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The influence of the trigeminal ganglion on carotid blood flow in anaesthetized guinea-pigs

[1]D.T. Beattie & H.E. Connor

Pharmacology II, Glaxo Research and Development Ltd, Park Road, Ware, Herts SG12 0DP

1 The influence of the trigeminal ganglion on the carotid circulation has been investigated by measuring electrical stimulation-induced alterations in carotid arterial blood flow and resistance in anaesthetized guinea-pigs. The effects of several receptor antagonists were assessed to determine which neurotransmitters are involved in regulating carotid blood flow.

2 Arterial blood pressure and carotid vascular resistance were reduced by electrical stimulation (0.5 mA, 1 ms, 5 Hz, 60 s) of the trigeminal ganglion ipsilateral to the carotid artery from which flow was measured. No consistent effect of electrical stimulation on carotid blood flow was observed. However, when guinea-pigs were pretreated with guanethidine (30 mg kg^{-1}, s.c., 24 h prior to experiments), stimulation produced little change in blood pressure, while carotid blood flow was increased and vascular resistance decreased, consistent with vasodilatation in the cranial circulation. Stimulation of the trigeminal ganglion contralateral to the carotid artery from which blood flow was measured, had little effect on either carotid blood flow or vascular resistance.

3 In animals pretreated with guanethidine, intravenous administration of the vasoactive intestinal polypeptide (VIP) receptor antagonist, [p-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1}) significantly attenuated the increase in carotid blood flow and decrease in carotid vascular resistance evoked by trigeminal ganglion stimulation. Responses evoked by trigeminal ganglion stimulation were, however, unaffected by intravenous injection of the tachykinin NK_1 receptor antagonists, GR82334 (0.3 mg kg^{-1}) and CP-99,994 (0.4 mg kg^{-1}), calcitonin gene-related peptide (CGRP) receptor antagonist, $CGRP_{8-37}$ (0.9 mg kg^{-1}) and the ganglion blocking agent, hexamethonium (10 mg kg^{-1}).

4 It is concluded that in the guanethidine-pretreated guinea-pig, electrical stimulation of the trigeminal ganglion increases carotid blood flow and produces an accompanying decrease in carotid vascular resistance, consistent with the dilatation of carotid blood vessels. The transmitter mediating this effect is most likely to be VIP.

Keywords: Trigeminal ganglion; carotid blood flow; electrical stimulation; VIP; NK_1; CGRP; hexamethonium; neuropeptide receptor antagonists

Introduction

The trigeminal nerve may be involved in the regulation of cranial blood flow (McCulloch *et al.*, 1986; Suzuki *et al.*, 1990). Stimulation of either the trigeminal ganglion or the nasociliary branch of its ophthalmic division increases blood flow in both cerebral and dural blood vessels of anaesthetized cats and rats (Hardebo *et al.*, 1991; Goadsby, 1993). The increase in blood flow arises from dilatation of cranial blood vessels, particularly those of the extracerebral circulation (Goadsby *et al.*, 1986). The vasodilatation has been shown in cats to be mediated in part by a parasympathetic reflex involving the seventh cranial nerve and the sphenopalatine and otic ganglia (Lambert *et al.*, 1984). Consistent with this mechanism, sectioning the seventh cranial nerve or administration of the ganglion blocking agent, hexamethonium, prevents the increases in carotid arterial blood flow and decreases in carotid vascular resistance which are evoked by trigeminal ganglion stimulation in cats and which reflect vasodilatation in the cranial vasculature (Lambert *et al.*, 1984; Goadsby & Macdonald, 1985). The neurotransmitter mediating this reflex vasodilatation and subsequent increase in cranial blood flow is likely to be vasoactive intestinal polypeptide (VIP) (Goadsby & Shelley, 1990). Cranial blood vessels receive a dense distribution of VIP-containing nerve fibres and stimulation of the efferent parasympathetic nerve releases VIP-like immunoreactivity. In addition, pretreatment with VIP antiserum blocks the increase in flow caused by trigeminal ganglion stimulation in anaesthetized cats (Larsson *et al.*, 1976; Goadsby & Macdonald, 1985; Goadsby & Shelley, 1990).

The neuropeptides, substance P and calcitonin gene-related peptide (CGRP) may also be important in the regulation of cranial blood flow (Suzuki *et al.*, 1990; Goadsby, 1993). Cranial blood vessels possess a dense distribution of substance P and CGRP-like immunoreactive nerve fibres originating in the trigeminal ganglion and stimulation of these fibres results in antidromic release of substance P and CGRP from perivascular nerve terminals (Edvinsson *et al.*, 1983; Liu-Chen *et al.*, 1983; Skofitsch & Jacobowitz, 1985). Both neuropeptides are potent dilators of cranial blood vessels. Moreover, recently it was demonstrated that increases in cerebral blood flow evoked by stimulation of the nasociliary nerve in cats are inhibited by human α $CGRP_{8-37}$, the putative $CGRP_1$ receptor antagonist (Jansen, 1992), indicating that CGRP may have a role in mediating this effect (Goadsby, 1993).

The relative importance of these neurotransmitters in the regulation of cranial blood flow remains to be clearly defined; previous studies have been hindered by the lack of selective and potent receptor antagonists for the transmitters implicated. The use of the CGRP receptor antagonist, human α $CGRP_{8-37}$, and the VIP and tachykinin receptor antagonists now available, such as [*p*-Cl-D-Phe^6,Leu^{17}]-VIP (Pandol *et al.*, 1986) and GR82334 (Hagan *et al.*, 1991) respectively, should allow the roles of these neuropeptides in the trigeminal nerve-mediated increase in carotid blood flow to be clarified.

The objectives of the present study were two fold. Firstly, it was intended to determine the effects of trigeminal ganglion stimulation on the carotid circulation, as measured by alterations in carotid arterial blood flow and vascular resistance (Lambert *et al.*, 1984; Spokes & Middlefell, 1993) and

[1] Author for correspondence.

secondly, to characterize pharmacologically the influence of selective VIP, CGRP and NK_1 receptor antagonists on responses evoked by trigeminal ganglion stimulation to determine which transmitters were implicated. The use of these antagonists may also identify the neurotransmitters which regulate, under resting conditions, carotid vascular tone and blood flow. Guinea-pigs were used in the present study as in this species, the NK_1 and $CGRP_1$ receptor subtypes have been identified as those responsible for the cranial vasodilatation mediated by substance P or CGRP respectively (Jansen *et al.*, 1991; Nilsson *et al.*, 1992; Beattie *et al.*, 1993).

Methods

Adult male Dunkin-Hartley guinea-pigs (300–450 g) were anaesthetized (i.p.) with ketamine (50 mg kg^{-1}) and pentobarbitone (25 mg kg^{-1}). The trachea was cannulated and animals artifically respired (60 strokes min^{-1}, 12 ml kg^{-1}) with room air supplemented by oxygen. The right carotid artery and jugular vein were cannulated to permit, respectively, the continuous measurement of arterial blood pressure and administration of drugs. Arterial blood flow was recorded by a Doppler flow probe (Bioengineering Department, University of Iowa, model 545C-4), placed around the left common carotid artery. Flow was recorded as a d.c. voltage (0.5 V per kHz Doppler shift). Carotid vascular resistance, equivalent to the mean arterial blood pressure divided by the carotid blood flow was monitored continuously by passing the pressure and flow signals to a peripheral resistance meter (Bioengineering Department, Glaxo Research and Development Ltd). The peak changes in blood pressure, carotid blood flow and vascular resistance were recorded. Anaesthesia was maintained by the infusion of pentobarbitone (15 mg kg^{-1} h^{-1}, i.p.). Rectal temperature was monitored with a thermistor (CFP 8185) and maintained at 37–38°C with a heated blanket.

Guinea-pigs were placed in a stereotaxic frame (David Kopf) and a longitudinal incision made in the scalp. Two burr holes were drilled in the skull and a bipolar stimulating electrode (Rhodes NE-200) was lowered by a micromanipulator into each trigeminal ganglion, 0.37 cm dorsal to bregma, ± 0.45 cm lateral from the midline and 1.05 cm below the dural surface. Electrode placements in the trigeminal ganglia were confirmed visually at the end of each experiment; the results described below are only those from animals in which the electrodes were located in the trigeminal ganglia. After lowering the electrodes into the trigeminal ganglia, 30 min were allowed to lapse to achieve stable resting levels in blood pressure, carotid arterial flow and vascular resistance before stimulation. In most experiments, the trigeminal ganglion ipsilateral to the carotid artery from which blood flow measurements were made, was subjected to two periods of electrical stimulation (0.5–1.5 mA, 1 ms, 5 Hz, 60 s), separated by 1 h. The first stimulation period served as a control for the second. In one group of experiments, the contralateral trigeminal ganglion was stimulated (1.0 mA, 1 ms, 5 Hz, 60 s). Drugs were dissolved in saline (0.9% w:v) immediately before use and administered (0.5 ml kg^{-1}, i.v.) approximately 5 min prior to the second stimulation period. Drug vehicle was injected as control, in a separate group of animals.

Drugs and solutions

The following drugs were used in this study: human α calcitonin gene-related peptide (CGRP), human α $CGRP_{8-37}$, [*p*-Cl-D-Phe^6,Leu^{17}]-porcine VIP (Bachem UK Ltd), guanethidine monosulphate (Sigma), hexamethonium bromide (Sigma) and substance P methyl ester (SPOMe; Cambridge Research Biochemicals Ltd). GR82334 ([D-Pro^9[Spiro-γ-Lactam]Leu^{10},Trp^{11}]physalaemin(1–11) and CP-99,994 (2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine) were synthesized in the Medicinal Chemistry Department, Glaxo Research and Development Ltd, Greenford. Peptides were dissolved in acetic acid (0.1 mM) and stock solutions (1 or 10 mM) stored in aliquots at − 20°C until needed. Solutions were diluted to the final concentrations in saline (0.9% w:v).

Statistical analysis

The effects of trigeminal ganglion stimulation on mean arterial blood pressure, carotid arterial blood flow and carotid vascular resistance were expressed as a percentage change from pre-stimulation levels. Values shown are means ± s.e.mean and *n* values quoted refer to the number of animals used. Comparisons between drug treatments were made by Student's *t* test for paired or unpaired samples, as appropriate, and a '*P*' value of less than 0.05 taken to indicate a significant difference between treatments.

Results

Guinea-pigs had a mean resting arterial blood pressure of 49.6 ± 2.1 mmHg ($n = 23$) and left carotid arterial blood flow of 12.0 ± 1.3 ml min^{-1} ($n = 20$). Electrical stimulation (0.5 mA, 1.0 ms, 5 Hz, 60 s) of the left trigeminal ganglion produced reductions in arterial blood pressure and left carotid vascular resistance with no consistent alteration in left carotid blood flow (changes from resting levels of − 29.8 ± 3.5, − 13.4 ± 3.9 and 3.2 ± 14.1 (each $n = 7$) respectively). A second period of stimulation, 1 h after the first, produced similar effects, both qualitatively and quantitatively, on blood pressure, carotid arterial blood flow and vascular resistance (changes from resting levels of − 34.4 ± 4.2, − 4.6 ± 11.6 and − 10.1 ± 4.5 (each $n = 7$) respectively). Stimulation, however, failed to evoke any response when the electrodes were placed outside the trigeminal ganglion.

Resting blood pressure and carotid blood flow were not significantly different in guinea-pigs pretreated with guanethidine (30 mg kg^{-1}, s.c.) compared to untreated animals. However, trigeminal ganglion stimulation (0.5–1.5 mA, 1.0 ms, 5 Hz, 60 s) in the pretreated animals produced little effect on blood pressure, but caused a consistent increase in carotid arterial blood flow and decrease in carotid vascular resistance (Figure 1). These effects were again reproducible when 1 h was left between stimulation periods (Table 1). The magnitude of the changes in carotid blood flow and vascular resistance evoked by trigeminal ganglion stimulation increased with increasing stimulation amplitude (Figure 1). Stimula-

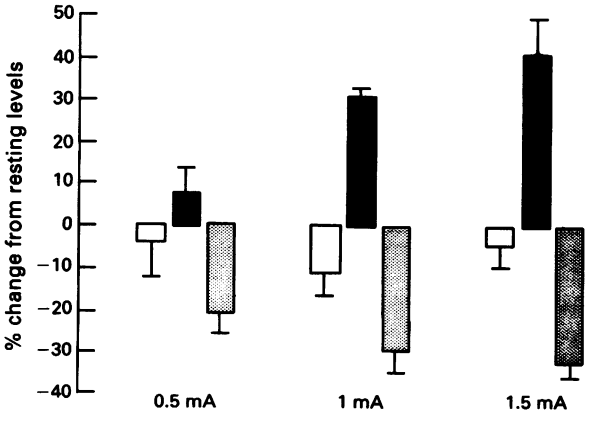

Figure 1 The effect of trigeminal ganglion stimulation (0.5, 1.0 and 1.5 mA, 1 ms, 5 Hz, 60 s) on mean arterial blood pressure (open columns), carotid blood flow (solid columns) and vascular resistance (cross-hatched columns) in guinea-pigs pretreated with guanethidine (30 mg kg^{-1}, s.c.). Results are expressed as a percentage change from resting levels ($n = 5$).

Table 1 Effects of two periods of trigeminal ganglion stimulation, separated by 1 h, on blood pressure and carotid blood flow and vascular resistance in guanethidine-pretreated guinea-pigs

	1st Stimulation (1.0 ms, 5 Hz, 60 s)			*2nd Stimulation* (1.0 ms, 5 Hz, 60 s)		
	0.5 mA	*1.0 mA*	*1.5 mA*	*0.5 mA*	*1.0 mA*	*1.5 mA*
% change in blood pressure	− 3.7 ± 8.2	− 10.9 ± 4.8	− 4.4 ± 5.0	− 3.0 ± 8.8	− 10.9 ± 4.1	− 10.5 ± 4.8
% change in carotid blood flow	7.3 ± 6.8	30.6 ± 1.5	40.9 ± 8.4	7.1 ± 5.8	24.3 ± 9.9	34.8 ± 6.7
% change in carotid vascular resistance	− 20.4 ± 5.1	− 29.5 ± 5.1	− 31.9 ± 3.5	− 17.2 ± 8.6	− 26.6 ± 4.2	− 33.4 ± 5.1

$n = 4-6$.

Table 2 Changes in blood pressure, carotid blood flow and vascular resistance evoked by trigeminal ganglion stimulation before (I) and following (II) administration of peptide receptor antagonists.

Drug treatment	*% change in blood pressure*		*% change in carotid blood flow*		*% change in carotid vascular resistance*	
	I	II	I	II	I	II
Saline vehicle (0.5 mg kg^{-1}, i.v.)	− 1.7 ± 7.9	− 7.9 ± 5.3	32.2 ± 11.6	26.9 ± 8.4	− 27.8 ± 3.4	− 26.6 ± 4.2
Hexamethonium (10 mg kg^{-1}, i.v.)	3.2 ± 4.4	− 2.1 ± 0.7	25.4 ± 2.3	26.2 ± 5.2	− 18.9 ± 3.8	− 22.0 ± 3.6
[*p*-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1})	− 3.2 ± 3.1	− 6.2 ± 2.2	30.0 ± 9.3	9.7 ± 6.5*	− 24.8 ± 4.6	− 11.0 ± 3.7*
$CGRP_{8-37}$ (0.9 mg kg^{-1})	− 5.6 ± 7.4	− 12.6 ± 3.9	26.9 ± 8.5	19.7 ± 10.5	− 20.8 ± 2.4	− 20.1 ± 4.8
GR82334 (0.3 mg kg^{-1})	3.7 ± 5.8	− 3.0 ± 6.6	37.6 ± 9.8	34.7 ± 17.3	− 23.6 ± 3.7	− 23.2 ± 9.7
CP-99,994 (0.4 mg kg^{-1})	− 2.2 ± 3.1	− 6.4 ± 0.5	29.3 ± 9.4	29.8 ± 1.0	− 23.9 ± 3.1	− 27.0 ± 0.9

$n = 4-6$.
*$P < 0.05$ compared to the first stimulation.

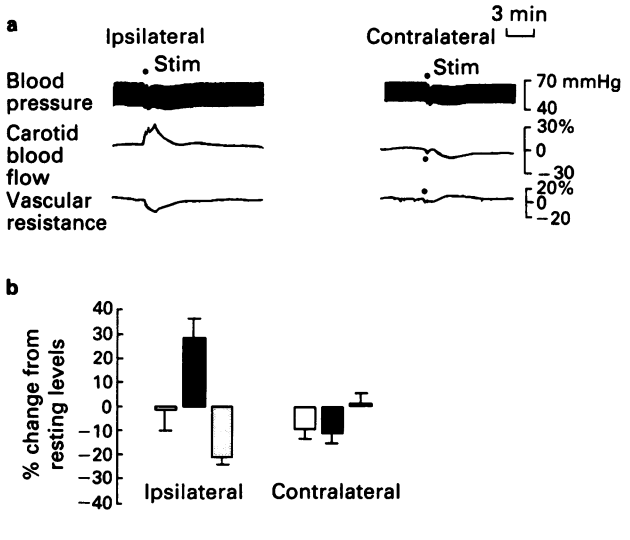

Figure 2 (a) Responses to stimulation (1 mA, 1 ms, 5 Hz, 60 s) of the trigeminal ganglion, ipsilateral and contralateral to the carotid artery from which flow was measured in guinea-pigs pretreated with guanethidine (30 mg kg^{-1}, s.c.). (b) A comparison of the effect of electrical stimulation (1 mA) of the ipsilateral and contralateral trigeminal ganglia on blood pressure (open columns), carotid blood flow (solid columns) and vascular resistance (cross-hatched columns), alterations expressed as a percentage change from resting levels ($n = 5$).

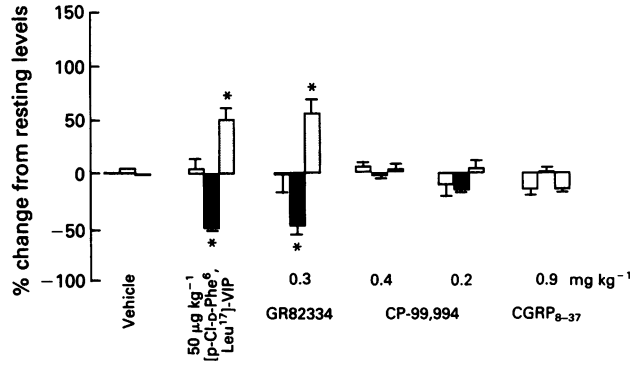

Figure 3 The effects of vehicle (0.5 ml kg^{-1}), [*p*-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1}), the NK_1 receptor antagonists, GR82334 (0.3 mg kg^{-1}) and CP-99,994 (0.4 and 2.2 mg kg^{-1}), and $CGRP_{8-37}$ (0.9 mg kg^{-1}) following i.v. administration, on blood pressure (open columns), carotid blood flow (solid columns) and carotid vascular resistance (cross-hatched columns) in anaesthetized guinea-pigs. [*p*-Cl-D-Phe^6,Leu^{17}]-VIP and GR82334, but not CP-99,994 significantly (*$P < 0.05$ compared to vehicle) increased vascular resistance and decreased flow ($n = 3-6$).

tion (1 mA), however, of the trigeminal ganglion contralateral to the carotid artery from which blood flow was measured, failed either to increase carotid blood flow or decrease vascular resistance in guanethidine-pretreated guinea-pigs (Figure 2). In all further studies, animals were pretreated with guanethidine (30 mg kg^{-1}, s.c.) 24 h prior to experiments.

Injection of saline vehicle (0.5 ml kg^{-1}, i.v.) 5 min before the second period of stimulation had no effect on mean arterial blood pressure, carotid blood flow or carotid vascular resistance in its own right (Figure 3), nor on respones evoked by trigeminal ganglion stimulation (Table 2). The ganglion blocking agent, hexamethonium (10 mg kg^{-1}, i.v.) on its own produced transient reductions, of 23.9 ± 0.8 and 33.5 ± 17% ($n = 4$), in blood pressure and carotid blood flow respectively. Carotid vascular resistance was little affected (an alteration of 3.6 ± 13.0% ($n = 4$) from the pre-injection level).

Responses to trigeminal ganglion stimulation (1 mA), 5 min later, when blood pressure and carotid blood flow had returned to basal levels, were not however significantly affected by hexamethonium (Table 2). The VIP receptor antagonist, [*p*-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1}, i.v.) had no significant effect on basal blood pressure (an alteration of 3.9 ± 9.3% (n = 6) from the resting level). However, carotid arterial blood flow was decreased and vascular resistance increased in all animals by [*p*-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1}) (peak changes from pre-injection levels of − 47.5 ± 4.5 and 48.7 ± 13.1% respectively (n = 6); Figure 3). When these effects had plateaued (approximately 5 min), the trigeminal ganglion was stimulated. The stimulation-induced alterations in carotid flow and vascular resistance, following [*p*-Cl-D-Phe^6,Leu^{17}]-VIP (50 μg kg^{-1}, i.v.) administration, were significantly ($P <$ 0.05) attenuated (Table 2).

The CGRP receptor antagonist, CGRP$_{8-37}$ (0.9 mg kg^{-1}, i.v.) reduced transiently (duration <2 min) blood pressure and carotid vascular resistance, but had no effect on carotid blood flow; the peak changes from resting levels were − 14.5 ± 5.8, − 12.9 ± 4.7 and 1.7 ± 3.6% respectively (n = 6; Figure 3). Responses evoked by trigeminal ganglion stimulation, however, when blood pressure and vascular resistance had returned to basal levels, were not significantly affected by CGRP$_{8-37}$ (Table 2). The NK$_1$ receptor antagonist, GR82334 (0.3 mg kg^{-1}, i.v.), but not CP-99,994 (0.4 and 2.2 mg kg^{-1}, i.v.), produced a marked and longlasting (in excess of 30 min) decrease in carotid blood flow and an accompanying increase in vascular resistance (Figure 3). Despite the alterations in carotid blood flow and vascular resistance, responses to trigeminal ganglion stimulation were unaffected by either GR82334 (0.3 mg kg^{-1}) or CP-99,994 (0.4 mg kg^{-1}) (Table 2).

Discussion

The trigeminal ganglion provides the principal sensory innervation of cranial blood vessels and is implicated in the regulation of blood flow in both cerebral and dural blood vessels (McCulloch *et al.*, 1986; Suzuki *et al.*, 1990; Hardebo *et al.*, 1991). The neurotransmitter(s) which may be responsible include substance P and CGRP, released antidromically from trigeminal nerve terminals, and VIP, released via a centrally-mediated parasympathetic reflex (Goadsby & Macdonald, 1985; Suzuki *et al.*, 1990). Each peptide is localized in nerve terminals innervating cranial blood vessels and their release and vasodilator activity have been demonstrated following trigeminal nerve activation (Edvinsson *et al.*, 1983; Goadsby & Shelley, 1990; Skofitsch & Jacobowitz, 1985; Buzzi *et al.*, 1991). The present study investigated the influence of the trigeminal ganglion on carotid blood flow in anaesthetized guinea-pigs by monitoring stimulation-induced changes in carotid arterial blood flow and vascular resistance and attempted to identify the neurotransmitters involved in this response by the use of selective antagonists.

Trigeminal ganglion stimulation reduced arterial blood pressure in anaesthetized guinea-pigs, a response similar to that observed in the cat (Lambert *et al.*, 1984) and monkey (Goadsby *et al.*, 1986), but contrasting with the pressor response reported in the rat (Spokes & Middlefell, 1993). Pretreatment of guinea-pigs with guanethidine significantly attenuated the hypotensive response suggesting that trigeminal ganglion stimulation in untreated animals may inhibit a sympathetic vascular tone. Moreover, only in guanethidine-pretreated guinea-pigs was trigeminal ganglion stimulation observed to increase carotid arterial blood flow. This enhancement of carotid blood flow occurred only following stimulation of the trigeminal ganglion ipsilateral to the carotid artery from which flow was measured, and is consistent with a localized dilatation of the cranial vasculature; stimulation of the contralateral ganglion resulted in small reductions in carotid arterial flow and blood pressure, but had no effect on carotid vascular resistance.

An aim of the study was to determine which neurotransmitter was responsible for the increase in carotid arterial blood flow and decrease in carotid vascular resistance evoked by trigeminal ganglion stimulation. The VIP receptor antagonist, [p-Cl-D-Phe^6,Leu^{17}]-VIP (Pandol *et al.*, 1986), significantly inhibited responses evoked by trigeminal nerve activation at a dose similar to that which inhibits VIP-induced coronary vasodilatation in the dog (Quebbemann *et al.*, 1991). The inhibition of the evoked responses in this study is unlikely to be due to physiological antagonism as the NK$_1$ receptor antagonist, GR82334, had no inhibitory action, despite having a similar effect to [*p*-Cl-D-Phe^6,Leu^{17}]-VIP on basal carotid blood flow and vascular resistance. The ganglion blocking agent, hexamethonium, at a dose (10 mg kg^{-1}, i.v.) which prevents the effects of trigeminal ganglion stimulation on carotid blood flow in cats (Lambert *et al.*, 1984), failed to influence the electrically-evoked responses implying that in the guinea-pig, ganglionic transmission is not involved in this process. The results suggest, therefore, that while VIP is responsible for the increased carotid blood flow produced by trigeminal ganglion stimulation in the guinea-pig, as in the cat (Goadsby & Macdonald, 1985), the nerve pathways may differ in the two species. It is possible that in the guinea-pig, VIP is released directly from sensory afferent trigeminal nerve terminals in the cranial vasculature, although to our knowledge there is no immunohistochemical evidence to suggest this; VIP-like immunoreactivity appears to be localised exclusively in parasympathetic efferent nerve fibres (Hara *et al.*, 1985; Uemura *et al.*, 1988). The precise mechanism underlying the VIP-induced increase in carotid blood flow requires further investigation.

The inability of CGRP$_{8-37}$ (0.9 mg kg^{-1}, i.v.), GR82334 (0.3 mg kg^{-1}, i.v.) and CP-99,994 (0.4 mg kg^{-1}, i.v.) (Chiba *et al.*, 1989; Hagan *et al.*, 1991; Desai *et al.*, 1992) to inhibit the changes in carotid blood flow and vascular resistance evoked by trigeminal ganglion stimulation suggests that neither CGRP$_1$ nor tachykinin NK$_1$ receptors mediate these responses. CGRP$_{8-37}$, at similar doses to that used in the present study (0.9 mg kg^{-1}), blocks cardiovascular effects of CGRP in conscious rats (Gardiner *et al.*, 1991) and increases in rat hindlimb blood flow evoked by saphenous nerve stimulation (Delay-Goyet *et al.*, 1992). Moreover, in the present study, in a different group of guinea-pigs, vasodepressor responses to CGRP (0.4 μg kg^{-1}, i.v.) were abolished by CGRP$_{8-37}$ (0.9 mg kg^{-1}, i.v.). Responses to trigeminal ganglion stimulation were also unaffected by doses of GR82334 (0.3 mg kg^{-1}) and CP-99,994 (0.4 mg kg^{-1}) which antagonized the vasodepressor activity of the tachykinin NK$_1$ receptor agonist, SPOMe (40 ng kg^{-1}, i.v.). While it is unlikely that either CGRP$_1$ or NK$_1$ receptors mediated the observed effects on carotid blood flow and vascular resistance in this study, the method used may not have been sensitive enough to detect any involvement of the peptides in discrete vascular beds, such as the cerebral or dural vasculature. Indeed, CGRP$_{8-37}$ has been shown to attenuate the increase in cerebral blood flow in cats following nasociliary nerve stimulation (Goadsby, 1993).

While the putative CGRP$_1$ receptor antagonist, CGRP$_{8-37}$, had only small effects on blood pressure, carotid blood flow or vascular resistance in its own right, the NK$_1$ antagonist, GR82334, and the VIP receptor antagonist, [*p*-Cl-D-Phe^6, Leu^{17}]-VIP, had profound effects on these parameters. Carotid blood flow was reduced and vascular resistance increased by both agents, effects consistent with antagonism of a resting NK$_1$ and VIP-regulated tone in the cranial circulation. While there may indeed be a regulatory role for VIP, the absence of any effect with CP-99,994 argues against involvement of NK$_1$ receptors. Although the NK$_1$ receptor antagonist, GR82334, has little affinity for other peptide receptors, such as those activated by neurokinins A and B, cholecystokinin, bradykinin or bombesin (Hagan, personal communication), another, as yet unidentified, mechanism would seem to

be responsible for its effects on carotid blood flow. This merits further investigation.

The results from this study provide evidence that the trigeminal ganglion is involved in the regulation of cranial blood flow in guinea-pigs, as in other species. The principal neurotransmitter mediating this process is likely to be VIP, rather than CGRP or substance P. It remains possible, however, that CGRP or substance P regulate blood flow in discrete regions of the carotid vascular bed, but that the changes in flow which they produce, represent only a small fraction of the blood flowing in the carotid artery.

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