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CP-93,129, sumatriptan, dihydroergotamine block c-fos expression within rat trigeminal nucleus caudalis caused by chemical stimulation of the meninges

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1 The effects of intravenously administered 5-HT_{1B} receptor agonists were examined on c-fos-like immunoreactivity, an indicator of neuronal activation, within the brain stem. C-fos was induced by injecting an algesic, vasoconstrictor substance (0.3 ml of autologous blood) or a pro-inflammatory molecule, carrageenin (1 mg in 0.1 ml saline) into the cisterna magna of pentobarbitone-anaesthetized Sprague-Dawley rats and was visualized in serial sections (50 μm) by use of a polyclonal antiserum.

2 As previously reported, the injection of blood caused significant labelling within laminae I, II_o of the trigeminal nucleus caudalis, a major nociceptive brain stem nucleus, as well as within nucleus of the solitary tract and area postrema. A similar pattern of expression with fewer cells per section was detected after carrageenin instillation. The number of expressing cells was reduced by 54% in trigeminal nucleus caudalis but not within the nucleus of the solitary tract or area postrema when blood was injected in adult rats after neonatal capsaicin treatment.

3 Pretreatment with 5-HT_1 agonists with some selectivity for the 5-HT_{1B} receptor, CP-93,129 (460 nmol kg^{-1} × 2, i.v.), sumatriptan (720 nmol kg^{-1} × 2, i.v.) or dihydroergotamine (86 nmol kg^{-1} × 2, i.v.) reduced positive cells by 39%, 31%, and 33% respectively in trigeminal nucleus caudalis but not in nucleus of the solitary tract or area postrema after blood instillation. Pretreatment with the analgesic morphine (15 μmol kg^{-1}, s.c.) also decreased the number of positive cells by 63% in trigeminal nucleus caudalis.

4 CP-93,129 (460 nmol kg^{-1} × 2, i.v.) reduced the number of c-fos labelled cells by 47% within lamina I, II_o after carrageenin instillation.

5 Drug-induced blockade appeared to be tissue-dependent. Pretreatment with sumatriptan (720 nmol kg^{-1} × 2, i.v.) did not block c-fos expression in trigeminal nucleus caudalis following formalin application to the nasal mucosa.

6 Drug-induced blockade may be mediated by an action on primary afferent (trigeminovascular) fibres in as much as CP-93,129 (460 nmol kg^{-1} × 2, i.v.) did not reduce the number of expressing cells within the trigeminal nucleus caudalis following blood instillation in rats treated as neonates with capsaicin.

7 We infer from these results that the analgesic actions of agonists at 5-HT_{1B} receptors (the receptor subtype analogous to 5-HT_{1D} in man) need not depend upon the presence of vasodilatation and, that 5-HT_{1D} receptor-mediated blockade of neurotransmission contributes significantly to the analgesic effects of these drugs in headache.

8 Based on the demonstrated effects of 5-$HT_{1B/D}$ agonists against the actions of two chemically-unrelated meningeal stimulants, we suggest that treatment with 5-HT_{1D} agonists may be useful for the alleviation of pain in other headache conditions associated with meningeal irritation. Bacterial, viral (including AIDS meningovascular inflammation) and other forms of chemical meningitis merit further investigation.

Keywords: Sumatriptan; CP-93,129; dihydroergotamine; trigeminovascular system; subarachnoid haemorrhage; c-fos expression; 5-$HT_{1B/D}$ receptor agonists; headache

Introduction

The mechanism by which sumatriptan and dihydroergotamine abort or ameliorate acute vascular head pain is not well understood. Both drugs are 5-HT_{1D} receptor agonists (Peroutka, 1990) and exhibit nanomolar binding affinities to 5-HT_{1D} recognition sites as well as to binding sites in rat brain membranes (5-HT_{1B}) which are analogous to the 5-HT_{1D} binding sites in man (Middlemiss & Hutson, 1990).

Explanations for the analgesia have been proposed based on prejunctional (Saito *et al.*, 1988; Buzzi & Moskowitz, 1990; Buzzi *et al.*, 1991a) and postjunctional mechanisms (Saxena & Verdouw, 1985; Humphrey *et al.*, 1990) in cephalic blood vessels. At prejunctional receptors, 5-HT_{1D} and probably 5-HT_{1B} agonists inhibit neuropeptide release from perivascular axons and block the development of neuroinflammation within the meninges and, by so doing, inhibit sensitization of innervating primary afferent fibres. Neuroinflammation in the meninges is characterized by mast cell degranulation, platelet aggregation, and endothelial activation (Buzzi *et al.*, 1992; Dimitriadou *et al.*, 1992). At postjunctional receptors, 5-$HT_{1B/D}$ receptor agonists constrict intracranial vascular smooth muscle (Connor *et al.*, 1989; Feniuk *et al.*, 1989). Pain may be alleviated by constricting the dilatation, the proposed mechanism for pain in this model. The triggering events for vascular headaches are unknown, although there is general agreement that meningeal blood vessels and their associated small unmyelinated nerve

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fibres generate the pain of most headaches (Moskowitz, 1984).

We have recently adapted an animal model to address the possibility that drugs with some selectivity for the 5-$HT_{1B/D}$ subtype exhibit important neurogenic mechanisms as a basis for their actions. The model is based on injecting autologous blood (Delgado *et al.*, 1985; Jackowski *et al.*, 1990) or the sulphated mucopolysaccharide, carrageenin into the cisterna magna and subarachnoid space of Sprague-Dawley rats. Blood in the subarachnoid space in man is quite noxious and causes significant pain, vasoconstriction and reductions in cerebral blood flow. Carrageenin is a potent inflammatory agent when injected into the central nervous system. As in other models of pain and inflammation, injection of a noxious substance promotes c-fos protein expression within cells of lamina I, II_o of the trigeminal nucleus caudalis (TNC) or dorsal horn of the spinal cord (Hunt *et al.*, 1987). TNC (laminae I, II_o) contains the synapses of primary afferent fibres concerned with the transmission of nociceptive information from trigeminal receptive fields and second and higher order neuorones which transmit nociceptive information to rostral centres (Willis, 1985; Menétrey & Basbaum, 1987). In a previous study, the number of positive cells in this nucleus correlated with the amount of injected blood (Nozaki *et al.*, 1992). Similarly, the number of positive cells in the dorsal horn correlated with a nociceptive behavioural response after formalin injection into the hind paw of a rat. In the formalin experiments, the number of expressing cells decreased after morphine administration (Presley *et al.*, 1990; Gogas *et al.*, 1991) in a dose-dependent, naloxone-sensitive manner. Chronic sectioning of the trigeminal nerve innervating the meninges, or destroying small unmyelinated C-fibres by neonatal capsaicin treatment significantly reduced the number of cells expressing c-fos within TNC after blood injection into the cisterna magna (Nozaki *et al.*, 1992).

We chose to address the possible importance of neurogenic mechanisms to the actions of 5-$HT_{1B/D}$ receptors by determining whether CP-93,129, sumatriptan or dihydroergotamine block expression of a marker of sensory activation when given prior to noxious chemical stimulation of the meninges. Our data show that 5-HT_{1B} receptor agonists block selectively the expression of a marker of activation within TNC after noxious trigeminovascular stimulation. These findings suggest a mechanism independent of vasoconstriction for the therapeutic effects of the drugs in alleviating headaches.

Methods

Blood and carrageenin instillation into the subarachnoid space

We chose to study the rat instead of the guinea-pig, a species possessing the 5-HT_{1D} receptor, because of the expected high mortality in the latter and because of the opportunity to monitor physiological parameters more easily. Male Sprague-Dawley rats (250–300 g, Charles River Laboratories, Wilmington, MA, U.S.A.) were anaesthetized with pentobarbitone (45 mg kg^{-1}, i.p.). Polyethylene catheters (22 Ga, 0.7 mm internal diameter; Intracath, Deseret, Sandy, Utah, U.S.A.) were inserted into the femoral artery and vein for blood withdrawal and drug administration. A midline skin incision was made from the occipital protuberance to $cervical_7$ area in order to introduce a soft catheter (PE-10, internal diameter 0.28; Intramedic, Clay Adams, Parsippany, NJ, U.S.A.) into the cisterna magna. Anaesthesia was maintained by periodic injections (i.p. or i.v.) of pentobarbitone (10 mg kg^{-1}) approximately every 2 h. Animals were kept in a prone position. Six hours after catheter placement, arterial blood (0.4 ml) was removed from the femoral catheter and an equivalent volume of saline was injected intra-arterially. Within 1 or 2 min following withdrawal, 0.3 ml of autologous non-heparinized arterial blood or artificial CSF (Na 150 mEq l^{-1}, K 3 mEq l^{-1}, Ca 2.5 mEq l^{-1}, Mg 1.2 mEq l^{-1}, Cl 132 mEq l^{-1}, glucose 3.7 mM, urea 6 mM, HCO_3 25 mEq l^{-1}, pH adjusted to 7.4) was injected into the cisterna magna over about 1 min via a tuberculin syringe (1 ml). Sham animals were treated identically but received no intracisternal injection.

In the carrageenin experiments, animals were treated as described above except that 0.1 ml of carrageenin solution (10 mg ml^{-1} in saline) was injected into the cisterna magna via a 27 gauge needle and tuberculin syringe.

Animals were then kept at 30° head-down for 30 min in order to facilitate the settling of blood or carrageenin in the basal cisterns after which they remained in the horizontal prone position. Animals received no additional anaesthetics.

In selected (chosen at random) animals, arterial PCO_2, PO_2, pH (Corning 178 Blood Gas Analyzer, Ciba Corning Diag Corp., Medford, MA, U.S.A.), haematocrit, blood glucose, and direct arterial blood pressure (Grass Model 7B Polygraph Grass Instrument Co., Quincy, MA, U.S.A.) were monitored along with heart rate and respiratory rate. Intracranial pressure was recorded in animals (administered blood only) equipped with a closed cranial window via an outlet from the cranial window to a pressure transducer (DTX/Plus™ Disposable Transducer Kit, Viggo-Spectramed, Oxnard, CA, U.S.A.) attached to a chart recorder (Gould model RS 3200, Gould Inc., Cleveland, Ohio, U.S.A.) (Morii *et al.*, 1986). Core body temperature was maintained between 36–37°C with a homeothermic blanket system (Harvard Apparatus, Natick, MA, U.S.A.).

Formalin application to the nasal mucosa

Formalin (5% in saline) was applied to the right nasal mucosa. Animals were lightly anaesthetized with pentobarbitone (20 mg kg^{-1}, i.p.) and 50 µl of 5% formalin solution was then dripped onto the right nasal cavity from a Hamilton syringe.

Capsaicin neonatal treatment

Rat pups within the first 48 h of birth were injected with capsaicin (50 mg kg^{-1}, s.c.) to destroy small unmyelinated sensory fibres as previously described (Markowitz *et al.*, 1987) and raised to adulthood. Before experiments, a small amount of capsaicin (1 µM–1 mM) was applied topically to the cornea to confirm that the animals were desensitized to this stimulus.

Drug treatment

Intracisternal blood-CP-93,129 (46, 140 or 460 nmol kg^{-1}), sumatriptan (240 or 720 nmol kg^{-1}) or dihydroergotamine (86 nmol kg^{-1}) (1 ml kg^{-1}) were injected intravenously 60 and 10 min before blood instillation. Drug dosages were chosen to approximate amounts previously shown to activate prejunctional receptors and block neurogenic extravasation within the meninges (Buzzi *et al.*, 1991b; Matsubara *et al.*, 1991). Morphine sulphate (15 µmol kg^{-1}) (0.67 ml kg^{-1}) was injected subcutaneously 20 min before blood instillation (see Presley *et al.*, 1990).

Carrageenin injection CP-93,129 (460 nmol kg^{-1} × 2) was injected 60 and 10 min before carrageenin instillation.

Formalin injection-Sumatriptan (720 nmol kg^{-1}) was injected intravenously 60 and 10 min before formalin application into the right nasal mucosa.

C-fos immunohistochemistry

All animals survived for 2 h after which they were anaesthetized and killed with an overdose of pentobarbitone (60 mg kg^{-1}, i.p.) and perfused through the ascending aorta

with 150 ml of saline (0.9%), followed by 200 ml of paraformaldehyde (4%) in 0.1 M phosphate buffer (PB, pH 7.3). The perfused brain stems with attached upper cervical spinal cords were kept in the same fixative overnight, and then placed in 20% sucrose and 30% ethylene glycol in 0.1 M PB for 48 h at 4°C before sectioning. The brain stem with upper cervical spinal cords were sectioned coronally (50 μm) on a freezing microtome.

Free-floating sections were processed immunohistochemically with the avidin-biotin procedure using commercially available kits (Vectastain ABC; Vector Labs Burlingame, CA) as described previously (Uemura *et al.*, 1991). Briefly, sections were incubated with 10% normal goat serum and 0.03% hydrogen peroxide in 0.1 M phosphate buffered saline (PBS, pH 7.3) for 30 min at room temperature with gentle agitation. Subsequently, sections were incubated with primary antisera for c-fos protein (1:5000 dilution) in PBS with 0.3% triton X-100 (Sigma Labs, St Louis, MO, U.S.A.) overnight at room temperature under gentle agitation. The rabbit polyclonal antiserum was directed against an *in vitro* translated product of c-fos gene (kindly provided by Dr Dennis Slamon, the Department of Hematology and Oncology at University of California, Los Angeles, U.S.A.), and was preabsorbed against acetone-dried rat liver powder overnight to reduce non-specific background staining. The staining pattern produced by this antibody is comparable to a commercially available monoclonal antibody raised against a synthetic peptide which consists of residue 4–17 of the c-fos protein. In some experiments, antisera were reused (maximum of 5 times) and collected and stored at 4°C after use.

After washes in PBS (15 min × 3), sections were placed in biotinylated anti-rabbit IgG antiserum (1:200 dilution in PBS, Vector Labs, Burlingame, CA, U.S.A.) at room temperature for 2 h with gentle agitation. After PBS washes (15 min × 3), sections were placed in ABC-peroxidase complex (Vector Lab) for 2 h at room temperature with gentle agitation. After further PBS washes (15 min × 3) sections were placed in a solution of 3,3'-diaminobenzidine tetrahydrochloride (40 mg%; Sigma Labs) and 0.003% hydrogen peroxide in 50 mM Tris-HCl buffer (pH 7.6) for about 20 min. After the diaminobenzidine reaction, sections were mounted on gelatin-coated slides, air-dried and coverslipped.

Every second section was processed for immunohistochemistry and approximately 150 sections were examined in each animal. Serial sections were evaluated and quantitated for c-fos expression bilaterally with a Zeiss microscope. Anatomical boundaries were determined by co-ordinates established by the Paxinos & Watson rat brain atlas. In order to quantitate c-fos protein expression, cells showing c-fos like immunoreactivity within lamina I, II$_o$ of TNC, nucleus of the solitary tract (NTS) and area postrema (AP) were counted by a 'blind' observer (P.B.) and these results were confirmed independently by K.N. The extent of TNC examined in this study was from AP to cervical 1 segment (about 50 sections in each animal), and those of NTS and AP were from 0.2 mm caudal–0.8 mm rostral to obex (in which the majority of positive cells were observed; about 10 sections in each animal) and from obex–0.5 mm rostral to obex (about 5 sections in each animal) respectively. Because re-use of primary antisera caused an apparent reduction in the number of c-fos expressing cells following blood instillation, it was especially important to include appropriate controls in each experiment.

Statistics

Data are expressed as the number of cells within each 50 μm section (mean ± s.d.) or in the case of carrageenin, the total number of labelled cells per nucleus. Statistical comparisons were performed by unpaired Student's *t* test. Probabilities of less than 0.05 were considered significant.

Drugs

Sumatriptan (Glaxo Ltd, Hertfordshire, England) and dihydroergotamine (DHE) (Sandoz; supplied as D.H.E.45 ampoules [1 mg ml^{-1}]) were diluted in saline; CP-93,129 {3-(1,2,5,b-tetrahydropyrid-4-yl)pyrrolo [3,2-b]pyrid-5-one} (Pfizer, Inc., Groton, CT, U.S.A.) was dissolved in dimethylsulphoxide:saline 1:19. Capsaicin was solubilized in saline:Tween 80 8:1:1 and purchased from Polysciences Inc., Wilmington, PA, U.S.A. Pentobarbitone was obtained from Anthony Products Co, Aracadia, CA, U.S.A. Morphine sulphate (Elkins-Sinn, Inc., Cherry Hill, NJ, U.S.A., [15 mg ml^{-1}]) was used without dilution. Lambda-carrageenin (Sigma Chemical Co, St. Louis, MO, U.S.A.) was mixed in saline.

Results

The mortality following anaesthesia and blood injection was less than 2%. Extensive blood clots were observed around the brain stem and in the basal cistern when rats were killed 2 h after blood instillation.

Physiological parameters

In both vehicle- and drug-treated [CP-93,129 (460 nmol kg^{-1} × 2, $n = 4$, Figure 1a) or sumatriptan (720 nmol kg^{-1} × 2, $n = 3$, Figure 1b)] animals, similar increases in femoral arterial blood pressure and decreases in pulse rate and respiratory rate were observed 1 min after blood injection, and returned to baseline by 20 min. Heart rate and femoral arterial blood pressure remained at baseline levels for the remaining 2 h, although respiratory rate increased slowly. Intracranial pressure increased up to 30–35 mmHg from baseline (4–12 mmHg) 5 min after blood instillation, and slowly returned towards baseline. At 1 h after blood instillation, intracranial pressure was 10–18 mmHg. There were no significant differences in the above physiological parameters between vehicle- and drug-treated animals. Similarly, arterial Pco_2, Po_2, pH, haematocrit or blood glucose did not differ between groups when measured just before blood instillation and after 30 min and 2 h (data not shown).

C-fos expression

In sham animals, few c-fos positive cells were detected in the brain stem. The number of c-fos immunoreactive cells per section in TNC (lamina I, II$_o$), NTS or AP was 6 ± 3, 4 ± 2 or 6 ± 4 ($n = 5$) respectively. In animals which were injected with 0.3 ml of artificial CSF, c-fos positive cells were sparsely observed in the same brain stem nuclei. The number of positive cells per section in TNC (lamina I, II$_o$), NTS or AP was 13 ± 5, 17 ± 5 or 17 ± 6 ($n = 12$), respectively.

C-fos-like immunoreactivity was found bilaterally and most intensely within cells of TNC (lamina I, II$_o$), NTS and AP in animals which were injected with 0.3 ml of blood. The numbers of c-fos containing cells per section in TNC (lamina I, II$_o$), NTS, AP were 39 ± 5, 35 ± 9, 39 ± 10 ($n = 12$), respectively. In adult animals which were treated as neonates with capsaicin, c-fos positive cells were reduced in number by 54% within TNC (lamina I, II$_o$), but not within NTS or AP (Figure 2).

Treatment with CP-93,129 or sumatriptan prior to blood injection dose-dependently reduced the numbers of c-fos positive cells within TNC. CP-93,129 (460 nmol kg^{-1} × 2) or sumatriptan (720 nmol kg^{-1} × 2) reduced the numbers by 39% [38 ± 4 ($n = 11$) versus 23 ± 3 ($n = 7$) in vehicle versus drug treatment, respectively] or 31% [39 ± 6 ($n = 9$) versus 27 ± 2 ($n = 7$) in vehicle versus drug treatment, respectively] (Figures 3 and 4), but not in NTS or AP (Figure 5a,b). Pretreatment with dihydroergotamine (86 nmol kg^{-1} × 2) also reduced the numbers of positive cells in TNC by 33%

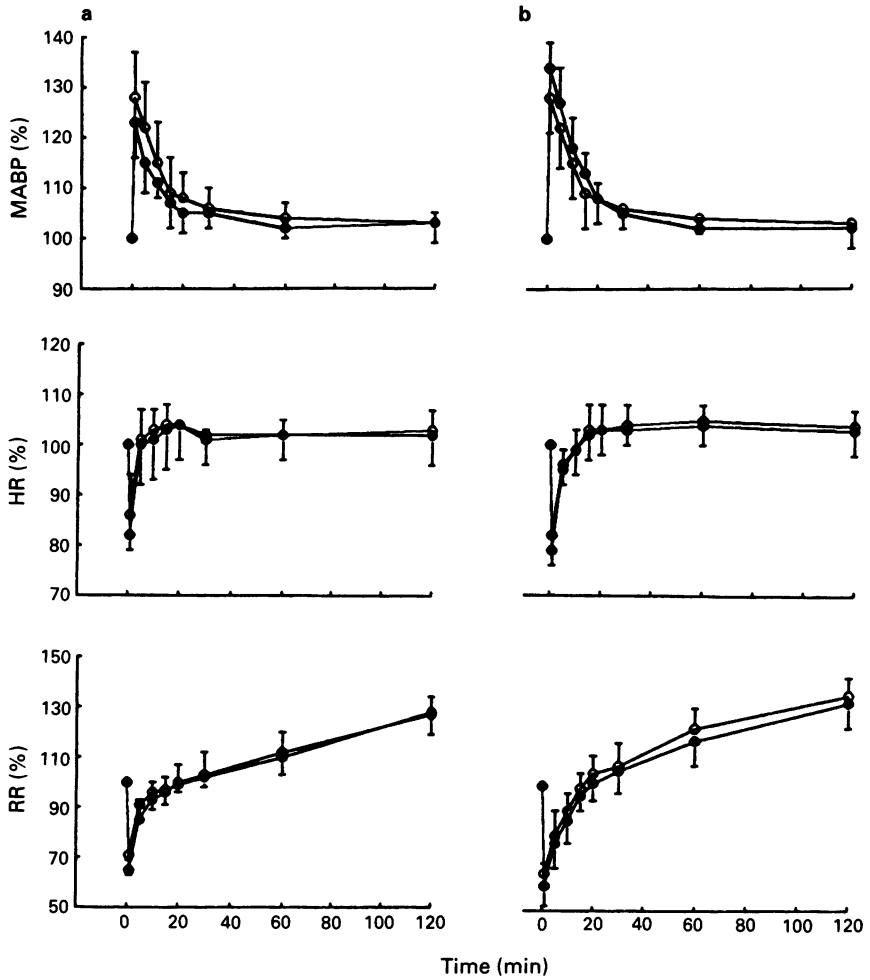

Figure 1 Time-dependent changes in mean arterial blood pressure (MABP), heart rate (HR), and respiratory rate (RR) following intracisternal injection of 0.3 ml of blood in animals which were pretreated with (a) CP-93,129 (460 nmol $kg^{-1} \times 2$, $n = 4$, ●) or vehicle ($n = 4$, ○) and (b) sumatriptan (720 nmol $kg^{-1} \times 2$, $n = 3$, ●) or vehicle ($n = 3$, ○). Data are expressed as percentage of baseline (with s.d. shown by vertical bars). Baseline values of MABP, HR and RR in CP-93-129- or vehicle-treatment were 93 ± 3 or 93 ± 3 mmHg, 383 ± 28 or 403 ± 27 min^{-1} and 68 ± 6 or 71 ± 6 min^{-1}, respectively. Baseline values of MABP, HR and RR in sumatriptan- or vehicle-treatment were 94 ± 5 or 96 ± 6 mmHg, 397 ± 16 or 404 ± 24 min^{-1} and 72 ± 7 or 73 ± 7 min^{-1}, respectively.

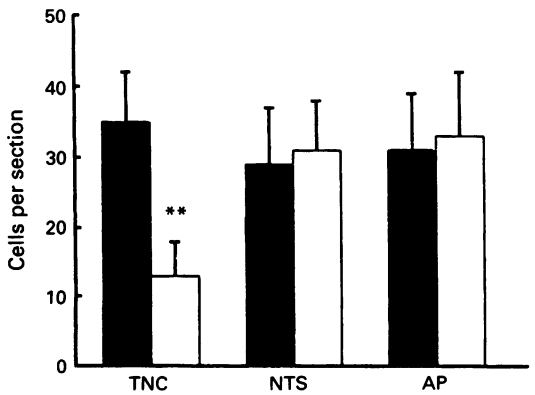

Figure 2 Capsaicin neonatal treatment selectively decreases c-fos expression in trigeminal nucleus caudalis (TNC) after subarachnoid blood injection. Numbers of c-fos immunoreactive cells per 50 μm sections are shown in TNC (lamina I, II_o), nucleus of the solitary tract (NTS) and area postrema (AP) 2 h after intracisternal injection of 0.3 ml of blood to adult animals treated during the neonatal period with vehicle (closed columns, $n = 7$) or capsaicin (open columns, $n = 6$). The numbers of c-fos positive cells per section observed in sham animals (6 ± 3 for TNC, 4 ± 2 for NTS, 6 ± 4 for AP, $n = 5$) are subtracted from each number.
$^{**}P < 0.01$.

[45 ± 3 ($n = 3$) versus 30 ± 5 ($n = 4$) in vehicle versus drug treatment, respectively], but not in AP or NTS (Figure 5c). Pretreatment with morphine (15 μmol kg^{-1}) markedly decreased the number of c-fos positive cells by 63% in TNC [38 ± 10 ($n = 4$) versus 14 ± 4 ($n = 5$) in vehicle versus drug treatment, respectively] and moderately within NTS (Figure 5d).

Pretreatment with CP-93,129 (460 nmol $kg^{-1} \times 2$) did not further reduce blood-induced c-fos expression within TNC in adult animals treated as neonates with capsaicin. The number of c-fos positive cells within TNC in CP-93,129- or vehicle-treated animals was 23 ± 6 ($n = 3$) or 22 ± 8 ($n = 6$), respectively (Figure 6).

Carrageenin instillation was accompanied by c-fos expression within lamina I, II_o of TNC (Figure 7) as well as within AP and NTS (data not shown). The number of labelled cells in each nucleus was significantly reduced as compared to the response following blood injection, which could have been due to the amount of injected carrageenin or to the nature of the stimulus (e.g., carrageenin is viscous and did not distribute as widely as the injected blood). Pretreatment with CP-93,129 (460 nmol $kg^{-1} \times 2$) significantly attenuated the c-fos response in the seven examined animals (531 ± 175 total cells in TNC after vehicle treatment versus 280 ± 171 after CP-93,129 treatment). C-fos labelled cells were not counted within NTS or AP as part of this latter experiment.

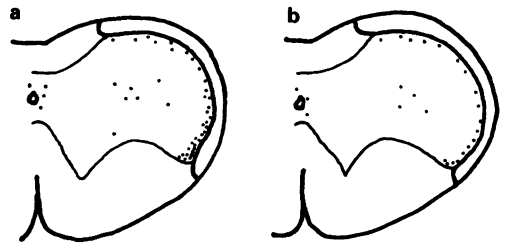

Figure 3 Camera lucida drawings of coronal sections of upper cervical spinal cord showing localization of c-fos immunoreactive cells (dots) within superficial lamina of trigeminal nucleus caudalis (TNC) (3.0 mm below obex) in animals which were pretreated with (a) vehicle or (b) sumatriptan (720 nmol kg^{-1}) 60 and 10 min before intracisternal blood injection. A few positive cells were found within the vicinity of the central canal and diffusely in deeper nuclei.

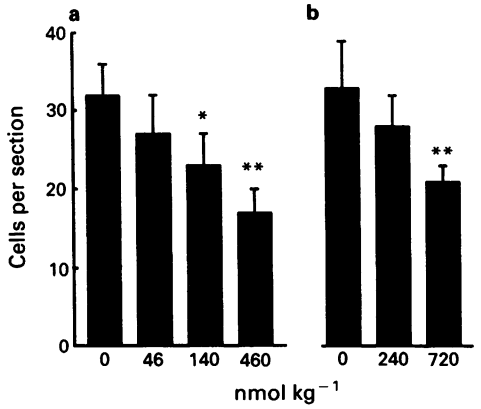

Figure 4 CP-93,129 and sumatriptan decrease the numbers of c-fos immunoreactive cells per 50 μm section within trigeminal nucleus caudalis (TNC) (lamina I, II$_o$) after intracisternal blood injection. (a) CP-93,129: vehicle ($n = 11$), 46 nmol kg^{-1} ($n = 3$), 140 nmol kg^{-1} ($n = 5$), 460 nmol kg^{-1} ($n = 7$); (b) sumatriptan: vehicle ($n = 9$), 240 nmol kg^{-1} ($n = 3$), 720 nmol kg^{-1} ($n = 7$) 60 and 10 min before blood injection. The number of c-fos positive cells per section observed in sham animals (6 ± 3, $n = 5$) is subtracted from each number.
*$P < 0.05$; **$P < 0.01$ compared with vehicle treatment.

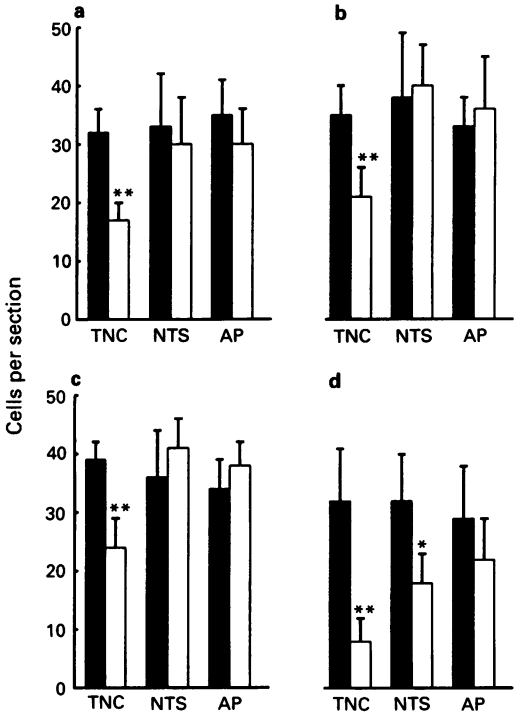

Figure 5 Drug-induced inhibition of c-fos expression within brain stem nuclei. C-fos immunoreactive cells were counted per 50 μm section within trigeminal nucleus caudalis (TNC) (lamina I, II$_o$), nucleus of the solitary tract (NTS) and area postrema (AP) in animals which were pretreated with (a) CP-93,129 (vehicle, closed columns, $n = 8$; 460 nmol kg^{-1} × 2; open columns, $n = 7$), (b) sumatriptan (vehicle, closed columns, $n = 6$; 720 nmol kg^{-1} × 2, open columns, $n = 7$), (c) dihydroergotamine (vehicle, closed columns, $n = 3$; 86 nmol kg^{-1} × 2, open colums, $n = 4$), and (d) morphine (vehicle, closed columns, $n = 4$; 15 μmol kg^{-1}, open columns, $n = 5$) before intracisternal blood injection. The numbers of c-fos positive cells per section observed in sham animals (6 ± 3 for TNC, 4 ± 2 for NTS, 6 ± 4 for AP) are subtracted from each number.
*$P < 0.05$; **$P < 0.01$ compared with vehicle.

Formalin (5%) injected into the right nasal mucosa increased c-fos expression mostly in ipsilateral TNC (lamina I, II$_o$), and in NTS and AP, as well. Treatment with sumatriptan (720 nmol kg^{-1} × 2) before formalin application did not reduce the number of c-fos positive cells in TNC (Figure 8).

Discussion

The expression of c-fos immunoreactivity within brain and spinal cord is useful for mapping the spatial distribution of neurones activated by noxious as well as non-noxious stimulation (Hunt *et al.*, 1987; Presley *et al.*, 1990). The experiments described herein, however, concern noxious stimulation of the meninges because, (a) small calibre, unmyelinated sensory axons which transmit nociceptive information (not larger calibre myelinated afferents) predominate within the pia mater of animals and man, (b) meningeal blood vessels cause pain (and no other sensation) during electrical or mechanical stimulation, (c) blood in the subarachnoid space is noxious in man and causes severe headache and pain, (d) c-fos labelling was most intense in lamina I and II$_o$ of TNC, a region that processes nociceptive information, (e) surgical or chemical sectioning of meningeal afferents reduces the number of labelled neurones in TNC, and (f) the analgesic, morphine, reduced c-fos expression in TNC as did the 5-HT$_1$ agonists.

5-HT$_{1B}$ is probably the receptor subtype mediating inhibition of c-fos expression in this rat subarachnoid haemorrhage model. CP-93,129 is a potent, selective and specific agonist for the 5-HT$_{1B}$ receptor subtype. CP-93,129 exhibits high nanomolar or micromolar affinities for all other 5-HT recognition sites (Macor *et al.*, 1990) and for dopamine, noradrenaline and adenosine binding sites as well. Sumatriptan binds with high affinity to 5-HT$_{1B}$ and 5-HT$_{1D}$ recognition sites, although it seems to be more potent as an agonist at 5-HT$_{1D}$ receptors. Dihydroergotamine exhibits nanomolar affinities at both 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor sites and is the least selective agent among the three. Because 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors are species-specific (Hoyer & Middlemiss, 1989; Matsubara *et al.*, 1991), 5-HT$_{1B}$ receptor-mediated c-fos inhibition in rat may be analogous to the 5-HT$_{1D}$ inhibition of vascular head pain in man.

5-HT$_{1B}$ receptors which inhibit c-fos expression appear closely associated with or directly on perivascular afferent fibres (Buzzi & Moskowitz, 1990; Buzzi *et al.*, 1991a,b; Moskowitz, 1991). The spatial distribution of expressing cells was similar after destruction of primary afferent fibres and after sumatriptan, CP,93-129 or dihydroergotamine pretreatment. Neither blocked cell labelling in NTS or AP after blood injection. A 5-HT receptor on brain stem neurones is less likely to mediate the observed effects because CP-93,129 did not inhibit c-fos expression in capsaicin-treated rats, and because sumatriptan did not block the expression after for-

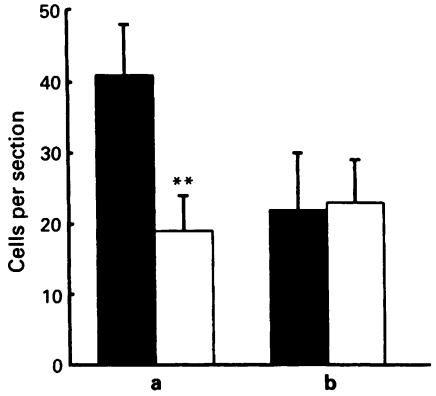

Figure 6 CP-93,129 does not decrease c-fos expression within trigeminal nucleus caudalis (TNC) in adult animals treated as neonates with capsaicin. (a) C-fos immunoreactive cells were counted per 50 μm section within TNC (lamina I, II_o) 2 h after intracisternal blood injection in adult animals after neonatal vehicle or capsaicin pretreatment (closed columns in a, $n = 6$ or open columns in a, $n = 5$, respectively). (b) CP-93,129 (460 nmol $kg^{-1} \times 2$, open columns, $n = 3$) or vehicle (closed columns, $n = 6$) was given 60 and 10 min before blood injection in adult animals after neonatal capsaicin pretreatment.
$^{**}P < 0.01$.

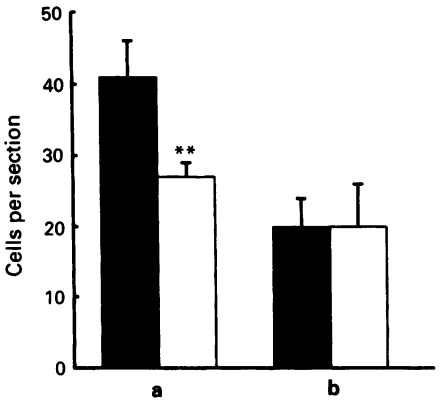

Figure 8 Sumatriptan did not block c-fos expression within trigeminal nucleus caudalis (TNC) (lamina I,II_o) after formalin application to the nasal mucosa. (a) Sumatriptan (720 nmol $kg^{-1} \times 2$, open columns, $n = 7$) or vehicle (closed columns, $n = 6$) was administered 60 and 10 min before intracisternal blood injection. (b) Sumatriptan (720 nmol $kg^{-1} \times 2$, open columns, $n = 3$) or vehicle (closed columns, $n = 3$) was administered 60 and 10 min before formalin application to the right nasal mucosa. The numbers of c-fos immunoreactive cells were counted per 50 μm sections.
$^{**}P < 0.01$.

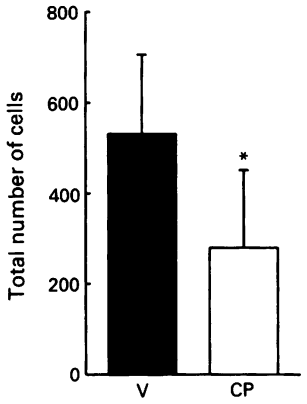

Figure 7 CP-93,129 (460 nmol $kg^{-1} \times 2$) attenuates the expression of c-fos within trigeminal nucleus caudalis (TNC) following the instillation of carrageenin (1 mg in 0.1 ml saline). Vehicle (V) or CP-93,129 (CP) were administered 60 and 10 min before carrageenin administration, and the animals were killed 2 h later ($n = 7$ per group). The data are expressed as the total number of cells (with s.d. shown by vertical bars) within TNC. The brains were processed as described in Methods.
$^{*}P < 0.05$ as compared to vehicle-treated group.

malin application to the nasal mucosa.

Activation of $5\text{-}HT_{1B}$ receptors on vascular smooth muscle does not mediate inhibition of c-fos expression within TNC. In man and animals, blood in the subarachnoid space is accompanied by intense cerebroarterial constriction and reductions in cerebral blood flow (50% lower than normal when tested in our own experiments using laser-Doppler flowmetry). If inhibition of c-fos expression does reflect reduction of a nociceptive signal, then dilated blood vessels are not a requisite for drug-induced blockade of neural transmission in this animal model. If constriction of dilated vessels is a mechanism for analgesia in the clinical situation (which is doubtful; Moskowitz, 1992), then our studies suggest that sumatriptan and dihydroergotamine possess a second and distinct antinociceptive mechanism. Our data show that $5\text{-}HT_{1B/D}$ receptors are coupled to at least two important functions which may diminish pain and sensitization in the meninges (Buzzi & Moskowitz, 1990; Buzzi *et al.*, 1991a,b; Matsubara *et al.*, 1991). The drugs block neural transmission within trigeminovascular fibres (as reflected by inhibition of c-fos protein expression), and also block the neuroinflammatory response by inhibiting neuropeptide release. The threshold doses for both effects are within a similar range and both are related to prejunctional mechanisms. The molecular basis remains to be determined.

The studies reported herein have focused on pain mechanisms related to the irritants blood or carrageenin in the subarachnoid space. The data suggest the possibility that $5\text{-}HT_{1D}$ agonists may be useful in treating other conditions characterized by meningeal pain and inflammation such as viral, bacterial or chemical meningitis.

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