascular system in producing dilation of dural blood vessels. Capsazepine ($3\,\mathrm{mg\,kg^{-1}}$) was given to study the effect of the VR1 receptor in dural vessel dilation produced by either electrical stimulation, CGRP ($1\,\mu\mathrm{g\,kg^{-1}}$) or capsaicin ($7\,\mu\mathrm{g\,kg^{-1}}$) bolus injection. We also looked at the effect of the CGRP blocker αCGRP$_{8-37}$ ($300\,\mu\mathrm{g\,kg^{-1}}$) on capsaicin-induced dilation so that we could see if the results found in vitro could also be found in vivo.

4 Electrical stimulation of the dura mater produced a robust vasodilator response between 130 and 137% of baseline diameter that was no different across four repeat stimuli ($F_{3,18} = 0.6$, $P = 0.61$). CGRP similarly produced a dilatation of 99–111% that was no different across four baseline infusions ($F_{3,15} = 2.4$, $P = 0.113$). Capsaicin also produced a consistent dilation of between 112 and 120% of baseline across three injections ($F_{2,10} = 0.6$, $P = 0.567$).

5 Capsazepine did not inhibit the dilation brought about by either electrical stimulation or CGRP injection, while it was able to inhibit the dilation brought about by capsaicin ($t_5 = 3.4$, $P < 0.05$). αCGRP$_{8-37}$ also inhibited the capsaicin-induced dilation ($t_5 = 7.4$, $P < 0.05$) probably inhibiting the action of released CGRP at the CGRP receptor.

6 The study demonstrates that capsaicin can repeatedly induce dural vessel dilation in vivo, presumably through inducing CGRP release from trigeminal sensory nerve fibres, while C-fibres may have been desensitised. The data imply that the VR1 receptor plays only a minor role in trigeminovascular-induced dural vessel dilation.
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Introduction

Capsaicin is the pungent ingredient in hot chilli peppers that activates the vanilloid type 1 receptor (VR1) (Caterina et al., 1997). VR1 receptors are located on small- and medium-sized neurons that are either unmyelinated C-fibres or thinly myelinated Aδ-fibres (Joo et al., 1969). VR1 receptors are found on neurons in the trigeminal and dorsal root ganglia (Guo et al., 1999; Ichikawa & Sugimoto, 2001). Intravenous capsaicin promotes the release of the proinflammatory neuropeptides, substance P (SP) and neurokinin A from trigeminal neurons and causes dural extravasation in the rat (Markowitz et al., 1987). It is thought that this action is C-fibre dependent as the destruction of C-fibres in neonate rats prevents this action of capsaicin as an adult (Markowitz et al., 1987) and because SP is predominantly contained within

C-fibres, and also, almost always colocalised with calcitonin gene-related peptide (CGRP) (Lee et al., 1985; O'Connor & van der Kooy, 1988). The potent vasodilator, CGRP is also released, probably via the activation of both C- and Aδ-fibres in the trigeminal region (Lee et al., 1985; O'Connor & van der Kooy, 1988; Escott et al., 1995a). The trigeminal nerve innervates pain-producing intracranial structures, so that the physiology and pharmacology of the trigeminovascular system are pivotal to understanding primary headaches (Goadsby et al., 2002).

VR1 receptors are also activated by acid and moderate heat, with a threshold $\sim 43^{\circ}\mathrm{C}$ (Caterina et al., 1997). The VR1 receptor belongs to a subgroup of the transient receptor potential (TRP) superfamily of ion channels called TRPV, so named because the VR1 receptor was the first mammalian member of the subgroup identified (Montell et al., 2002). They contain six transmembrane segments, with a re-entrant pore

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separating the last two segments (Gunthorpe *et al.*, 2002; Montell *et al.*, 2002). There are other members of the TRP superfamily that are also temperature sensitive covering a range of temperatures from cool to hot. They tend to be initially categorised by their sensitivity to capsaicin (a brief overview of the heat-sensitive TRP superfamily of ion channels is presented in Table 1).

Recently, VR1-immunoreactivity has been found in 16% of total neuronal cell bodies in human trigeminal ganglia; of this 16%, there was a small proportion that showed colocalisation with CGRP (Hou *et al.*, 2002). CGRP is believed to be a marker of small- to medium-sized neurons in sensory ganglia (Ju *et al.*, 1987), and it is on this type of neuron that VR1 receptors are expressed. Therefore, it seems likely that VR1 receptors are colocalised with CGRP in the trigeminovascular system and cause the release of CGRP when they are activated by capsaicin.

Neurogenic dural inflammation uses high intensity, sustained electrical stimulation that activates C-fibres and causes the release of SP and CGRP (Lee *et al.*, 1985; Markowitz *et al.*, 1987; O'Connor & van der Kooy, 1988). Relative to this, neurogenic dural vasodilatation is induced by much lower electrical intensity stimulation for only a brief period, and it is believed to only recruit trigeminal $A\delta$ sensory nerve fibres (Escott *et al.*, 1995b; Williamson *et al.*, 1997a). The evidence for this comes from the fact that the SP/NK$_1$ antagonist, RP 67580, was unable to attenuate neurgenic dural vasodilatation, and we report above that SP is predominantly contained within C-fibres, indicating the C-fibres are probably not activated (Williamson *et al.*, 1997b). Trigeminal sensory nerve fibres innervate the cranial blood vessels and they contain the neuropeptide, CGRP (Edvinsson *et al.*, 1989; Jansen *et al.*, 1991). When these trigeminal sensory nerve fibres become activated, they release CGRP into the cranial circulation (Goadsby *et al.*, 1988). Indeed, it is thought that neurogenic dural vasodilatation is caused by the release of CGRP from activated $A\delta$-fibres, and CGRP then acts on CGRP receptors on the smooth muscle of dural arteries to cause the dilation (Williamson *et al.*, 1997a).

Capsaicin causes potent vasodilation by activating VR1 receptors *in vitro* (Sams-Nielsen *et al.*, 2001), and the literature indicates that VR1 receptors are present on $A\delta$ and C-fibres in the trigeminovascular system (Guo *et al.*, 1999; Ichikawa & Sugimoto, 2001). Neurogenic dural vasodilatation, a model that is believed to activate $A\delta$-fibres solely, has been found to be a highly predictive model for identifying acute antimigraine drugs and helps dissect migraine pathophysiology. The purpose of the study was firstly to see if capsaicin could cause vasodilatation in our *in vivo* model, in the same way that it is able to cause vasodilation *in vitro*, presumably by activating C-fibres, and provoking CGRP release (Saito & Goto, 1986; Markowitz *et al.*, 1987; Maggi *et al.*, 1988; Martling *et al.*, 1988; Wardle *et al.*, 1997; Flores *et al.*, 2001). It was the second aim of this study, having observed the response of capsaicin *in vivo*, to examine whether the VR1 receptor is also involved in $A\delta$-fibre specific mediated neurogenic dural vasodilatation. We used the *in vivo* model of intravital microscopy that allows direct study of dural vessel diameter. We examined the effect of a VR1 receptor antagonist, capsazepine, on neurogenically dilated dural blood vessels, and on dilation caused by both capsaicin and CGRP. We also looked at the effect of a CGRP blocker, CGRP$_{8-37}$, on the activated trigeminal VR1 receptor response with capsaicin.

Methods

Surgical preparation

All experiments were conducted under UK Home Office (Scientific Procedures) Act (1986). Male Sprague–Dawley rats (285–430 g) were anaesthetised throughout the experiments with sodium pentobarbitone (60 mg kg^{-1} i.p. and then 18 mg kg^{-1} h i.v. infusion). The left femoral artery and vein were cannulated for blood pressure recording, and intravenous infusion of anaesthetic and test compounds, respectively. Temperature was maintained throughout using a homeothermic blanket system. The rats were placed in a stereotaxic

Table 1 Summary of the temperature-sensitive TRP superfamily of ion channels

Nomenclature from the TRP superfamily (Montell et al., 2002)	Former receptor name	Temperature threshold (°C)	Agonist	Antagonist	References
TRPV1	VR1	∼43	Capsaicin, anandamide	Capsazepine (specific)	Caterina et al. (1997)
TRPV2	VRL-1	∼52	Capsaicin insensitive	Ruthenium red (nonspecific TRPV antagonist)	Caterina et al. (1999)
			Insulin-like growth factor		
TRPV3	VRL-3	∼39	Capsaicin insensitive	Ruthenium red	Peier et al. (2002b), Smith et al. (2002) Xu et al. (2002)
TRPV4	OTROC4, VRL-2, VR-OAC, TRP 12	27–42	Capsaicin insensitive	Ruthenium red	Guler et al. (2002)
			Hypotonic solution Phorbol derivatives		
ANKTM1 (mammalian TRP-like receptor)		12–24	Icilin (potent cooling compound)	Ruthenium red	Story et al. (2003)
TRPM8	CMR1	18–28	Capsaicin-insensitive menthol, icilin	Not determined	McKemy et al. (2002), Peier et al. (2002a)

frame and ventilated with oxygen-enriched air, 3–5 ml, 60–80 strokes per minute (Small Rodent Ventilator – Model 683, Harvard Instruments, U.K.). End-tidal CO_2 was monitored (Capstar-100, CWE Inc., U.S.A.) and kept between 3.5 and 4.5% and blood pressure was monitored continually. This allows continuous monitoring of respiration and blood pressure for changes due to medium-term anaesthetic. The skull was exposed and the right or left parietal bone thinned by drilling with a saline-cooled drill until the blood vessels of the dura were clearly visible through the intact skull.

Intravital microscopy

The cranial window was covered with mineral oil (37°C) and a branch of the middle meningeal artery was viewed using an intravital microscope (Microvision MV2100, U.K.) and the image displayed on a television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyser (Living Systems Instrumentation, U.S.A.) and displayed with blood pressure on an online data analysis system (Spike4 v2, Cambridge Electronic Design).

Experimental protocols

Defining electrical stimulation parameters Electrical stimulation was used to evoke dilation of the dural blood vessels with a bipolar stimulating electrode (NE 200X, Clark Electromedical) that was placed on the surface of the cranial window approximately 200 μm from the vessel of interest. The surface of the cranial window was stimulated at 5 Hz, 1 ms for 10 s (Grass Stimulator S88, Grass Instrumentation) with increasing voltage until maximal dilation was observed. Subsequent electrically induced responses in the same animal were then evoked using that voltage (Williamson *et al.*, 1997b; Akerman *et al.*, 2002b).

CGRP and capsaicin-induced dilation In the preparations where CGRP was used to dilate dural blood vessels, CGRP was given as an intravenous bolus of 1 μg kg^{-1}, which has been shown to produce a maximal dilation (Williamson *et al.*, 1997a). In those preparations where capsaicin was used to dilate the dural blood vessels, capsaicin was given as an intravenous bolus of up to 7 μg kg^{-1} until a maximal dilation was observed.

Effects of the vanilloid antagonist capsazepine on evoked dilation in rats

The effects of the VR1 receptor antagonist capsazepine were studied. Capsazepine (1 mg kg^{-1}) was administered intravenously at least 10 min after the control response to either electrical stimulation, bolus of CGRP or capsaicin. The electrical stimulation, bolus of CGRP or capsaicin was then repeated after 10 min. Then at least a further 10 min later an increased dosage of capsazepine (3 mg kg^{-1}) was administered followed by another electrical stimulation, bolus of CGRP or capsaicin after 10 min.

Effects of αCGRP$_{8-37}$ on capsaicin-induced dilation The effects of the CGRP peptide fragment αCGRP$_{8-37}$, a CGRP blocker, was studied. αCGRP$_{8-37}$ (300 μg kg^{-1}) was administered intravenously at least 10 min after the control

response to capsaicin, and then the control response to capsaicin was repeated 2 min later. At 20 min after the dose of αCGRP$_{8-37}$ the control response to capsaicin was again repeated.

Data analysis

The effects of electrical stimulation, bolus of CGRP or capsaicin on dural vessel diameter were calculated as a percentage increase from the prestimulation baseline diameter. During the experimental set-up selecting the optimal vessel results in the magnification, as visualised, being different in each animal making it impossible to standardise the dural vessel measurement. The dural vessel diameter was, therefore, measured in arbitrary units. All data are expressed as mean $\pm$ s.e.m. Statistical analysis was performed using an ANOVA for repeated measures and *post hoc* comparison made with a Student's paired *t*-test (SPSS v10.0). The reproducibility of the neurogenic vasodilator and CGRP responses has been tested previously using four consecutive saline-controlled stimuli (Akerman *et al.*, 2002b) to determine whether there was any systematic effect of test compounds over time in the meningeal circulation. We also completed a series of saline-controlled vasodilator repetitions for comparison with capsaicin. For these baseline studies, the consecutive determinations of the neurogenic dilator response, CGRP or capsaicin-induced dilations were compared to the control dilation used for the pharmacological interventions using an ANOVA with repeated measures and a two-factor design, responses across consecutive stimuli and between the two groups. Significance was assessed at the $P < 0.05$ levels.

Drugs

The infusion of anaesthetic and experimental drugs were all *via* the same catheter; however, the line was always flushed with saline first, several minutes before administering the different compound. Capsazepine (Tocris Cookson, U.K.) was dissolved in a couple of drops of DMSO (Sigma-Aldrich, U.K.) and further diluted in a 1 : 1 : 8 solution of Tween 80 (polyoxyethylene-sorbitan mono-oleate, Sigma-Aldrich, U.K.); ethanol: 0.9% NaCl. (*E*)-capsaicin (Tocris Cookson, U.K.) was also made up in the solution of Tween 80, ethanol and saline. CGRP and αCGRP$_{8-37}$ (Sigma-Aldrich, U.K.) were both dissolved in deoxygenated water and aliquoted and frozen until required and then redissolved further in 0.9% NaCl.

Results

Baseline group

The results for the series of control dilations where saline was used as a pretreatment to electrical stimulation and CGRP-induced dilation showed no difference across the cohort (Akerman *et al.*, 2002b). Similarly, three consecutive bolus capsaicin injections produced mean dilations that showed no difference across the cohort, 119.6 $\pm$ 11, 115.3 $\pm$ 16 and 111.6 $\pm$ 12%, respectively ($F_{2,10} = 0.6$, $P = 0.567$). These control effects were compared using an ANOVA to the control

dilations used in the pharmacological studies, and there were no significant differences.

Effect of a vanilloid receptor antagonist on electrical stimulation, bolus of CGRP and capsaicin-induced dilation

In rats treated with capsazepine (1 and $3\,\mathrm{mg\,kg^{-1}}$, $n = 6$) there was no difference in dural blood vessel diameter after electrical stimulation of the cranial window when compared with the control dilation, 119 ± 6 to $126 \pm 12\%$ ($1\,\mathrm{mg\,kg^{-1}}$) and $134 \pm 19\%$ ($3\,\mathrm{mg\,kg^{-1}}$, $F_{1,5} = 1.19$, $P = 0.325$). Increases in dural blood vessel diameter evoked by CGRP ($1\,\mu\mathrm{g\,kg^{-1}}$, i.v.) also showed no significant difference between the control dilation and CGRP-induced dilation after capsazepine (1 and $3\,\mathrm{mg\,kg^{-1}}$, $n = 6$) intervention, 121 ± 12 to $122 \pm 13\%$ ($1\,\mathrm{mg\,kg^{-1}}$) and $128 \pm 12\%$ ($3\,\mathrm{mg\,kg^{-1}}$, $F_{1,5} = 0.148$, $P = 0.716$). Dural blood vessel dilation brought about by capsaicin showed a significant reduction after pretreatment with capsazepine ($3\,\mathrm{mg\,kg^{-1}}$) when compared to the control dilation, 134 ± 11 to $102 \pm 11\%$ ($P < 0.05$, $n = 7$, $t_5 = 3.4$). There was no significant reduction with $1\,\mathrm{mg\,kg^{-1}}$ capsazepine (Figure 1). Capsazepine injections ($n = 31$) caused a significant increase in blood pressure, $8 \pm 2\,\mathrm{mmHg}$ ($P < 0.05$) that was accompanied by a significant drop in blood vessel diameter of $28 \pm 5\%$ ($P < 0.05$), both were restored to preinjection levels before control dilations were repeated.

Effect of a CGRP blocker on capsaicin-induced dilation

In rats treated with $\alpha\mathrm{CGRP}_{8-37}$ ($1\,\mu\mathrm{g\,kg^{-1}}$, i.v., $n = 6$) there was significant inhibition of the capsaicin ($7\,\mu\mathrm{g\,kg^{-1}}$)-induced dilation when compared with the control dilation, 112 ± 14 to $10 \pm 6\%$ ($P < 0.05$, $t_6 = 7.3$). The capsaicin-induced dilation returned significantly after a delay of $20\,\mathrm{min}$, 10 ± 6 to $67 \pm 12\%$ ($P < 0.05$, $t_5 = 7.4$). Although there is a significant return of the dilation after a gap of $20\,\mathrm{min}$, this dilation is still significantly different from the control dilation, 112 ± 14 and $67 \pm 12\%$ ($P < 0.05$, $t_5 = 5.9$) see Figure 2.
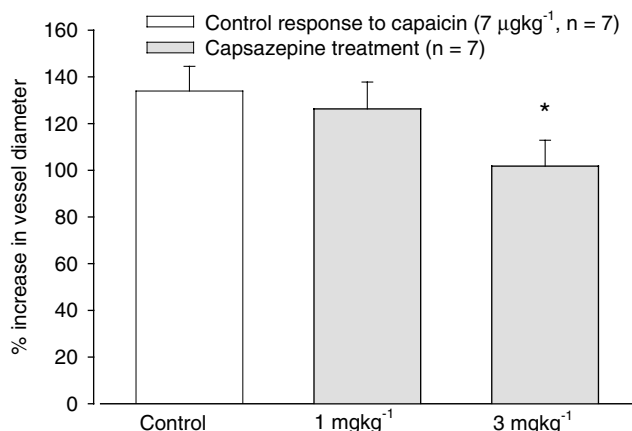

Figure 1 Effects of repeated capsaicin (up to $7\,\mu\mathrm{g\,kg^{-1}}$) injection with capsazepine treatment (grey) on dural blood vessel diameter. Following control responses rats were injected capsazepine (1 and $3\,\mathrm{mg\,kg^{-1}}$) and capsaicin injection repeated. *$P < 0.05$ significance compared to the control response.
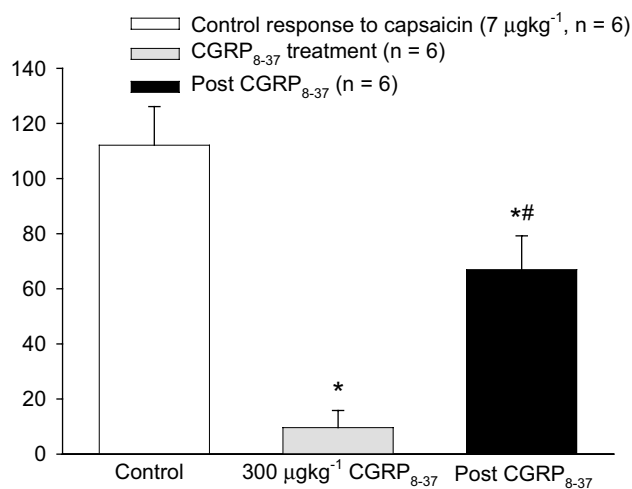

Figure 2 Effects of repeated capsaicin ($7\,\mu\mathrm{g\,kg^{-1}}$) injection with CGRP_{8-37} (grey) on dural blood vessel diameter. Following control responses rats were injected with CGRP_{8-37} ($300\,\mu\mathrm{g\,kg^{-1}}$) and capsaicin injection repeated. *$P < 0.05$ significance compared to the control response. #$P < 0.05$ significance compared to the inhibitory response of CGRP_{8-37}.

Effect of capsazepine on the blood pressure changes induced by CGRP and capsaicin

The four consecutive CGRP injections as previously described (Akerman *et al.*, 2002b) and the control group of the Results section produced decreases in mean blood pressure of 41 ± 7, 38 ± 5, 37 ± 7 and $33 \pm 3\,\mathrm{mmHg}$, respectively. There was no difference across the cohort ($F_{3,12} = 1.492$, $P = 0.283$). Our previous control effects (Akerman *et al.*, 2002b) were compared with an ANOVA for repeated measures to the control dilations used in the pharmacological studies, and there were no significant difference. These blood pressure changes were not altered by pretreatment for capsazepine ($F_{2,6} = 5.48$, $P = 0.8$). The blood pressure response to capsaicin has a triphasic effect, as has been previously shown (Chahl & Lynch, 1987), and the blood pressure decreases that accompanied the vasodilations, as stated above in the 'Control Groups' of the results section, were 44 ± 5, 38 ± 4 and $31 \pm 3\,\mathrm{mmHg}$, respectively. The blood pressure drop that accompanied the second and third capsaicin injections was significantly less than the first injection ($t_5 = 3.6$ and 3.152, $P < 0.05$, respectively). Similarly, when capsazepine was used as a pretreatment for capsaicin, the decrease in blood pressure was significantly less for the third capsaicin injection compared to the first ($t_6 = 2.973$, $P < 0.05$).

Discussion

Electrical stimulation, CGRP and capsaicin bolus injections were each capable of creating reproducible dural vessel dilation measured with intravital microscopy in rat, but only the capsaicin-induced dilation was inhibited by the VR1 antagonist capsazepine. Capsaicin-induced dilation was also inhibited by the CGRP receptor blocker, CGRP_{8-37} which has been shown previously to inhibit both CGRP and electrically induced dilation of dural blood vessels (Williamson *et al.*,

1997a, b). These data suggest that VR1 receptor activation plays a minor role in the Aδ-fibre trigeminovascular-mediated dural vasodilation and predicts there is little or no role in migraine pathophysiology.

Capsaicin was able to cause dural vessel dilation repeatedly when given as an intravenous bolus in our *in vivo* model. The VR1 receptor antagonist capsazepine and CGRP receptor blocker, $CGRP_{8-37}$, were able to inhibit capsaicin-induced dural blood vessel dilation. Capsaicin administration potently causes the release of CGRP from trigeminal sensory nerve terminals, in a capsazepine (VR1 receptor antagonist) reversible manner both *in vitro* and *in vivo* (Saito & Goto, 1986; Maggi *et al.*, 1988; Martling *et al.*, 1988; Wardle *et al.*, 1997; Flores *et al.*, 2001). In another *in vitro* study $CGRP_{8-37}$, a CGRP blocker, as well as capsazepine, was found to inhibit the capsaicin-induced vasodilation in guinea-pig basilar artery (Sams-Nielsen *et al.*, 2001). Although capsaicin administration also causes the release of SP and neurokinin A, it is CGRP release that causes the vasodilation of arteries (Franco-Cereceda & Rudehill, 1989; Jansen-Olesen *et al.*, 1996). Capsaicin binding to VR1 receptors triggers calcium influx, and elevated intracellular calcium levels stimulate exocytosis and CGRP release (Caterina *et al.*, 1997). In the *in vivo* model of trigeminovascular activation used here, it was demonstrated that a capsaicin-induced dilation of dural blood vessels is blocked by both capsazepine and $CGRP_{8-37}$, while capsazepine was unable to block the CGRP-induced dural vessel dilation. It seems likely that the dilation brought about by capsaicin is caused by activation of VR1 receptors on trigeminovascular C-fibres, causing the release of CGRP from perivascular trigeminal sensory fibres and consequent dilation. Evidence from the neurogenic dural vasodilatation study will highlight any Aδ-fibre component to the action of VR1 receptors on dural vessel calibre. Capsazepine probably inhibits capsaicin-induced dilation prejunctionally by inhibiting the release of CGRP from C-fibres, while $CGRP_{8-37}$ is likely to be acting postsynaptically to block the CGRP from CGRP receptors on the smooth muscle of the dura mater.

Neurogenic dural vasodilatation, as monitored by intravital microscopy, is likely to be a result of CGRP release *via* activation of perivascular trigeminal sensory Aδ-fibres (Escott *et al.*, 1995b; Williamson *et al.*, 1997a) and the distension of the dural blood vessels results in activation of trigeminal afferents that project to the trigeminal nucleus caudalis, a key relay centre in pain transmission (Moskowitz, 1984). The CGRP receptor antagonist, $CGRP_{8-37}$, inhibits the effects of neurogenic dural vasodilatation by 85% and the 'Triptans', acute antimigraine compounds, also significantly attenuate neurogenic dural vasodilatation response (Williamson *et al.*, 1997a–c). Our results show that the VR1 receptor antagonist, capsazepine was unable to significantly inhibit the neurogenic dural vasodilatation despite VR1 receptor presence in the trigeminovascular pathway. Therefore, it seems that the VR1 receptors do not play a major role in causing CGRP release *via* Aδ-fibre activation from an electrical stimulus.

Direct intravenous administration of CGRP causes dilation through CGRP receptors located on the smooth muscle of dural blood vessels (Jansen *et al.*, 1992; Edvinsson *et al.*, 1998; Williamson *et al.*, 1997a, b). The VR1 receptor antagonist capsazepine was unable to inhibit or attenuate the dilation brought about by CGRP administration. This lack of effect suggests that capsazepine does not act on $CGRP_1$ receptors,

and that there is not a postsynaptic component to the action of capsazepine on these neurons.

There is recent evidence indicating that VR1 receptors are present in the trigeminovascular system. VR1-immunoreactive sensory neurons are present in the rat trigeminal ganglion and trigeminal nucleus (Guo *et al.*, 1999; Ichikawa & Sugimoto, 2000; Mezey *et al.*, 2000) and the human trigeminal ganglion (Hou *et al.*, 2002). Approximately 16% of total neuronal cell bodies in the human trigeminal ganglion had VR1 receptor-like immunoreactivity and that, of those cells, 10% were coexpressed with CGRP (Hou *et al.*, 2002). Despite this recent evidence of localisation in the trigeminal region, it would appear that activation of VR1 receptors does not contribute to neurogenic dural vasodilatation, or that the few VR1 receptors that are located in the trigeminal region on Aδ sensory nerve fibres do not contribute significantly to neurogenic dural dilation. The low intensity electrical stimulation that was used in these studies is not capable of significantly recruiting C-fibres (Williamson *et al.*, 1997b). It seems likely that the majority of VR1 receptors in the trigeminal region are situated on C-fibres, and these fibres were not activated by the electrical stimulus used in this study, therefore, capsazepine had little effect as the VR1 receptors were not sufficiently activated. It is important to note that the dose of capsazepine used was biologically effective in the rat as it was able to inhibit the capsaicin-induced dilation.

In our studies, dural vessel dilation can be readily reproduced with repeated injection of a capsaicin bolus over time. A reproducible dilation with capsaicin was also shown in the guinea-pig basiliar artery *in vitro* (Sams-Nielsen *et al.*, 2001). Capsaicin administration produces an activation of primary afferent C and Aδ-fibres and the release of sensory neuropeptides, such as SP, neurokinin A and CGRP (Jancso *et al.*, 1967; Holzer, 1988; Dray *et al.*, 1989; Dray, 1992a, b; 1996), through calcium entry in the cells (Dray *et al.*, 1989). Repeated administration of capsaicin leads to a desensitisation and inactivation of C-fibre sensory neurons and contributes to the antinociceptive properties of capsaicin, as there is prolonged inactivation of transmitter release (Jancso *et al.*, 1967; Jancso-Gabor *et al.*, 1970; Dray *et al.*, 1989; Lynn, 1990). Why then is it that repeated capsaicin administration in this study was still able to produce dural vessel dilation on each occasion? Reproducible dilation implies that no desensitisation of dural or basilar arteries occurs to capsaicin administration. VR1 receptors have been found to be present on small- and medium-sized neurons with unmyelinated C-fibres and thinly myelinated Aδ-fibres (Joo *et al.*, 1969) in the trigeminal and dorsal root ganglia (Guo *et al.*, 1999; Ichikawa & Sugimoto, 2001). CGRP is believed to be a marker of small- to medium-sized neurons in sensory ganglia that the VR1 receptors are expressed on (Ju *et al.*, 1987). C-fibres that are normally activated with capsaicin, and contribute to plasma extravasation, may be desensitised by repeated administration. In contrast, the amount of capsaicin administered in the dural vessel dilation in our study and the basilar artery dilation previously reported (Sams-Nielsen *et al.*, 2001) may have been sufficient to mediate the activation of the small amount of VR1 receptors on the Aδ-fibres that are known to release CGRP when activated (Lee *et al.*, 1985; McCarthy & Lawson, 1990; Williamson *et al.*, 1997b). It may be interesting in the future to compare whether C-fibre desensitisation, perhaps measured by markers of inflammation, can occur

concurrently with Aδ-fibre activation, resulting in dural vessel dilation continues in the same animal. It may also be useful to electrical stimulate the dura to invoke dural vasodilatation after the desensitisation of C-fibres to show again that there is no C-fibre contribution to this vasodilation.

Both vasodilators that were administered had effects on blood pressure. CGRP is known to cause a dose-dependent drop in blood pressure (Brain *et al.*, 1993; Williamson *et al.*, 1997a), while capsaicin has been shown to cause a triphasic change in blood pressure, a fall, a big increase and then a fall again to baseline, which is thought to be partially mediated by the vagal nerve (Chahl & Lynch, 1987). Both of these effects were observed in this study. The response of both CGRP and capsaicin on blood pressure is short-lived compared to the dural vasodilator response, but it raises the question whether the blood pressure effect causes the vasodilation, or whether the vasodilation is a direct action on receptors in the trigeminal system? In a study that looked at the effect of CGRP in the cardiovascular system, it showed that CGRP caused greater increases in blood flow in the heart than the brain. However, when the same studies were conducted in the presence of β-adrenoceptor blockade, the changes in heart and brain blood flow were similar, indicating that the myocardial blood flow increases result from a reflex-mediated mechanism rather than from a direct effect at the CGRP receptors located in the coronary vasculature (Shen *et al.*, 2001). This implies that the vasodilatory action is *via* CGRP, acting at the CGRP receptor on the smooth muscle, rather than a compensatory effect of the cardiovascular changes. Similarly, the increase in blood pressure with capsaicin appears to be predominantly mediated by adrenergic mechanisms, α-adrenoceptor antagonists blocked the blood pressure response and propranolol blocked

the drop in heart rate (Chahl & Lynch, 1987). This would indicate a sympathetic basis for these changes. Although it cannot be ruled out that the vasodilatation is caused by a response to these blood pressure changes without replicating the blocking of the cardiovascular changes whilst observing dilation, it is likely that the vasodilatation is caused by action on receptors in the trigeminovascular system, rather than a response to the cardiovascular changes which are a result of a sympathetic reflex.

In summary, the new study demonstrates that capsaicin can produce repeated dural vessel dilation *in vivo* inducing the release of CGRP, probably by activating VR1 receptors on trigeminal sensory nerve C-fibres. The nature of this CGRP release is unclear, since we do not know whether VR1 receptors activate CGRP release directly, or *via* an indirect mechanism. VR1 receptors play a minor role in Aδ-fibre trigeminovascular-mediated dural vasodilatation as they are unable to inhibit neurogenic dural vasodilatation. It is possible that VR1 receptors on both Aδ- and C-fibres are involved in capsaicin-induced CGRP release as C-fibres become desensitised with repeated capsaicin administration, therefore, VR1 receptors on Aδ-fibres may play a role when this occurs. The data do not support a significant role for VR1 receptor modulation of the trigeminovascular system.

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