ELSEVIER

European Journal of Pharmacology 284 (1995) 69–76

ejp

The modulation of the increase in rat facial skin blood flow observed after trigeminal ganglion stimulation

K. Jane Escott [a,*], David T. Beattie [b], Helen E. Connor [b], Susan D. Brain [a]

[a] *Pharmacology Group and Vascular Biology Research Centre, King's College, Manresa Road, London SW3 6LX, UK*
[b] *Pharmacology II, Glaxo Research & Development Ltd., Park Road, Ware, Hertfordshire, SG12 ODP, UK*

Received 2 February 1995; revised 16 May 1995; accepted 16 June 1995

Abstract

Electrical stimulation of the trigeminal ganglion causes an increase in facial skin blood flow in the anaesthetised rat, as measured by laser Doppler flowmetry. We investigated the modulation of this neurogenic vasodilator response using selective receptor agonists for putative prejunctional inhibitory receptors, as well as other pharmacological agents to further characterise this response. [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO, a μ-opioid receptor agonist) inhibited the vasodilator response in a dose-related (0.058–5.8 μmol/kg i.v.) and naloxone-sensitive manner. A similar inhibitory response was observed with the local anaesthetic lignocaine (2% w/v, s.c. 20 μl). In contrast, the histamine H_3-receptor agonist α-methylhistamine (15 or 35 μmol/kg, i.v.) and the 5-HT_{1D} receptor agonists sumatriptan (0.24 or 2.4 μmol/kg, i.v.) and CP 122,288 (0.0003–3 μmol/kg, i.v.) had no effect on these responses. Similarly, atropine (1.5 μmol/kg, i.v.) and indomethacin (28 μmol/kg, i.v.) did not alter the vasodilatation observed in this model. In conclusion, only μ-opioid receptor activation and local anaesthetic had any inhibitory action on the neurogenic vasodilatation observed in this model.

Keywords: Neurogenic inflammation; Trigeminal ganglion; Sensory nerve; μ-Opioid receptor agonist; 5-HT_{1D} receptor agonist; Histamine H_3 receptor agonist

1. Introduction

Electrical stimulation of the trigeminal ganglion leads to plasma extravasation in the rat and guinea-pig dura mater (Markowitz et al., 1987) and increases cerebral blood flow in the anaesthetised cat (Goadsby and Edvinsson, 1993). These responses are evoked by the efferent release of sensory neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP), from sensory afferent nerves. These pro-inflammatory processes have been proposed to develop within the cranial vasculature during migraine headache and may amplify or sustain migraine pain (Moskowitz, 1993). More recently, it has been demonstrated that the trigeminovascular system is activated during cluster headache in humans (Goadsby and Edvinsson, 1994). Therefore, neurogenic inflammation may be important in both migraine and cluster headaches.

We have recently shown that electrical stimulation of the trigeminal ganglion elicits an increase in microvascular blood flow in rat facial skin (Escott et al., 1995). This neurogenic vasodilator response was unaffected by the tachykinin NK_1 receptor antagonist, RP67580, or the vasoactive intestinal peptide (VIP) receptor antagonist, [*p*-Cl-D-Phe^6,Leu^{17}]VIP. In contrast, the CGRP receptor antagonist, CGRP (8–37), significantly reduced the neurogenic vasodilator response. These results suggest that CGRP is the major neuropeptide involved in the neurogenic vasodilator response elicited in the rat facial skin by trigeminal ganglion stimulation.

Neurogenic inflammation may be modulated in several ways: by inhibiting neuropeptide release from peripheral sensory nerve terminals and by inhibiting the postjunctional inflammatory action of neuropeptides. There is increasing evidence to suggest that activation of prejunctional receptors on sensory nerve terminals, including opioid receptors (μ and κ), histamine (H_3) receptors, 5-HT (5-$HT_{1D/1B}$) receptors, α_2-adrenoceptors and somatostatin receptors, may reduce efferent neuropeptide release. Activation of prejunctional histamine H_3 receptors (with α-methylhistamine), α_2-

* Corresponding author. Tel. 0171-333 4731, fax 0171-376 8150.

Elsevier Science B.V.
SSDI 0014-2999(95)00367-3

adrenoceptors (with UK-14,304) and somatostatin receptors (with SMS 201–995) inhibits dural plasma extravasation evoked by trigeminal ganglion stimulation in anaesthetised rats and guinea-pigs (Matsubara et al., 1992). Similarly, the 5-HT_{1D} receptor agonists, sumatriptan and the conformationally restricted sumatriptan analogue CP 122,288 reduce guinea-pig dural plasma extravasation (Buzzi and Moskowitz, 1990; Lee and Moskowitz, 1993). Also, the μ-opioid receptor agonists [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO) and lofentanil attenuate plasma extravasation and vasodilatation in rat hind paw skin evoked by saphenous nerve stimulation (Barber, 1993; Lembeck and Donnerer, 1985).

The aim of this study was to investigate the modulation of neurogenic vasodilator responses elicited in the rat facial skin by trigeminal ganglion stimulation using (a) agents which have been proposed to act at the prejunctional level to inhibit neuropeptide release (DAGO, α-methylhistamine, sumatriptan and CP 122,288) and (b) those which act elsewhere on other systems, for example, sensory nerve conduction (lignocaine), prostaglandin synthesis (indomethacin) and the parasympathetic nervous system (atropine). Microvascular blood flow in the rat facial skin was continuously measured by laser Doppler flowmetry (Vongsavan and Matthews, 1993).

2. Materials and methods

2.1. Animals and surgery

Male Wistar rats (180–200 g) were pretreated 24 h prior to trigeminal ganglion stimulation with 20 mg/kg s.c. guanethidine in order to reduce possible sympathetic vasoconstriction evoked by trigeminal ganglion stimulation (Gamse and Saria, 1987). On the day of the experiment, rats were anaesthetised with sodium pentobarbitone, initially 60–80 mg/kg i.p. and maintained throughout the experiment with an infusion of 30 mg/kg/h i.p. Body temperature was maintained at 36–38°C by an automated heating pad. Each side of the face was shaved and depilated using a commercial cream (Immac, Reckitt & Colman). The femoral artery and trachea were cannulated for the continuous measurement of blood pressure and heart rate, and for artificial ventilation (60 strokes/min), respectively. The tail vein was cannulated for intravenous drug administration. Neuromuscular blockade was achieved using pancuronium bromide (induction with 0.5 mg/kg, i.v. followed by an infusion 0.25 mg/kg per h i.p.). Blood pressure and heart rate were continuously monitored throughout the course of the experiment to assess the level of anaesthesia and to monitor the cardiovascular effects of the drugs used in this study.

2.2. Electrical stimulation of the trigeminal ganglion

The anaesthetised rat was placed in a stereotaxic frame and a midline incision made in the scalp to expose the skull. Two burr holes were drilled in the skull and bipolar electrodes (Rhodes NE-200) were positioned bilaterally into the trigeminal ganglion, using a micromanipulator and stereotaxic coordinates for the trigeminal ganglion (0.32 cm dorsal to bregma, $\pm$0.29 cm lateral from the midline and 0.92 cm below the dural surface). Correct placement of the electrodes in the trigeminal ganglion was confirmed prior to paralysis by a brief (1 s) electrical stimulation of the trigeminal ganglion which results in jaw movements due to the activation of the motor nerves of the ganglion. Also, the position of the electrodes was checked visually post mortem following removal of the brain. Only one trigeminal ganglion was electrically stimulated at 10 V, 5 Hz, 1 ms for 30 s. These parameters are similar to those used by our group and others to stimulate the trigeminal ganglion (Escott et al., 1995; Beattie and Connor, 1994).

2.3. Measurement of facial skin blood flow

Microvascular blood flow changes in the facial skin of the rat, evoked by trigeminal ganglion stimulation, were measured by laser Doppler flowmetry using a Moor dual laser Doppler flow meter (MBF3D). Laser Doppler flowmetry monitors changes in both nutritional and non-nutritional capillary blood flow in the skin, measuring blood cell movements 1–2 mm below the skin surface (Vongsavan and Matthews, 1993). Laser Doppler probes were positioned on either side of the face approximately 0.5 cm below the middle of the eye, an area innervated by the maxillary division (V2) of the trigeminal nerve. Facial skin blood flow was monitored throughout the experiment and blood flow changes were measured as arbitrary units of flux (Escott and Brain, 1993). Following surgery, the anaesthetised animal was allowed to stabilise for 30 min and was then subjected to two periods of electrical stimulation, separated by a 30 min interval. In all groups, a control blood flow response was obtained following the first stimulation and the effect of the drugs or vehicles on the blood flow response to the second stimulation were assessed. The first stimulation was used as a control for the second. [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO, a μ-opioid receptor agonist), α-methylhistamine (H_3-receptor agonist), the 5-HT_{1D} receptor agonists sumatriptan and CP 122,288, atropine or the appropriate vehicles were administered i.v. 10 min prior to the second stimulation. Naloxone and indomethacin were administered i.v. 15 min prior to the second stimulation. All the above drugs or an equivalent volume of the appropriate vehicles were injected as a bolus dose

i.v. (1 ml/kg). Lignocaine or saline were given locally by s.c. injection (20 μl volume) in the facial skin directly under the laser Doppler probe, 10 min prior to the second stimulation.

2.4. Materials

Guanethidine monosulphate (Ismelin) was obtained from Ciba-Geigy, U.K.. [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO; Bachem), naloxone (Sigma, U.K.), α-methylhistamine and sumatriptan (synthesised by Glaxo Research and Development, UK) and atropine (Sigma, UK) were all dissolved in physiological saline. CP 122,288 (5-methyl-aminosulfonylmethyl-3-(*N*-methylpyrrolidin-2*R*-yl-methyl)-1*H*-indole; synthesised by Glaxo Research and Development, UK) was dissolved in 10% 1 M HCl in saline at 3 μmol/ml and further dilutions were made with saline. Indomethacin (Sigma, UK) was dissolved in 0.5% Na_2CO_4 in saline and lignocaine (Lignavet, UK) was used at 2% w/v in saline.

2.5. Statistical analysis

The raw blood flow data was calculated in two ways. (1) The initial peak increase in facial skin blood flow (arbitrary units of flux) following stimulation was obtained from laser Doppler recordings and (2) the area under the curve (mm^2) was measured by computerised planimetry for up to 15 min after stimulation. The small increase in flux or the area under the curve on the contralateral side (unstimulated) of the face was subtracted from the larger response observed on the ipsilateral (stimulated) side (Fig. 1a). Results were then expressed as the % inhibition (peak increase in flux or the area under the curve) of the second stimulation compared to the first stimulation and values given were means ± S.E.M. Comparisons between the first and second stimulation within the same group were made using Student's paired *t*-test and the difference between drug and vehicle treated groups were assessed using ANOVA followed by Bonferroni's modified *t*-test. All statistical tests were carried out on the raw data and statistical difference was accepted when $P < 0.05$.

3. Results

3.1. Effects of trigeminal ganglion stimulation

Electrical stimulation (10 V, 5 Hz, 1 ms for 30 s) of the trigeminal ganglion produced a long-lasting (up to 20 min) increase in facial skin blood flow ipsilateral to the side of stimulation as previously described by our group (Escott et al., 1995) (Fig. 1a). A smaller and more transient rise (30 s) in facial blood flow was also observed on the contralateral side to stimulation and was probably due to the increase in blood pressure observed during electrical stimulation. Data pooled from saline-treated animals showed that there was no significant difference, in the peak increase in flux or the area under the curve, between the first and second stimulation (first stimulation: peak increase in flux 92.9 ± 6.8, area under the curve 964.6 ± 57.4 mm^2; second stimulation: peak increase in flux 94.9 ± 7.3, area under the curve 954.1 ± 61.4 mm^2, using paired *t*-test $P > 0.05$, $n = 25$). Therefore, the vasodilator response evoked by trigeminal ganglion stimulation could be reproduced twice in the same animal.

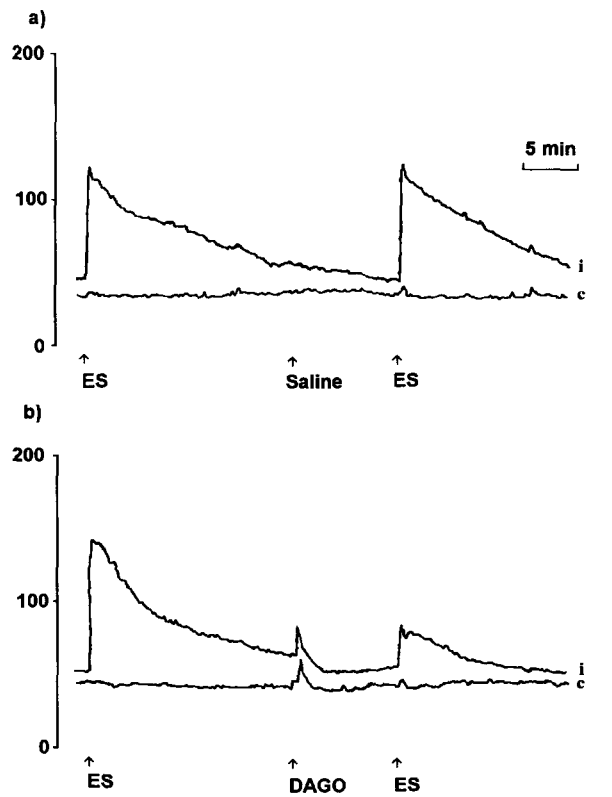

Fig. 1. Representative tracings of facial skin blood flow responses to electrical stimulation (ES) of the trigeminal ganglion (10 V, 5 Hz, 1 ms for 30 s). The tracings show the blood flow changes observed in the rat facial skin, ipsilateral (i) and contralateral (c) to the side of stimulation. The ordinate is shown in arbitrary units of flux. The blood flow response to two consecutive stimulations, with a 30 min interval is shown in (a). (a) also shows the effect of saline (1 ml/kg i.v.) and (b) illustrates the effect of [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO, 5.8 μmol/kg i.v.) on the vasodilator response evoked by the second stimulation.

3.2. Effect of drugs on resting blood pressure, heart rate and facial skin blood flow

The mean arterial blood pressure and heart rate of the animals used in this study were all within normal

physiological ranges for anaesthetised rats (96.9 ± 1.2 mmHg and 311 ± 5.4 beats per min, respectively; n = 96). Administration of i.v. bolus saline, vehicles or naloxone had no effect on resting blood pressure, heart rate or facial skin blood flow (Table 1). However some of the test agents, at the highest doses used in this study, altered the resting values of these parameters as illustrated in Table 1. These cardiovascular effects were transient (20 s to a maximum of 2 min) and returned to basal pre-injection values prior to the second stimulation. Both saline and lignocaine, administered s.c. in a volume of 20 μl, caused a small reduction in facial skin blood flow from resting levels (−17.5 ± 4.6% and −19 ± 8.0% respectively). This was probably an experimental artefact caused by the s.c. administration of the 20 μl injection volume to the facial skin, moving the probe slightly away from the skin.

3.3. Effect of a μ-opioid receptor agonist, DAGO

The vasodilatation evoked by trigeminal ganglion stimulation was inhibited in a dose-related manner by [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO, at 0.058–5.8 μmol/kg, administered i.v. 10 min prior to the second stimulation) as illustrated in Figs. 1 and 2. The peak increase in flux and the area under the curve were significantly inhibited at 0.58, 2 and 5.8 μmol/kg DAGO when compared to saline treated animals (P < 0.05, n = 4–6) (Fig. 2). Naloxone alone (2.7 μmol/kg, i.v. 15 min prior to the second stimulation) had no significant effect on the response elicited by trigeminal ganglion stimulation when compared to saline-treated animals (P > 0.05, n = 4–6). However, the inhibitory effect of DAGO (2 μmol/kg, i.v.), on both the peak increase in flux and the area under the curve, was significantly reversed in the presence of naloxone (2.7 μmol/kg, i.v., P < 0.01, n = 4–6) (Fig. 3). Similarly, the inhibitory effect of DAGO (2 μmol/kg, i.v.) could be fully reversed by local intradermal injection of naloxone (27 nmol/20 μl, i.d.), injected directly into the facial skin under the laser Doppler probe. The peak increase in flux was significantly reversed from 66.1 ± 8.3% inhibition with DAGO alone to 20.7 ± 6.1% in the presence of both DAGO and intradermal naloxone (P < 0.05, n = 4). The area under the curve was also reversed from 75.1 ± 6.5% inhibition with DAGO alone to 10.6 ± 4.4% inhibition in the presence of intradermal naloxone (P < 0.05, n = 4). However, intradermal injection of the saline (20 μl) or naloxone (27 nmol/20 μl) alone had little effect on the vasodilator response evoked by trigeminal ganglion stimulation (peak increase in flux: 9.7 ± 2.2% or 14.8 ± 1.0% inhibition respectively).

Systemic administration of DAGO (2 μmol/kg, i.v.) also had no effect on vasodilatation induced by intradermal CGRP (10 pmol/20 μl, i.d.) (unpublished observation, data not shown).

Table 1
Effect of drugs on resting blood pressure, heart rate and facial skin blood flow

Treatment		% Change in blood pressure		% Change in heart rate		% Change in skin flux		n
		(a) t = 0.5 min	(b) t = 10 min	(a) t = 0.5 min	(b) t = 10 min	(a) t = 0.5 min	(b) t = 10 min	
Saline		2.7 ± 2.1	0.2 ± 1.0	−1.2 ± 0.7	−1.8 ± 1.2	0.2 ± 1.7	−3.1 ± 2.1	6
DAGO	5.8 μmol/kg	−37 ± 2.3 [d]	5.8 ± 4.3	−19.5 ± 2.4 [d]	−9.2 ± 2.9	15.6 ± 8.0	7.8 ± 5.1	5
Naloxone	2.7 μmol/kg	4.5 ± 1.2	−1.3 ± 2.0	−3 ± 1.1	−4 ± 2.3	4.3 ± 4.3	4.3 ± 4.3	4
Saline		2.8 ± 2.4	1.2 ± 1.9	−0.5 ± 0.5	−3.2 ± 1.5	−2.7 ± 3.5	−3.7 ± 3.5	6
α-Methylhistamine	35 μmol/kg	−20.0 ± 4.9 [c]	−0.5 ± 1.5	4.8 ± 1.2	1.7 ± 2.3	−5.0 ± 7.3	3.2 ± 5.4	4
Saline		2.8 ± 2.4	1.2 ± 1.9	−0.5 ± 0.5	−3.2 ± 1.5	−2.7 ± 3.5	−3.7 ± 3.5	4
Sumatriptan	2.4 μmol/kg	−20.0 ± 4.9 [c]	−0.5 ± 1.5	4.8 ± 1.2	1.7 ± 2.3	−5.0 ± 7.3	3.2 ± 5.4	4
Vehicle		1.0 ± 1.0	0.2 ± 0.5	−0.6 ± 0.6	−0.6 ± 0.6	1.0 ± 1.0	4.5 ± 4.9	5
CP 122,288	3 μmol/kg	−14.5 ± 2.1 [c]	−2.9 ± 1.2	−2.0 ± 1.2	−0.6 ± 0.6	6.5 ± 8.3	4.3 ± 7.3	4
Saline		3.5 ± 1.5	0.3 ± 0.3	−0.8 ± 0.8	1 ± 1	4.3 ± 4.3	1 ± 6.2	4
Atropine	1.5 μmol/kg	−7.8 ± 1.9 [c]	−1.5 ± 0.6	−2.3 ± 0.8	−3 ± 1.2	−4.5 ± 3.1	−6.5 ± 2.7	4
Vehicle		2.8 ± 4.4	1.3 ± 2.8	−2.3 ± 1.4	−4.5 ± 0.9	7 ± 4.7	0 ± 3.9	4
Indomethacin	28 μmol/kg	−20.8 ± 3.8 [c]	2.5 ± 4.2	−2.3 ± 1.4	−3 ± 1.7	15.8 ± 8.1	−1.3 ± 4.6	4

The table shows the changes in mean arterial blood pressure, heart rate and facial skin blood flow at various times after bolus i.v. administration of vehicle or drugs. Results are expressed as % change from pre-injection values. For each cardiovascular parameter % change is expressed in two ways (a) the initial change following drug administration (t = 0.5 min) or (b) the change 10 min after drug administration, i.e. immediately prior to the second stimulation. Each value represents mean ± S.E.M. and n represents the number of animals in each group. Statistical significance is represented by [c]P < 0.05 or [d]P < 0.01 compared to the appropriate vehicle-treated group, otherwise differences between drug-treated groups and vehicle-treated groups were statistically non-significant.

3.4. Effect of an histamine H_3 receptor agonist, α-methylhistamine

α-Methylhistamine, administered i.v. at 15 or 35 μmol/kg 10 min prior to the second stimulation ($t = -10$ min), had no significant effect on the peak increase in flux or the area under the curve evoked by trigeminal ganglion stimulation when compared to saline treated animals ($P > 0.05$, Table 2). α-Methylhistamine was used at similar doses to those used in previous studies which inhibited dural plasma extravasation (Matsubara et al., 1992).

3.5. Effect of 5-HT_{1D} receptor agonists: sumatriptan and CP 122,288

Sumatriptan (0.24 or 2.4 μmol/kg) and CP 122,288 (0.0003–3 μmol/kg) were also administered i.v. ($t = -10$ min). However, neither of these two drugs had any significant ($P > 0.05$) effect on blood flow responses elicited by trigeminal ganglion stimulation (Table 2). The doses of sumatriptan and CP 122,288 used in this study were chosen because they have previously been shown to inhibit plasma extravasation in the dura mater following trigeminal ganglion stimulation (Lee and Moskowitz, 1993).

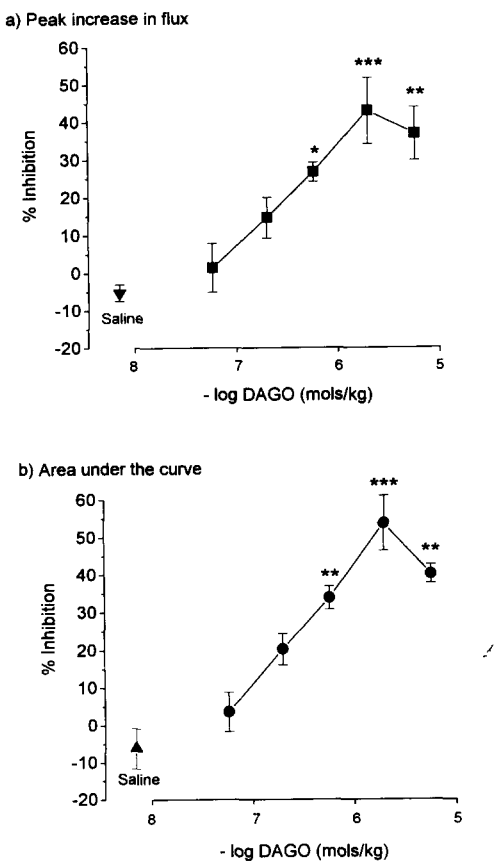

Fig. 2. The inhibitory effect of [D-Ala^2,Me-Phe^4,Gly^5-ol]enkephalin (DAGO, 0.058–5.8 μmol/kg, i.v.) on a) the peak increase in flux and (b) the area under the curve following trigeminal ganglion stimulation (10 V, 5 Hz, 1 ms for 30 s). A saline-treated control group is represented by the filled triangles and DAGO-treated groups are shown by the filled squares or circles. Results are expressed as % inhibition of the second response compared to the first response, means ± S.E.M., $n = 4$–6. Statistical significance is represented by $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared to the saline-treated group.

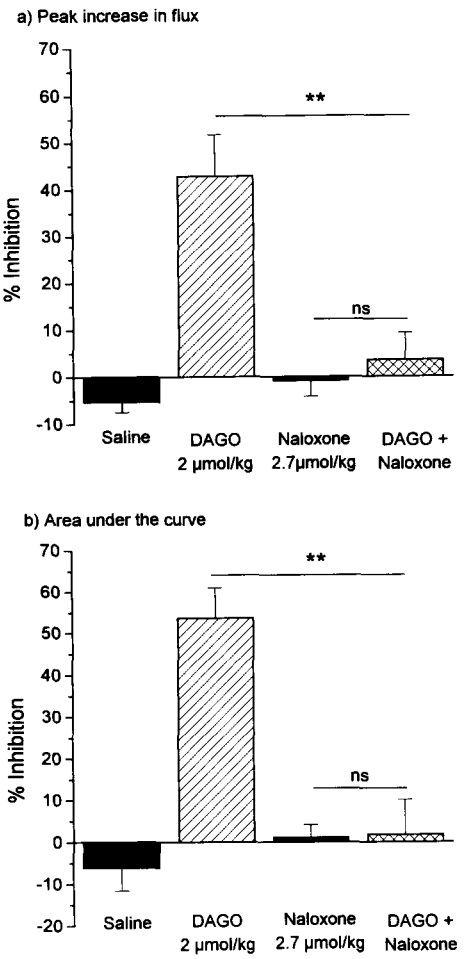

Fig. 3. The inhibitory effect of DAGO (2 μmol/kg i.v.) on (a) the peak increase in flux and (b) the area under the curve, and reversal by naloxone (2.7 μmol/kg i.v.). Results are expressed as % inhibition of the second response compared to the first response, means ± S.E.M., $n = 4$–6. A statistically significant difference between DAGO (2 μmol/kg i.v.) alone- and DAGO (2 μmol/kg i.v.) + naloxone (2.7 μmol/kg i.v.)-treated groups is represented by $^{**}P < 0.01$. No significant difference between naloxone (2.7 μmol/kg i.v.) alone and DAGO (2 μmol/kg i.v.) + naloxone (2.7 μmol/kg i.v.) is shown by 'ns'.

3.6. Other modulators

Lignocaine (2% w/v, 20 μl, s.c., $t = -10$ min) significantly inhibited the peak increase in flux and the area under the curve by 72% and 68% respectively

Table 2
Modulation of facial skin blood flow responses

Treatment		% Inhibition		n
		(a) peak increase in flux	(b) area under the curve	
(a) Proposed presynaptic modulators				
Saline		−4.8 ± 2.7	4.1 ± 4.1	6
α-methylhistamine	15 μmol/kg	−7.6 ± 2.7	0.8 ± 5.7	4
	35 μmol/kg	−3.7 ± 5.6	3.1 ± 11.2	4
Saline		5.5 ± 1.1	−0.3 ± 4.0	4
Sumatriptan	0.24 μmol/kg	−8.4 ± 11.6	0.8 ± 5.5	3
	2.4 μmol/kg	−20.6 ± 10.6	4.5 ± 1.6	4
Vehicle		0.4 ± 3.6	4.6 ± 6.5	5
CP122,288	0.0003 μmol/kg	−2 ± 3.8	0.5 ± 15	3
	0.003 μmol/kg	−2.4 ± 6.3	−5.4 ± 9.7	3
	0.3 μmol/kg	−0.2 ± 6.0	−4.9 ± 5.6	3
	3 μmol/kg	3.6 ± 6.1	5.1 ± 11.5	4
(b) Other modulators				
Saline	s.c. 20 μl	11.7 ± 4.3	1.2 ± 5.7	6
Lignocaine	(2% w/v) s.c. 20 μl	72 ± 8.7 [d]	68.4 ± 8.9 [d]	6
Saline		−0.3 ± 5.3	1.5 ± 2.8	4
Atropine	1.5 μmol/kg	−2.7 ± 2.8	8.8 ± 4.3	4
Vehicle		−0.4 ± 7.3	−5 ± 13.6	4
Indomethacin	28 μmol/kg	4.0 ± 9.4	3.0 ± 10.8	4

The effect of various drugs on (a) the peak increase in flux and (b) the area under the curve evoked by trigeminal ganglion stimulation (10 V, 5 Hz, 1 ms for 30 s) are illustrated below. Results are expressed as % inhibition of the second response compared to the first response, means ± S.E.M. and n represents the number of animals in each group. Statistical significance is represented by $^{d}P < 0.01$, otherwise there was no significant difference between drug- and vehicle-treated groups.

when compared to s.c. saline (20 μl) ($P < 0.01$, Table 2). Atropine (1.5 μmol/kg, i.v., $t = -10$ min) at a dose which previously reduced other skin vasodilator responses evoked by sensory nerve stimulation (Lembeck and Holzer, 1979), had no effect on trigeminal evoked vasodilator responses. Similarly, indomethacin (28 μmol/kg, i.v., $t = -15$ min) had no effect on these responses. Indomethacin inhibits prostaglandin synthesis and a variety of inflammatory responses at similar doses to those used in this study (Garcia Leme et al., 1973).

4. Discussion

Electrical or chemical (capsaicin) stimulation of primary afferent sensory nerves causes neurogenic inflammation due to the release of inflammatory mediators, including substance P and CGRP, from peripheral nerve terminals. Previously we have shown that electrical stimulation of the trigeminal ganglion leads to an increase in microvascular facial skin blood flow which is inhibited by the CGRP receptor antagonist, CGRP-(8–37) (Escott et al, 1995). CGRP-(8–37) (40–400 nmol kg^{-1}, i.v.) caused a dose-related inhibiton of both the peak increase in flux (1.2 ± 4.9% to 55.1 ± 8.4% inhibition) and the area under the curve (21.8 ± 16.6% to 81.6 ± 7.9% inhibition) (Escott unpublished). Using this model, we have now investigated the action of several pharmacological agents which have been proposed to modulate neurogenic inflammation in order to further characterise the mechanisms involved in this vasodilator response. These agents include those which may act prejunctionally to inhibit neuropeptide release and others which act on nerve conduction, prostaglandin synthesis and the parasympathetic nervous system.

Opioids modulate neurogenic inflammation in a variety of tissues, including lung and skin, via the activation of prejunctional opioid receptors located on sensory nerve terminals which inhibit neuropeptide release (Maggi, 1991). In this study, we have shown that the μ-opioid receptor agonist DAGO reduced facial skin vasodilatation induced by trigeminal ganglion stimulation in the rat, inhibiting both the peak increase as well as the long duration of the vasodilator response. The inhibitory action of DAGO was dose-related and naloxone-sensitive. It is unlikely that the modulatory action of DAGO was caused by the reduction in heart rate and blood pressure initially observed upon systemic application of DAGO, because at all the

doses used in this study the cardiovascular effects were transient and returned to basal levels prior to the second stimulation. Also, DAGO had no effect on the vasodilator action of exogenous CGRP. Moreover, other agents reduced blood pressure and yet had no effect on vasodilator responses evoked by trigeminal ganglion stimulation. These results are similar to those in other studies where systemic application of the μ-opioid-selective agonists, lofentanil (Lembeck and Donnerer, 1985) or the enkephalin analogue [D-Met^2,Pro^5]enkephalinamide (Lembeck et al., 1982; Gamse and Saria, 1987), inhibited neurogenic extravasation or vasodilatation in rat hind paw skin elicited by saphenous nerve stimulation. These inhibitory responses could also be reversed with naloxone. In a more recent study, the μ-receptor agonists (morphine and DAGO) and the κ-receptor agonists (U 50488H, ICI 197067 and ICI 204448) inhibited saphenous nerve-induced plasma extravasation (Barber, 1993). This study and our work, also showed that the inhibitory action of the μ-opioid agonists was due to a peripheral mechanism of action because the activity of the systemically applied DAGO was blocked by naloxone injected locally into the paw or the facial skin.

The action of other agents proposed to act at the prejunctional level to inhibit neuropeptide release were also investigated. In our study, the H_3 receptor agonist α-methylhistamine had no effect on vasodilatation induced by trigeminal ganglion stimulation. This is in contrast to studies in guinea-pig airways where α-methylhistamine inhibited nonadrenergic noncholinergic neural bronchoconstriction and plasma extravasation in vivo (Ichinose and Barnes, 1989; Ichinose et al., 1990).

Interestingly, α-methylhistamine has also been shown to reduce dural plasma extravasation elicited by electrical or capsaicin stimulation of the rat/guinea-pig trigeminal ganglion, but has no effect on plasma extravasation induced by systemic application of substance P (Matsubara et al., 1992). This latter study suggests that blockade of the neurogenic response is mediated via inhibitory prejunctional H_3 receptors located on trigeminal nerves in the dura. In our model of neurogenic vasodilatation it is possible that the negative results may be explained by the possible lack of inhibitory H_3 receptors on peripheral trigeminal nerve terminals located in the facial skin.

The 5-HT_{1D} agonists, sumatriptan and CP 122,288, had no effect on facial skin blood flow responses evoked by trigeminal ganglion stimulation. Both these drugs inhibit plasma extravasation evoked by trigeminal ganglion stimulation in guinea-pigs (Lee and Moskowitz, 1993). Furthermore, Goadsby and Edvinsson (1993) reported a prejunctional inhibitory action of sumatriptan on neurogenic vasodilatation. This study showed that sumatriptan inhibited the increase in cerebral blood flow and circulating CGRP levels observed following trigeminal ganglion stimulation in the cat. Our results suggest that in this model of neurogenic vasodilatation 5-HT_{1D} receptors do not inhibit the release of the vasodilator neuropeptide, CGRP. This is similar to previous studies where sumatriptan displayed a lack of inhibitory effect on neuropeptide release in isolated guinea-pig pulmonary artery (Butler et al., 1993) and basilar artery (O'Shaughnessy et al., 1993) in vitro. These results are in keeping with the suggestion that inhibitory prejunctional 5-HT_{1D} receptors may be localised to very distinct areas, such as intracranial blood vessels but not in the facial skin or other peripheral tissues (Buzzi and Moskowitz, 1990).

The negative effect of α-methylhistamine, sumatriptan and CP 122,288 may be due to the lack of inhibitory prejunctional receptors (histamine H_3 or 5-HT_{1D}) on peripheral nerve terminals in the facial skin. CP 122,288 may also inhibit plasma extravasation via another unknown mechanism or receptor subtype which is not present on peripheral terminals of the trigeminal ganglion. Another possibility is that there are different mechanisms governing the release of oedema-forming mediators (substance P) compared to vasodilators (CGRP) from peripheral nerve terminals. There are very few reports that these agents, which inhibit neuropeptide release, actually reduce vasodilatation following antidromic sensory nerve stimulation. Most studies have shown prejunctional inhibition of mediators which cause plasma extravasation, possibly suggesting different control mechanisms of neuropeptide release.

Other pharmacological agents were used to further characterise the mediators involved in the neurogenic vasodilator response to trigeminal ganglion stimulation. A cholinergic involvement in antidromic vasodilatation has previously been described (Holzer, 1992). Atropine produces a small reduction in the vasodilatation evoked by saphenous nerve stimulation in rat hind paw skin (Lembeck and Holzer, 1979). In this study, we investigated the role of cholinergic nerves in trigeminal-evoked vasodilatation using atropine. However, atropine had no effect on neurogenic vasodilatation suggesting that this response probably does not involve the parasympathetic nervous system. Another possible mediator or modulator of the increase in blood flow observed following trigeminal ganglion stimulation are the vasodilator prostaglandins (PGE_2, PGI_2). The cyclo-oxygenase inhibitor, indomethacin did not alter neurogenic vasodilatation excluding a role for prostaglandins. This finding is in line with the results of Lembeck and Holzer (1979) who showed that prostaglandins are not involved in vasodilatation or plasma extravasation in rat hind paw skin following saphenous nerve stimulation. However, the local anaesthetic lignocaine, which blocks nerve conduction

in sensory nerve fibres, significantly reduced the response to electrical stimulation of the trigeminal ganglion providing more evidence that the vasodilator agent responsible is neuronal in origin.

In conclusion, the results of this study show that activation of μ-opioid receptors inhibits neurogenic vasodilatation in rat facial skin evoked by trigeminal ganglion stimulation. This inhibitory action is probably mediated by a reduction in neuropeptide release (CGRP) from sensory nerves via the activation of prejunctional μ-opioid receptors. Moreover, activation of histamine H_3 or 5-HT_{1D} receptors with selective receptor agonists had no effect on vasodilator responses observed in this model. These findings are in contrast to studies which have shown that H_3 or 5-HT_{1D} receptor agonists inhibit neurogenic plasma extravasation evoked in the dura mater by trigeminal ganglion stimulation. In addition, it is also suggested that parasympathetic nerves and prostaglandins are not involved in the increase in facial skin blood flow observed after trigeminal ganglion stimulation in the rat.

Acknowledgements

K. Jane Escott is the recipient of a Medical Research Council/Glaxo (UK) collaborative PhD studentship.

Note added in proof

It is now published that CP 122,288 inhibits neurogenic oedema formation and blood flow in rat paw skin (Kajekar et al., 1995, Br. J. Pharmacol. 115, 1–2; Br. J. Pharmacol. 115, 8P).

References

Barber, A., 1993, μ- and κ-opioid receptor agonists produce peripheral inhibition of neurogenic plasma extravasation in rat skin, Eur. J. Pharmacol. 236, 113.

Beattie, D.T. and H.E. Connor, 1994, The influence of the trigeminal ganglion on cranial blood flow in anaesthetised guinea-pigs, Br. J. Pharmacol. 112, 262.

Butler, A., S.P. Worton, C.T. O'Shaughnessy and H.E. Connor, 1993, Sensory nerve-mediated relaxation of guinea-pig isolated pulmonary artery: prejunctional modulation by α_2-adrenoceptor agonists but not sumatriptan, Br. J. Pharmacol. 109, 126.

Buzzi, M.G. and M.A. Moskowitz, 1990, The antimigraine drug, sumatriptan, GR43175, selectively blocks neurogenic plasma extravasation from blood vessels in dura mater, Br. J. Pharmacol. 99, 202.

Escott, K.J. and S.D. Brain, 1993, Effect of a calcitonin gene-related peptide antagonist ($CGRP_{8-37}$) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve, Br. J. Pharmacol. 110, 772.

Escott, K.J., D.T. Beattie, H.E. Connor and S.D. Brain, 1995, Trigeminal ganglion stimulation increases facial skin blood flow in the rat: a major role for calcitonin gene-related peptide, Brain Res. 669, 93.

Gamse, R. and A. Saria, 1987, Antidromic vasodilatation in the rat hindpaw measured by laser Doppler flowmetry: pharmacological modulation, J. Auton. Nerv. Syst. 19, 105.

Garcia Leme, J., L. Hamamura, M.P. Leite and M. Rocha e Silva, 1973, Pharmacological analysis of the acute inflammatory process induced in the rat's paw by local injection of carrageenin and by heating, Br. J. Pharmacol. 48, 88.

Goadsby, P.J. and L. Edvinsson, 1993, The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats, Ann. Neurol. 33, 48.

Holzer, P., 1992, Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds, Rev. Physiol. Biochem. Pharmacol., 121, 70.

Ichinose, M. and P.J. Barnes, 1989, Histamine H_3-receptors modulate nonadrenergic noncholinergic neural bronchoconstriction in guinea-pig in vivo, Eur. J. Pharmacol. 174, 49.

Ichinose, M., M.G. Belvisi and P.J. Barnes, 1990, Histamine H_3-receptors inhibit neurogenic microvascular leakage in airways, J. Appl. Physiol. 68 (1), 21.

Lee, W.S. and M.A. Moskowitz, 1993, Conformationally restricted sumatriptan analogues, CP-122,288 and CP-122,638 exhibit enhanced potency against neurogenic inflammation in dura mater, Brain Res. 626, 303.

Lembeck, F. and J. Donnerer, 1985, Opioid control of the function of primary afferent substance P fibres, Eur. J. Pharmacol. 114, 241.

Lembeck, F. and P. Holzer, 1979, Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation, Naunyn-Schmied. Arch. Pharmacol. 310, 175.

Lembeck, F., J. Donnerer and L. Bartho, 1982, Inhibition of neurogenic vasodilatation and plasma extravasation by substance P antagonists, somatostatin and [D-Met^2,Pro^5]enkephalinamide, Eur. J. Pharmacol. 85, 171.

Maggi, C.A., 1991, The pharmacology of the efferent function of the sensory nerves, J. Auton. Pharmacol. 11, 173.

Markowitz, S., K. Saito and M.A. Moskowitz, 1987, Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain, J. Neurosci. 7, 4129.

Matsubara, T., M.A. Moskowitz and Z. Huang, 1992, UK-14,304, $R(-)$-α-methyl-histamine and SMS 201-995 block plasma protein leakage within dura mater by prejunctional mechanisms, Eur. J. Pharmacol. 224, 145.

Moskowitz, M.A., 1993, Neurogenic inflammation in the pathophysiology and treatment of migraine, Neurology 43, S16.

O'Shaughnessy, C.T., G.J. Waldron. and H.E. Connor, 1993, Lack of effect of sumatriptan and UK-14, 304 on capsaicin-induced relaxation of guinea-pig isolated basilar artery, Br. J. Pharmacol. 108, 191.

Vongsavan, N. and B. Matthews, 1993, Some aspects of the use of laser Doppler flow meters for recording tissue blood flow, Exp. Physiol. 78, 1.