

# Topiramate inhibits trigeminovascular activation: an intravital microscopy study

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**1** Activation, or the altered perception of activation, of trigeminal nerves that innervate the cranial vasculature is considered to be a pivotal component of the pathophysiology of acute migraine.

**2** Calcitonin gene-related peptide (CGRP) levels are increased during migraine and after trigeminal nerve stimulation in the cat. Both CGRP and nitric oxide (NO) infusion causes headache and delayed migraine in migraineurs. Neurogenic stimulation of a cranial window, CGRP and NO injection all cause meningeal artery dilation in the rat when viewed using intravital microscopy.

**3** Topiramate is an antiepileptic drug with established efficacy as a migraine preventive, and has recently been shown to inhibit neurons of the trigeminocervical complex after superior sagittal sinus stimulation.

**4** In this study, we used intravital microscopy with neurogenic dural vasodilation, and CGRP- and NO-induced dilation to examine whether intravenous topiramate has effects on the trigeminovascular system.

**5** Topiramate was able to attenuate neurogenic dural vasodilation maximally after 15 min by 52% at 30 mg kg<sup>-1</sup> ( $t_5 = 6.78$ ,  $n = 6$ ); there was no significant inhibition at 10 mg kg<sup>-1</sup>.

**6** There was also significant attenuation of the NO-induced dilation maximally after 15 min, at both 10 and 30 mg kg<sup>-1</sup> by 21% ( $t_6 = 6.09$ ,  $n = 7$ ) and 41% ( $t_6 = 5.3$ ,  $n = 7$ ), respectively.

**7** CGRP-induced dilation was not inhibited at either dose of topiramate.

**8** The study demonstrates that topiramate is likely to inhibit neurogenic dural vasodilation by inhibiting the release of CGRP from prejunctional trigeminal neurons, thus attenuating the dural vasodilation. Topiramate is not able to act postsynaptically at the blood vessels themselves as the CGRP-induced dilation was not attenuated.

**9** The data are consistent with an effect of topiramate on trigeminovascular activation which may form part of its preventive antimigraine mechanisms of action.

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**Abbreviations:** AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoaxolepropionate; ANOVA, analysis of variance; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; GABA,  $\gamma$ -aminobutyric acid; GTN, glycerol trinitrate; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; TCC, trigeminocervical complex

## Introduction

The pathophysiology of migraine is still to be fully understood, but it is thought to involve activation of trigeminal afferents (Goadsby *et al.*, 2002). Trigeminal sensory nerve fibres that innervate cranial structures contain the neuropeptides calcitonin gene-related peptide (CGRP), substance P and neurokinin A (Uddman & Edvinsson, 1989). Activation of A $\delta$ -trigeminal nerve fibres causes the release of CGRP and dilation of dural arteries in animals (Goadsby *et al.*, 1988; Williamson *et al.*, 1997a), while CGRP levels in the blood plasma of migraineurs are increased during migraine (Goadsby *et al.*, 1990). Intravenous injection of CGRP causes a dull headache and subsequent migraine in humans (Lassen *et al.*, 2002) and dural blood vessel dilation in rats (Williamson *et al.*, 1997a). This all seems to indicate that CGRP may be a key transmitter in the pathogenesis of migraine, especially given that recent clinical

evidence suggests that blockade of CGRP has a potent acute antimigraine effect (Olesen *et al.*, 2004).

Another trigger of headache and migraine in migraineurs is nitric oxide (NO). NO is a potent endogenous vasodilator with an impressive array of biological actions (Moncada *et al.*, 1991). Intravenous infusion of glycerol trinitrate (GTN, an exogenous NO donor) causes an immediate headache in migraineurs and some control subjects, and it is also able to induce a delayed migraine attack, that fulfilled the International Headache Society criteria for migraine (Headache Classification Committee of The International Headache Society, 1988) several hours after the infusion of GTN had ceased (Iversen *et al.*, 1989; Olesen *et al.*, 1993; 1994). Similarly, it was found that NO infusion was able to cause a reproducible vasodilation of middle meningeal arteries in the rat (Akerman *et al.*, 2002b). It is believed that NO activates guanylate cyclase, catalysing the formation of cyclic guanosine monophosphate (cGMP); the activation of this pathway leads

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to the relaxation of the smooth muscle and vasodilation of the blood vessel (Moncada *et al.*, 1991). The mechanism by which NO causes a delayed migraine response is still unclear, but it is likely to involve activation at central sites that triggers a cascade of events in the brain that lead to migraine in susceptible persons.

Topiramate is a derivative of the naturally occurring monosaccharide D-fructose. It has antiepileptic activity, and established efficacy as a migraine preventive (Brandes *et al.*, 2004; Diener *et al.*, 2004; Silberstein *et al.*, 2004). It is not known by what mechanism topiramate acts as a migraine preventive. Topiramate has five potential mechanisms for its antiepileptic effects: through a modulation of voltage-gated sodium ion channels and voltage-gated calcium ion channels, blockade of excitatory glutamate transmission and potentiation of GABA inhibition, and by inhibition of carbonic anhydrase (Shank *et al.*, 2000). These mechanisms can influence the excitatory state of a cell by influences on receptor/channel protein complexes, including changes to their phosphorylation states. Such mechanisms are known to influence trigeminovascular activity.

Inhibition of neurogenic dural vasodilation using intravital microscopy is highly predictive of acute antimigraine efficacy, examples in the 'triptans', 5-HT<sub>1B/1D</sub> receptor agonists, opioids and CGRP receptor antagonists (Shepherd *et al.*, 1997; Williamson *et al.*, 1997b; 2001a, b). Also, given that both CGRP and NO are able to cause headache and migraine in patients (Iversen *et al.*, 1989; Olesen *et al.*, 1993; 1994; Lassen *et al.*, 2002), and that CGRP and NO are capable of causing reproducible meningeal vasodilation in rats (Williamson *et al.*, 1997a; Akerman *et al.*, 2002b), compounds that inhibit the response in rats may have potential at inhibiting the induced headache and subsequent migraine in patients. In this series of experiments, we wanted to examine the effect of topiramate on neurogenic dural vasodilation using intravital microscopy. We tested topiramate at both 10 and 30 mg kg<sup>-1</sup> doses against neurogenic dural vasodilation, CGRP- and NO-induced dilation of meningeal blood vessels. This method uses dilation of dural blood vessel as a measure of trigeminal nerve activation, and thus compounds that can inhibit the vasodilatory response have the potential to inhibit trigeminovascular nociceptive transmission and may explain topiramate's antimigraine mode of action.

## Methods

### *Surgical preparation*

All experiments were conducted under U.K. Home Office Animals (Scientific Procedures) Act (1986). Male Sprague–Dawley rats (260–400 g) were anaesthetised throughout the experiments with sodium pentobarbitone (60 mg kg<sup>-1</sup> i.p. and then 18 mg kg<sup>-1</sup> h<sup>-1</sup> 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 frame and ventilated with oxygen-enriched air, 3–5 ml, 60–80 strokes min<sup>-1</sup> (Small Rodent Ventilator – Model 683, Harvard Instruments, U.K.). End-tidal CO<sub>2</sub> 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 one to monitor for changes to respiration and blood pressure due to long-term anaesthetic maintenance. The rats were placed in a stereotaxic frame, the skull 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 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 analyzer (Living Systems Instrumentation, U.S.A.) and displayed with blood pressure on an online data analysis system (CED spike5v1 software).

### *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) 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.*, 1997a; Akerman *et al.*, 2002b).

**CGRP- and NO-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<sup>-1</sup>, and this has been shown previously to produce a maximal dilation (Williamson *et al.*, 1997a). In preparations where NO was used to dilate the dural blood vessel, sodium nitroprusside, the NO donor, was given as a 5-min infusion at 4–8 µg kg<sup>-1</sup> min<sup>-1</sup> via the femoral vein, to produce maximal dilation (Akerman *et al.*, 2002b).

The reproducibility of the vasodilator challenges has been demonstrated previously using either three or four consecutive saline-controlled stimuli for electrical stimulation and CGRP bolus (Akerman *et al.*, 2002a) and sodium nitroprusside (Akerman *et al.*, 2002b), in order to test whether there was any systematic effect of test compounds over time in the meningeal circulation. In each case there was no significant effect across the cohort. CGRP and sodium nitroprusside injections caused decreases in blood pressure that were not significantly different across the cohorts.

### *Effect of the topiramate on evoked dural vessel dilation*

A control response to dural electrical stimulation was performed and at least 10 min later topiramate (10 mg kg<sup>-1</sup>) was administered intravenously, and then the electrical stimulation was repeated 5, 10, 15, 30 and 60 min after the topiramate treatment. A similar protocol was used with a higher (30 mg kg<sup>-1</sup>) dose of topiramate, except that there was

also stimulation at 90 min. In a separate series of experiments a control response to CGRP ( $1 \mu\text{g kg}^{-1}$ ) was performed and at least 10 min later topiramate (either 10 or  $30 \text{ mg kg}^{-1}$ ) was administered and CGRP bolus repeated after 5, 15, 30 and 60 min.

NO-induced dural vessel dilation was tested with topiramate as well, using a similar protocol. A control response to sodium nitroprusside infusion ( $4\text{--}8 \mu\text{g kg}^{-1} \text{ min}^{-1}$ ) for 5 min was followed 10 min later by an intravenous dose of topiramate ( $10$  or  $30 \text{ mg kg}^{-1}$ ) and sodium nitroprusside infusion repeated after 5, 15, 30 and 60 min.

### Data analysis

The effects of electrical stimulation, bolus of CGRP and sodium nitroprusside infusion on dural vessel diameter were calculated as a percentage increase from the prestimulation baseline diameter. The nature of the experimental set-up, where the magnification of the dural vessel visualised was different in each set-up by virtue of selecting an appropriate target vessel, made it impossible to standardise the dural vessel measurement; therefore, the dural vessel diameter were measured in arbitrary units. The typical vessel diameter measured ranges from 120 to  $150 \mu\text{m}$ . All data are expressed as mean  $\pm$  s.e.m. Statistical analysis was performed using an ANOVA for repeated measures with Bonferroni *post-hoc* correction for multiple comparisons, followed by Student's paired *t*-test (SPSS v10.0). Significance was assessed at the  $P < 0.05$  levels. The experiments were not conducted in a blinded fashion although the data are digitised and the analysis automated to avoid bias.

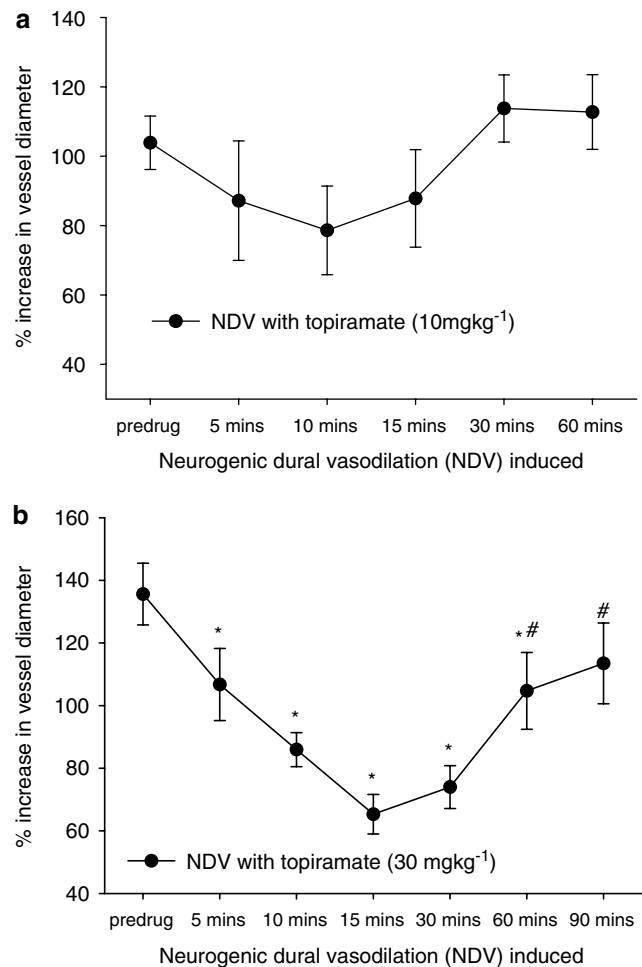
### Drugs

The infusion of anaesthetic and experimental drugs were all *via* the same femoral catheter; however, the line was always flushed with saline first, several minutes before administering the different compound. Topiramate was provided as a gift by Johnson and Johnson, U.S.A. It was dissolved in water for injection at a maximum concentration of  $10 \text{ mg kg}^{-1}$  and heated in an ultrasonic water bath to  $55^\circ\text{C}$  until fully dissolved. CGRP (Sigma-Aldrich, U.K.) was dissolved in deoxygenated water, aliquotted and frozen until required and then redissolved in 0.9% NaCl for use. Sodium nitroprusside (Sigma-Aldrich) was dissolved in 0.9% NaCl.

## Results

### Effects of topiramate on neurogenic dural vasodilation

Topiramate was not able to attenuate significantly neurogenic dural vasodilation at  $10 \text{ mg kg}^{-1}$  ( $F_{4,24} = 1.283$ ,  $P = 0.304$ ), but had a significant effect at  $30 \text{ mg kg}^{-1}$  ( $F_{6,30} = 10.225$ ,  $P = 0.002$ ,  $n = 6$ ), see Figure 1. At the  $30 \text{ mg kg}^{-1}$  dose neurogenic dural vasodilation was attenuated, compared to the control response, at 5, 10, 15, 30 and 60 min post-topiramate injection. Attenuation was greatest after 15 min,  $136 \pm 10\%$  compared to  $65 \pm 6\%$  ( $t_5 = 6.78$ ,  $P < 0.05$ ,  $n = 6$ ; see Table 1).



**Figure 1** Effects of topiramate treatment on neurogenic dural vasodilation. Following control responses to electrical stimulation, rats were injected with topiramate (a)  $10 \text{ mg kg}^{-1}$  or (b)  $30 \text{ mg kg}^{-1}$ , and electrical stimulation repeated after 5, 10, 15, 30, 60 and 90 min. \* $P < 0.05$ , significance compared to the control response. # $P < 0.05$ , significance compared to the response 15 min post topiramate.

### Effects of topiramate on CGRP-induced dilation

Topiramate was not able to significantly attenuate CGRP-induced dilation at  $10 \text{ mg kg}^{-1}$  ( $F_{4,24} = 0.91$ ,  $n = 6$ ,  $P = 0.423$ ) and  $30 \text{ mg kg}^{-1}$  doses ( $F_{4,24} = 2.8$ ,  $n = 6$ ,  $P = 0.098$ ; see Figure 2).

### Effects of topiramate on NO-induced dilation

Topiramate was able to attenuate significantly NO-induced dilation (caused by sodium nitroprusside infusions) at both  $10 \text{ mg kg}^{-1}$  ( $F_{4,24} = 11.55$ ,  $n = 7$ ,  $P = 0.000$ ) and  $30 \text{ mg kg}^{-1}$  ( $F_{4,24} = 8.19$ ,  $n = 7$ ,  $P = 0.002$ ; see Figure 3). At the  $10 \text{ mg kg}^{-1}$  dose, the NO-induced dilation was attenuated 5 and 15 min post-topiramate injection, at the  $30 \text{ mg kg}^{-1}$  dose the NO-induced dilation was attenuated 5, 15 and 30 min post-topiramate injection. At each dose the attenuation was greatest after 15 min,  $119 \pm 6\%$  compared to  $95 \pm 6\%$  ( $10 \text{ mg kg}^{-1}$ ,  $t_6 = 5.30$ ,  $P < 0.05$ ) and  $143 \pm 9\%$  compared to  $86 \pm 10\%$  ( $30 \text{ mg kg}^{-1}$ ,  $t_6 = 6.09$ ,  $P < 0.05$ ; see Table 2).

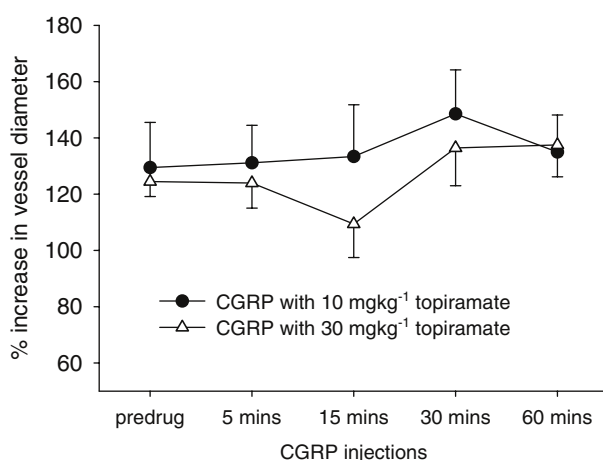
**Table 1** Effects of 30 mg kg<sup>-1</sup> topiramate on neurogenic dural vasodilation

Neurogenic dural vasodilation	Increase in dural vessel diameter (% age change)
Control response	135.6 ± 10%
5 min post 30 mg kg <sup>-1</sup> TPM	106.7 ± 12% <sup>a</sup> ( <i>t</i> <sub>5</sub> = 4.56, <i>n</i> = 6)
10 min post	85.9 ± 5% <sup>a</sup> ( <i>t</i> <sub>5</sub> = 6.23, <i>n</i> = 6)
15 min post	65.3 ± 6% <sup>a</sup> ( <i>t</i> <sub>5</sub> = 6.78, <i>n</i> = 6)
30 min post	74.0 ± 7% <sup>a</sup> ( <i>t</i> <sub>5</sub> = 7.61, <i>n</i> = 6)
60 min post	104.7 ± 12% <sup>a</sup> ( <i>t</i> <sub>5</sub> = 3.05, <i>n</i> = 6)
90 min post	113.5 ± 13% <sup>b</sup> ( <i>t</i> <sub>5</sub> = 2.94, <i>n</i> = 6)

TPM = topiramate.

<sup>a</sup>*P* < 0.05, significance compared to control response.

<sup>b</sup>*P* < 0.05, significance compared to vasodilation 15 min post TPM injection.

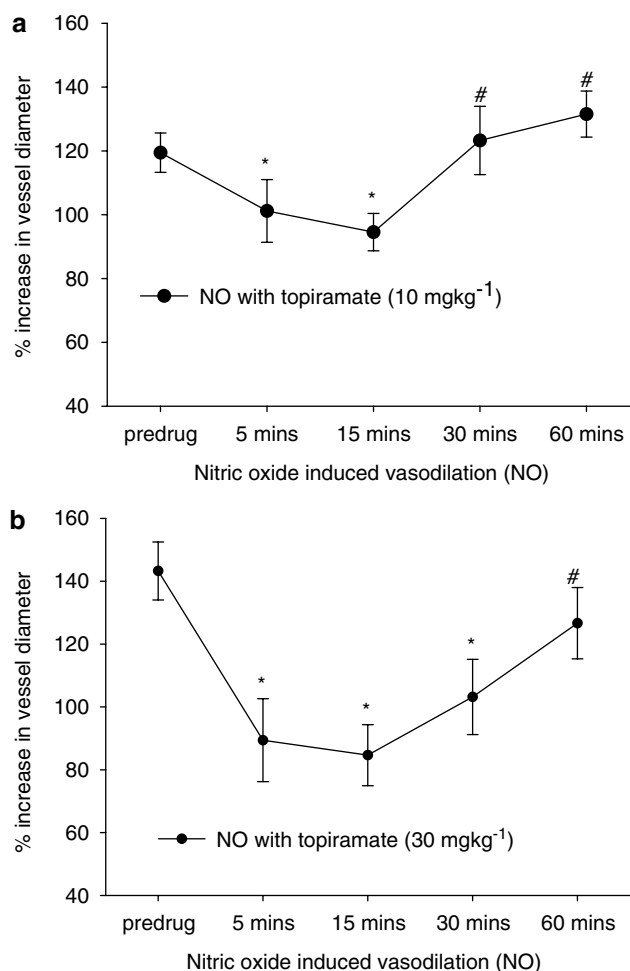


**Figure 2** Effects of topiramate treatment on CGRP-induced dilation. Following control responses to CGRP, bolus rats were injected with topiramate (10 or 30 mg kg<sup>-1</sup>) and CGRP bolus repeated after 5, 15, 30 and 60 min.

#### Effect of topiramate on mean arterial blood pressure, and the blood pressure effects caused by CGRP and sodium nitroprusside

Topiramate caused a significant reduction in mean arterial blood pressure at both 10 and 30 mg kg<sup>-1</sup> doses, of 4 ± 2 mmHg (10 mg kg<sup>-1</sup>, *t*<sub>15</sub> = 2.19, *P* < 0.05, *n* = 16) and 7.7 ± 1 mmHg (30 mg kg<sup>-1</sup>, *t*<sub>15</sub> = 5.5, *P* < 0.05, *n* = 16). This blood pressure change returned to preinjection levels before the next vasodilator challenge. There was no significant effect on dural blood vessel diameter.

CGRP caused a significant decrease in mean arterial blood pressure on each occasion it was given; when we looked at the entire cohort this was significantly reduced by administration of 10 mg kg<sup>-1</sup> topiramate (*F*<sub>4,24</sub> = 4.56, *P* < 0.05, *n* = 7), and specifically at 15 min (*t*<sub>6</sub> = 2.98, *P* < 0.05, *n* = 7) and 45 min (*t*<sub>6</sub> = 3.34, *P* < 0.05, *n* = 7), but was not significant with 30 mg kg<sup>-1</sup> topiramate (*F*<sub>4,24</sub> = 1.40, *P* = 0.284, *n* = 7); the data are summarised in Table 4. Sodium nitroprusside caused a significant decrease in mean arterial blood pressure on each occasion it was given; when we looked across the entire cohort the mean arterial blood pressure changes were not significantly



**Figure 3** Effects of topiramate treatment on NO-induced dilation. Following control responses to sodium nitroprusside infusion, rats were injected with topiramate (a) 10 mg kg<sup>-1</sup> or (b) 30 mg kg<sup>-1</sup>, and sodium nitroprusside infusion repeated after 5, 15, 30 and 60 min. \**P* < 0.05, significance compared to the control response. #*P* < 0.05 significance compared to the response 15 min post topiramate injection. †*P* < 0.05, significance compared to the response 5 min post topiramate injection.

different after topiramate intervention at both 10 mg kg<sup>-1</sup> (*F*<sub>4,24</sub> = 3.00, *P* = 0.67, *n* = 7) and 30 mg kg<sup>-1</sup> (*F*<sub>4,24</sub> = 0.95, *P* = 0.42, *n* = 7); the data are summarised in Table 4.

## Discussion

Topiramate was able to significantly inhibit neurogenic dural vasodilation at the 30 mg kg<sup>-1</sup> dose and it was able to significantly inhibit NO-induced dural blood vessel dilation at both 10 and 30 mg kg<sup>-1</sup> doses. CGRP-induced dural blood vessel dilation was unaffected by pretreatment with topiramate at either dose. Taken together, the data suggest a prejunctional effect for topiramate in the trigeminovascular system that would be consistent with its antimigraine effect as demonstrated in clinical trials. The prospect that preventive agents may influence the trigeminovascular system has not hitherto been widely expected (Edvinsson, 1999), but is an interesting outcome in the context of the development of new agents.

Topiramate has been shown previously to inhibit cell firing in the trigeminocervical complex (TCC) after stimulation of the superior sagittal sinus in cats (Storer & Goadsby, 2004). It was found to be effective at a range of doses, from 5 to 50 mg kg<sup>-1</sup>, with the 30 mg kg<sup>-1</sup> dose proving to be optimal. In the data presented here we show that topiramate is also able to inhibit neurogenic dural vasodilation, but only at the 30 mg kg<sup>-1</sup> dose. The dose is in line with effects on seizure activity and in a model of allodynia in rat (Wieczorkiewicz-Plaza *et al.*, 2004; Grabenstatter *et al.*, 2005), and thus is consistent with other biological effects in rat. Neurogenic dural

vasodilation is thought to activate perivascular trigeminal sensory nerve fibres, and cause CGRP release that result in dural blood vessel dilation (Williamson *et al.*, 1997a), and thus inhibition of this response, by, for example, topiramate, might be *via* a pre- or postsynaptic receptor.

CGRP acts directly at CGRP receptors on the smooth muscle of the dural blood vessels; therefore, any inhibition of CGRP-induced dilation is likely to be postsynaptic (Edvinsson *et al.*, 2000). Topiramate is unable to inhibit the CGRP-induced dural blood vessel dilation, and therefore it is unlikely to be acting postsynaptically.

The trigeminal ganglion is a bipolar ganglion whose neural fibres innervate the dural blood vessels in the periphery and project to the trigeminal nucleus caudalis centrally. In general, the pharmacology of the two poles mirror each other, and therefore evidence gained from studying evoked cell firing in the TCC may also apply to neural mechanisms involving the dural blood vessel. The 'triptans', serotonin 5-HT<sub>1B/1D</sub> receptor agonists (Storer & Goadsby, 1997; Williamson *et al.*, 1997b, c; Goadsby *et al.*, 2001), ergotamine and dihydroergotamine (Goadsby *et al.*, 2001; Williamson *et al.*, 2001a), opioids (Williamson *et al.*, 2001b; Storer *et al.*, 2003), calcium channel blockers (Akerman *et al.*, 2003a; Shields *et al.*, 2003) and CGRP receptor blockers (Williamson *et al.*, 1997a; Storer *et al.*, 2004) are all able to inhibit the activation of both peripheral trigeminal afferents and neurons of the trigeminal nucleus caudalis. It is, therefore, clear that evidence gleaned from trigeminal neurovascular studies and trigeminal electrophysiological studies may be mutually informative.

**Table 2** Effects of 30 mg kg<sup>-1</sup> topiramate on NO-induced dilation

NO-induced dilation	Increase in dural vessel diameter (% age change)
Control response	143.3 ± 9%
5 min post 30 mg kg <sup>-1</sup> TPM	89.4 ± 13% <sup>a</sup> ( <i>t</i> <sub>6</sub> = 4.55, <i>n</i> = 7)
15 min post	84.6 ± 10% <sup>a</sup> ( <i>t</i> <sub>6</sub> = 6.09, <i>n</i> = 7)
30 min post	103.2 ± 12% <sup>a</sup> ( <i>t</i> <sub>6</sub> = 2.58, <i>n</i> = 7)
60 min post	127.7 ± 11% <sup>b</sup> ( <i>t</i> <sub>6</sub> = 2.49, <i>n</i> = 7) <sup>c</sup> ( <i>t</i> <sub>6</sub> = 3.35, <i>n</i> = 7)

TPM = topiramate

<sup>a</sup>*P* < 0.05, significance compared to control response.

<sup>b</sup>*P* < 0.05, significance compared to vasodilation 15 min post TPM injection.

<sup>c</sup>*P* < 0.05, significance compared to vasodilation 30 min post TPM injection.

**Table 3** Effect of topiramate on the hypotensive effects of CGRP

Pretreatment	Mean arterial blood pressure and decreases (mmHg) after CGRP injection (1 µg kg <sup>-1</sup> )			
	10 mg kg <sup>-1</sup> TPM		30 mg kg <sup>-1</sup> TPM	
	Baseline BP	BP drop	Baseline BP	BP drop
CGRP control	123.5 ± 5	41.2 ± 3	125.8 ± 4	41.1 ± 3
Post TPM 5 min	119.5 ± 5	38.4 ± 4	121.6 ± 5	34.1 ± 5
Post TPM 15 min	118.8 ± 8	29.5 ± 3 <sup>a</sup>	118.5 ± 4	32.7 ± 3
		( <i>t</i> <sub>6</sub> = 2.98)		
Post TPM 30 min	117.5 ± 3	36.4 ± 4	121.5 ± 4	34.4 ± 4
Post TPM 45 min	123.6 ± 4	33.4 ± 3 <sup>a</sup>	123.5 ± 5	33.2 ± 4
		( <i>t</i> <sub>6</sub> = 3.34)		

BP = blood pressure.

TPM = topiramate.

CGRP = calcitonin gene-related peptide.

<sup>a</sup>*P* < 0.05 significance compared to the control response.

**Table 4** Effect of topiramate on the hypotensive effects of sodium nitroprusside

Pretreatment	Mean arterial blood pressure and decreases (mmHg) after SNP infusions (4–8 µg kg <sup>-1</sup> )			
	10 mg kg <sup>-1</sup> TPM		30 mg kg <sup>-1</sup> TPM	
	Baseline BP	BP drop	Baseline BP	BP drop
SNP control	124.2 ± 7	53.4 ± 10	108.3 ± 8	43.7 ± 7
Post TPM 5 min	122.4 ± 6	50.0 ± 8	122.7 ± 7	40.5 ± 5
Post TPM 15 min	121.3 ± 8	48.0 ± 8	119.3 ± 7	42.9 ± 7
Post TPM 30 min	119.0 ± 8	48.9 ± 10	118.6 ± 7	46.9 ± 8
Post TPM 45 min	115.3 ± 7	41.8 ± 10	116.3 ± 6	39.9 ± 9

BP = blood pressure.

TPM = topiramate.

SNP = sodium nitroprusside (a NO donor).

It has been shown previously in the trigeminovascular intravital model that L-, N- and P/Q-type voltage-dependent calcium channel blockers are able to inhibit neurogenic dural vasodilation (Akerman *et al.*, 2003b), and they are also able to inhibit superior sagittal sinus-activated neurons of the trigeminal nucleus caudalis (Shields *et al.*, 2003). It is therefore possible that modulation of voltage-gated calcium channels by topiramate is contributing to the inhibition of neurogenic dural vasodilation. It has been argued that inhibition of voltage-gated calcium channels prevents the release of transmitters by preventing calcium influx into cells and thus preventing exocytosis and the mechanism of transmitter release. It is established that GABA agonists do not appear to be able to inhibit neurogenic dural vasodilation (Williamson & Hargreaves, 2001); therefore it appears that this mechanism is not relevant. However, it remains possible that topiramate acts by promoting GABA inhibition in the trigeminal nucleus caudalis to cause inhibition of neurons activated by trigeminovascular nociceptive stimuli (Storer & Goadsby, 2004).

Evidence gained from studying the trigeminal nucleus caudalis indicates that inhibiting both *N*-methyl-D-aspartate (NMDA) and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoaxolepropionate (AMPA)/kainate receptors results in an inhibition in neuronal firing (Storer & Goadsby, 1999; Goadsby & Classey, 2000; Classey *et al.*, 2001). No studies have been carried out using neurogenic dural vasodilation, but it is possible that inhibition of glutamatergic transmission will also inhibit peripheral trigeminal afferents. This would provide another avenue for the effect of topiramate.

The only sodium channel modulator used against neurogenic dural vasodilation is flunarizine (Akerman *et al.*, 2002a), which has been used as a migraine preventive, and it was unsuccessful in causing any inhibition. Flunarizine acts on a large number of systems, including sodium/calcium channels, but it is not clear how it exerts its preventive action. Given the large array of sodium channels present in the brain and the variety of blockers and modulators that can be directed at them, it is difficult to discount an action of topiramate at sodium channels to inhibit neurogenic dural vasodilation. Perhaps the use of tetrodotoxin, a sodium channel blocker that defines the subtype of sodium channel, may be a useful starting point in the established models of trigeminovascular nociception. It is, however, worth noting that medicines with actions at sodium channels do not have convincing antimigraine effects, such as lamotrigine (Steiner *et al.*, 1997) and carbamazepine (Rompel & Bauermeister, 1970; Anthony & Lance, 1972).

Topiramate was also able to inhibit NO-induced dural blood vessel dilation. It is thought that NO activates trigeminovascular neurons and causes the release of CGRP from the nerve endings, which contributes to the dural blood vessel dilation, as well as the NO acting directly on the dural blood vessels themselves. NO-induced dural and cerebral vasodilation is inhibited by application of a CGRP receptor blocker (Wei *et al.*, 1992; Akerman *et al.*, 2004). Indeed, it is thought that CGRP and NO behave synergistically in promoting trigeminovascular activation, as NO synthase inhibitors are able to inhibit CGRP-induced dilation (Wei *et al.*, 1992; Akerman *et al.*, 2002b). The evidence from this study would seem to indicate the same: topiramate is able to attenuate the NO response, presumably inhibiting the release of CGRP from

trigeminal neurons, but there is still the NO-cGMP interaction that produces dilation of blood vessels. It seems unlikely that topiramate is acting directly on the dural blood vessels, as it is unable to inhibit CGRP-induced dilation.

Topiramate also affected the mean arterial blood pressure of the rats with a minor but significant reduction in blood pressure at both 10 and 30 mg kg<sup>-1</sup> doses. This was not accompanied by any change in blood vessel diameter, and the blood pressure returned to its baseline level before vasodilator challenges were repeated. Topiramate was also unable to affect the changes in mean arterial blood pressure caused by the NO donor. Taken together, the data indicate that topiramate does not affect the dural blood vessel diameter *per se*, nor the cardiovascular effects of the NO donor, but only the response of the activated trigeminal neurons. Curiously, topiramate (10 mg kg<sup>-1</sup>) was found to inhibit the blood pressure changes caused by CGRP after 15 and 45 min, despite no effects on the dural blood vessel diameter, and no effect of the higher dose of topiramate. Considering Table 3 *in toto*, this result seems anomalous and bears repeating before firm conclusions can be drawn.

A potential limitation of the study is that the doses used in this study and that of Storer & Goadsby (2004) are not comparable with that used in the clinic, where a standard dose is 100 mg day<sup>-1</sup> (Silberstein *et al.*, 2004). The dosing for this study was based on Storer's results (2004) and also in response to the observations made during experimentation, where similar levels of attenuation, 50%, experiment were found in each study. We also considered that we were measuring in the periphery while previous work studied the trigeminal nucleus caudalis (Storer & Goadsby, 2004) that is behind the blood-brain barrier. We have also shown previously that a measure of 30 mg kg<sup>-1</sup> was sufficient to get into the brain and inhibit cortical spreading depression, and it was more effective in the rat than the cat (Akerman & Goadsby, 2004). Other factors that need to be considered are that topiramate is used as a migraine preventive, which uses a dosing schedule over several weeks to succeed in reducing the number of headaches (Brandes *et al.*, 2004; Silberstein *et al.*, 2004). We only applied a single dose of topiramate to observe whether there is an effect on trigeminovascular neurons. This was achieved with the doses we used, and it is unlikely that we would learn any more from increasing the dose, particularly with respect to previous experiments. Therefore, it seems inappropriate to compare directly the dose in the clinic with the doses in these experiments. It would certainly be interesting to observe the effects of chronic topiramate treatment in the rat, although it would be more difficult to obtain good control data.

The current study demonstrates that topiramate is capable of inhibiting neurogenic dural vasodilation in an animal model. The results complement an electrophysiological study that demonstrates inhibition of trigeminocervical neurons activated by a nociceptive stimulus at comparable doses (Storer & Goadsby, 2004). It is feasible that trigeminovascular inhibition plays a role in the antimigraine effect of topiramate, although it is unlikely to be the full explanation for its effects. Considering well-established preventives like flunarizine are not effective in inhibiting neurogenic dural vasodilation (Akerman *et al.*, 2002a), and mechanisms at other parts of the neuraxis are also likely to be important, such as those in the thalamus (Shields & Goadsby, 2005). Broadly speaking,

understanding the extent to which preventive and acute attack treatments can alter trigeminovascular activation provides insights into the underlying mechanisms of both the medicines and into migraine itself.

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