Trigeminal nerve ganglion stimulation-induced neurovascular reflexes in the anaesthetized cat: Role of endothelin_B receptors in carotid vasodilatation

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1 The effects of intravenous administration of endothelin (ET) receptor antagonists SB-209670 (0.001–10.0 mg kg⁻¹), SB-217242, SB-234551 (0.01–10.0 mg kg⁻¹) and BQ-788 (0.001–1.0 mg kg⁻¹) were investigated on trigeminal nerve ganglion stimulation-induced neurovascular reflexes in the carotid vasculature of the anaesthetized cat. Comparisons were made with sumatriptan (0.003–3.0 mg kg⁻¹) and α -CGRP_{8–37} (0.001–0.1 mg kg⁻¹).

2 Trigeminal nerve ganglion stimulation produced frequency related increases in carotid blood flow, reductions in carotid vascular resistance and non-frequency related increases in blood pressure. Guanethidine (3 mg kg⁻¹, i.v.) blocked trigeminal nerve ganglion-induced increases in blood pressure but had no effect on changes in carotid flow or resistance. Maximal reductions in carotid vascular resistance was observed at 10 Hz, and this frequency was selected to investigate the effects of drugs on trigeminal nerve ganglion stimulation-induced responses in guanethidine treated cats.

3 Saline, α -CGRP_{8–37} SB-209670 and BQ-788 had little or no effect on resting haemodynamic parameters. SB-217242 (10 mg kg⁻¹, *n* = 3) produced a 56% reduction in arterial blood pressure whereas SB-234551 (10 mg kg⁻¹, *n* = 3) produced a 30% reduction in carotid vascular resistance. Sumatriptan produced dose-related reductions in resting carotid flow and increases (max. 104% at 0.3 mg kg⁻¹, *n* = 5) in vascular resistance.

4 SB-209670 (*n* = 6–7), SB-217242 (*n* = 3) and BQ-788 (*n* = 3) produced inhibition of trigeminal nerve ganglion stimulation-induced reductions in carotid vascular resistance. Saline, SB-234551, α -CGRP_{8–37} and sumatriptan had no effect.

5 These data demonstrate ET_B receptor blockade attenuates the vasodilator effects of trigeminal nerve ganglion stimulation in the carotid vascular bed of guanethidine pretreated anaesthetized cats.

Keywords: SB-209670; SB-234551; SB-217242; trigeminal nerve ganglion; ET_B receptor; sumatriptan; neurovascular reflex

Abbreviations: CdVR, carotid vascular resistance; CGRP, calcitonin gene-related peptide; TGN, trigeminal nerve ganglion stimulation; VIP, vasoactive intestinal polypeptide

Introduction

Electrical stimulation of the trigeminal nerve ganglion produces increases in blood flow (Goadsby & Duckworth, 1987; Goadsby & Edvinsson, 1993; 1994; Lambert *et al.*, 1984), protein extravasation (Markowitz *et al.*, 1987; Saito *et al.*, 1988) and increases in brain stem cFos, but not c-jun and jun-D (Uhl *et al.*, 1991; Shephard *et al.*, 1995). These experimental models have supported the concept of neurogenic inflammation as a mechanism for the development of migraine headache (Moskowitz & MacFarlane, 1993). Stimulation of sensory fibres appears to account for all the extravasation response (Markowitz *et al.*, 1987) and detailed investigation has shown that sumatriptan and other 5-HT_{1B/1D} agonists inhibit these effects (Buzzi & Moskowitz, 1990; Connor *et al.*, 1997; Martin *et al.*, 1997; Williamson *et al.*, 1997).

A variety of NK-1 receptor antagonists also inhibit trigeminal nerve ganglion stimulation-induced extravasation in rats (Lee *et al.*, 1994; O'Shaughnessy & Connor, 1994; Phebus *et al.*, 1997; Shephard *et al.*, 1993, 1995). Some species differences do exist as the NK-1 antagonist, GR82334 blocks neurogenic inflammation in rats and guinea-pigs, whereas the

calcitonin gene-related peptide (CGRP) receptor antagonist α CGRP_{8–37} is effective in guinea-pigs, but not rats (O'Shaughnessy & Connor, 1994). Other agents that inhibit neurogenic extravasation include endothelin receptor antagonists (Brandli *et al.*, 1995), sumatriptan analogues (Lee & Moskowitz, 1993) and a variety of other receptor agonists (Matsubara *et al.*, 1992). However, a number of agents which show activity in this model, such as bosentan (May *et al.*, 1996), CP-122288 (Roon *et al.*, 1997) and LY-303870 (Goldstein *et al.*, 1997) have not shown efficacy as acute migraine therapies in the clinic.

Trigeminal nerve ganglion stimulation also produces a reflex activation of autonomic ganglia which are involved in a reflex vasodilatation of the carotid vascular bed in the cat (Lambert *et al.*, 1984). Stimulation of the trigeminal nerve ganglion produces release of both sensory (CGRP) and parasympathetic (vasoactive intestinal polypeptide, VIP) neurotransmitters (Buzzi *et al.*, 1991; Goadsby & Edvinsson, 1994), but does not appear to involve the sympathetic nervous system (Lambert *et al.*, 1984). This interaction between afferent and efferent nervous systems is well documented in trigeminal nerve ganglion stimulation-induced reductions in common carotid vascular resistance in the cat (Lambert *et al.*, 1984). Section of the VII nerve (parasympathetic) or pre-treatment

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with hexamethonium inhibited these reflex pathways (Lambert *et al.*, 1984) which utilize VIP as the final mediator (Goadsby & MacDonald, 1985). A similar neurovascular reflex also operates in the intracranial circulation of the cat (Goadsby & Duckworth, 1987). In the rat, trigeminal fibres containing substance P and CGRP are present in parasympathetic ganglia forming basket-like arrangements with neurons (Suzuki *et al.*, 1989a) and provide an anatomical basis for this neurovascular reflex. This pathway may also have clinical implications as inhibition of parasympathetic nerve has been suggested to mediate the antimigraine actions of intranasal lidocaine (Maizels *et al.*, 1996).

It is known that sumatriptan produces complete inhibition of trigeminal nerve ganglion-induced extravasation in rats (Buzzi & Moskowitz, 1990), inhibits trigeminal nerve-ganglion-induced increases pial blood flow in cats (Goadsby & Edvinsson, 1993) and increases resting carotid vascular resistance (Perren *et al.*, 1989) in cats. In contrast, sumatriptan has little effect on trigeminal nerve ganglion-induced neurovascular reflexes involving the parasympathetic nerve in cats (Lambert & Michalick, 1996).

As endothelin-1 has been found in sensory nerves (Franco-Cereceda *et al.*, 1991; Giaid *et al.*, 1989) and ET receptor antagonists inhibit trigeminal nerve ganglion stimulation-induced extravasation (Brandli *et al.*, 1995) and block cranial arterial vasodilatation (Maurice *et al.*, 1997; Nilsson *et al.*, 1997), the aims of the present studies were to explore the effects of endothelin receptor blockade on trigeminal nerve ganglion-induced neurovascular reflexes in comparison to the effects of sumatriptan and α -CGRP₈₋₃₇ on these responses.

Some of these results have been published in abstract form (Raval *et al.*, 1996; 1997).

Methods

Experimental procedures

Animals This work was conducted in compliance with the Home Office Guidance on the operation of the Animals (Scientific Procedures) Act 1986, and was reviewed and approved by the SmithKline Beecham Procedures Review Panel.

Male cats (Hillgrove family farm), weight range 2.6–4.2 kg were housed in groups and allowed free access to food and water.

Surgical procedures

Cats were fasted overnight then anaesthetized with halothane (4%) in oxygen and maintained with intravenous administration of α -chloralose (120 mg kg⁻¹ in a volume of 3 ml kg⁻¹). The trachea was cannulated and cats were artificially ventilated with room air at a rate of 30–35 strokes min⁻¹ and tidal volume of 8 ml kg⁻¹ which maintained blood oxygen and carbon dioxide tensions within normal limits. The right femoral artery was cannulated for measurement of blood pressure and derived heart rate and the left femoral vein for the administration of drugs.

Animals were placed in the prone position in a stereotaxic frame (David Kopf, California, U.S.A.) and bipolar electrodes (Rhodes NEX-100, Clark Electromedical, Reading, U.K.) were placed through a burr hole craniotomy on the right trigeminal nerve ganglion, calculated relative to Bregma at –9.5 mm in the anterior posterior direction and +6.0 mm in the lateral direction. An electromagnetic flow probe (Statham

Gould diameter 1.5–2.0 mm) was placed around the right carotid artery and calibrated to a standard flow and recorded on a Gould flowmeter. Using the above co-ordinates, a bipolar electrode was advanced into the right trigeminal nerve ganglion which was stimulated briefly (10 Hz, 2 mA) using opposite polarity square wave pulses from 2 Grass constant current units (CCU, 1 A) driven by Grass stimulator isolation units (SIU, 5 A) and a Grass stimulator (S11). Correct placement of the electrode was indicated by strong lower jaw movements, salivation and an increase in carotid blood flow. This provides a useful marker of trigeminal nerve ganglion stimulation and was verified by direct current lesion of the trigeminal nerve in some animals.

In preliminary experiments, a frequency-response relationship was determined for changes in carotid blood flow and vascular resistance. The trigeminal nerve ganglion was stimulated (2 mA for 2 min) at 0.2, 0.5, 2, 5, 10 and 20 Hz and changes in blood pressure, heart rate, carotid blood flow and vascular resistance recorded following intravenous pretreatment with vehicle (saline) or guanethidine sulphate 3 mg kg⁻¹.

All subsequent experiments were conducted following administration of intravenous guanethidine sulphate 3 mg kg⁻¹. Two consecutive, reproducible control responses to trigeminal nerve stimulation were obtained at 30 min intervals before experiments were performed.

Physiological recordings

Physiological recordings were obtained using standard techniques as previously described by other groups in cats (Lambert *et al.*, 1984; Lambert & Michalick, 1996) and our Laboratory in dogs (Parsons *et al.*, 1997). Systolic and diastolic blood pressures were recorded *via* the arterial cannulae connected to a pressure transducer (Bell & Howell). Pulsatile arterial blood pressure recordings were used to derive heart rate. Carotid blood flow was measured by electromagnetic flow probes. Which were calibrated to a range of standard flows through a glass capillary in accordance with the manufacturer's specifications. Blood flow was displayed on a Gould Flowmeter and all parameters were recorded on an 8 channel Lectromed chart recorder.

Pharmacological characterization

In separate experiments, the trigeminal nerve ganglion was stimulated at 30 min intervals for up to 2.5 h following intravenous administration of saline (0.1 ml kg⁻¹) 5 min prior to stimulation. Increasing doses of either SB-209670 (0.001–1 or 0.001–10.0 mg kg⁻¹, see below), SB-217242 or SB-234551 (0.01–10.0 mg kg⁻¹), BQ 788 (0.001–1.0 mg kg⁻¹), α -CGRP₈₋₃₇ (0.001–0.1 mg kg⁻¹) or sumatriptan (0.0003–3.0 mg kg⁻¹) were administered as a bolus (0.1 ml kg⁻¹), 5 min before nerve ganglion stimulation (10 Hz, 2 mA, 2 min) at 30 min intervals.

To investigate the duration of action of SB-209670, it was administered as doses between 0.001–1.0 mg kg⁻¹ ($n=3$) and 0.001–10.0 mg kg⁻¹ ($n=3$) and its effect on trigeminal nerve ganglion stimulation-evoked responses investigated 30 min following the last administration of compound.

Confirmation of α -CGRP₈₋₃₇ activity in vivo

In a separate series of experiments, in guanethidine pretreated cats, we confirmed the blocking properties of α -CGRP₈₋₃₇ on CGRP-induced reductions in arterial blood pressure. In brief,

CGRP 100 ng kg⁻¹ was administered intravenously prior to, and 30 min post intravenous administration of the CGRP receptor antagonist α -CGRP₈₋₃₇ (0.1 mg kg⁻¹).

Materials

SB-209670 (1RS-2SR,3RS)-3-(2-carboxymethoxy-4-methoxyphenyl)-5-(prop-1-yloxy) indane-2-carboxylic acid), SB-217242 ((+)-(1S,2R,3S)-3-[2-(2-hydroxyeth-1-yloxy)-4-methoxyphenyl]-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indan-2-carboxylic acid), SB-234551 ((E)- α -[[1-butyl-5-[2-carboxyphenyl methoxy]-4-methoxyphenyl]-1H-pyrazol-4-yl]-methylene]-6-methoxy-1,3-benzodioxole-5-proanoic acid), α -CGRP₈₋₃₇ and guanethidine were synthesized at SmithKline Beecham Pharmaceuticals. α -Choralose was obtained from BDH (Germany). CGRP was obtained from Peninsula UK. Sumatriptan was extracted from commercially available sources by the Custom Synthesis Group, SmithKline Beecham. Imigran tablets were crushed and boiled with ethanol for 10 min, filtered whilst hot and allowed to cool and crystallize out of solution. The white solid was filtered off and dried *in vacuo*. The material was analysed and found to be >99% pure by h.p.l.c.. The structure of sumatriptan was confirmed by NMR. All drugs were dissolved in isotonic saline.

Data analysis

All data were entered into a PC and calculations performed using Excel. Carotid vascular resistance was calculated as mean arterial blood pressure divided by carotid blood flow. The following formula was used to calculate mean arterial blood pressure by ((systolic-diastolic blood pressure) $\times$ 1/3) + diastolic blood pressure.

Baseline mean arterial blood pressure, carotid flow and heart rate were measured before and after repeat administration of saline or each dose of compound under investigation and changes in carotid vascular resistance calculated. Trigeminal nerve ganglion stimulation-induced changes in mean arterial blood pressure, heart rate, carotid blood flow and calculated carotid vascular resistance were determined before and at the end of the 2 min stimulation period and the percentage change from control stimulation calculated.

Control changes in mean arterial blood pressure, heart rate and carotid vascular resistance after nerve stimulation were derived from the mean values for the two responses obtained before administration of any drug. Effects of drugs on nerve stimulation were expressed as per cent inhibition of the control response.

The effects of pharmacological agents on resting carotid vascular resistance, heart rate and blood pressure were expressed as per cent change from initial resting values prior to drug administration.

To rule out the small effects of trigeminal nerve ganglion stimulation induced changes in arterial blood pressure on carotid blood flow, the effects of drugs were investigated on changes in carotid vascular resistance alone.

Data points were obtained from two groups of animals each receiving SB-209670 (0.001 mg kg⁻¹–1.0 or 10 mg kg⁻¹), as no differences were observed between groups all data points were combined.

All data were subjected to ANOVA followed by Duncan's multiple range test with a significance level of $P < 0.05$.

Results

Frequency response characteristics of trigeminal nerve ganglion stimulation

Trigeminal nerve ganglion stimulation (0.2–20 Hz) produced a frequency related increase in carotid blood flow and reduction in carotid vascular resistance both in the absence and in the presence of guanethidine (3 mg kg⁻¹, i.v.) pre-treatment (Figure 1). The maximal change in carotid blood flow was greater ($P < 0.05$, Student's *t*-test) in control ($75 \pm 8.0\%$, $n = 4$) than guanethidine pre-treated cats ($59.0 \pm 2.3\%$, $n = 4$) and occurred at a stimulation frequency of 10 Hz in both groups. However, no significant differences in change in carotid vascular resistance were present between treatment groups with reduction of 34 ± 6.0 and $39 \pm 5.5\%$ in vehicle or guanethidine pre-treated animals respectively at this frequency. Trigeminal nerve ganglion stimulation produced marked but transient increases in mean arterial blood pressure which was abolished following guanethidine pre-treatment

Figure 1 Effects of trigeminal nerve ganglion stimulation on changes in mean arterial blood pressure (MABP), heart rate (HR), carotid flow and carotid vascular resistance in (a) vehicle treated and (b) guanethidine (3 mg kg⁻¹, i.v.) treated animals ($n = 4$ per group). Data points represent mean $\pm$ s.e. mean of per cent change from basal values in individual animals.

(Figure 1). Trigeminal nerve ganglion stimulation had little effect on heart rate in either group.

Therefore, the stimulation parameters of 2 mA at 10 Hz for 2 min were selected for further studies in the presence of guanethidine to remove effects of the sympathetic nervous system on mean arterial blood pressure.

Resting haemodynamic parameters

Resting haemodynamic variables in the presence of guanethidine are shown in Table 1. Mean arterial blood pressure, heart rate, carotid blood flow and calculated carotid vascular resistance were all in the expected range and not significantly different from animals treated with repeated saline (vehicle) administration (ANOVA) (not shown).

Effects of drugs on resting haemodynamic parameters

Saline (vehicle), α -CGRP₈₋₃₇ (0.001–0.1 mg kg⁻¹, i.v.) and BQ-788 (ET_B selective antagonist; 0.001–1 mg kg⁻¹, i.v.) had no significant effect on mean arterial blood pressure (Figure 2), heart rate (not shown), carotid blood flow (not shown) or carotid vascular resistance (Figure 3). SB-209670 (ET_{A/B} antagonist) produced a small, but statistically significant ($P < 0.05$, $F = 6.7$), dose related transient reduction in arterial blood pressure with a maximal decrease of $16 \pm 3.3\%$ following intravenous administration of 10 mg kg⁻¹ (Figure 2). In

contrast, SB-217242 produced dose related ($P < 0.05$; $F = 23.6$) transient (< 5 min) decreases in mean arterial blood pressure (Figure 2). At the highest intravenous dose administered, SB-217242 produced a $56.8 \pm 5\%$ reduction in mean arterial blood pressure. This reduction in blood pressure precluded collection of carotid flow data in two animals as the flow signal was transiently lost, probably due to movement of the flow probe around the carotid artery. Restoration of the carotid blood flow signal occurred within 5 min which allowed inclusion of these animals for stimulation experiments. SB-234551 at 10 mg kg⁻¹ also produced a lowering of mean arterial blood pressure ($27.0 \pm 4.4\%$, $n = 3$, $P < 0.05$; $F = 10.7$, Figure 2) and a decrease in carotid vascular resistance of $30.6 \pm 4.4\%$ ($n = 3$, $P < 0.05$; $F = 14.7$, Figure 3) with little effect on heart rate.

In contrast, sumatriptan had little effect on blood pressure (Figure 2) but produced marked dose-related increases ($P < 0.05$; $F = 3.1$) in carotid vascular resistance (Figure 3) with a maximal effect of $104.8 \pm 34.1\%$ observed at 0.3 mg kg⁻¹ ($n = 5$).

Effect of trigeminal nerve ganglion stimulation

Trigeminal nerve stimulation (10 Hz, 2 mA, 2 min) produced a small depressor response ($< 8\%$) with no effect on heart rate (Table 1). In contrast to the minor effects on the systemic circulation, trigeminal nerve ganglion stimulation produced an

Table 1 Mean $\pm$ s.e. mean blood pressure, heart rate, carotid blood flow and calculated carotid vascular resistance in anaesthetized cats pre- and post-trigeminal nerve ganglion stimulation (10 Hz, 2 mA for 2 min) prior to drug administration

Status	Arterial blood pressure (mmHg)	Heart rate (beats min ⁻¹)	Carotid blood flow (ml min ⁻¹)	Carotid vascular resistance (mmHg min ml ⁻¹)
Before stimulation (basal)	76 ± 2	159 ± 3	16 ± 1	5.5 ± 0.4
After nerve ganglion stimulation	$70 \pm 2^*$	158 ± 3	$27 \pm 2^*$	$3.0 \pm 0.2^*$

Data is pooled from 30 animals pre-treated with guanethidine used in the present investigations. No differences were found in the resting haemodynamic variables from groups of animals receiving, saline (vehicle) α -CGRP₈₋₃₇ (0.001–0.1 mg kg⁻¹), sumatriptan (0.003–3.0 mg kg⁻¹), SB-209670 (0.001–10.0 mg kg⁻¹), BQ-788 (0.001–1.0 mg kg⁻¹), SB-217242 or SB-234551 (0.01–10.0 mg kg⁻¹).

*Represents a significant difference from pre-stimulation baseline (paired *t*-test, $P < 0.05$).

Figure 2 Effects of (a) SB-209670 ($n = 3-7$), BQ-788 ($n = 3$), SB-217242 ($n = 3$), SB-234551 ($n = 3$) or (b) CGRP₈₋₃₇ ($n = 3$), sumatriptan ($n = 4-5$) or saline (time matched controls, $n = 2-3$) on resting mean arterial blood pressure (MABP). Data points represent means $\pm$ s.e. mean of per cent change from basal values (* $P < 0.05$, ANOVA with Duncan's multiple range test compared pre-drug values).

increase in mean $\pm$ s.e. mean carotid blood flow from 16 ± 1 to 27 ± 2 ml min⁻¹, which was associated with a concomitant reduction in mean $\pm$ s.e. mean vascular resistance from 5.5 ± 0.4 mmHg min⁻¹ ml⁻¹ to 3.0 ± 0.2 mmHg min⁻¹ ml⁻¹ (Table 1).

Pharmacological modulation of the trigeminal nerve ganglion stimulation-induced changes in carotid vascular resistance

Control responses to trigeminal nerve ganglion stimulation, before drug administration in each experimental group, produced reductions of carotid vascular resistance of $46.5 \pm 2.7\%$ in cats administered saline ($n=3$), $45.6 \pm 4.8\%$ in SB-209670 ($n=7$), $48.1 \pm 2.1\%$ in BQ-788 ($n=6$), $44.5 \pm 6.7\%$ in SB-217242 ($n=3$), $41.0 \pm 3.3\%$ in SB-234551 ($n=3$), $40.9 \pm 7.2\%$ in sumatriptan ($n=5$) and $54.0 \pm 7.4\%$ in α -CGRP₈₋₃₇ ($n=3$) treated animals, respectively. Trigeminal nerve ganglion stimulation induced changes in carotid blood flow and carotid vascular resistance, but not arterial blood

pressure were reproducible in saline (vehicle) treated animals over the experimental period. The effects of drug treatment on trigeminal nerve stimulation induced reductions in arterial blood pressure were not investigated further.

SB-209670 (0.001 – 10 mg kg⁻¹) dose-dependently inhibited trigeminal nerve ganglion stimulation-induced reductions ($P < 0.05$; $F = 4.2$) in carotid vascular resistance (Figure 4) with a maximum inhibition of $53.8 \pm 6.4\%$ ($P < 0.05$, $n=3$) at 10 mg kg⁻¹ (i.v.). SB-217242 (0.01 – 10 mg kg⁻¹) and BQ-788 (0.001 – 1 mg kg⁻¹) also inhibited trigeminal nerve ganglion stimulation-induced reduction in carotid vascular resistance at intravenous doses of 10 mg kg⁻¹ ($35 \pm 16.1\%$ inhibition, $n=3$, $P < 0.05$; $F = 2.3$) and 1 mg kg⁻¹ ($39.3 \pm 14.0\%$ inhibition, $n=3$, $P < 0.05$; $F = 1.9$) respectively (Figure 4). Comparison of the dose required to produce a 25% inhibition of the trigeminal ganglion stimulation-induced reduction of carotid vascular resistance shows the following rank order of antagonist potency (Figure 4). SB-209670 = BQ-788 > SB-217242. SB-234551 (0.01 – 1 mg kg⁻¹, i.v.), α -CGRP₈₋₃₇ (0.001 – 0.1 mg kg⁻¹) and sumatriptan (0.003 – 3 mg kg⁻¹, i.v.) had no

Figure 3 Effects of (a) SB-209670 ($n=3$ – 7), BQ-788 ($n=3$), SB-217242 ($n=3$: at 10 mg kg⁻¹, $n=1$), SB-234551 ($n=3$) or (b) CGRP₈₋₃₇ ($n=3$), sumatriptan ($n=4$ – 5) or saline (time matched controls, $n=2$ – 3) on resting carotid vascular resistance (CdVR). Data points represent means $\pm$ s.e. mean of per cent change from basal values (* $P < 0.05$). It should be noted that carotid flow values could not be recorded in two out of three animals following 10 mg kg⁻¹ SB-217242 as marked reductions in blood pressure occurred.

Figure 4 Effects of (a) SB-209670 ($n=3$ – 7), BQ-788 ($n=3$), SB-217242 ($n=3$), SB-234551 ($n=3$) or (b) CGRP₈₋₃₇ ($n=3$), sumatriptan ($n=4$ – 5) or saline (time matched controls, $n=2$ – 3) on the trigeminal nerve (10 Hz, 2 mA, 2 min) mediated reduction in carotid vascular resistance in the anaesthetized cat. Data points represent means $\pm$ s.e. mean of the per cent inhibition of trigeminal nerve ganglion stimulation (TGN)-induced response from individual animals (* $P < 0.05$, ANOVA with Duncan's multiple range test compared to control neurogenic response).

significant effects on trigeminal nerve ganglion-induced reductions in carotid vascular resistance (Figure 4).

Duration of pharmacological effect of SB-209670

The inhibitory effects of SB-209670 ($0.001\text{--}1\text{ mg kg}^{-1}$ or $0.001\text{--}10\text{ mg kg}^{-1}$) were also evaluated 30 min post administration. No significant effect on trigeminal nerve-mediated responses was observed (Figure 5) demonstrating a short duration of action of SB-209670 in this system.

Confirmation of αCGRP_{8-37} activity in vivo

In these experiments, resting arterial blood pressure was $66 \pm 8\text{ mmHg}$. CGRP (0.1 mg kg^{-1}) produced a mean reduction of $23 \pm 3\text{ mmHg}$ ($n=3$) which was attenuated by approximately 50% by pre-treatment with $\alpha\text{-CGRP}_{8-37}$ (0.1 mg kg^{-1}) to $10 \pm 1\text{ mmHg}$ ($n=3$) (paired t -test $P<0.05$). Administration of CGRP had little effect on heart rate, indicating a lack of sympathetic reflexes due to guanethidine pretreatment.

Discussion

This study confirms previous observations of trigeminal nerve ganglion-mediated reductions in carotid vascular resistance in the cat (Lambert *et al.*, 1984; Lambert & Michalick, 1996). The degree of reduction in carotid vascular resistance was greater in the present study compared to the results of Lambert & Michalick (1996), and may be a result of the different stimulation parameters used in each case. In the present study, high intensity stimulation (2 mA) was selected to provide compatibility with neurogenic extravasation experiments (1.2 mA) in rats (Brandli *et al.*, 1995; Buzzi & Moskowitz, 1990; O'Shaughnessy & Connor, 1994) and guinea-pigs (1.5 mA) (O'Shaughnessy & Connor, 1994). To investigate the trigeminal nerve ganglion-induced neurovascular reflex between the sensory (trigeminal nerve) and parasympathetic systems, we pretreated animals with guanethidine to remove any confounding effects of stimulation of the sympathetic nerve, such as alteration of systemic arterial blood pressure. Other groups have used surgical lesion of the cervical

sympathetic trunk as a routine procedure to achieve the same result in cats (Lambert *et al.*, 1984).

In the absence of guanethidine, trigeminal nerve stimulation produced a pressor response in addition to increases in carotid flow and reductions in resistance. The changes in arterial blood pressure were abolished by pre-treatment with guanethidine indicating activation of the sympathetic nervous system in these effects. Such increases in blood pressure have been observed by other workers in rabbits (Kumada *et al.*, 1977), rats (Spokes & Middlefell, 1995) and cats (Lambert *et al.*, 1984; Lambert & Michalick, 1996), the magnitude of which may be related to different stimulation parameters (Goadsby & Edvinsson, 1993). However, guanethidine had no effect on the change in carotid vascular resistance evoked by trigeminal nerve ganglion stimulation with a maximal increase in carotid flow obtained at a stimulation frequency of 10 Hz.

Trigeminal nerve ganglion stimulation has also been shown to enhance reductions in carotid vascular resistance in anaesthetized rats (Spokes & Middlefell, 1995). However, in anaesthetized guinea-pigs, trigeminal nerve ganglion stimulation only produced consistent reductions in carotid vascular resistance in guanethidine pretreated animals (Beattie & Connor, 1994) which may indicate a marked sympathetic involvement of this pathway in this species.

Effects of endothelin receptor antagonists on neurovascular reflexes

This study is the first to demonstrate that endothelin antagonists, which are effective inhibitors of trigeminal afferent fibre-induced extravasation (Brandli *et al.*, 1995) also block trigeminal nerve ganglion-induced neurovascular reflex changes in carotid vascular resistance. SB-209670 is a non-peptide mixed $\text{ET}_{\text{A/B}}$ receptor antagonist (Ohlstein *et al.*, 1994) with a binding affinity of 18 nM at human cloned ET_{B} receptors. The peptide ET_{B} receptor antagonist, BQ-788 shows selective binding to human ET_{B} receptor ($\text{IC}_{50}\text{ }1.2\text{ nM}$ in human heart) (Ishikawa *et al.*, 1994), whereas SB-217242 has 100 fold (Ohlstein *et al.*, 1996) and SB-234551 more than 500 fold selectivity (Ohlstein *et al.*, 1998) for human cloned ET_{A} receptors over human cloned ET_{B} receptors. The rank order of antagonist potency in producing inhibition of the trigeminal nerve-induced reductions in carotid vascular resistance was $\text{SB-209670} = \text{BQ-788} > \text{SB-217242}$. SB-234551 had no effect. This rank order of potency is consistent with a role for ET_{B} receptor activation mediating these effects in the anaesthetized cat.

Effects of endothelin receptor antagonists on resting haemodynamic parameters

It is important to note that SB-209670, BQ-788 and SB-217242 produced inhibition of trigeminal nerve-induced reductions in carotid vascular resistance with no effect on resting tone, indicating a direct effect on neuroeffector coupling with no local vascular effect. However, SB-217242 produced marked reductions in mean arterial blood pressure in the cat, whereas in the rat, SB-217242 ($0.3\text{--}30\text{ mg kg}^{-1}$, i.v.) had no significant haemodynamic effects (Ohlstein *et al.*, 1996). SB-209670 and SB-234551 had a small effect on arterial blood pressure. This profile of activity does not parallel activity at the human cloned ET_{A} receptor with pK_i values of 12, 111 and 500 nM for SB-209670 (Ohlstein *et al.*, 1994), SB-217242 (Ohlstein *et al.*, 1996) and SB-234551 (Ohlstein *et al.*, 1998) respectively. However, activity of these agents at feline endothelin ET_{A} receptors is not known. SB-234551 also produced a small

Figure 5 Effects of SB-209670 (1.0 or 10 mg kg^{-1}) 5 or 30 min post administration on the trigeminal nerve ganglion stimulation-induced reduction in carotid vascular resistance (CdVR). All animals received lower doses of SB-209670 as part of the ascending dose response investigation. Data points represent means $\pm$ s.e. mean of change in carotid vascular resistance ($n=3$ per group) (* $P<0.05$, ANOVA followed by Duncan's multiple range test).

decrease in baseline carotid vascular resistance which raises the possibility of a modulatory role for endothelin ET_A receptor activation in the maintenance of vascular tone in the guanethidine treated anaesthetized cat.

Potential inhibitory mechanisms of endothelin receptor antagonists on neurovascular reflexes

Despite inhibiting trigeminal evoked responses, SB-209670 (0.001–1 and 0.001–10 mg kg⁻¹) produced only transient blockade and had little effect on trigeminal nerve ganglion stimulation-induced responses 30 min post administration. This may reflect a limited exposure of SB-209670 in the cat or tolerance to the effects of endothelin receptor blockade over time. Further experiment will be required to verify this observation.

Endothelin has been identified in both sensory neurones and glial cells (Giaid *et al.*, 1989; Franco-Cereceda *et al.*, 1991) and ET-3 release has been demonstrated from trigeminal sensory nerves (Brandli *et al.*, 1995). ET_B receptor activation increases cholinergic nerve-mediated contraction in the bronchus (Fernandes *et al.*, 1996). Possible explanations for the inhibitory effects of ET_B antagonists in this study therefore include inhibition of the vascular effects of released endothelins and/or inhibition of release of transmitters from parasympathetic or sensory nerves. Inhibition of ET_B receptor-mediated potentiation of parasympathetic nerve activity may produce a reduction in trigeminal nerve ganglion neurovascular reflexes.

Effects of calcitonin gene-related peptide receptor antagonist, CGRP₈₋₃₇

In the present study, we confirmed the pharmacological activity of CGRP₈₋₃₇ (0.1 mg kg⁻¹) which produced an approximate 50% inhibition of CGRP (100 ng kg⁻¹)-induced reductions in mean arterial blood pressure. However, CGRP₈₋₃₇ had little effect on trigeminal nerve ganglion stimulation-induced neurovascular responses in the carotid vascular bed. In contrast the same dose of CGRP₈₋₃₇ completely inhibited sensory nerve-induced (neurogenic) protein extravasation in the meninges of the guinea-pig (O'Shaughnessy & Connor, 1994). Similar doses of CGRP₈₋₃₇ also inhibited trigeminal nerve ganglion-induced increases in facial skin blood flow (Escott *et al.*, 1995) in rats. Lower doses of the antagonist (100 µg) also inhibited nasociliary nerve-induced increases in cerebral blood flow (Goadsby, 1993) in cats.

Since the dose of CGRP receptor antagonist used in the present study was pharmacologically active, these data demonstrate that CGRP does not play a major role in trigeminal nerve induced neurovascular responses in the carotid circulation. Similar results were obtained in the guinea-pig (Beattie & Connor, 1994). In these studies, CGRP₈₋₃₇ (0.9 mg kg⁻¹) had no effect on trigeminal nerve ganglion-induced reductions in carotid vascular resistance, whereas a VIP receptor antagonist abolished this response (Beattie & Connor, 1994).

Collectively, these data show that stimulation of trigeminal nerve tracts may produce different responses dependent on whether the nerve (e.g. nasociliary) or nerve ganglion has been stimulated and the nature of the response investigated (e.g. dilatation or extravasation).

Effects of sumatriptan

Sumatriptan had no sustained effect on mean arterial blood pressure but produced marked increases in carotid vascular

resistance as reported by Perren *et al.* (1989). It had no effect on trigeminal nerve-induced reductions in carotid vascular resistance. This observation is consistent with the results of Lambert & Michalick (1996) who demonstrated that sumatriptan had no effect on changes in carotid vascular resistance or meningeal vascular resistance over a range of nerve stimulation frequencies. Similar results were obtained in rats where sumatriptan inhibited trigeminal nerve ganglion stimulation-induced extravasation but not reductions in carotid vascular resistance (Spokes & Middlefell, 1995).

However, sumatriptan has been shown to inhibit trigeminal nerve ganglion-induced increases in cortical blood flow in the cat (Goadsby & Edvinsson, 1993) when measured by laser doppler flowmetry. These observations again illustrate marked differences in pharmacological properties of agents modulating neuroeffector function. The overall response to an inhibitory agent may therefore depend on the local and neurovascular reflexes initiated by nerve stimulation.

Neurotransmitters and trigeminovascular reflexes

A number of neurotransmitter systems may be involved in sensory nerve induced neurogenic extravasation by antidromic release of neurotransmitters. For example, both NK-1 antagonists (GR 82334) and CGRP antagonists (CGRP₈₋₃₇) produce maximal inhibition of neurogenic extravasation in the guinea-pig (O'Shaughnessy & Connor, 1994). In the rat, administration of an ET_B receptor agonist, sarafotoxin S6c, produced marked protein extravasation which was inhibited by the neurokinin receptor antagonist, spantide, supporting an indirect effect of endothelin receptor agonists on inflammatory transmitter release from the trigeminal nerve (Brandli *et al.*, 1995).

VIP mediates extracranial vasodilatation in the cat (Goadsby & MacDonald, 1985) via the parasympathetic nerve. In cats, trigeminal nerve ganglion stimulation-induced reductions in carotid vascular resistance and increases in cerebral blood flow were inhibited by lesion of the VIIth nerve and blocked by hexamethonium in most animals (Goadsby & Duckworth, 1987; Lambert *et al.*, 1984). In guinea-pigs, VIP appears to mediate trigeminal nerve ganglion-induced reductions in carotid vascular resistance which are not blocked by hexamethonium (Beattie & Connor, 1994). The lack of effect of hexamethonium was taken to indicate a lack of ganglionic transmission in this response (Beattie & Connor, 1994). However, Substance P/CGRP containing cells forming basket-like structures around cell bodies of the sphenopalatine ganglion have been suggested to form a reflex arc which may not utilize acetylcholine as an excitatory transmitter (Suzuki *et al.*, 1989b). Excitation of sensory nerve endings may therefore involve trigeminal nerve reflexes, which also involve parasympathetic pathways. The present study clearly demonstrates the capability of ET receptor antagonists to modulate this pathway.

It is apparent that ET receptor antagonists modulate both trigeminal nerve-induced extravasation (Brandli *et al.*, 1995) and trigeminal nerve-induced neurovascular reflexes (this study). However, a degree of redundancy appears to be apparent in these pathways as a number of neurotransmitters have been shown to play a role in these responses. This suggestion may partly explain the failure of ET receptor antagonist (May *et al.*, 1996) and NK-1 receptor antagonists (Goldstein *et al.*, 1997) in the clinic and indicates that modulation of post-synaptic mechanisms may not provide adequate efficacy as an antimigraine agent. Future strategies

may be better directed to pre-synaptic inhibition of these pathways.

We conclude that ET_B receptor activation plays a significant role in modulation of trigeminal nerve ganglion induced-neurovascular responses in the carotid vascular bed of anaesthetized cats. Stimulation of 5-HT_{1B/1D} receptors by sumatriptan or blockade of CGRP receptors has no effect.

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