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Role of opioid receptors in neurogenic dural vasodilation and sensitization of trigeminal neurones in anaesthetized rats

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1 Migraine headache is thought to be caused by a distension of meningeal blood vessels, the activation of trigeminal sensory neurones and the the development of a central sensitization within the trigeminal nucleus caudalis (TNC). It has been proposed that clinically effective 5-$HT_{1B/1D}$ agonists act peripherally to inhibit the release of calcitonin gene-related peptide (CGRP) and neurogenic dural vasodilation, and to attenuate nociceptive neurotransmission within the TNC. Since opioids are also effective anti-migraine agents the present studies investigated the role of opioids within the trigemino-vascular system in anaesthetised rats.

2 Electrical stimulation of the dura mater evoked neurogenic dural vasodilation which was significantly inhibited by morphine (1 mg kg^{-1}) the selective μ-opioid agonist DAGO (10 μg kg^{-1}) and the mixed agonist/antagonist butorphanol (1 mg kg^{-1}) but not by the κ- and δ-opioid agonists ($\pm$) U50488H (100 μg kg^{-1}) and DPDPE (1 mg kg^{-1}). Morphine had no effect on CGRP-evoked dural vasodilation.

3 In electrophysiological studies morphine (1–10 mg kg^{-1}) significantly attenuated brainstem neuronal activity in response to electrical stimulation of the dura by 65% at 10 mg kg^{-1}. Morphine (3 mg kg^{-1}) also inhibited the TNC neuronal sensitization following CGRP-evoked dilation.

4 The present studies have demonstrated that opioids block the nociceptive neurotransmission within the trigeminal nucleus caudalis and in addition inhibit neurogenic dural vasodilation *via* an action on μ-opioid receptors located on trigeminal sensory fibres innervating dural blood vessels. These peripheral and central actions are similar to those of the 'triptan' 5-$HT_{1B/1D}$ agonists and could account for the anti-migraine actions of opioids.
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Abbreviations: DAGO, [D-Ala2, N-MePhe4, Gly5-ol]enkephalin; DPDPE, [D-Pen2,5]enkephalin; MMA, middle meningeal artery; TNC, trigeminal nucleus caudalis

Introduction

Migraine headache is thought to result from activation of the trigeminal nerve leading to painful distension of cerebral and meningeal blood vessels. This hypothesis is supported by studies showing that cranial blood vessels are richly innervated by perivascular trigeminal sensory nerves which contain the potent vasodilator calcitonin gene-related peptide (CGRP; Uddman & Edvinsson, 1989; Edvinsson, 1991; Jansen *et al*., 1992) and that elevated levels of CGRP have been detected in the jugular blood during acute migraine attacks in humans (Goadsby *et al*., 1990). Clinically effective anti-migraine 5-$HT_{1B/1D}$ receptor agonists such as sumatriptan are thought to abort migraine attacks *via* direct constriction of distended cranial and meningeal blood vessels (Humphrey & Feniuk, 1991) and/or inhibition of neuropeptide release from trigeminal sensory fibres innervating these blood vessels (Moskowitz, 1992). In addition, it has been proposed that brain penetrant 5-$HT_{1B/1D}$ receptor agonists may also inhibit neurotransmission *via* an action within the trigeminal nucleus caudalis, a key relay centre for nociceptive neurotransmission (Kaube *et al*., 1993; Shepheard *et al*., 1995).

Morphine and other opioid analgesics such as pethidine, are sometimes used in the clinic to treat severe cases of migraine headache, particularly when other agents are ineffective or contraindicated, such as during pregnancy or in patients with coronary heart disease (Goadsby, 1994; Silberstein & Lipton, 1994). Although these agents are preferential μ-opioid receptor agonists, it is reported that a nasal spray formulation of the mixed κ-opioid agonist/μ-opioid antagonist butorphanol is also a safe and effective treatment for acute migraine attacks (Gillis *et al*., 1995).

As with the 5-$HT_{1B/1D}$ agonists, opioids are reported to have inhibitory actions within the trigeminal nucleus caudalis and inhibit neuropeptide release from trigeminal sensory neurones innervating peripheral structures. Opioids interact with three receptor types termed μ, δ and κ, although it is now thought that there may be further subtypes of these receptors (Martin, 1983; Millan, 1986; Raynor *et al*., 1994). Morphine has been shown to inhibit c-fos expression within

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the trigeminal nucleus caudalis evoked by application of capsaicin to the meninges (Nozaki *et al*., 1992). Similarly the selective μ- and δ-opioid agonists [D-Ala2, N-MePhe4, Gly5-ol]enkephalin (DAGO) and [D-Pen2,5]enkephalin (DPDPE) have been shown to inhibit the activity of sensory neurones within the trigeminal nucleus caudalis, suggesting a modulatory role of δ- and μ-opioids within the brainstem (Wang *et al*., 1996).

Opioids have also been shown to have inhibitory effects on peripheral structures innervated by the trigeminal nerve. Plasma protein extravasation evoked in the dura mater by electrical stimulation of the trigeminal ganglion or intravenous capsaicin has been shown to be blocked by morphine (Moskowitz & MacFarlane, 1993) and the selective μ-opioid agonist lofentanil (Saito *et al*., 1988). Similarly, electrical stimulation of the trigeminal ganglion has been shown to evoke increases in facial blood flow mediated *via* CGRP (Escott *et al*., 1995a) which were inhibited by pretreatment with DAGO (Escott *et al*., 1995b). Consistent with these findings, Li *et al*. (1998) have recently demonstrated the colocalisation of μ-opioid receptor-like immunoreactivity in neurones containing CGRP in rat trigeminal ganglion. Furthermore, PCR studies on rat brain and peripheral sensory nerves have demonstrated the presence of κ-opioid (Schäfer *et al*., 1994) and δ-opioid (Buzas & Cox, 1997) mRNA in small diameter neurones within the trigeminal ganglion.

We have previously shown that electrical stimulation of the dura mater can evoke a peripheral neurogenic dural vasodilation (Williamson *et al*., 1997a,b) and activate neurones in the trigeminal nucleus caudalis (Cumberbatch *et al*., 1997). Each of these responses was inhibited by the clinically effective anti-migraine 5-$HT_{1B/1D}$ agonist rizatriptan suggesting that these mechanisms may be relevant to anti-migraine properties of 5-$HT_{1B/1D}$ agonists. Recently we have demonstrated that dilation of dural blood vessels, by electrical stimulation or intravenous CGRP, produced a facilitation of convergent sensory inputs from the face which was seen as a sensitization within the trigeminal nucleus caudalis (Williamson *et al*., 1998; Cumberbatch *et al*., 1999a). Such a sensitization may underlie an important mechanism in the development of migraine headache and explain the associated symptoms of facial pain and hypersensitivity that often accompany migraine. These observations are supported by clinical studies which showed that intravenous infusion of glyceryl trinitrate into healthy volunteers resulted in a mild headache that was accompanied by reduced facial nociceptive thresholds (Thomsen *et al*., 1996). In animal studies, a central sensitization within the TNC, evoked by a CGRP-mediated dural vasodilation, was blocked by pretreatment with the $5HT_{1B/1D}$ agonist, L-741604 suggesting an additional mechanism of action of the triptans (Cumberbatch *et al*., 1999a).

Since μ opioid agonists and butorphanol are also effective in the treatment of migraine the purpose of these studies was to firstly examine the effects of morphine, butorphanol and selective opioid receptor agonists DAGO (μ), ($\pm$)U-50,488 (κ) and DPDPE (δ) on neurogenic dural vasodilation in anaesthetized rats. Additional objectives were to examine the effects of morphine on the activity of trigeminal neuronal responses evoked by electrical stimulation of the dura mater and the central sensitization of brainstem neurones to CGRP-evoked dural vasodilation in anaesthetized rats. Some of these data have been published previously in abstract form (Williamson *et al*., 1999; Cumberbatch *et al*., 1999b).

Methods

General surgery

All experiments were conducted and terminated under general anaesthesia in accordance with a project licence issued by the U.K. Home Office under the 'Animals (Scientific Procedures) Act 1986'. Male Sprague-Dawley rats (300–400 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). The trachea was cannulated to provide artificial ventilation and the femoral artery and veins were cannulated to monitor blood pressure and to administer drugs and/or anaesthetic. Body temperature was maintained at approximately 37°C using a heated blanket connected to a rectal probe thermistor. Animals were placed in a stereotactic frame and in all experiments the skull was thinned using a saline cooled drill to form a transparent layer of intact bone over the middle meningeal artery (MMA). A tungsten bipolar stimulating electrode was positioned on the thinned surface of the skull adjacent to the MMA using a micromanipulator. All exposed surfaces were covered with mineral oil to prevent desiccation. In electrophysiological studies neuromuscular blockade was initiated and maintained with pancuronium bromide (1 mg kg^{-1} h^{-1}). General anaesthesia was monitored throughout and was ensured by the absence of any cardiovascular and pupillary responses to noxious stimuli.

Dural blood vessel diameter measurements

The methodology for dural blood vessel diameter measurements and neurogenic dural vasodilation has previously been described in detail (Williamson *et al*., 1997b). Briefly, a branch of the middle meningeal artery was viewed using an intravital microscope (Microvision MV2100, U.K.) and the image displayed on a television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyser (Living Systems Instrumentation, U.S.A.) and recorded with blood pressure on a data analysis system (MI^2, Modular Instruments, U.K.). In neurogenic dural vasodilation studies the surface of the cranial window was stimulated at 5 Hz, (1 ms pulse width for 10 s, Grass S88 stimulator, Grass Instruments, U.S.A.) with increasing voltage until an intensity was reached at which a maximal dilation was observed. Subsequent studies were then evoked using this pre-defined stimulation intensity.

Brainstem single unit extracellular recordings

The methodology for electrophysiological recording of trigeminal nucleus caudalis neurones has previously been described in detail (Cumberbatch *et al*., 1997). Briefly, the brainstem was exposed by retracting the overlying muscle and removing the atlanto-occipital membrane and extracellular action potentials were recorded from single caudal trigeminal neurones using a glass microelectrode filled with 0.5 M NaCl (4–5 MΩ). The signal was amplified (1 k) and filtered (500 Hz–5 kHz band-width) and action potentials were displayed on oscilloscopes to enable accurate isolation

of a single unit from adjacent cell activity. Extracellular single unit action potentials were evoked by electrical stimulation of the cranial window with single square wave pulses (100–200 μs pulse width; threshold intensities of 1–3 mA) applied at 0.2–0.3 mA above threshold at 1 Hz for 20 s; this was repeated every 200 s. Action potentials were counted using a window discriminator and the total counts over the 20 s of stimulation was used to calculate drug effects.

Simultaneous blood vessel diameter and brainstem electrophysiology recordings

The methodology for these studies has previously been described in detail (Cumberbatch *et al*., 1999a). Cells were selected for study that responded to mechanical stimulation of the vibrissae. After determining the facial receptive field an electrical stimulus (1–3 mA; 100–200 μs) was applied to the dura-mater, through the closed cranial window, to determine if the cell received convergent input. Vibrissal stimuli were applied in 1 min cycles (trains of 10×100 ms air-jet pulses at 1 Hz) using a directable air jet until responses were stable.

Experimental protocols

Protocol 1: Effects of opioid agonists on neurogenic and CGRP-evoked dural dilation In experiments examining the effects of opioid agonists on neurogenic dural vasodilation, a control response to electrical stimulation was performed and 5 min later, morphine (100 μg kg^{-1}, i.v.), DAGO (1 or 10 μg kg^{-1}, i.v.), butorphanol (1 or 10 mg kg^{-1}, i.v.)$\pm$ U50,488 (100 μg kg^{-1}, i.v.) or DPDPE (1000 μg kg^{-1}, i.v.) was administered and the electrical stimulation repeated after 15 min. Using the same protocol, a second dose of morphine (1 mg kg^{-1}, i.v.) or DAGO (0.1 or 1 mg kg^{-1}, i.v.) was given followed by a third electrical stimulation. In the neurogenic vasodilation studies with morphine, DAGO and butorphanol the reversibility of the inhibition was examined by intravenous administration of naloxone (1 mg kg^{-1}, i.v.) and an electrical stimulation repeated 5 min later.

In studies examining the effects of morphine (1 mg kg^{-1}, i.v.) on rat-αCGRP (0.3 μg kg^{-1}, i.v.) the same protocol was employed as above, with the control dilation to CGRP compared to that evoked after a 15 min pretreatment with morphine. The effects of opioids on neurogenic or rat-αCGRP evoked dural dilation were calculated as percentage increases in dural blood vessel diameter and compared to the dilation evoked prior to drug. The effects of drugs on dural blood vessel diameter and mean arterial blood pressure were also monitored.

Protocol 2: To determine the effects of morphine on the activity of trigeminal neurones in response to electrical stimulation of the dura mater The second protocol examined the effects of morphine on responses to noxious stimulation of the dura-mater. Regular cycles of noxious electrical stimuli (trains of 20 stimuli at 1–3 mA and 100–200 μs) were applied to the duramater every 200 s until responses were stable. Morphine was administered in a cumulative dose regimen of 1, 3 and 10 mg kg^{-1}, i.v. followed by naloxone (1 mg kg^{-1}, i.v.) and the drug effects were calculated as a percentage of the mean of the three pre-morphine control responses.

Protocol 3: To determine the effects of morphine on the central sensitization following CGRP evoked dural vasodilation In separate experiments vehicle or morphine (3 mg kg^{-1}, i.v.) was administered as a pre-treatment immediately after surgery and prior to locating a neurone. Trigeminal neuronal activity was monitored in response to air-jet stimulation of the vibrissa. When the responses were stable rat-αCGRP (1 μg kg^{-1}) was administered to evoke a dilation of the middle meningeal artery. Any changes in neuronal activity following the vasodilation were calculated as a percentage of the mean of the three control responses immediately before rat-αCGRP administration. For each cell the effects of rat-αCGRP were calculated from the three responses that coincided with the recovery phase of the vasodilation.

Statistics

All data are expressed as mean$\pm$s.e.mean. Statistical tests (BMDP statistical software) on brainstem neuronal and dural vasodilation responses following electrical stimulation of the cranial window were performed using an analysis of variance (ANOVA) with repeated measures (treatment was the within-subject effect) with planned contrast analysis. In studies investigating the effects of morphine on CGRP-mediated sensitization of brainstem neurones the overall number of spikes for the response to CGRP were calculated for each animal in the vehicle and morphine treated groups and comparisons between groups made using a *t*-test; $P \leqslant 0.05$ was considered to be significant.

Drugs

Morphine hydrochloride, naloxone hydrochloride, butorphanol tartrate, (Sigma, U.K.), DAGO ([D-Ala2, N-MePhe4, Gly5-ol]enkephalin), DPDPE [D-Pen2,5]enkephalin), ($\pm$)U-50,488 methanosulphate and rat-αCGRP (RBI, U.K.) were dissolved in isotonic saline. All doses refer to free base weight and drugs were injected in a volume of 1 ml kg^{-1}.

Results

Effects of morphine on neurogenic and rat-αCGRP dural vasodilation

In rats pretreated with morphine (1 mg kg^{-1}, i.v.) the response to electrical stimulation (50–300 μA, 5 Hz, 1 ms for 10 s) was significantly reduced from a 78$\pm$6% to a 31$\pm$7% increase in blood vessel diameter ($n=7$, $F_{3,18}=14.2$, $P<0.05$) and was completely reversed (92$\pm$11%, $P<0.05$) by naloxone (1 mg kg^{-1}, i.v. Figure 1a). In contrast, increases in dural blood vessel diameter evoked by rat-αCGRP (0.3 μg kg^{-1}, i.v.) of 91$\pm$10% were unaffected by morphine (1 mg kg^{-1}, i.v.) pretreatment (109$\pm$14%, $n=6$, Figure 1b).

Effects of DAGO on neurogenic dural vasodilation

In two separate experiments, electrical stimulation of the cranial window evoked a 119$\pm$10% and 89$\pm$15% increase in dural blood vessel diameter. In rats treated with DAGO (1 and 10 μg kg^{-1}, i.v., $n=6$) or (10 and 100 μg kg^{-1}, i.v., $n=5$) these responses were significantly inhibited to 57$\pm$21%

($F_{3,15}=12.3$) and 44±11% respectively, after 10 μg kg^{-1}, i.v. ($P<0.05$) and to 18±7% after 100 μg kg^{-1}, i.v. ($F_{3,12}=8$, $P<0.05$, Figure 2). The inhibitory effects of DAGO were fully reversed to control values by naloxone (1 mg kg^{-1}, i.v., $P<0.05$).

Effects of butorphanol on neurogenic dural vasodilation

Following treatment with butorphanol the response to electrical stimulation was significantly reduced from 116±11% to 83±10% after 1 mg kg^{-1}, i.v. ($P<0.05$, $n=8$, Figure 3a) and 117±16 to 29±5 after 10 mg kg^{-1}, i.v. ($F_{2,14}=11.6$, $P<0.05$, $n=8$, Figure 3b). Administration of naloxone (1 mg kg^{-1}, i.v.) in animals treated with 10 mg kg^{-1} butorphanol partially reversed the response to electrical stimulation to 88±25%, although this failed to reach statistical significance ($P=0.07$).

Effects of DPDPE on neurogenic dural vasodilation

Intravenous administration of DPDPE (1000 μg kg^{-1}) did not inhibit neurogenic dural vasodilation; prior to DPDPE electrical stimulation evoked a 143±8% increase in diameter which was unaffected (137±14%, $n=3$) by DPDPE pretreatment.

Effects of (±)U50,488 on dural blood vessel diameter and neurogenic dural vasodilation

Intravenous injection of (±)U50,488 (100 μg kg^{-1}, i.v.) evoked a small and sustained increase in dural blood vessel diameter from 40±2 to 42±1 arbitrary units, AU ($n=4$) and at 1000 μg kg^{-1}, i.v., a sustained increase in diameter to 49±3 AU ($P=0.06$, $n=4$). Electrical stimulation of the cranial window evoked a 102±16% increase in diameter which was not significantly blocked (87±15%) by (±)U50,488 (100 μg kg^{-1}, i.v.). The higher dose of (±)U50,488 also did not block the response to electrical stimulation. However, analysis of the actual values following electrical stimulation demonstrated that blood vessel diameter increased from 40±2 to 81±6 AU prior to (±)U50,488 and 49±3 to 81±8 AU after 1000 μg kg^{-1}, i.v. and represented no block in the magnitude of dilation following electrical stimulation.

Effects of opioid agonists on dural blood vessel diameter and MABP

With the exception of (±)U50,488 (see above) none of the opioid agonists had any lasting effects on dural blood vessel diameter, typically evoking a small reduction in blood vessel diameter which returned to baseline within 5 min. Intra-

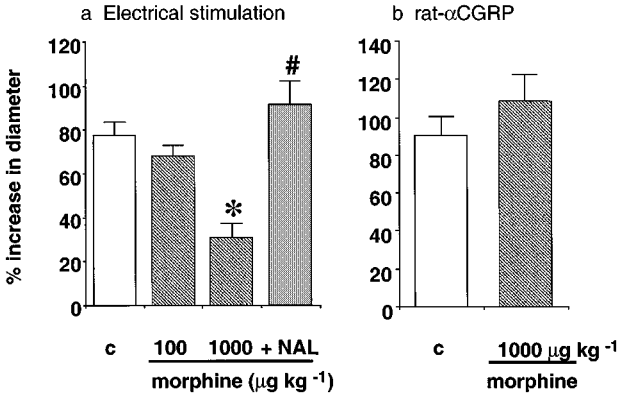

Figure 1 Effects of morphine on (a) neurogenic and (b) CGRP-evoked dural dilation in anaesthetized rats. Following a control dilation (c) morphine was administered and the dilator challenges repeated. In neurogenic dural vasodilation studies naloxone (1 mg kg^{-1}) was administered and the electrical stimulation repeated. Each column represents the mean percentage increase in dural blood vessel diameter±s.e.mean of (a) seven and (b) six rats. Significantly different from control: *$P<0.05$; significantly different from morphine (1 mg kg^{-1}): #$P<0.05$.

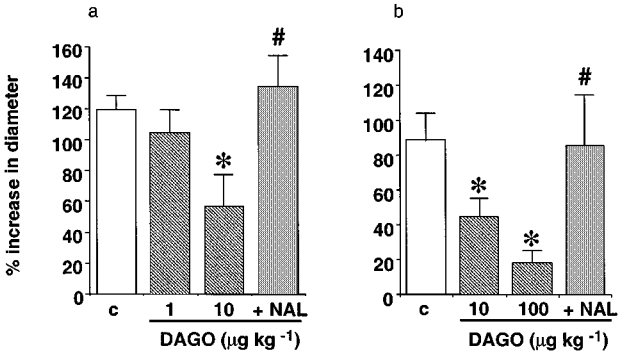

Figure 2 Effects of DAGO (a) 1 and 10 μg kg^{-1} or (b) 10 and 100 μg kg^{-1} on neurogenic dural dilation in anaesthetized rats. Following a control dilation (c) DAGO was administered and an electrical stimulation repeated after each dose. Finally naloxone (1 mg kg^{-1}) was administered and the electrical stimulation repeated. Each column represents the mean percentage increase in dural blood vessel diameter±s.e.mean of (a) six and (b) five rats. Significantly different from control: *$P<0.05$: significantly different from DAGO (1 mg kg^{-1}): #$P<0.05$.

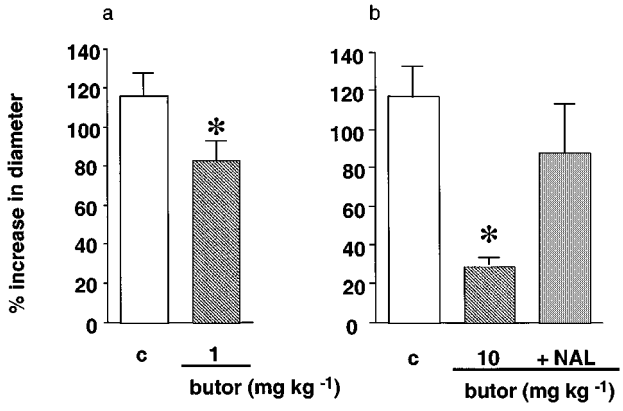

Figure 3 Effects of butorphanol (a) 1 mg kg^{-1} or (b) 10 mg kg^{-1} on neurogenic dural dilation in anaesthetized rats. Following a control dilation (c) butorphanol was administered and an electrical stimulations repeated after each dose. Finally in (b) naloxone (1 mg kg^{-1}) was administered and the electrical stimulation repeated. Each column represents the mean percentage increase in dural blood vessel diameter±s.e.mean of (a) eight and (b) eight rats. Significantly different from control: *$P<0.05$.

venous injection of butorphanol over 2 min produced a profound and sustained reduction in MABP of 36±3% ($P<0.05$) after 10 mg kg^{-1} which was only modestly reversed (28±1%) by administration of naloxone (1 mg kg^{-1}). Intravenous injection of (±)U50,488 also produced a sustained and significant decrease in MABP of 37±5% ($P<0.05$) 15 min after 1 mg kg^{-1}.

Effects of morphine on the activity of trigeminal neurones in response to electrical stimulation of the dura mater

Following vehicle treatment, noxious electrical stimulation of the dura mater (trains of 20 stimuli at 1–3 mA, 100–200 μs in 200 s cycles) evoked a mean number of 19.5±0.3 action potentials with a mean latency of 4±1.7 ms. Intravenous administration of morphine produced a dose dependent inhibition of durally evoked responses reaching a maximum 65±2% inhibition (7±0.5 action potentials per 20 stimuli; $F_{4,8}=10.6$, $P<0.05$, $n=3$, Figure 4) following 10 mg kg^{-1}. The inhibitory actions of morphine were significantly reversed by administration of 3 mg kg^{-1} naloxone (14±3.6 action potentials per 20 stimuli).

Effects of morphine on the central sensitization following CGRP evoked dural vasodilation

Extracellular electrophysiological recordings were made from 24 cells in the trigeminal nucleus caudalis of 14 rats. Cells were located 1–2 mm caudal to obex at a depth of 400–1200 μm from the surface of the pia mater. All cells responded to electrical stimulation of the dura mater (1–3 mA, 200 μs pulse width) with latencies of 2–11 ms and also had convergent input from the face. Cells were classified as low threshold mechanoreceptive and responded to mechanical stimulation of the vibrissae with an air jet.

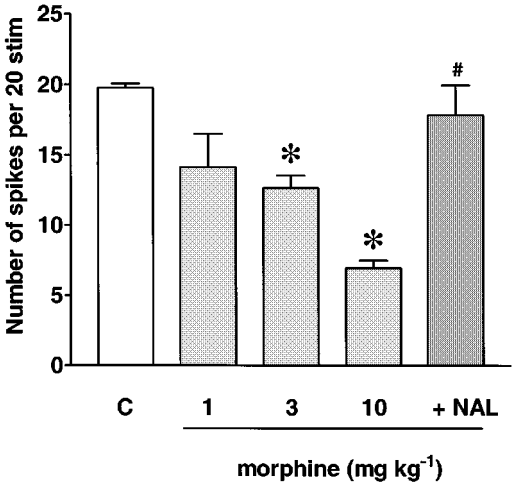

Figure 4 Effects of morphine on the activity of trigeminal nucleus caudalis in response to electrical stimulation of the dura mater. Following the control response morphine (1–10 mg kg^{-1}) was administered and electrical stimulations repeated after each dose. Finally naloxone (1 mg kg^{-1}) was administered and the electrical stimulation repeated. Each column represents the mean number of action potentials generated±s.e.mean per 20 stimuli applied to the dura in three rats. Significantly different from control: *$P<0.05$: significantly different from morphine (10 mg kg^{-1}): #$P<0.05$.

Innocuous stimulation of the vibrissae evoked reproducible and stable firing of brainstem neurones so that baseline responses prior to CGRP varied by only±5%. Intravenous injection of rat-αCGRP (1 μg kg^{-1}) evoked a 90±7% increase in dural blood vessel diameter lasting 5.5±0.3 min and evoked an increase in the response to vibrissal stimulation by a maximum of 37±7% ($P<0.05$, $n=12$ cells from 11 rats, Figure 5a) 4 min after CGRP injection. This facilitated response was sustained 3 min after the dural blood vessel diameter normalized and then returned to pre-CGRP baseline values. In a separate group of rats treated with morphine (3 mg kg^{-1}, i.v.) intravenous administration of rat-αCGRP evoked a dural blood vessel vasodilation (192±36% of pre-CGRP control) but there was no facilitation of the brainstem neuronal activity (99±0.8% of pre-CGRP control) and this was significantly inhibited compared to the facilitated response in control animals ($P<0.05$, $n=12$ cells from five rats, Figure 5b).

Discussion

Opioids are clinically effective anti-migraine agents and are used when other treatments are not appropriate or are not

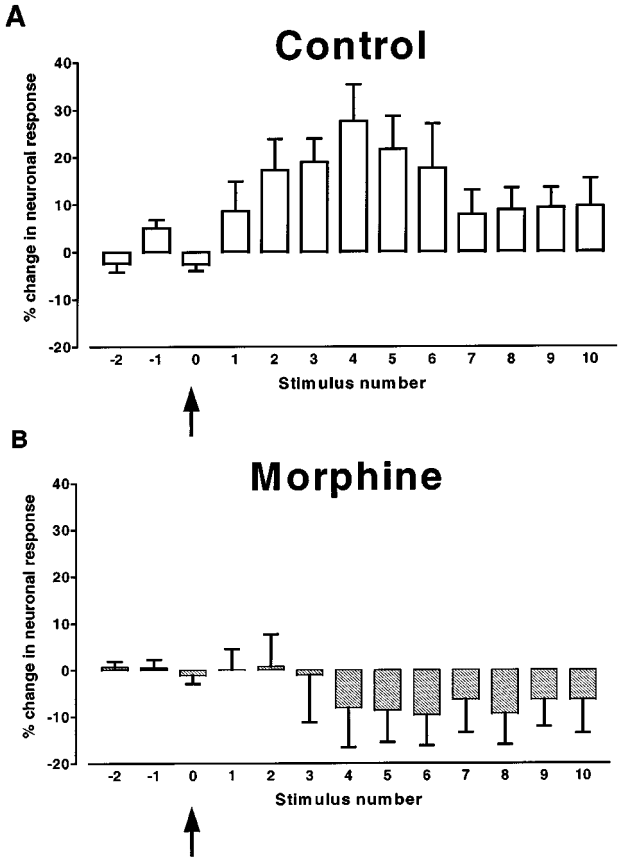

Figure 5 Intravenous injection of rat-αCGRP (1 μg kg^{-1}) as indicated by the arrow evoked increases in trigeminal nucleus caudalis neuronal activity in animals treated with vehicle (control). In contrast there was no increase in neuronal activity upon CGRP administration in animals treated with 3 mg kg^{-1} morphine (b). Bars are the percentage increase in neuronal activity±s.e.mean from pre-CGRP baseline activity in 12 cells per group.

effective. The present studies demonstrate that opioids have potent inhibitory actions within the trigeminal system acting both peripherally, to inhibit neurogenic dural vasodilation and centrally to inhibit brainstem neuronal activity in response to stimulation of the dura and meningeal vasodilation. Although the doses of morphine, butorphanol and naloxone are higher than used clinically, the actions of the opioids within the trigeminal system are similar to the inhibitory effects of the clinically effective antimigraine 5-$HT_{1B/1D}$ receptor agonists such as sumatriptan and rizatriptan (Cumberbatch *et al*., 1997; 1999a; Williamson *et al*., 1997a, b).

Effects of opioids on neurogenic dural vasodilation

The present study demonstrates the dural neurogenic vasodilation was inhibited by morphine, DAGO and butorphanol but not by DPDPE or (±)U50,488. The inhibitory effects of morphine were completely reversed by the opioid receptor antagonist naloxone, indicating the effects of morphine were mediated specifically *via* activation of opioid receptors. Morphine blocked vasodilation evoked by electrical stimulation but not that evoked by intravenous rat-αCGRP suggesting that it acts to inhibit CGRP release in this preparation. It is therefore likely that the inhibitory actions of morphine reflect an action at opioid receptors located on the terminals of perivascular trigeminal sensory fibres innervating dural blood vessels.

Although morphine has highest affinity at the μ-opioid receptor it also has appreciable affinity at κ- and δ-opioid receptor subtypes. In contrast, the opioid agonists DAGO, (±)U50,488 and DPDPE are much more selective for μ, κ and δ binding sites, respectively (see Table 1). The present studies suggest that the effects of morphine on neurogenic dural vasodilation are mediated *via* activation of μ-opioid receptors and further highlights an important role of μ-opioid receptors within the trigeminal system, as has been suggested previously (Escott *et al*., 1995b; Moskowitz & Macfarlane, 1993; Saito *et al*., 1988).

The effects of the κ-opioid agonists (±)U50,488 and butorphanol on neurogenic vasodilation are more difficult to interpret. Intravenous injection of (±)U50,488 up to 1 mg kg^{-1} produced a sustained reduction in MABP and an increase in baseline dural vessel diameter. The baseline blood vessel diameter changes confounded the data analysis since percentage increases in diameter were reduced after (±)U50,488, but the actual maximum level of dilation after electrical stimulation was the same as the control dilation. Thus it is likely that the contribution of κ-opioid receptors to the modulation of dural neurogenic vasodilation is minimal in the rat since (±)U50,488 is a potent and selective agonist and has been shown to block hindlimb neurogenic extravasation at similar doses to those tested in this assay (Barber, 1993). The observation that butorphanol blocks neurogenic vasodilation in the present studies and that its inhibitory effects were partially reversed by naloxone also suggests that butorphanol may be acting *via* an opioid receptor mechanism. It could be possible that the marked and sustained hypotensive effects may in some way interfere with the mechanisms involved in the production of neurogenic vasodilation, but this is unlikely since administration of naloxone only reversed the hypotensive effects by a modest amount yet reversed the inhibition of neurogenic dural vasodilation to a much greater degree. Although the involvement of κ-opioid receptors cannot be ruled out, the lack of effect of the highly potent κ agonist (±)U50,488, which is approximately equipotent with butorphanol at the κ-binding site, makes this unlikely. Butorphanol has been claimed to be a κ-opioid receptor agonist with antagonist activity at μ and δ receptors but more recently it has been suggested that under certain experimental conditions butorphanol acts as a partial μ-opioid receptor agonist (Horan & Ho, 1989). Recent studies show the antinociceptive effect of butorphanol in the mouse radiant heat test to be antagonised by the selective μ-opioid receptor antagonist beta-funaltrexamine (Garner *et al*., 1997). Thus, a partial agonist activity of butorphanol at μ-opioid receptors may best explain the effects of butorphanol on dural neurogenic vasodilation in the rat.

Table 1 Binding affinities of opioid agonist at μ-, δ- and κ-opioid receptors on rat brain membranes

	Ki (nM)			
Drug	μ	δ	κ	*References*
Morphine	1.8	90	300	Magnan *et al*. (1982)
DAGO	1.9	345	6090	Leslie (1987)
DPDPE	710	2.7	>15 000	Leslie (1987)
±U-50 488	941	8790	0.72	Leslie (1987)
Butorphanol	5	13	1.7	Seeman (1993)

Effects of morphine on brainstem neuronal activity following electrical stimulation of the dura mater

These data show that morphine inhibits central nociceptive neurotransmission arising from noxious stimulation of the dura mater *via* specific opioid receptors within the brainstem. It is likely that, as with the peripheral inhibitory actions of the opioids, morphine inhibits nociceptive neurotransmission via an action on the pre-synaptic terminals of central trigeminal projections.

Although the receptor subtype mediating the inhibitory effects of morphine on the neurones within TNC was not investigated using the selective opioid agonists in the present studies, it is notable that all three opioid receptor subtypes have been detected within the trigeminal ganglion (Xie *et al*., 1999). Furthermore Li *et al*. (1998) demonstrated μ-opioid receptor-like immunoreactivity in rat trigeminal ganglion neurones that was co-localized with substance P and CGRP in axon terminals in laminae I and II of the trigeminal nucleus caudalis where trigeminal primary afferents are known to synapse with second order neurones. Whether morphine inhibits the activation of brainstem neurones to stimulation of the dura *via* an action at μ, δ or κ receptors remains to be determined.

Effects of morphine on the central sensitization following CGRP-evoked dural vasodilation

Intravenous administration of rat-αCGRP facilitated the response of brainstem neurones to innocuous stimulation of the vibrissae with cells that received convergent input from the dura mater. In animals pre-treated with morphine the vasodilator response to rat-αCGRP was unaffected yet the

facilitation of brainstem neuronal activity was completely inhibited, suggesting that morphine blocks the development of the central sensitization evoked by meningeal vasodilation. This central sensitization, which facilitates non-nociceptive neurotransmission, may be analogous to the common associated symptoms of migraine headache such as extracranial allodynia and facial tenderness. This concept is supported by clinical evidence demonstrating decreased nociceptive thresholds in facial tissues during nitroglycerin induced headache (Thomsen *et al*., 1996) and in migraineurs (Burstein *et al*., 2000).

In summary, the present experiments have demonstrated that opioids such as morphine, which are effective in the treatment of migraine, have potent inhibitory effects within the trigeminovascular system *via* both peripheral and central mechanisms. Morphine blocked neurogenic dural vasodilation *via* an action at inhibitory μ-opioid receptors located on trigeminal fibres innervating the dural blood vessels presumably resulting in the inhibition of CGRP release. This peripheral inhibitory action of the opioids on neurogenic dural vasodilation is similar to those observed with the clinically effective 5-$HT_{1B/1D}$ agonists such as rizatriptan (Williamson *et al*., 1997a). Thus it is possible that the anti-migraine actions of opioids may be similar to those of the triptans in that they inhibit neurogenic release of CGRP and reduce the degree of meningeal vasodilation.

In addition to the peripheral actions of the opioids, the present studies also demonstrate that morphine has profound inhibitory actions within the trigeminal nucleus caudalis, to inhibit meningeal nociception and prevent the development of the central sensitization following meningeal vasodilation. These too are shared with 5-$HT_{1B/1D}$ agonists. Thus it is possible that the anti-migraine actions of opioids and 5-$HT_{1B/1D}$ agonists are mediated *via* the same neuronal mechanisms. Since opioids are not vasoconstrictor, unlike 5-$HT_{1B/1D}$ agonists, this may highlight the importance of neuronal versus vascular mechanisms as future potential anti-migraine targets.

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