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Capsaicin-insensitive sensory-efferent meningeal vasodilatation evoked by electrical stimulation of trigeminal nerve fibres in the rat

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1 Antidromic vasodilatation and plasma extravasation to stimulation of the trigeminal ganglion or its perivascular meningeal fibres was investigated by laser-Doppler flowmetry and ^{125}I-labelled bovin serum albumin in the dura mater and in exteroceptive areas (nasal mucosa, upper eyelid) of anaesthetized rats pretreated with guanethidine and pipecuronium.

2 Trigeminal stimulation at 5 Hz for 20 s elicited unilateral phasic vasodilatation in the dura and lasting response in the nasal mucosa. Resiniferatoxin (1–3 μg kg^{-1} i.v.), topical (1%) or systemic capsaicin pretreatment (300 mg kg^{-1} s.c. plus 1 mg kg^{-1} i.v.) did not inhibit the meningeal responses but abolished or strongly inhibited the nasal responses. Administration of vinpocetine (3 mg kg^{-1} i.v.) increased both basal blood flow and the dural vasodilatation to perivascular nerve stimulation.

3 Dural vasodilatation to trigeminal stimulation was not inhibited by the calcitonin gene-related peptide-1 receptor (CGRP-1) antagonist hCGRP$_{8-37}$ (15 or 50 μg kg^{-1} i.v). or the neurokinin-1 receptor antagonist RP 67580 (0.1 mg kg^{-1} i.v.) although both antagonists inhibited the nasal response. Neither mucosal nor meningeal responses were inhibited by atropine (5 mg kg^{-1} i.v.), hexamethonium (10 mg kg^{-1} i.v.) or the vasoactive intestinal polypeptide (VIP) antagonist (p-chloro-D-Phe^6-Leu1^7)VIP (20 μg kg^{-1} i.v.).

4 Plasma extravasation in the dura and upper eyelid elicited by electrical stimulation of the trigeminal ganglion was almost completely abolished in rats pretreated with resiniferatoxin (3 μg kg^{-1} i.v.).

5 It is concluded that in the rat meningeal vasodilatation evoked by stimulation of trigeminal fibres is mediated by capsaicin-insensitive primary afferents, while plasma extravasation in the dura and upper eyelid and the vasodilatation in the nasal mucosa are mediated by capsaicin-sensitive trigeminal fibres.

Keywords: Capsaicin; resiniferatoxin; microcirculation; vasodilatation; trigeminal ganglion; dura mater; nasal mucosa; hCGRP$_{8-37}$; vinpocetine

Abbreviations: Arrow, injection of drug; arrowheads, electrical stimulations; AU, arbitrary unit; AUC, area under the curve; BP, blood pressure; CGRP, calcitonin gene-related peptide; c.p.m., count per minute; ER, extravasation ratio; hCGRP$_{8-37}$, 8–37 fragment of human calcitonin gene-related peptide; MBF, meningeal blood flow; NBF, nasal blood flow; NK, neurokinin; N.S., non-significant; RTX, resiniferatoxin; s.e.m., standard error of mean; SP, substance P; VIP, vasoactive intestinal polypeptide

Introduction

In the skin and internal organs neurogenic inflammatory plasma extravasation and vasodilatation can be evoked by antidromic stimulation of afferent nerve fibres (Jancsó *et al.*, 1967; Maggi, 1995; Pintér & Szolcsányi, 1995; Szolcsányi, 1996; Lundberg, 1996). These responses are abolished by capsaicin pretreatment and therefore they are manifestations of the sensory-efferent function of a capsaicin-sensitive subpopulation of primary afferent neurones. Neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) are released from their peripheral nerve terminals and are the principle mediators of the inflammatory and vasodilator responses, respectively (Holzer, 1988; Maggi, 1995). Electrical or chemical stimulation of trigeminal afferent fibres elicits plasma leakage from blood vessels in the dura mater and in extracranial tissues such as facial skin, nasal mucosa and conjunctiva supplied by the trigeminal nerve (Markowitz *et al.*, 1988; Jancsó *et al.*, 1967; Jancsó-Gábor & Szolcsányi, 1972).

Neurogenic inflammation of the dura mater has been implicated in the pathogenesis of migraine and other headaches (Goadsby & Edvinsson, 1993). The dura mater encephali of the rat is densely innervated by SP and CGRP immunoreactive trigeminal fibres (Keller & Marfurt, 1991; Messlinger *et al.*, 1993) and dural plasma leakage at the postcapillary venules is due to activation of NK$_1$ tachykinin receptors (Shepheard *et al.*, 1993). Plasma extravasation in the rat dura mater induced by electrical or chemical trigeminal stimulation is assumed to be mediated by capsaicin-sensitive fibres because neonatal capsaicin pretreatment has been shown to abolish the response. After neonatal capsaicin pretreatment, however, profound secondary changes during maturation occurs. Therefore it is not a specific tool to decide which primary afferent neurones are sensitive to capsaicin in adult animals (Szolcsányi *et al.*, 1994; Szolcsányi, 1993), consequently a firm evidence for exclusive mediation of neurogenic inflammation by capsaicin-sensitive fibres in the dura is still lacking.

Stimulation of trigeminal nerve fibres increases cerebral (Goadsby, 1993; Goadsby & Edvinsson, 1993) and dural microcirculation (Kurosawa *et al.*, 1995). Vasodilator re-

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sponses were described also in extracranial areas including facial skin (Couture & Cuello, 1984, Escott *et al*., 1995a) and nasal mucosa (Lundblad *et al*., 1983a; Stjärne *et al*., 1991). Evidence for a mediator role for CGRP was obtained in the rat facial skin (Escott *et al*., 1995a), dura mater (Kurosawa *et al*., 1995) and also in the cat brain (Escott *et al*., 1995b; Goadsby, 1993). To our knowledge, no study has addressed the question whether afferent fibres mediating trigeminal vasodilatation in the dura mater are capsaicin-sensitive or not.

The aim of the present study was to investigate with laser Doppler technique the mechanism of dural vasodilatation evoked by electrical trigeminal nerve stimulation with special emphasis on the capsaicin sensitivity of the afferent fibres involved. In addition, we wished to examine the pharmacological modulation of dural vasodilatator responses by using an example drug known to enhance cerebral blood flow. For this purpose we first utilized the method of Kurosawa *et al*. (1995), but we developed also a new model which allows for simultaneous comparison of microcirculatory blood flow changes in the meninges and the nasal mucosa, i.e. in intracranial *vs* extracranial tissues, in response to stimulation of the trigeminal ganglion.

Methods

General procedures

The experiments were carried out on male Wistar rats weighing 250–400 g. The animals were housed at the Laboratory Animal Centre of the University Medical School of Pécs under pathogen free conditions at 24–25°C and provided with standard rat chow and water *ad libitum*. All procedures used in this study are in agreement with the rules of the Ethics Committee on Animal Research of the University Medical School of Pécs. The animals were anaesthetized with thiopentone sodium (Trapanal, 100 mg kg^{-1} i.p.), additional doses being given every 30 min (5 mg kg^{-1} i.v.). The right femoral artery was cannulated for measuring arterial blood pressure with a Statham pressure transducer. The responses were recorded on a polygraph (Type RM, Beckman). A fine polyethylene cannula introduced into the right femoral vein served for drug administration. The cannula inserted into the trachea was connected to a small animal respirator (SAR-830/P, IITC Inc./Life Science Instruments) by which the animals were artificially ventilated after skeletal muscle paralysis with pipecuronium bromide (Arduan, 0.3 mg kg^{-1} i.v., additional doses being given when necessary). In the first experiments using non-paralyzed animals the level of anaesthesia evoked by 100 mg kg^{-1} thiopentone sodium was checked ($n=5$) for up to 6 h by painful pinchings of the extremities. No signs of movements or rise in blood pressure indicative of inadequate anaesthesia were observed in these animals. In experiments employing muscle paralysis the muscle relaxant was administered only if the animal was unresponsive to such test stimuli. These experiments lasted for no more than 4 h. Rectal temperature was kept at 37 ± 0.5°C by a controlled infrared lamp (Experimetria Ltd). At the end of the experiments, the animals were killed by an overdose of thiopentone sodium (100 mg kg^{-1} i.v.).

Electrical stimulation of the dura mater and measuring meningeal blood flow

The method developed by Kurosawa *et al*. (1995) was used. The animal's head was fixed in a stereotaxic frame. The skull was exposed by a midline incision. The skin, periosteum and the dorsal part of the masseter were removed. On the left side of the top of the skull a parasagittal burr hole of about 2×6 mm was drilled for electrical stimulation of the dura mater encephali. The parasagittal opening was filled with liquid paraffin after a pair of stimulating platinum electrodes had been lowered onto the dural surface. The dura was electrically stimulated with rectangular pulses of a constant length of 0.5 ms. Stimulus strength was 15 V, the frequency was 5 Hz and the duration was 20 s. For measuring blood flow a round hole with a diameter of 3 mm was drilled on the left, parietal side-wall of the skull. A rectangular needle type probe of a dual laser-Doppler flowmeter (MBF3D, Moor Instruments) was placed over the area supplied by middle meningeal artery without touching it, and the hole was covered with pieces of gauze soaked with 0.9% NaCl solution. In some cases blood flow was measured in a similar way contralateral to the side of stimulation, i.e. on the right side. In all experiments blood flow changes were recorded on the polygraph, expressed in arbitrary units and percentage changes of the responses were determined by the area under the curve method (Messlinger *et al*., 1997).

Electrical stimulation of the trigeminal ganglion and measuring meningeal and nasal mucosal blood flow

The animal's head was fixed in a stereotaxic frame and the skull was exposed by a midline incision. The skin, periosteum and the dorsal part of the masseter were removed. A burr hole (2 mm diameter) was drilled on the left side of the top of the skull at 3.2 mm lateral to the sagittal suture and 3.7 mm posterior to the bregma for the placement of bipolar stimulating electrodes. The electrodes were lowered into the left trigeminal ganglion to a depth of 9.5 mm from the dura mater overlying the dorsal surface of the brain. The left trigeminal ganglion was electrically stimulated (15 V, 0.5 ms, 0.5–10 Hz, 100 impulses). In order to diminish sympathetic pressor reflexes evoked by trigeminal ganglion stimulation animals were given the adrenergic neurone blocking agent guanethidine (8 mg kg^{-1} i.v.) in the beginning of each experiment. Blood flow was monitored in two areas innervated by the trigeminal nerve: in the meninges as described above and in the nasal mucosa. In the latter case another rectangular needle type probe of the flowmeter was gently placed on the mucosa of the nasal septum close to the aperture in the left nasal cavity. In some cases meningeal and mucosal blood flow was measured contralateral to the side of stimulation, i.e. on the right side. After surgical preparation the animals were allowed to rest until all physiological parameters were stable, at which time the measurement was started. Meningeal and mucosal blood flow values expressed in arbitrary units were recorded on the polygraph.

Capsaicin/resiniferatoxin (RTX) pretreatments

The first group of rats was pretreated systemically with a total dose of 300 mg kg^{-1} of capsaicin in order to impair the function of capsaicin-sensitive primary afferent neurones (Szolcsányi, 1993). Capsaicin was administered in daily s.c. injections given over 4 consecutive days under sodium pentobarbitone (Nembutal) anaesthezia (40 mg kg^{-1} i.p.) at doses of 30, 50, 100 and 120 mg kg^{-1}, respectively, the last dose being given 3 days before the experiments. In addition, for achieving a more complete blockade of capsaicin-sensitive primary afferents a further 1 mg kg^{-1} dose of capsaicin (twice increasing doses from 5 μg kg^{-1} to 20 μg kg^{-1} then

50 μg kg^{-1} + 3 × 100 μg kg^{-1} + 3 × 200 μg kg^{-1} to reach a total dose of 1 mg kg^{-1}) was given i.v. during the experiments. In another group of animals local acute capsaicin pretreatment was induced by application of a 1% solution for 30 min onto the surface of the meninges or nasal mucosa in the close vicinity of the laser Doppler probe. In the third group of animals acute administration of RTX (1 – 3 μg kg^{-1} i.v.) was utilized for blocking the vascular responses evoked by capsaicin-sensitive primary afferents (Pórszász & Szolcsányi 1994).

Plasma extravasation evoked by electrical stimulation of the trigeminal ganglion

After intravenous administration of ^{125}I-labelled bovine serum albumin (70 μCi kg^{-1}) and RTX (3 μg kg^{-1}) or vehicle (0.9% NaCl, 1.0 ml) the electrodes were lowered into the left trigeminal ganglion as described earlier. Ten minutes later the trigeminal ganglion was electrically stimulated (25 V, 0.5 ms, 5 Hz for 5 min) and after a further 10 min period the animals were exsanguinated. The dura was dissected free on both sides of the skull and the surrounding area of the electrode penetration was discarded. Correct electrode placement was confirmed by the presence of electrode pin prick marks in the trigeminal ganglion. The upper eyelids from both sides were taken as an extracranial trigeminal innervated area. Samples were rinsed, dried overnight, weighed and radioactivity counted to quantify extravasation. The amount of extravasation in the stimulated and nonstimulated sides was calculated and expressed as an extravasation ratio (ER) of stimulated *vs* nonstimulated sides. The percentage of inhibition of extravasation in drug treated animals was calculated with respect to a vehicle treated group.

Drugs

Guanethidine, atropine, hexamethonium, capsaicin, hCGRP$_{8-37}$, VIP, (p-chloro-D-Phe^6-Leu1^7)VIP and [^{125}I]-BSA were purchased from Sigma, RP 67580 from RBI, vinpocetine and pipecuronium from Richter Gedeon, RTX from LC Laboratories and thiopentone sodium from BYK. All drugs were dissolved and diluted in saline except capsaicin [saline: ethanol: Tween 80 (8 : 1 : 1)].

Data analysis

In the text and figure legends *n* means the number of the animals used in each group. In every experiment the average of

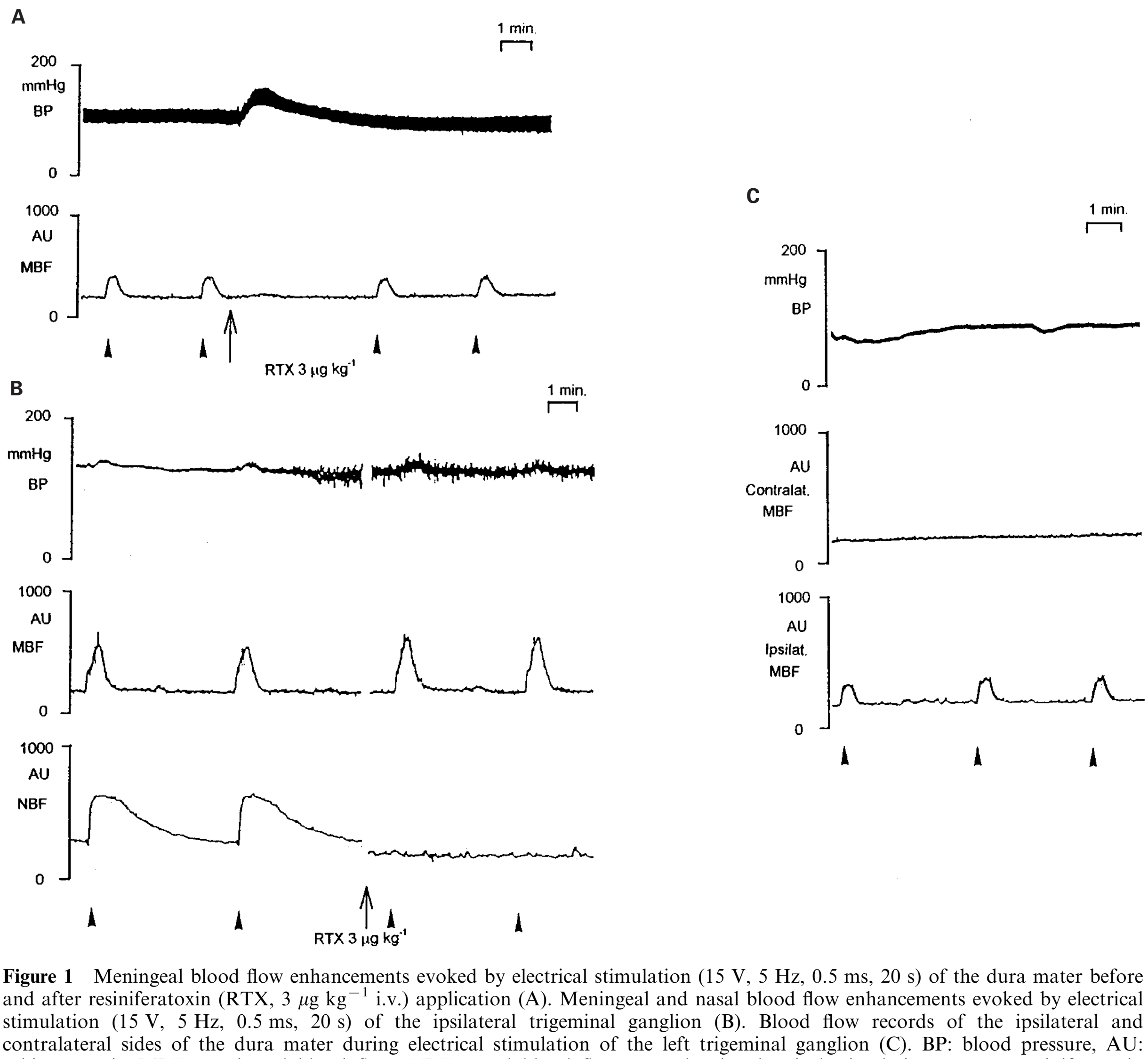

Figure 1 Meningeal blood flow enhancements evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the dura mater before and after resiniferatoxin (RTX, 3 μg kg^{-1} i.v.) application (A). Meningeal and nasal blood flow enhancements evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion (B). Blood flow records of the ipsilateral and contralateral sides of the dura mater during electrical stimulation of the left trigeminal ganglion (C). BP: blood pressure, AU: arbitrary unit, MBF: meningeal blood flow, NBF: nasal blood flow, arrowheads: electrical stimulations, arrow: resiniferatoxin injection.

the three control responses was taken as the control value (100%). All values presented in the text give the mean and its standard error (s.e.m.mean). One-way analysis of variance and a two-tailed *t*-test were used for statistical analysis with a significance limit of $P<0.05$.

Results

Electrical stimulation of the dura mater

Surface electrical stimulation of the dura mater (15 V, 0.5 ms, 5 Hz, 20 s) evoked an ipsilateral increase in meningeal blood flow without a change in arterial blood pressure (Figure 1A). This blood flow enhancement was not inhibited by the sensory neurone blocking agent RTX (1–3 μg kg^{-1} i.v.; Szolcsányi *et al.*, 1990) and its value was 105.4% $\pm$ 12.3 ($n=10$) of the control response. Nevertheless, our method seems to be suitable to detect drug-induced microcirculatory changes since infusion of vinpocetine (3 mg kg^{-1} i.v. at a rate of 0.1 mg kg^{-1} min^{-1} for 30 min), a drug which has been described to enhance cerebral microcirculation (Imamoto *et al.*, 1984; Kiss & Kárpáti 1996), increased basal blood flow by 72.6% $\pm$ 17.8 as well as the hyperaemic response to electrical stimulation of the dura by 141.5% $\pm$ 23.2 (Figure 2.; $n=6$). Similar infusion of the solvent did not alter these parameters (Figure 2; $n=4$). The mean blood pressure before and after vinpocetine infusion was 105.2 $\pm$ 12.4 and 97.7 $\pm$ 8.9 mmHg, respectively, the difference being non-significant ($P>0.05$).

Electrical stimulation of the trigeminal ganglion

Electrical stimulation of the trigeminal ganglion evoked an ipsilateral enhancement of both meningeal and nasal mucosal blood flow accompanied by a transient (in most cases lasting for 20–25 s) increase in arterial blood pressure, which was absent or strongly inhibited after guanethidine treatment (Figure 1B). In the contralateral side of stimulation blood flow both in the meninges (Figure 1C) and nasal mucosa (data not shown) remained unaltered ($n=5$). For sham trigeminal stimulation the stimulating electrode was lowered 3–4 mm above the trigeminal ganglion. Stimulation at this electrode position failed to evoke blood flow changes either in the meninges or nasal mucosa ($n=5$). Trigeminal stimulation with 100 pulses at 0.5 or 1 Hz induced blood flow enhancement in the nasal mucosa but not in the meninges. The frequency optimum of the meningeal response was 5–10 Hz whereas it was only 2 Hz in the nasal mucosa (Figure 3). The latency and recovery of the enhancement in blood flow was shorter in the meninges than in the nasal mucosa (Table 1). Furthermore, when basal blood flow in the nasal mucosa was relatively high, a transient decrease of blood flux lasting

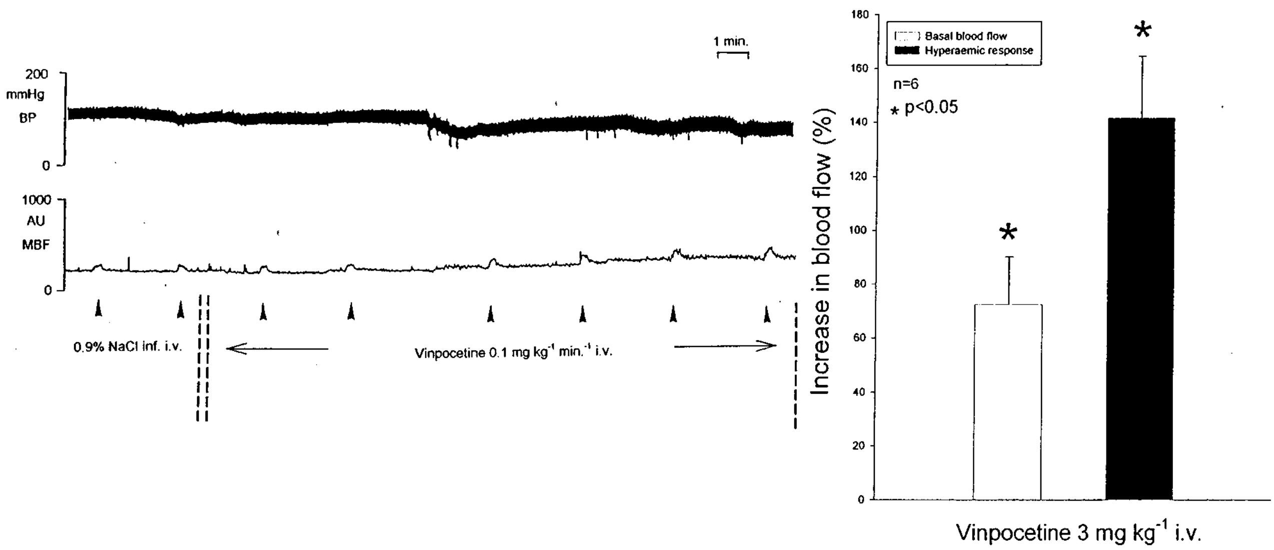

Figure 2 Left: Effect of vinpocetine infusion (3 mg kg^{-1} i.v. at a rate of 0.1 mg kg^{-1} min^{-1} for 30 min) on meningeal blood flow responses evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the dura mater. The terminal part of a similar infusion of the solvent is also shown. Right: Per cent increase in blood flow values at the end of vinpocetine infusion [area under the curve (AUC); $n=6$; mean $\pm$ s.e.mean $*P<0.05$]. BP: blood pressure, AU: arbitrary unit, MBF: meningeal blood flow, arrowheads: electrical stimulations.

Table 1 Comparison of the characteristics of blood flow enhancements in the meninges and nasal mucosa evoked by electrical stimulation of the trigeminal ganglion

	Latency (s)	*$T_{1/2}$ of recovery* (s)	*Inhibition by 3 μg kg^{-1} RTX*	*Inhibition by capsaicin pretreatment*	*Inhibition by 50 μg kg^{-1} $hCGRP_{8-37}$*
Meninges	2.1 $\pm$ 0.06	40.2 $\pm$ 1.86	No	No	No
Nasal mucosa	7.2 $\pm$ 0.30**	73.8 $\pm$ 7.20*	Yes	Yes	Yes

Stimulation parameters were 15 V, 0.5 ms, 5 Hz and 20 s. Values represent means $\pm$ s.e.mean of six experiments. Significance levels refer to comparisons between corresponding meningeal and mucosal values. $**P<0.001$; $*P<0.005$.

20–30 s appeared before the enhancement in six animals out of 18 guanethidine-pretreated rats.

Effect of capsaicin or resiniferatoxin treatment

The effect of three types of capsaicin treatments and that of i.v. injection of RTX was investigated on the blood flow changes in the nasal mucosa and dura mater in response to unilateral trigeminal nerve stimulation (15 V, 0.5 ms, 5 Hz, 20 s).

After systemic capsaicin treatment (total s.c. dose 300 mg kg^{-1}, last dose being given 3 days before the experiment; $n=6$) the nasal response was reduced to 32.5% $\pm$ 6.4 of the control responses obtained in untreated animals. After subsequent cumulative i.v. injections of a total dose of 1 mg kg^{-1} capsaicin ($n=6$) the response was completely abolished (Figures 4 and 5). In the second group of rats 10–30 min after topical application of 1% capsaicin solution to the recording site for 30 min the

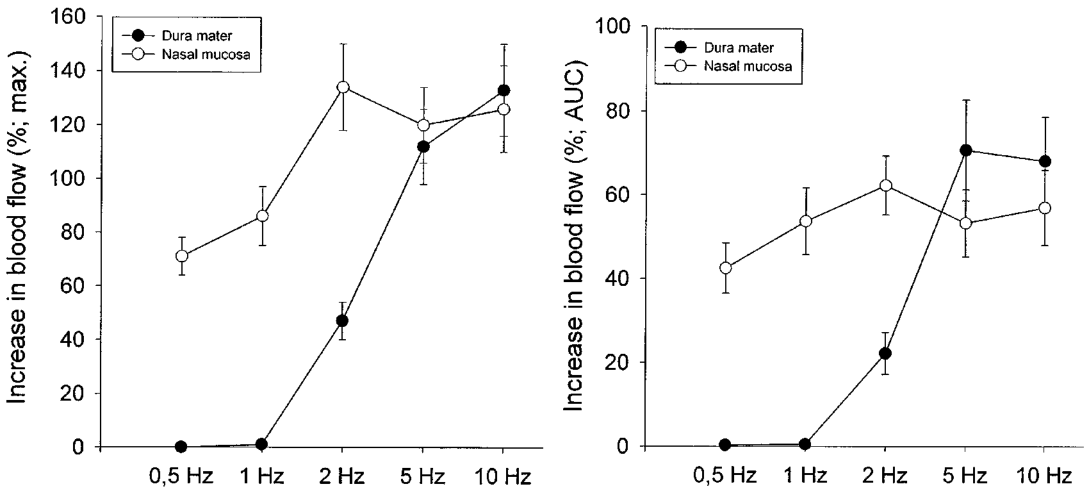

Figure 3 Frequency-response curves of meningeal and nasal blood flow enhancements (means $\pm$ s.e.mean) evoked by electrical stimulation (15 V, 0.5 ms, 100 impulses) of the ipsilateral trigeminal ganglion ($n=6$; max: response maximum; AUC: area under the curve).

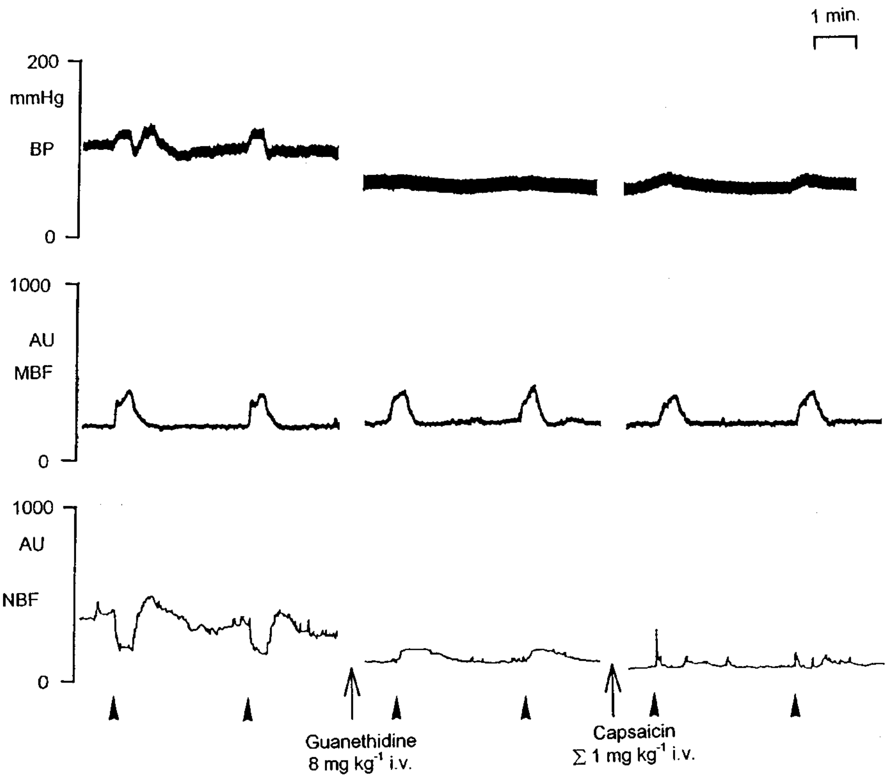

Figure 4 Effect of guanethidine (8 mg kg^{-1} i.v.) and capsaicin (cumulative dose of 1 mg kg^{-1} i.v.) on blood pressure and on meningeal and nasal blood flow enhancements evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion in a capsaicin pretreated (total dose: 300 mg kg^{-1}) rat. BP: blood pressure, AU: arbitrary unit, MBF: meningeal blood flow, NBF: nasal blood flow, arrowheads: electrical stimulations, arrows: injection of drugs.

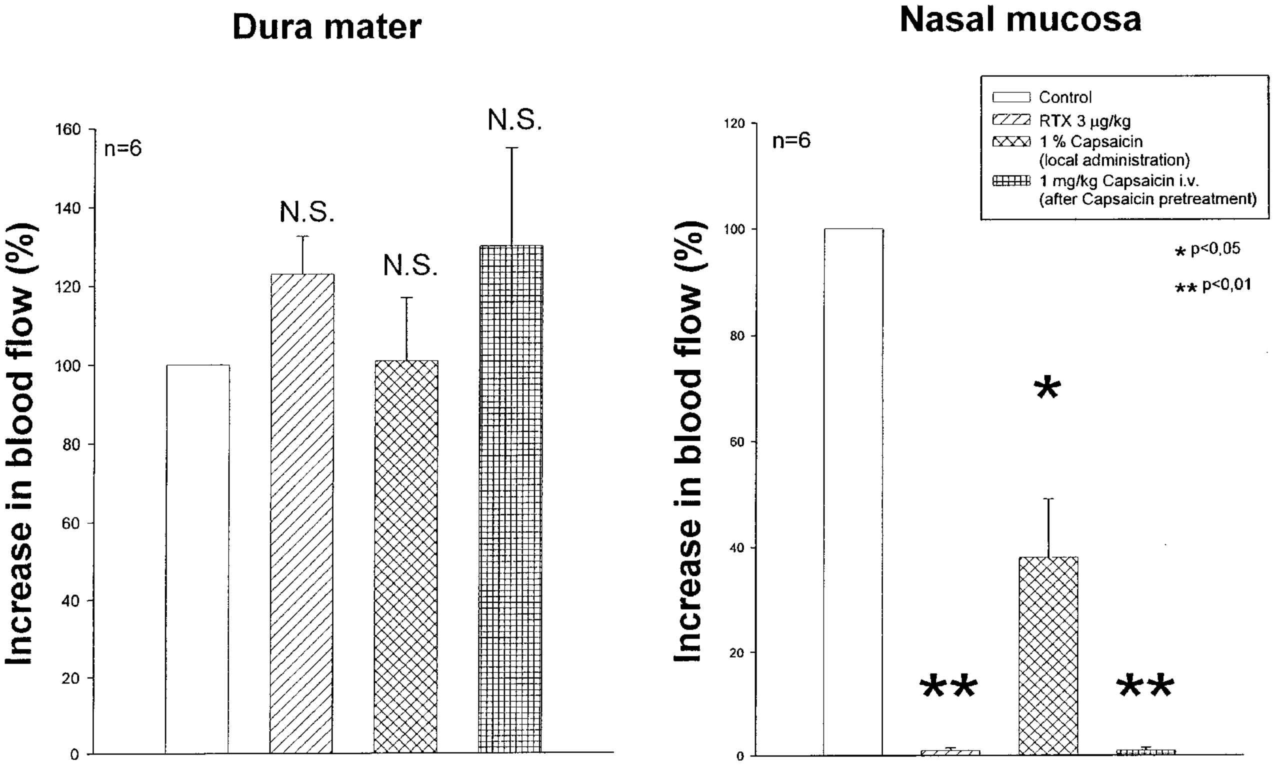

Figure 5 Quantitative evaluation of the effect of resiniferatoxin (RTX, 3 μg kg^{-1} i.v.; $n=15$), local (1%; $n=6$) or systemic application of capsaicin (cumulative dose of 1 mg kg^{-1} i.v. in capsaicin pretreated rats with a total dose of 300 mg kg^{-1}; $n=6$) on dural and nasal blood flow enhancement evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion. Responses are expressed as percentage deviation from control values (means±s.e.m). $*P<0.05$, $**P<0.01$. N.S., non significant.

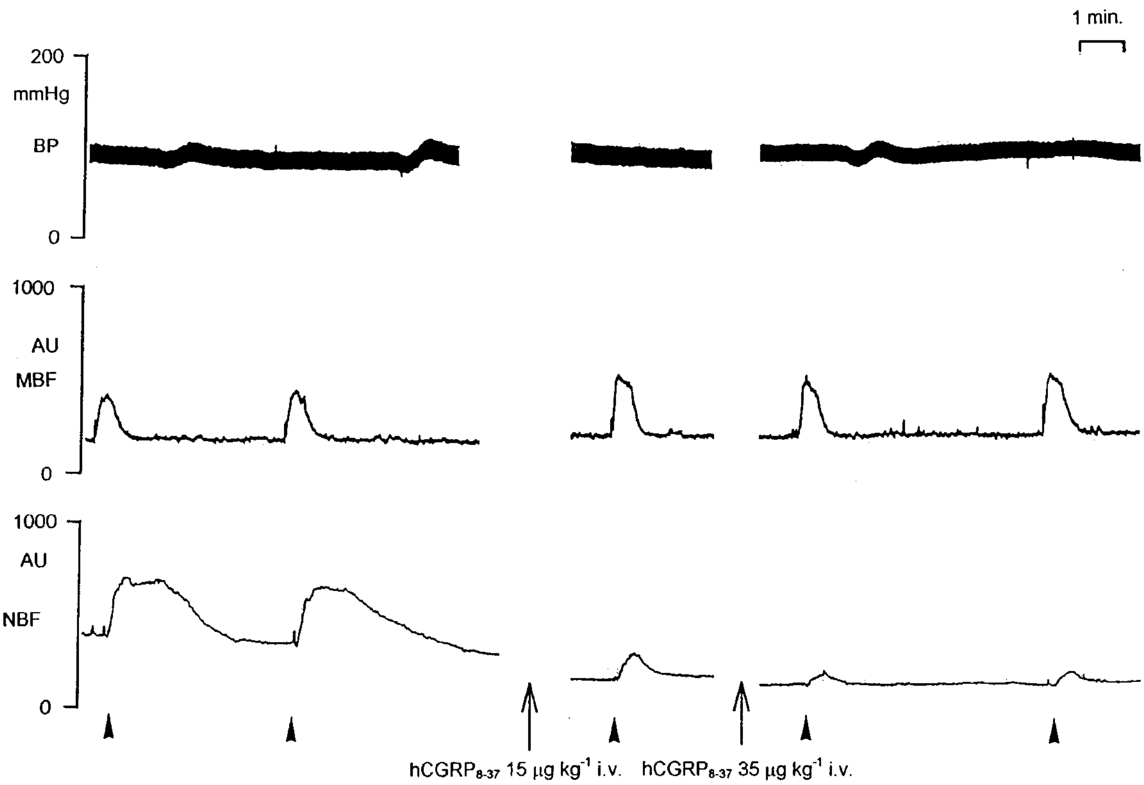

Figure 6 Effect of hCGRP$_{8-37}$ (15+35 μg kg^{-1} i.v.) on blood flow enhancement in the dura and nasal mucosa evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion. BP: blood pressure, AU: arbitrary unit, MBF: meningeal blood flow, NBF: nasal blood flow, arrowheads: electrical stimulations, arrows: injection of drug.

increase in nasal blood flow to trigeminal nerve stimulation was reduced to 38.4% ± 11.7 of the control values (n = 6, Figure 5). None of these treatments inhibited the increase in blood flow in the dura mater evoked by trigeminal nerve stimulation and the respective values after i.v. and topical application of capsaicin were 102.9% ± 9.8 and

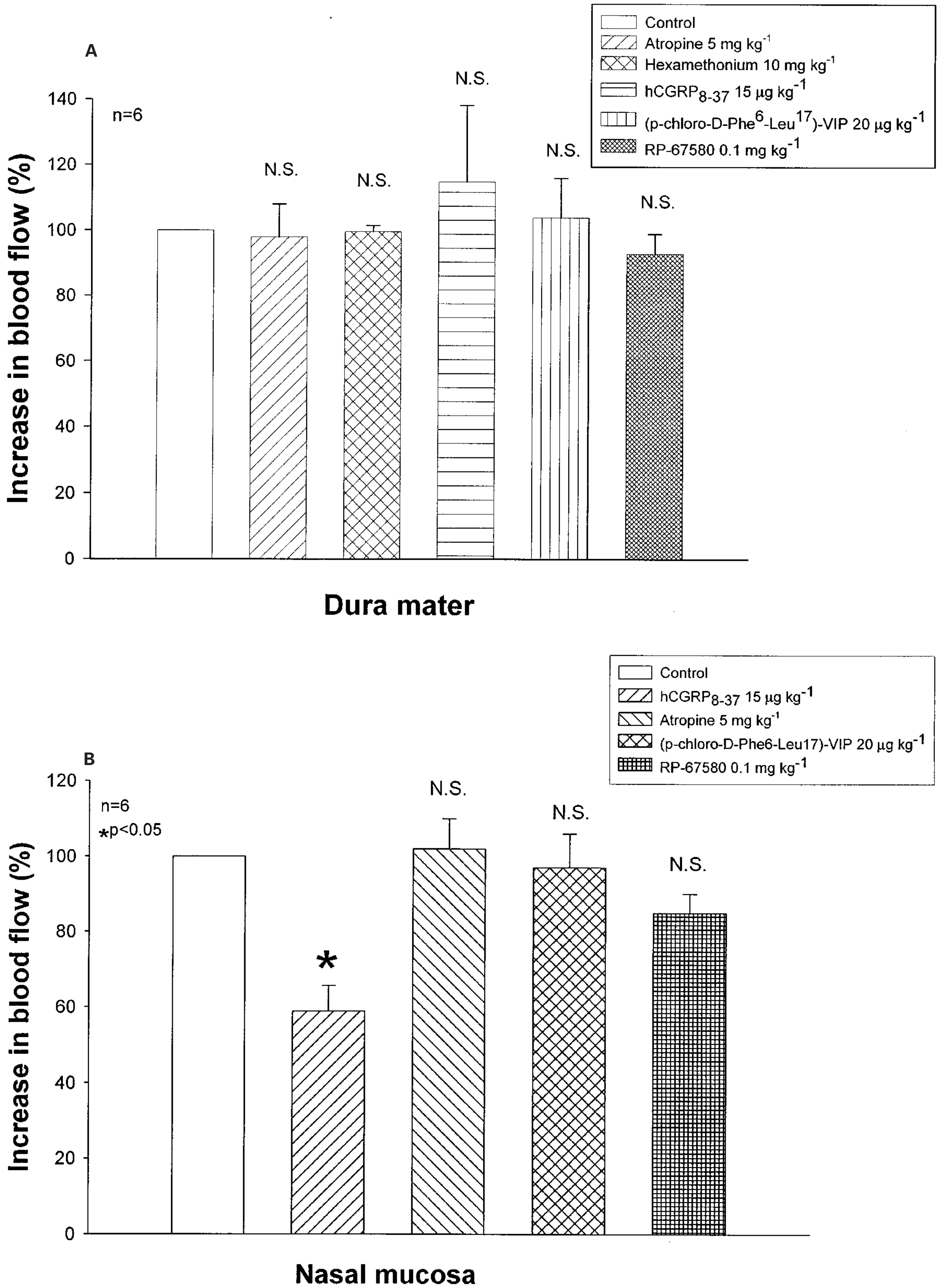

Figure 7 (A) Quantitative evaluation of the effect of hCGRP$_{8-37}$ (15 μg kg^{-1} i.v.; n = 6), atropine (5 mg kg^{-1} i.v.; n = 6), hexamethonium (10 mg kg^{-1} i.v.; n = 6), p-chloro-D-Phe^6-Leu1^7-VIP (20 μg kg^{-1} i.v.; n = 6) and RP 67580 (0.1 mg kg^{-1} i.v.; n = 18) on the blood flow increase in the dura mater evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion. (B) Quantitative evaluation of the effect of hCGRP$_{8-37}$ (15 μg kg^{-1} i.v.; n = 6), atropine (5 mg kg^{-1} i.v.; n = 6), p-chloro-D-Phe^6-Leu1^7-VIP (20 μg kg^{-1} i.v.; n = 6) and RP 67580 (0.1 mg kg^{-1} i.v. n = 12) on the blood flow increase in the nasal mucosa evoked by electrical stimulation (15 V, 5 Hz, 0.5 ms, 20 s) of the ipsilateral trigeminal ganglion. Responses are expressed as percentage deviation from control values (means ± s.e.mean). *P < 0.05, N.S., non significant.

109.5% ± 13.3 ($n = 6$) as compared to their control values. (Figures 4 and 5).

Administration of RTX (1 – 3 μg kg^{-1} i.v.; $n = 15$) completely abolished the nasal blood flow enhancement but even the dose of 3 μg kg^{-1} RTX did not induce a significant change (110.6% ± 10.6 of the controls) in the dural blood flow response (Figures 1B and 5). The effect of RTX on the response of the nasal mucosa was long lasting without any sign of recovery during a 60 – 90 min period of observation. RTX (10 or 100 μg kg^{-1}, $n = 4$) administered i.v. also failed to inhibit the meningeal response which was 121.2% ± 17.5 of its control value.

Effect of neuropeptide antagonists, atropine and hexamethonium

Systemically applied hCGRP$_{8-37}$ (15 + 35 μg kg^{-1} i.v.), a CGRP receptor antagonist, failed to reduce the blood flow enhancement in the meningeal tissue which was 114.8% ± 23.8 of the control value after the total dose of 50 μg kg^{-1} ($n = 6$, Figure 6). The control response in the nasal mucosa was reduced after these two doses to 58.8% ± 6.8 and 38.4 ± 7.3, respectively ($P > 0.05$, $n = 6$; Figure 6). The difference between the degree of inhibition of the nasal response produced by these doses of hCGRP$_{8-37}$ was non-significant ($P > 0.05$) and increasing the dose of hCGRP$_{8-37}$ to 200 μg kg^{-1} i.v. ($n = 2$) produced no further inhibition (42.1%). The effect of hCGRP$_{8-37}$ was relatively short lasting, with signs of partial recovery seen within 15 min after the 50 μg kg^{-1} total dose. After administration of the non-peptide NK$_1$-receptor antagonist RP 67580 (Shepheard *et al.*, 1993) at a dose of 0.1 mg kg^{-1} i.v. the meningeal blood flow response remained unaltered (93.1% ± 6.3, $n = 18$; Figure 7). The enhancement of nasal blood flow in response to trigeminal nerve stimulation was slightly inhibited by RP 67580 (0.1 mg kg^{-1} i.v.) in rats where – owing to a lower basal blood flow level – no vasoconstrictor phase preceeded the increase in blood flow response. After the treatment the responses were 85.7% ± 5.3 of their control values ($n = 12$; $P > 0.05$). In six animals in which mucosal basal blood flow was higher and a transient vasoconstrictor phase preceeded the vasodilator response, RP 67580 (0.1 mg kg^{-1} i.v.) enhanced this initial vasoconstrictor phase and reduced the vasodilator response to 31.9% ± 6.2 s.e.mean ($P < 0.05$) of the control value during a 60 s period.

Atropine (5 mg kg^{-1} i.v.; $n = 6$) or hexamethonium (10 mg kg^{-1} i.v.; $n = 6$) administration did not alter the hyperaemic response in the dura mater (97.8% ± 10.4 and 99.7 ± 1.8, respectively) and nasal mucosa (104.1% ± 12.4 and 97.8% ± 8.7; ($n = 3$, data not shown), respectively) to electrical stimulation of the trigeminal ganglion, while hexamethonium decreased the systemic blood pressure by 18.2 ± 3.4 mmHg. The vasoactive intestinal polypeptide (VIP) antagonist (p-chloro-D-Phe^6-Leu1^7)-VIP (20 μg kg^{-1} i.v.) was administered for revealing the possible contribution of VIP-ergic efferent fibres to the responses under study. The antagonist did not cause inhibition, and the magnitude of the responses in the dura mater remained 102.7% ± 8.3 and in the nasal mucosa 96.8% ± 9.7 of the control values (Figure 7). Intravenous infusion of VIP (0.1 μg kg^{-1} min^{-1} for 1 min; $n = 4$) evoked a decrease in blood pressure (21.5 ± 3.2 mmHg). In the presence of the VIP antagonist (20 μg kg^{-1} i.v.) this response of VIP infusion was inhibited by 53% (10.1 ± 1.9 mmHg; $P < 0.01$). During the VIP infusion no change was observed in basal blood flow and evoked responses in the dura mater and nasal mucosa.

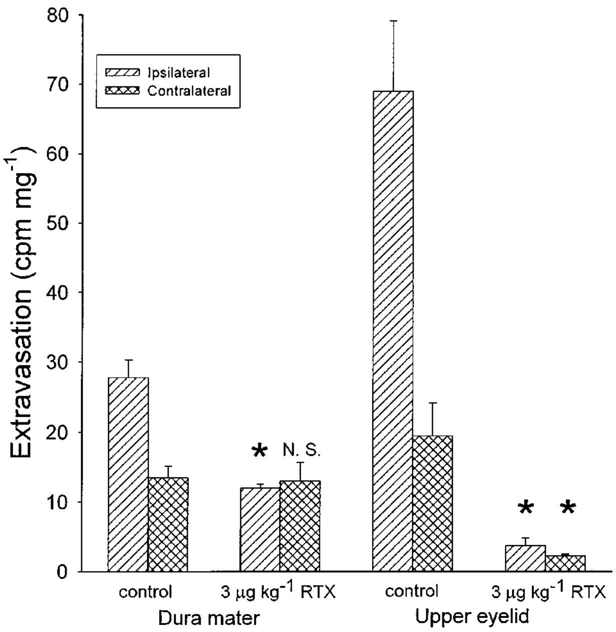

Figure 8 Effect of resiniferatoxin (RTX, 3 μg kg^{-1} i.v. 10 min. before electrical stimulation; $n = 6$) on plasma extravasation in the dura mater and in the upper eyelid evoked by electrical stimulation (25 V, 5 Hz, 0.5 ms, 5 min) of the ipsilateral trigeminal ganglion (means ± s.e.mean; c.p.m.: count per minute; *$P < 0.01$; N.S., non significant).

Effect of resiniferatoxin on plasma extravasation evoked by electrical stimulation of the trigeminal ganglion

Electrical stimulation (25 V, 0.5 ms, 5 Hz, 5 min) of the trigeminal ganglion in the control animals ($n = 6$) induced an ipsilateral enhancement of plasma extravasation in the dura mater and upper eyelid (Figure 8). The respective enhancement as expressed by the extravasation ratio (ER) of the stimulated *vs* nonstimulated sides was 2.13 ± 0.1 and 4.40 ± 0.76. In those rats where the electrodes were lowered into the trigeminal ganglion without nerve stimulation there was no significant extravasation in the dura mater and upper eyelid (ER: 1.1 ± 0.25 and 1.03 ± 0.18, respectively; $n = 6$).

Administration of RTX (3 μg kg^{-1} i.v.) but not the vehicle almost completely abolished the plasma extravasation in the dura mater and upper eyelid (Figure 8). and the respective ER's were 1.08 ± 0.26 and 1.58 ± 0.42 ($n = 6$).

Discussion

It has been shown earlier in several vascular areas that local or systemic capsaicin treatment abolishes antidromic vasodilatation and various effector tissue responses elicited by stimulation of sensory fibres. Hence, sensory-efferent vascular and other tissue responses were described as a characteristic feature of the capsaicin-sensitive subgroup of afferent fibres (Szolcsányi, 1984, 1996; Holzer, 1988; Maggi, 1995). The present study has provided evidence for the first time that a sensory-efferent response, the meningeal vasodilatation evoked by trigeminal stimulation, is mediated by capsaicin-insensitive afferent fibres. This conclusion is supported by results obtained with three ways of capsaicin/

RTX pretreatments and two methods of nerve stimulation. The dural stimulation method developed by Kurosawa *et al.* (1995) excites both sensory and autonomic perivascular nerve fibres but the advantage of this method is the lack of effect on blood pressure. Our technique of electrical stimulation of the trigeminal ganglion which selectively excites trigeminal afferent fibres, enables the comparison of blood flow changes in extracranial and intracranial tissues. Owing to the stimulation of the whole ganglion, however, reflex increases in blood pressure often occur, therefore sympathetic blockade was applied. Guanethidine at the applied dose abolished or strongly inhibited these blood pressure changes by blockade of both noradrenergic and neuropeptide Y mediated sympathetic transmission (Pintér *et al.*, 1997). Beside guanethidine, the role of autonomic reflex responses was excluded by the lack of effect of atropine and the nicotinic receptor blocking agent hexamethonium.

Meningeal vasodilatation evoked by perivascular or trigeminal ganglionic stimulation was very similar with respect to time course (latency and recovery rate) and capsaicin-insensitivity of the fibres involved. This indicates that both stimulation techniques activate identical populations of meningeal trigeminal afferent fibres. On the contrary, nasal mucosal vasodilatation induced by stimulation of the trigeminal ganglion is strikingly different from the meningeal response: (1) The frequency optimum of the meningeal response was higher, the onset and recovery was faster than those of the nasal mucosal response; (2) Meningeal vasodilatation was mediated by capsaicin-insensitive fibres, while the mucosal response was capsaicin-sensitive; (3) The mucosal response was inhibited by a CGRP receptor antagonist ($hCGRP_{8-37}$) while the meningeal response was not inhibited by this drug.

RTX causes an acute and selective functional blockade of capsaicin-sensitive primary afferent neurones (Szolcsányi *et al.*, 1990). At a similar dose as used in the present study RTX abolished or strongly inhibited antidromic vasodilatation recorded with laser Doppler flowmetry in several tissues of the rat including the skin and striated muscle (Pórszász & Szolcsányi, 1994; Pintér *et al.*, 1997). In the present study systemic RTX or capsaicin pretreatment abolished and topical capsaicin application strongly inhibited mucosal vasodilatation but none of these treatments inhibited the meningeal vasodilatation evoked by trigeminal stimulation. The lack of evidence for an involvement of capsaicin-sensitive afferents in the meningeal vasodilatation of the rat is a surprising new observation since all sensory-efferent responses studied so far proved to be mediated by capsaicin-sensitive afferents (Holzer, 1988; Maggi, 1995; Szolcsányi, 1996). This result is particularly striking since dural plasma extravasation induced by trigeminal stimulation is mediated by capsaicin-sensitive primary afferents according to the present and earlier data (Markowitz *et al.* 1988). Immunohistochemical observations indicated that capsaicin pretreatment did not deplete SP containing trigeminal fibres in the rat leptomeninges, whereas in the guinea-pig depletion was seen for both SP and CGRP (Geppetti *et al.*, 1990).

The different effectiveness of the CGRP antagonist $hCGRP_{8-37}$ in meningeal *vs* mucosal vasodilatation indicates a further difference between the responses. Mucosal hyperaemia was considerably inhibited but not abolished by $hCGRP_{8-37}$, hence this response is mediated in part by CGRP acting on CGRP-1 receptors, although the involvement of CGRP-2 receptors, – which are not sensitive to $hCGRP_{8-37}$ (Xu & Wiesenfeld-Hallin, 1996) – and other mediators should also be considered. It has been shown that meningeal vasodilatation induced by perivascular electrical stimulation of the dura mater of the rat was inhibited by topical application of $hCGRP_{8-37}$ at high concentrations (Kurosawa *et al.*, 1995). The present results indicate that a systemic dose of $hCGRP_{8-37}$ which strongly inhibited the nasal vasodilatation was ineffective in the meningeal response.

In accordance with earlier data (Shepheard *et al.*, 1993; Carmody *et al.*, 1996) activation of NK_1 receptors is unlikely to be involved in the meningeal vasodilatation since the selective NK_1 receptor antagonist, RP 67580, did not inhibit the response. In the nasal mucosa stimulation of NK_1 receptors seems to contribute to the initial phase of the response in those cases in which basal blood flow is high, as evidenced by the inhibitory effect of RP 67580. The most probable source of released CGRP and SP in the rat nasal mucosa is trigeminal capsaicin-sensitive primary afferent fibres which have been shown to store both SP and CGRP (Lundblad *et al.*, 1983b; Fusco *et al.*, 1994). The present functional results obtained with RTX and capsaicin pretreatments also support this assumption. It is worth mentioning that in the cat electrical or capsaicin-induced stimulation of the trigeminal nerve evokes an atropine and hexamethonium resistant vasodilatation in the nasal mucosa (Lundblad *et al.*, 1983a). Similarly, antidromic or capsaicin-induced trigeminal stimulation in the pig leads to vasodilatation in the nasal mucosa, the latter response being strongly inhibited by $hCGRP_{8-37}$ but only slightly by NK_1 receptor antagonists (Stjärne *et al.*, 1991; Rinder & Lundberg, 1996).

A contribution of autonomic efferent fibres to either the meningeal or nasal vasodilatation responses to stimulation of the trigeminal ganglion or its dural fibres is unlikely. The role of mediators released from sympathetic postganglionic fibres can be ruled out since the experiments were made after guanethidine pretreatment. The fact that administration of hexamethonium, a ganglion blocking agent in this and a previous study (Izumi & Karita, 1991) failed to inhibit vasodilatation in the dura mater evoked by electrical stimulation of the trigeminal ganglion provides further evidence against the possibility that the autonomic nervous system plays any role in the response. Parasympathetic efferent fibres releasing acetylcholine or VIP can also been ruled out, since neither atropine nor a VIP antagonist inhibited these responses. Whereas cutaneous hyperaemia induced by trigeminal or facial nerve stimulation in cats was attributed to VIP-ergic nerve fibres (Lambert *et al.*, 1984; Goadsby & MacDonald, 1985; Goadsby & Duckworth, 1987). Cutaneous hyperaemic responses to trigeminal stimulation in the rat were not affected by a VIP antagonist as shown here and by Escott *et al.* (1995a), indicating marked species differences in this respect. It is worth mentioning that infusion of VIP in a dose sufficient to produce a decrease in blood pressure did not enhance the evoked blood flow responses in the dura and nasal mucosa.

The present study provides evidence that the method of Kurosawa *et al.* (1995) is suitable for investigation of the effects of drugs on meningeal microcirculation. Our results confirm with laser Doppler flowmetry the enhancement induced by vinpocetine (Kiss & Kárpáti, 1996) of the meningeal blood flow and the hyperaemic response induced by stimulation of the dura mater.

In conclusion, the present study has revealed that vasodilatation evoked by electrical trigeminal stimulation is remarkably different in meningeal and exteroceptive areas in respect to frequency optimum of stimulation, response kinetics, capsaicin sensitivity of the mediating fibres and susceptibility to CGRP receptor antagonism. Hence extrapolation from data obtained in trigeminally innervated extracranial vascular beds towards meningeal vascular mechanisms might be misleading and, therefore, direct investigation of meningeal blood flow regulation is necessary in migraine research. Furthermore, these data provide the first evidence for the existence of a vascular region where antidromic vasodilatation is mediated by sensory fibres which are resistant to the sensory blocking effect of capsaicin and RTX. The striking capsaicin-insensitivity of trigeminal sensory fibres mediating meningeal vasodilatation has been shown with three different types of pretreatments under control conditions, therefore, they might be useful in further research to reveal a possible neural response mediated by capsaicin-insensitive fibres. Pharmacological analysis of the sensory mechanisms in the dural vascular phenomena seems to be promising owing to their special pharmacological features and clinical significance.

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