Pain, 64 (1996) 545–552

PAIN 2981

Excitatory amino acid antagonists in the rostral ventromedial medulla inhibit mesencephalic morphine analgesia in rats

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(Received 10 May 1995, revised version received 25 August 1995, accepted 31 August 1995)

Summary Supraspinal opioid analgesia is mediated in part by connections between the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). Morphine analgesia elicited from the PAG is respectively decreased by selective serotonergic and opioid receptor antagonists administered into the RVM, and increased by RVM neurotensin antagonists. Since glutamate and excitatory amino acid (EAA) receptors are also active in the RVM, the present study evaluated whether either competitive (AP7) or non-competitive (MK-801) *N*-methyl-D-aspartate (NMDA) antagonists or a kainate/AMPA (CNQX) antagonist microinjected into the RVM altered morphine (2.5 μg) analgesia elicited from the PAG as measured by the tail-flick and jump tests. Mesencephalic morphine analgesia was markedly reduced on both tests after RVM pretreatment with either AP7 (0.01–1 μg, 0.08–7.8 nmol) or MK-801 (0.03–3 μg, 0.04–4.4 nmol). In contrast, small but significant reductions in mesencephalic morphine analgesia occurred on the jump test following CNQX (0.5 μg, 2.2 nmol) in the RVM. NMDA antagonists did not markedly alter either basal nociceptive thresholds following RVM administration, or mesencephalic morphine analgesia following administration into medullary placements lateral or dorsal to the RVM. These data implicate EAA and particularly NMDA receptors in the RVM in modulating the transmission of opioid pain-inhibitory signals from the PAG.

Key words: Morphine analgesia; Periaqueductal gray; Rostral ventro-medial medulla; Excitatory amino acid; MK-801; AP7; CNQX; NMDA receptor

Introduction

Supraspinal opioid analgesia appears to be modulated in part by a neural system connecting the midbrain periaqueductal gray (PAG) and the rostral ventro-medial medulla (RVM) which includes the nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis (NRGC) and NRGC, pars alpha (see reviews: Fields and Basbaum 1978; Basbaum and Fields 1984). Analgesia occurs following intracerebral administration of opiates in either structure (e.g., Jacquet and Lajtha 1973; Yaksh et al. 1976; Azami et al. 1982; Satoh et al. 1983a; Jensen and Yaksh 1986; Bodnar et al. 1988; Smith et al. 1988; Urban and Smith 1993, 1994). Direct PAG-NRGC projections and direct PAG-NRM projections have been described (Abols and Basbaum 1981; Beitz et al. 1983; VanBockstaele et al. 1989, 1991; VanBockstaele and Aston-Jones 1992), and analgesic synergy between the PAG and RVM has been observed using morphine, mu-selective agonists and delta$_2$-selective agonists (Rossi et al. 1993, 1994). Serotonergic receptors in the RVM participate in the mediation of morphine analgesia elicited from the PAG since either general (methysergide), 5HT$_{2A}$ (ritanserin) or 5HT$_3$ (ICS205930) antagonists reduced mesencephalic morphine analgesia on the tail-flick and jump tests (Kiefel et al. 1992a,b). Opioid receptors in the RVM also modulate mesencephalic morphine analgesia since either general (naltrexone), mu (beta-funaltrexamine) or delta (naltrindole) antagonists reduced mesencephalic morphine analgesia on both nociceptive tests (Kiefel et al. 1993). In contrast, a selective neurotensin antagonist in the RVM enhances mesencephalic morphine analgesia on the tail-flick test (Urban and Smith 1993). These effects were quite selective since: (1) the antagonists failed to alter baseline

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SSDI 0304-3959(95)00192-1

nociceptive latencies or thresholds, and (2) medullary cannula placements that are lateral, dorsal or ventral to the RVM failed to antagonize mesencephalic morphine analgesia (Kiefel et al. 1992a,b, 1993).

Excitatory amino acid (EAA) receptors have been implicated in this supraspinal analgesic pathway since either L-glutamate or *N*-methyl-D-aspartate (NMDA) elicits analgesia following PAG (e.g., Behbehani and Fields 1979; Jacquet 1988; Siegfried and Nunes deSouza 1989) or RVM (Satoh et al. 1983b; Jensen and Yaksh 1984a,b; van Praag and Frenk 1990; McGowan and Hammond 1993a,b) administration. PAG neurons containing glutamate and aspartate (Clements et al. 1987) project to the RVM (Wiklund et al. 1988; Beitz 1990). Aimone and Gebhart (1986) found that EAA receptor antagonists administered into the RVM increased the intensity of electrical stimulation elicited from the PAG. Further, vanPraag and Frenk (1990) found that morphine analgesia elicited from the PAG was significantly reduced by RVM pretreatment of the EAA antagonists, 1-(*p*-chlorobenzoyl)-piperazine-2,3-dicarboxylate (PCB) or DL-2-amino-5-phosphono-valerate (APV). Since PCB was more potent than APV in reducing PAG morphine analgesia, van Praag and Frenk (1990) suggested that kainate-quisqualate receptors modulated analgesic processes in the RVM more than NMDA receptors. To explore further the relationship between medullary EAA receptors in modulating morphine analgesia elicited from the PAG, the present study evaluated whether RVM pretreatment of either the non-competitive NMDA antagonist, MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine maleate), the competitive NMDA antagonist, AP-7 ((-)-2-amino-7-phosphono-heptanoate) or the kainate/AMPA antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), altered analgesia on the tail-flick and jump tests following morphine administered into the PAG. Since NMDA antagonists may potentially exert their effects by diffusing into structures outside of the injection area (Nasstrom et al. 1993a,b), additional groups of rats with misplaced medullary cannulae aimed dorsal and lateral to the RVM were tested in the same paradigm.

Materials and methods

Subjects, surgery and histology

Adult male albino Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA; 80–120 days of age) were housed individually and maintained on a 12-h light/dark cycle with food and water available ad libitum. Following anesthesia with chlorpromazine (3 mg/kg, i.p.) and ketamine HCl (100 mg/kg, i.m.), two stainless steel guide cannulae (26 gauge, Plastic Products, Roanoke, VA) were placed stereotaxically (Kopf Instruments) into the PAG and RVM of rats (Bodnar et al. 1988; Kiefel et al. 1992a,b, 1993). Stereotaxic coordinates were: incisor bar (−5 mm), PAG: 0.3–0.6 mm anterior to the lambda suture, 1.5–2.0 mm lateral to the sagittal suture, 6.5–7.0 mm from the top of the skull, and angled towards the sagittal suture at 12°, and RVM: 10.8–11.3 posterior to the bregma suture, 0–0.7 mm lateral to the midline, and 10.0–11.0 mm from the top of the skull. An additional control group was stereotaxically implanted with cannuale aimed at the PAG and medullary placements that were lateral (1.5–2.0 mm lateral to the midline) and dorsal (9.5–10.0 mm from the top of the skull) to the RVM. Cannulae were secured to anchor screws with dental acrylic. All animals were allowed 1 week to recover and clear anesthetic. After testing, cannulae placements were examined in anesthetized (Euthanasia, H. Schein) rats who received a trans-cardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. Coronal (40 μm) sections, stained with Cresyl violet were examined by light microscopy by an observer unfamiliar with the behavioral data; only animals with confirmed cannulae placements were included in the data analyses.

Nociceptive tests

In a given session, each animal was tested on the tail-flick *and* jump tests in that order to minimize carryover effects between tests. A tail-flick analgesiometer (IITC) produced a radiant heat source that was mounted 8 cm above a photocell upon which the rat's tail was placed. Radiant heat was applied 3–9 cm proximal to the tip of the rat's tail; removal of the tail activated the photocell and determined the latency (0.01 sec accuracy). The thermal intensity of the radiant heat source was set to produce baseline tail-flick latencies between 2 and 3.5 sec. Each session consisted of 3 latency determinations at different points on the tail at 10-sec intertrial intervals. To avoid tissue damage, a trial was automatically terminated if a response did not occur within 12 sec. Rats were tested on the jump test immediately after tail-flick testing. Electric shock was delivered to the feet of the rat by a shock generator (BRS/LVE) and shock scrambler (Campden Instruments). The jump threshold was defined in milliamperes as the lowest of two consecutive ascending intensities in which the animal simultaneously removed both hindpaws from the grids. Each of six trials began with the animal receiving a 300-msec footshock at a current intensity of 0.10 mA with subsequent shocks increased in 0.05 mA steps at 10-sec intervals until the jump threshold was determined. Baseline latencies and thresholds were determined for at least 4 days before experimental testing began to insure stability of responding. All animals displayed consistent latencies and thresholds in baseline and vehicle testing that did not appear subject to desensitization.

Drugs

All microinfusions were administered in 1 μl volumes at a rate of 0.2 μl every 10 sec through a stainless steel internal cannula (33 gauge, Plastic Products) which was connected to a Hamilton microsyringe by polyethylene tubing. Morphine (Pennick Labs), MK-801 (Research Biochemicals, MW = 383.7) and AP7 (Research Biochemicals, MW = 225.2) were dissolved in normal saline. CNQX (Research Biochemicals, MW = 232.2) was dissolved in 45% (w/v) aqueous 2-hydroxypropyl-β-cyclodextrin buffer (Research Biochemicals) in distilled water. Medullary microinjections of the antagonists preceded mesencephalic microinjections by 20 min.

Protocols

Separate groups of rats received a maximum of five pairs of microinjection conditions at weekly intervals. Tail-flick latencies *and* jump thresholds were determined at 30, 60, 90 and 120 min following the second microinjection of each pair. Table I summarizes the treatments, doses and sample sizes used to assess the effects of either MK-801 (A), AP7 (B) or CNQX (C) administered into the RVM upon morphine analgesia elicited from the PAG, while the D section summarizes treatments of control rats with medullary placements lateral and dorsal to the RVM. Higher doses of CNQX could not be used because of solubility problems and limitations to a 1 μl volume. Animals receiving particular antagonist doses were matched on the basis of their basal nociceptive latencies and thresholds as well as the degree of mesencephalic morphine analgesia. A morphine dose of 2.5 μg was chosen because of its

TABLE I

SUMMARY OF EXPERIMENTAL GROUPS

The control group had medullary placements that were lateral or dorsal to the RVM. RVM = rostral ventral medulla; PAG = periaqueductal gray.

RVM condition	PAG condition	n
A: MK-801 group		
Vehicle	Vehicle	6
Vehicle	Morphine (2.5 μg)	6
MK-801 (0.03 μg)	Morphine (2.5 μg)	6
MK-801 (0.3 μg)	Morphine (2.5 μg)	6
MK-801 (3.0 μg)	Morphine (2.5 μg)	6
MK-801 (3.0 μg)	Vehicle	6
B: AP7 group		
Vehicle	Vehicle	9
Vehicle	Morphine (2.5 μg)	9
AP7 (0.01 μg)	Morphine (2.5 μg)	8
AP7 (0.1 μg)	Morphine (2.5 μg)	8
AP7 (1.0 μg)	Morphine (2.5 μg)	9
AP7 (1.0 μg)	Vehicle	8
C: CNQX group		
Vehicle	Vehicle	6
Vehicle	Morphine (2.5 μg)	6
CNQX (0.5 μg)	Morphine (2.5 μg)	6
CNQX (0.5 μg)	Vehicle	6
D: Control group		
Vehicle	Vehicle	5
Vehicle	Morphine (2.5 μg)	5
MK-801 (3 μg)	Morphine (2.5 μg)	5
AP7 (1 μg)	Morphine (2.5 μg)	5

significant, but not maximal analgesic effects following PAG microinjection, and the weekly interval between conditions minimized possible tolerance effects (e.g., Bodnar et al. 1988; Kiefel et al. 1992a,b, 1993).

Statistical analyses

Analyses of variance indicated that significant differences in latencies and thresholds failed to occur between baseline and vehicle-vehicle conditions (data not shown); the latter were used for subsequent comparisons with agonist and antagonist effects. Separate split-plot analyses of variance were performed for each of the antagonist and control protocols. Each split-plot analysis of variance assessed significant effects among conditions (between-groups factor) and across the time course (within-groups factor) with Dunnett comparisons ($P < 0.05$) determining morphine analgesia relative to vehicle/vehicle treatment. Dunn comparisons

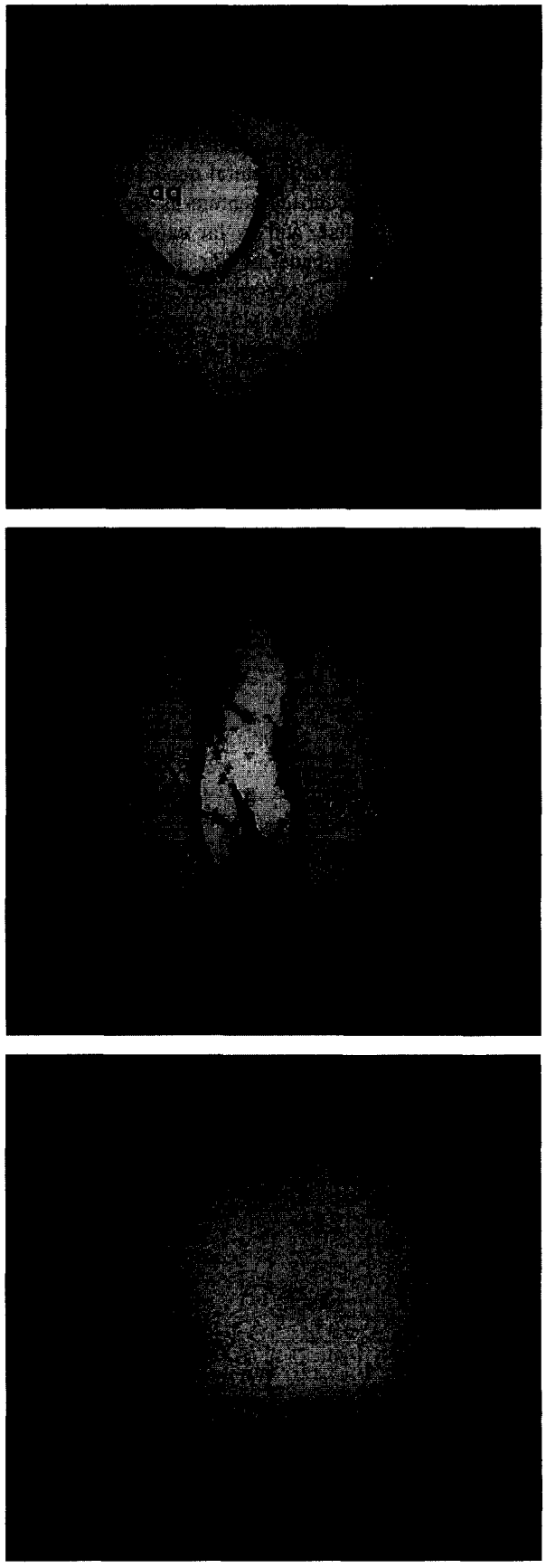

Fig. 1. The top panel is a representative photomicrograph of a mesencephalic cannula placement in the ventrolateral quadrant of the periaqueductal gray (PAG) which elicited analgesia following morphine microinjections. The middle panel is a representative photomicrograph of a medullary cannula placement in the nucleus raphe magnus (RMg)/nucleus reticularis gigantocellularis pars alpha (GiA) through which excitatory amino acid antagonists significantly reduced mesencephalic morphine analgesia. The bottom panel is a representative photomicrograph of a medullary cannula placement dorsal to the nucleus of the seventh cranial nerve (VII) through which excitatory amino acid antagonists failed to alter mesencephalic morphine analgesia. aq: cerebral aqueduct; IV: nucleus of the fourth cranial nerve; py: pyramidal tract. The bar denoted in the bottom panel = 500 μm.

($P < 0.05$) determined antagonist effects relative to vehicle/ morphine treatment.

Results

Histological verifications

Mesencephalic cannula placements were all localized in the lateral, ventral and ventrolateral quadrants of the PAG and immediately adjacent tegmentum as far rostral as the III cranial nerve nucleus and as far caudal as the dorsal raphe nucleus. The top panel of Fig. 1 is a representative photomicrograph of a PAG placement. RVM cannula placements were localized in either the NRM, NRGC or NRGC, pars alpha as far rostral as the genu of the VII cranial nerve and as far caudal as the nucleus of the VII cranial nerve. Misplaced medullary cannula placements were localized along the rostro-caudal extent of RVM placements, but were located lateral and dorsal to this area. The middle and bottom panels of Fig. 1 are representative photomicrographs of proper and misplaced RVM placements, respectively. There was both anatomical overlap and similar analgesic potency for mesencephalic cannula placements of rats with RVM and misplaced medullary cannula placements.

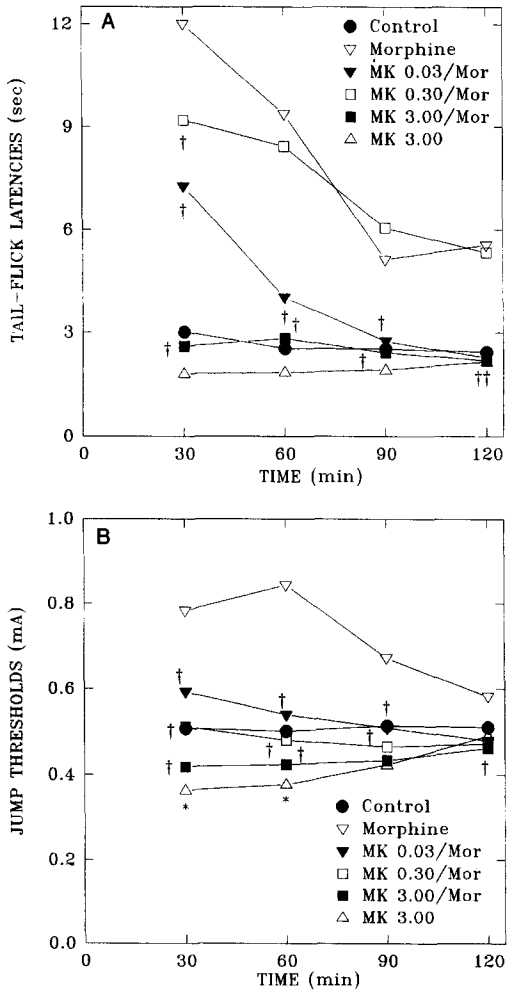

Fig. 2. Alterations in tail-flick latencies (left panel) and jump thresholds (right panel) in rats microinjected with vehicle (control) or morphine in the mesencephalic PAG region 20 min after pretreatment with MK-801 at doses of 0, 0.03, 0.3 or 3 (4.4 nmol) μg microinjected into the RVM. Significant alterations in morphine analgesia by MK-801 are denoted by crosses, and significant alterations in basal latencies and thresholds by MK-801 are denoted by stars.

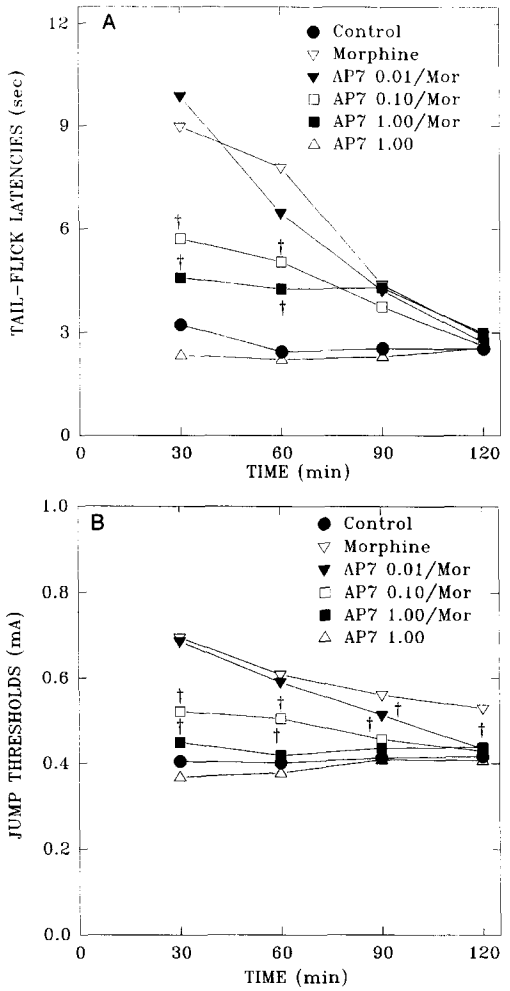

Fig. 3. Alterations in latencies (left panel) and thresholds (right panel) in rats microinjected with vehicle (control) or morphine in the PAG 20 min after pretreatment with AP7 at doses of 0, 0.01, 0.1 or 1 (7.8 nmol) μg microinjected into the RVM.

RVM MK-801 and mesencephalic morphine analgesia

Significant differences were observed among conditions (tail-flick: $F_{5,30} = 20.65$, $P < 0.0001$; jump: $F = 12.02$, $P < 0.0001$), across times (tail-flick: $F_{3,90} = 19.57$, $P < 0.0001$; jump: $F = 1.60$, ns) and for their interaction (tail-flick: $F_{15,90} = 4.09$, $P < 0.0001$; jump: $F = 3.94$, $P < 0.0001$). PAG morphine significantly increased latencies (30–120 min, Fig. 2A) and thresholds (30–90 min, Fig. 2B). In rats receiving RVM microinjections of the lowest (0.03 μg, 0.08 nmol) and highest (3 μg, 7.8 nmol) MK-801

doses, mesencephalic morphine analgesia was significantly reduced across the time course on the tail-flick test to within vehicle-vehicle levels. RVM microinjections of the middle (0.3 μg, 0.8 nmol) MK-801 dose significantly reduced mesencephalic morphine analgesia only after 30 min on the tail-flick test. All MK-801 doses in the RVM significantly reduced mesencephalic morphine analgesia across the time course on the jump test to within the range of vehicle-vehicle values. MK-801 (3 μg) in the RVM significantly decreased basal jump thresholds (30–60 min), but not basal tail-flick latencies relative to vehicle-vehicle values.

RVM AP7 and mesencephalic morphine analgesia

Significant differences were observed among conditions (tail-flick: $F_{5,48} = 9.64$, $P < 0.0001$; jump: $F = 30.14$, $P < 0.0001$), across times (tail-flick: $F_{3,144} = 42.11$, $P < 0.0001$; jump $F = 26.51$, $P < 0.0001$) and for their interaction (tail-flick: $F_{15,144} = 7.57$, $P < 0.0001$; jump: $F = 10.13$, $P < .0001$). PAG morphine significantly increased latencies (Fig. 3A) and thresholds (Fig. 3B) for up to 90 min. In rats receiving RVM microinjections of the middle (0.1 μg, 0.4 nmol) and highest (1 μg, 4.4 nmol) AP7 doses, mesencephalic morphine analgesia was significantly reduced for up to 60 min on the tail-flick test; the low (0.01 μg, 0.04 nmol) AP7 dose failed to exert effects. Whereas RVM microinjections of the middle and highest AP7 doses significantly reduced mesencephalic morphine analgesia across the time course on the jump test, the low AP7 dose significantly reduced mesencephalic morphine analgesia after 90 and 120 min. RVM microinjections of AP7 failed to alter basal latencies or thresholds.

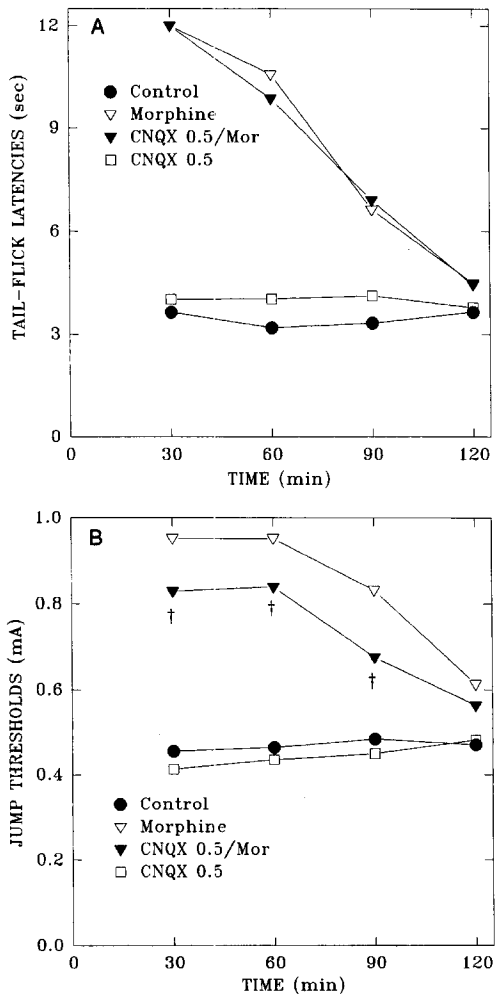

Fig. 4. Alterations in latencies (left panel) and thresholds (right panel) in rats microinjected with vehicle (control) or morphine in the PAG 20 min after pretreatment with CNQX at a dose of 0.5 μg (2.2 nmol) microinjected into the RVM.

TABLE II

ALTERATIONS IN MESENCEPHALIC MORPHINE ANALGESIA BY PRETREATMENT OF EITHER MK-801 OR AP7 IN MEDULLARY SITES LATERAL AND DORSAL TO THE ROSTRAL VENTRAL MEDULLA

Veh = Vehicle; Mor = Morphine (2.5 μg); MK-801 = (3 μg); AP7 = (1 μg). The first injection was administered into the misplaced medullary placement and the second injection was administered into the periaqueductal gray.

Condition	Post-injection time (min)			
	30	60	90	120
Tail-flick latencies (sec)				
Veh/Veh	3.25	2.64	3.12	3.05
Veh/Mor	12.00 *	10.51 *	7.29 *	3.75
MK-801/Mor	12.00 *	11.24 *	6.47 *	4.88 *
AP7/Mor	12.00 *	10.80 *	7.82 *	4.41
Jump Thresholds (mA)				
Veh/Veh	0.363	0.338	0.343	0.358
Veh/Mor	0.930 *	0.828 *	0.703 *	0.608 *
MK-801/Mor	0.785 *+	0.726 *+	0.583 *+	0.525 *
AP7/Mor	0.833 *+	0.750 *	0.600 *+	0.507 *+

* Significant difference relative to corresponding Veh/Veh (Dunnett comparison, $P < 0.05$).

\+ Significant difference relative to corresponding Veh/Mor (Dunn comparison, $P < 0.05$).

RVM CNQX and mesencephalic morphine analgesia

Significant differences were observed among conditions (tail-flick: $F_{3,20} = 41.94$, $P < 0.0001$; jump: $F = 42.13$, $P < 0.0001$), across times (tail-flick: $F_{3,60} = 32.78$, $P < 0.0001$; jump: $F = 15.46$, $P < 0.0001$) and for their interaction (tail-flick: $F_{9,60} = 10.80$, $P < 0.0001$; jump: $F = 8.58$, $P < 0.0001$). PAG morphine significantly increased latencies (30–90 min, Fig. 4A) and thresholds (30–120 min, Fig. 4B). While RVM microinjections of CNQX (0.5 μg, 2.2 nmol) failed to alter mesencephalic morphine analgesia on the tail-flick test, it produced small but significant reductions in mesencephalic morphine analgesia for up to 90 min on the jump test.

Mesencephalic morphine analgesia and NMDA antagonists in control placements

Significant differences were observed among conditions (tail-flick: $F_{3,16} = 37.29$, $P < 0.0001$; jump: $F = 17.40$,

$P < 0.0001$), across times (tail-flick: $F_{3,48} = 88.93$, $P < 0.0001$; jump: $F = 45.98$, $P < 0.0001$) and for their interaction (tail-flick: $F_{9,48} = 10.74$, $P < 0.0001$; jump: $F = 5.25$, $P < 0.0001$). PAG morphine significantly increased latencies (30–90 min) and thresholds (30–120 min) (Table II). RVM microinjections of the highest MK-801 or AP7 doses failed to significantly alter mesencephalic morphine analgesia on the tail-flick test, but produced small, but significant reductions in analgesia on the jump test.

Discussion

The present study found that RVM administration of either the non-competitive (MK-801) or competitive (AP7) NMDA antagonist significantly inhibited mesencephalic morphine analgesia. Mesencephalic morphine analgesia was eliminated by MK-801 on both tests, and was potently reduced by AP7. These effects were highly selective for three reasons. First, if either MK-801 or AP7 was microinjected into medullary cannula placements that were lateral and dorsal to the RVM, they failed to alter mesencephalic morphine analgesia on the tail-flick test, and produced small, but significant reductions on the jump test. This control suggests that the inhibitory actions of MK-801 and AP7 upon mesencephalic morphine analgesia are acting largely at the RVM injection sites, and not non-specifically through diffusion (e.g., see Nasstrom et al. 1993a,b). Second, RVM microinjections of the highest MK-801 dose failed to alter basal latencies, but produced small, but significant reductions in basal thresholds. However, the magnitude of this hyperalgesia could not account for the observed reductions in mesencephalic morphine analgesia. Further, RVM microinjections of the highest AP7 dose failed to alter basal latencies or thresholds. These data indicate that the reductions in mesencephalic morphine analgesia were not the result of offsetting hyperalgesic properties of NMDA antagonists in the RVM. Third, RVM microinjection of the AMPA/kainate antagonist, CNQX failed to alter mesencephalic morphine analgesia on the tail-flick test, but produced small, but significant reductions in mesencephalic morphine analgesia on the jump test. However, the CNQX effects were less than competitive or non-competitive NMDA antagonist effects even though the doses were of comparable molecular weights. This control suggests specificity in EEA receptor antagonist effects in the RVM upon mesencephalic morphine analgesia.

Administration of either L-glutamate or aspartate elicits analgesia from several brain sites, including the PAG (Behbehani and Fields 1979; Jacquet 1988; Siegfried and Nunes de Souza 1989) and RVM (Satoh et al. 1983b; Jensen and Yaksh 1984a,b; vanPraag and Frenk 1990; McGowan and Hammond 1993a,b). A subset of glutamate-containing and asparatate-containing cells in the PAG (Clements et al. 1987) project to the RVM (Wiklund et al. 1988; Beitz 1990). EAA transmitters and receptors modulate analgesic responses within and between the PAG and RVM. Whereas analgesia elicited by PAG NMDA was reduced by PAG AP7 (Jacquet 1988), analgesia elicited by PAG glutamate was reduced by PAG PCB, but not APV (vanPraag and Frenk 1990). Intracerebroventricular (i.c.v.) administration of MK-801 and CNQX also produce differential effects upon different forms of i.c.v. opioid analgesia in mice (Suh et al. 1995a,b). Whereas i.c.v. MK-801 blocked analgesia elicited by i.c.v. mu (morphine), $kappa_1$ (U50488H) and $delta_1$ (D-Pen^2, D-Pen^5-enkephalin: DPDPE) agonists, but not beta-endorphin analgesia, i.c.v. CNQX only blocked morphine analgesia, but not analgesia elicited by U50488H, DPDPE or beta-endorphin.

VanPraag and Frenk (1990) previously demonstrated that RVM administration of either PCB or APV significantly reduced PAG morphine analgesia. Since PCB was more potent on a molar basis than APV in exerting these effects, and since PCB, but not APV blocked RVM glutamate analgesia, it appeared that the kainate/quisqualate receptor subtype was primarily responsible for EAA modulation of analgesic responses in the RVM. The present study came to a different conclusion based upon the potent effectiveness of either competitive (AP7) or non-competitive (MK-801) NMDA receptor antagonists in the RVM to block PAG morphine analgesia as compared to RVM microinjections of CNQX, a selective AMPA/kainate antagonist. There were several procedural and methodological differences between studies. vanPraag and Frenk (1990) utilized a high (10 μg) dose of morphine in the PAG that elicited maximal analgesia at a short (7 sec) cut-off latency immediately after opiate administration. In contrast, our laboratory used a smaller (2.5 μg) dose of morphine analgesia in the PAG that produced pronounced, yet submaximal analgesia (Bodnar et al. 1988; Smith et al. 1988; Kiefel et al. 1992a,b, 1993; Rossi et al. 1993, 1994; Urban and Smith 1993, 1994). Further, we used two nociceptive tests with higher cut-off latencies and thresholds across a far longer (120 min) time course. Whereas the former procedure yields maximal analgesia that may be somewhat impervious to physiological manipulations, the latter procedure yields potent analgesia which can be more sensitive to alterations induced by physiological manipulations (e.g., Kiefel et al. 1992a,b, 1993; Urban and Smith 1993). This is consistent with the dose range of EAA antagonists employed in the two studies. Whereas micromolar doses of PCB and APV in the RVM were needed to elicit reductions of 50–70% of PAG morphine (10 μg) analgesia (vanPraag and Frenk 1990), low nmol doses of MK-801 and AP7 in the RVM were capable of virtually eliminating PAG morphine (2.5 μg) analgesia. Further, these far lower doses of EAA antagonists failed to exert effects when the medullary placement was dorsal and lateral to the RVM. It is unknown whether diffusion factors of the far higher EAA antagonist doses (see Nasstrom et al. 1993a,b) may have produced some of the effects found by vanPraag and

Frenk (1990). Given these provisos, it appears that EAA transmitters and receptors modulate supraspinal analgesic processes in general, and that NMDA receptors in the RVM modulate morphine analgesia elicited from the PAG in particular.

The integrity of the RVM is important for the full expression of analgesic responses elicited from the PAG based upon lesion studies (Behbehani and Fields 1979; Mohrland et al. 1982; Prieto et al. 1983; Morton et al. 1984) and administration of reversible neuronal blocking agents (Proudfit 1980; Gebhart et al. 1983; Sandkuhler and Gebhart 1984; Urban and Smith 1994). The neurochemical substrates mediating mesencephalic morphine analgesia in the RVM are complex, and include GABA (Heinricher et al. 1991; McGowan and Hammond 1993a,b), serotonin (Kiefel et al. 1992a,b), opioids (Heinricher et al. 1992; Morgan et al. 1992; Kiefel et al. 1993; Heinricher et al. 1994) and neurotensin (Urban and Smith 1993). The present and previous (Aimone and Gebhart 1986; vanPraag and Frenk 1990) studies indicate that EAA transmitters and receptors in the RVM are involved in the modulation of opioid analgesic processing from the PAG as well.

Acknowledgements

This research was supported in part by PSC/CUNY Grants 664055 and 665218. We thank Pennick Laboratories for morphine sulfate.

References

Abols, I.A. and Basbaum, A.I., Afferent connections of the rostral medulla of the cat: a neural substrate for midbrain-medullary interactions, J. Comp. Neurol., 201 (1981) 285–297.

Aimone, L.D. and Gebhart, G.F., Stimulation-produced spinal inhibition from the midbrain in the rat is mediated by an excitatory amino acid transmitter in the medial medulla, J. Neurosci., 6 (1986) 1803–1813.

Azami, J., Llewelyn, M.B. and Roberts, M.H.T., The contribution of nucleus reticularis paragigantocellularis and nucleus raphe magnus to the analgesia produced by systemically administered morphine, investigated with the microinjection technique, Pain, 12 (1982) 229–246.

Basbaum, A.I. and Fields, H.L., Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry, Annu. Rev. Neurosci., 7 (1984) 309–338.

Behbehani, M.M. and Fields, H.L., Evidence that an excitatory connection between the periaqueductal gray and the nucleus raphe magnus mediates stimulation produced analgesia, Brain Res., 170 (1979) 85–93.

Beitz, A.J., Relationship of glutamate and aspartate to the periaqueductal gray-raphe magnus projection: analysis using immunocytochemistry and microdialysis, J. Histochem. Cytochem., 38 (1990) 1755–1765.

Beitz, A.J., Mullett, M.A. and Weiner, L.L., The periaqueductal gray projections to the rat spinal trigeminal, raphe magnus, gigantocellualr pars alpha, and paragigantocellular nuclei arise from separate neurons, Brain Res., 288 (1983) 307–314.

Bodnar, R.J., Williams, C.L., Lee, S.J. and Pasternak, G.W., Role of mu_1 opiate receptors in supraspinal opiate analgesia: a microinjection study, Brain Res., 447 (1988) 25–34.

Clements, J.R., Madl, J.E., Johnson, R.L., Larson, A.A. and Beitz, A.J., Localization of glutamate, glutaminase, aspartate and aspartate aminotransferase in the rat midbrain periaqueductal gray, Exp. Brain Res., 67 (1987) 594–602.

Fields, H. and Basbaum, A.I., Brain control of spinal pain transmission neurons, Annu. Rev. Physiol., 40 (1978) 217–248.

Gebhart, G.F., Sandkuhler, J., Thalhammer, J.G. and Zimmerman, M., Inhibition of spinal nociceptive information by stimulation in midbrain of the cat is blocked by lidocaine microinjected in nucleus raphe magnus and medullary reticular formation, J. Neurophysiol., 50 (1983) 1446–1458.

Heinricher, M.M., Haws, C.M. and Fields, H.L., Evidence for GABA-mediated control of putative nociceptive modulating neurons in the rostral ventromedial medulla: iontophoresis of bicuculline eliminates the off-cell pause, Somatosens. Motor Res., 8 (1991) 215–225.

Heinricher, M.M., Morgan, M.M. and Fields, H.L., Direct and indirect actions of morphine on medullary neurons that modulate nociception, Neuroscience, 48 (1992) 533–543.

Heinricher, M.M., Morgan, M.M., Tortrici, V. and Fields, H.L., Disinhibition of off-cells and antinociception produced by an opioid action within the rostral ventromedial medulla, Neuroscience, 63 (1994) 279–288.

Jacquet, Y., The NMDA receptor: central role in pain inhibition in the periaqueductal gray, Eur. J. Pharmacol., 154 (1988) 271–276.

Jacquet, Y. and Lajtha, A., Morphine action at central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose, Science, 182 (1973) 490–491.

Jensen, T.S. and Yaksh, T.L., Glutamate-induced analgesia: effects of spinal serotonin, norepinephrine and opioid antagonists, Adv. Pain Res. Ther., 9 (1984a) 513–518.

Jensen, T.S. and Yaksh, T.L., Spinal monoamine and opiate systems partly mediate the antinociceptive effects produced by glutamate at brainstem sites, Brain Res., 321 (1984b) 287–289.

Jensen, T.S. and Yaksh, T.L., I. Comparison of antinocieptive action of morphine in the periaqueductal gray, medial and paramedial medulla in rat, Brain Res., 363 (1986) 99–113.

Kiefel, J.M., Cooper, M.L. and Bodnar, R.J., Inhibition of mesencephalic morphine analgesia by methysergide in the medial ventral medulla of rats, Physiol. Behav., 51 (1992a) 201–205.

Kiefel, J.M., Cooper, M.L. and Bodnar, R.J., Serotonin receptor subtype antagonists in the medial ventral medulla inhibit mesencephalic opiate analgesia, Brain Res., 597 (1992b) 331–338.

Kiefel, J.M., Rossi, G.C. and Bodnar, R.J., Medullary mu and delta opioid receptors modulate mesencephalic morphine analgesia in rats, Brain Res., 624 (1993) 151–160.

McGowan, M.K. and Hammond, D.L., Antinociception produced by microinjection of L-glutamate into the ventromedial medulla of the rat: mediation by spinal GABA-A receptors, Brain Res., 620 (1993a) 86–96.

McGowan, M.K. and Hammond, D.L., Intrathecal GABA-B antagonists attenuate the antinociception produced by microinjection of L-glutamate into the ventromedial medulla of the rat, Brain Res., 607 (1993b) 39–46.

Mohrland, J.S., McManus, D.Q. and Gebhart, G.F., Lesions in the nucleus gigantocellularis: effect on the antinociception produced by microinjection of morphine and focal electrical stimulation in the periaqueductal gray matter, Brain Res., 231 (1982) 143–152.

Morgan, M.M., Heinricher, M.M. and Fields, H.L., Circuitry linking opioid-sensitive nociceptive modulatory systems in periaqueductal gray and spinal cord with rostral ventromedial medulla, Neuroscience, 47 (1992) 863–874.

Morton, C.R., Duggan, A.W. and Zhao, Z.Q., The effects of lesions of medullary midline and lateral reticular areas on inhibition in the dorsal horn produced by periaqueductal gray stimulation in the cat, Brain Res., 301 (1984) 121–130.

Nasstrom, J., Boo, E., Stahlberg, M. and Berge, O.-G., Tissue distribution of two NMDA receptor antagonists, [^{3}H]CGS19755 and [^{3}H]MK-801,

after intrathecal injection in mice, Pharmacol. Biochem. Behav., 44 (1993a) 9–15.

Nasstrom, J., Karlsson, U. and Berge, O.-G., Systemic or intracerebroventricular injection of NMDA receptor antagonists attenuates the antinociceptive activity of intrathecally administered NMDA receptor antagonists, Brain Res., 623 (1993b) 47–55.

Prieto, G.J., Cannon, J.T. and Liebeskind, J.C., Nucleus raphe magnus lesions disrupt stimulation produced analgesia from ventral but not dorsal midbrain areas in the rat, Brain Res., 261 (1983) 53–57.

Proudfit, H.K., Reversible inactivation of raphe magnus neurons: effects on nociceptive threshold and morphine-induced analgesia, Brain Res., 201 (1980) 459–464.

Rossi, G.C., Pasternak, G.W. and Bodnar, R.J., Synergistic brainstem interactions for morphine analgesia, Brain Res., 624 (1993) 171–180.

Rossi, G.C., Pasternak, G.W. and Bodnar, R.J., Mu and delta opioid synergy between the periaqueductal gray and the rostro-ventral medulla, Brain Res., 665 (1994) 85–93.

Sandkuhler, J. and Gebhart, G.F., Relative contributions of the nucleus raphe magnus and adjacent medullary reticular formation to the inhibition by stimulation in the periaqueductal gray of a spinal nociceptive reflex in the pentobarbital-anesthetized rat, Brain Res., 305 (1984) 77–87.

Satoh, M., Kubota, A., Iwama, T., Wada, T., Yasui, M., Fujibayashi, K. and Takagi, H., Comparison of analgesic potencies of mu, delta and kappa agonists locally applied to various CNS regions relevant to analgesia in rats, Life Sci., 33 (1983a) 689–692.

Satoh, M., Oku, R. and Akaike, A., Analgesia produced by microinjection of L-glutamate into the rostral ventromedial bulbar nuclei of the rat and its inhibition by intrathecal alpha-adrenergic blocking agents, Brain Res., 261 (1983b) 361–364.

Siegfried, B. and Nunes de Souza, R.L., NMDA receptor blockade in the periaqueductal grey prevents stress-induced analgesia in attacked mice, Eur. J. Pharmacol., 168 (1989) 239–242.

Smith, D.J., Perotti, J.M., Crisp, T., Cabral, M.E.Y., Long, J.T. and Scalziti, J.N., The mu receptor is responsible for descending pain inhibition originating in the periaqueductal gray region of the rat brain, Eur. J. Pharmacol., 156 (1988) 47–54.

Suh, H.-W., Choi, Y.-S., Yoo, J.-S., Song, D.-K., Kim, Y.-H. and Tseng, L.-F., Non-NMDA receptor antagonist attenuates antinociception induced by morphine, but not beta-endorphin, D-Pen^2-D-Pen^5-enkephalin, and U50,488H administered intracerebroventricularly in mice, Neuropeptides, 28 (1995a) 125–129.

Suh, H.-W., Song, D.-K., Kim, Y.-H. and Tseng, L.-F., Differential effects of MK-801 in antagonizing antinociception induced by morphine, beta-endorphin, D-Pen^2-D-Pen^5-enkephalin and U-50,488H administered intracerebroventricularly in mice, Eur. J. Pharmacol., 263 (1995b) 217–221.

Urban, M.O. and Smith, D.J., Role of neurotensin in the nucleus raphe magnus in opioid-induced antinociception from the periaqueductal gray, J. Pharmacol. Exp. Ther., 265 (1993) 580–586.

Urban, M.O. and Smith, D.J., Nuclei within the rostral ventromedial medulla mediating morphine antinociception from the periaqueductal gray, Brain Res., 652 (1994) 9–16.

VanBockstaele, E.J. and Aston-Jones, G., Distinct populations of neurons in the ventromedial periaqueductal gray project to the rostral ventral medulla and abducens nucleus, Brain Res., 576 (1992) 59–67.

VanBockstaele, E.J., Aston-Jones, G. and Pierbone, V.A., Subregions of the periaqueductal gray topographically innervate the rostral ventral medulla in the rat, J. Comp. Neurol., 309 (1991) 305–327.

VanBockstaele, E.J., Pieribone, V.A. and Aston-Jones, G., Diverse afferents converge on the nucleus paragigantocellularis in the rat ventrolateral medulla: retrograde and anterograde tracing studies, J. Comp. Neurol., 290 (1989) 561–584.

vanPraag, H. and Frenk, H., The role of glutamate in opiate descending inhibition of nociceptive spinal reflexes, Brain Res., 524 (1990) 101–105.

Wiklund, L., Behzadi, G., Kalen, P., Headley, P.M., Nicolopoulos, L.S., Parsons, C.G. and West, D.C., Autoradiographic and electrophysiological evidence for excitatory amino acid transmission in the periaqueductal gray projection to nucleus raphe magnus in the rat, Neurosci. Lett., 93 (1988) 158–163.

Yaksh, T.L., Yeung, J.A. and Rudy, T.A., Systematic examination in the rat of brain sites sensitive to the direct application of morphine: observations of differential effects within the periaqueductal gray, Brain Res., 114 (1976) 83–103.