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Region specific expression of NMDA receptor NR1 subunit mRNA in hypothalamus and pons following chronic morphine treatment

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Abstract

The NMDA receptor has been implicated in opioid tolerance and physical dependence. Using in situ hybridization techniques, the effects of chronic morphine treatment on the expression of mRNAs encoding the NMDA receptor subunits NR1, NR2A, and NR2B were investigated. A significant increase in the level of the NR1 subunit mRNA was found in the locus coeruleus and the hypothalamic paraventricular nucleus following 3 days of intracerebroventricular (i.c.v.) morphine infusion (26 nmol μl^{-1} h^{-1}) through osmotic minipumps. No changes were detected in expression of the NR1 mRNA in the frontal cortex, caudate–putamen, nucleus accumbens, amygdala, CA1, CA2, and the dentate gyrus of the hippocampus, and in the central grey after morphine treatment. The expression of NR2A and NR2B subunit mRNAs did not change after morphine treatment in any brain region. These results suggest that changes in gene expression of the NR1 subunit of the NMDA receptor are involved in the development of morphine tolerance and dependence.

Keywords: NMDA receptor; Morphine; In situ hybridization; Tolerance; Physical dependence

1. Introduction

Chronic treatment with opioid drugs such as morphine leads to the development of both tolerance and dependence. Tolerance is a decreased sensitivity to the effects of the drug, leading to the requirement for a larger dose to achieve the desired effect. Dependence is a continued need for a drug in order to avoid physical discomfort (withdrawal symptoms). The neuropharmacological basis for opioid tolerance and dependence is demonstrably multifactorial and incompletely understood. Nevertheless, an abundance of data has implicated a critical role for excitatory amino acid, and particularly glutamatergic, systems in these two phenomena. Microdialysis studies have shown an increase in the extracellular levels of glutamate within the pontine locus coeruleus during withdrawal from morphine (Aghajanian et al., 1994; Zhang et al., 1994). Furthermore, direct intracerebroventricular (i.c.v.) or locus coeruleus injection of glutamate dose-dependently induced withdrawal signs in opioid-dependent animals (Tokuyama et al., 1996, 1998).

The *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor is widely distributed in the mammalian central nervous system and is important in a variety of forms of synaptic plasticity, including long-term potentiation (Collingridge and Bliss, 1987). In particular, they have been proposed to contribute to the neural plasticity underlying opioid addiction (Trujillo and Akil, 1991). For example, NMDA receptor antagonists have been reported to attenuate the development of opioid tolerance and dependence when they are co-administered with morphine (Marek et al., 1991a,b; Trujillo and Akil, 1991; Fundytus and Coderre, 1994), and pretreatment with an antisense oligonucleotide to the NMDA-R1 (NR1) subunit attenuated certain withdrawal signs from morphine (Zhu and Ho, 1998).

Studies based on molecular cloning have demonstrated that NMDA receptor complexes are made up of various combinations of subunits. All NMDA receptors contain at least one obligatory NR1 subunit that is combined with one or more kinds of NMDA-R2 subunits (NR2A-D) (Monyer et al., 1992; Nakanishi, 1992). Experiments in vitro have shown that NR1 subunits can form a homo-oligomeric structure that has channel activity, whereas NR2 subunits produce functional receptors only when they

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are coexpressed with NR1 (Kutsuwada et al., 1992; Monyer et al., 1992; Nakanishi, 1992). NMDA receptors with different NR1 and NR2 subunit combinations have different electrophysiological and pharmacological properties (Nakanishi, 1992). The NR1 subunit is expressed ubiquitously throughout the central nervous system, while NR2 subunits display specific distributions in the brain (Nakanishi, 1992).

The present experiment examined the effects of chronic morphine treatment on the expression of the mRNAs encoding the NMDA receptor subunits NR1, NR2A, and NR2B using an in situ hybridization technique.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 250–275 g were purchased and housed in groups of three or four animals in each cage. They were maintained in the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved facility under conditions of 21 ± 2°C and a 12–12 h light–dark cycle with free access to food and water for a week before surgery. All experimental protocols were reviewed and approved by the University of Mississippi Medical Center Institutional Animal Care and Use Committee.

2.2. Chemicals

Reagents including [α-^{35}S]dATP (1250 Ci/mmol), an oligonucleotide 3′ end labeling kit and the purified oligodeoxynucleotide probes complementary to rat NMDA receptor subunit cDNA of NR1 (a mixture of NR1-1: 5′-CGT GCG AAG GAA ACT CAG GTG GAT ACT CTT GTC AGA GTA GAT GGA-3′; residues 375–420, and NR1-2: 5′-ATA GTT GGC AAA CTT CCG GTC CCC ATC CTC ATT GAA TTC CAC ACG-3′; residues 1011–1056), NR2A (5′-AGA AGG CCC GTG GGA GCT TTC CCT TTG GCT AAG TTT C-3′; residues 567–579), and NR2B (5′-GGG CCT CCT GGC TCT CTG CCA TCG GCT AGG CAC CTG TTG TAA CCC-3′; residues 557–572) were obtained from DuPont-NEN (Boston, MA). All other chemicals were obtained from Sigma (Saint Louis, MO) or Research Biochemicals International (Natick, MA).

2.3. Induction of morphine dependence

Rats were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \times 7H_2O$, 0.972 g sodium pentobarbital, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make a final volume of 100 ml), 0.3 ml/100 g body weight, i.p., and then were placed in a stereotaxic apparatus. A stainless steel guide cannula (21 gauge, 10 mm long) was implanted into the right lateral cerebral ventricle (AP: −0.5 mm, LAT: +1.3 mm, and DV: −4.5 mm; Paxinos and Watson, 1986) of each rat. The presence of cerebrospinal fluid in the guide cannula was examined as verification of proper placement. Dental acrylic cement (Lang Dental, Wheeling, IL) was applied to the surface of the skull, and a protective aluminum cap was placed around the cannula and anchored to the skull by three screws. A stylet (26 gauge stainless steel tubing) was placed into the guide cannula to ensure that the cannula would remain patent. After surgery, rats were given 300,000 units of procaine penicillin G, s.c., to prevent infection, and were allowed at least a week to recover.

Morphine dependence was induced by continuous i.c.v. infusion with morphine sulfate (26 nmol μl^{-1} h^{-1}) for 3 days through osmotic minipumps (Alzet 2001, Alza, Palo Alto, CA). According to previous studies (Horan and Ho, 1991), this dose and period of morphine infusion can successfully produce physical dependence. A control group received an i.c.v. infusion of saline (1 μl/h). Before introduction into the pump, the solutions were passed through 0.2 μm sterile Acrodisk™ filters (Gelman Science, Ann Arbor, MI). The minipumps were primed overnight at 35°C in sterile saline so that the nominal flow rate (1 μl/h) was attained prior to implantation. Under halothane anesthesia, rats were implanted s.c. with osmotic minipumps between the scapulae. A 4-cm piece of Tygon tubing (0.38 mm inner diameter, Cole–Palmer, Chicago, IL) was used to connect the outlet of the minipump to a piece of 'L'-shaped stainless steel injector tubing (26 gauge, 20 mm long), which was placed into the i.c.v. guide cannula.

2.4. In situ hybridization

After 3 days of i.c.v. infusion, the connecting tube between the i.c.v. cannula and the outlet of the minipump was disconnected. Animals were sacrificed by rapid decapitation. The brains were dissected from the skull, frozen in liquid nitrogen and stored at −80°C. Coronal sections of 14 μm thickness were made using an AMES microtome-cryostat II (Miles Laboratories, Naperville, IL) at −20°C and thaw mounted on gelatin-coated microscope slides. The slides were dried at room temperature and stored at −80°C until used.

The oligonucleotide probe was labeled on its 3′ end using terminal deoxynucleotidyl transferase and [α-^{35}S]dATP. Ten picomoles of the probe in the tailing buffer (0.1 M potassium cacodylate, 25 mM Tris base, 1.0 mM cobalt chloride, and 0.2 mM dithiothreitol, pH 7.0) and 50 pmol [α-^{35}S]dATP were incubated with 36 units of terminal deoxynucleotidyl transferase at 37°C for 30 min. The reaction was terminated by adding 400 μl cold (4°C) Nensorb reagent A (0.1 M Tris–HCl, pH 7.7, 10 mM triethylamine, 1 mM EDTA). The labeled probe was puri-

fied using Nensorb 20 column chromatography and was eluted in 50% ethanol. The probe solution was diluted with hybridization buffer [50% formamide, 10% dextran sulfate, 1% Denhardt's solution, 100 mM dithiothreitol, 0.025% tRNA from *Escherichia coli* and 0.05% DNA from salmon testes in 4 × standard sodium citrate buffer (SSC, 1 × SSC: 0.15 M NaCl and 15 mM sodium citrate, pH 7.0).

Frozen slides were dried at room temperature for 10 min, and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 10 min. The slides were

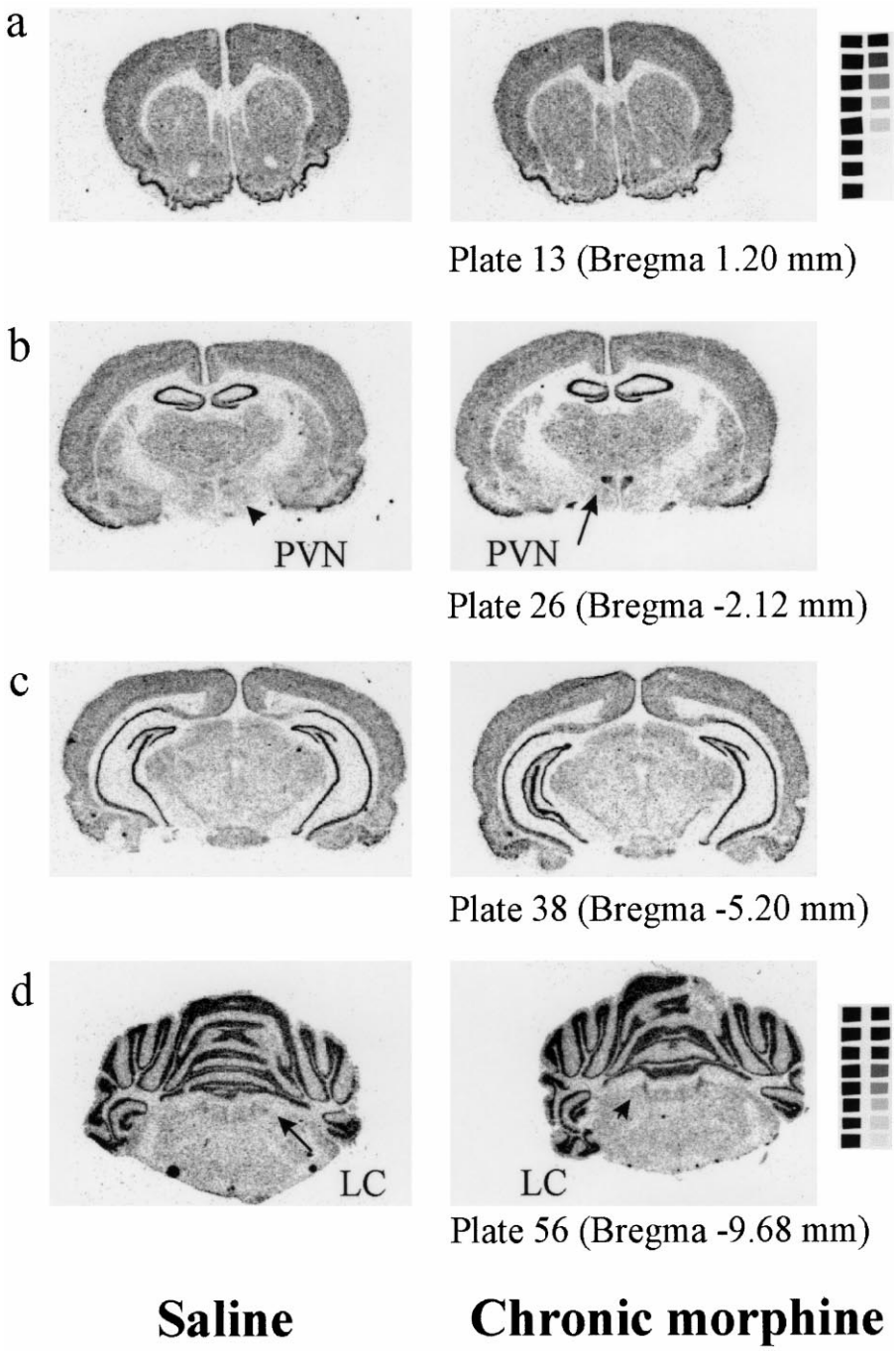

Fig. 1. Representative autoradiograms of NMDA receptor NR1 subunit mRNA distribution in 14 μm coronal sections from saline control (left column) and chronic morphine-treated (right column) rat brains. Arrows point to sections containing the hypothalamic paraventricular nucleus and the locus coeruleus. Note the increased densities in sections from the morphine-treated rat. Sections correspond (from a to d) to approximately plate numbers 13, 26, 38, and 56, respectively, from the atlas of Paxinos and Watson (1986). The inserts show the autoradiograms of ^{14}C-plastic standard sets. The exposure time of a, b, and c was 2 weeks. The exposure time of d was 3 weeks. PVN, the hypothalamic paraventricular nucleus; LC, locus coeruleus.

Table 1
The effects of chronic morphine treatment on the expression of NMDA receptor NR1 subunit mRNA

Regions	mRNA levels (pCi/mg tissue)	
	Saline	Chronic morphine
Forebrain		
Frontal cortex	87.5 ± 3.7 ($n = 6$)	95.7 ± 3.7 ($n = 6$)
Caudate–putamen	70.5 ± 3.1 ($n = 6$)	72.8 ± 4.4 ($n = 6$)
Nucleus accumbens	68.8 ± 2.1 ($n = 6$)	75.1 ± 6.2 ($n = 6$)
Diencephalon		
Amygdala	72.8 ± 2.8 ($n = 5$)	75.6 ± 4.2 ($n = 5$)
PVN	82.4 ± 1.6 ($n = 5$)	115.0 ± 15.9 ($n = 5$)[a]
Hippocampus		
CA1	226.3 ± 10.9 ($n = 6$)	191.9 ± 9.7 ($n = 6$)
CA2	244.8 ± 10.8 ($n = 6$)	241.6 ± 20.5 ($n = 6$)
Dentate gyrus	178.2 ± 6.2 ($n = 6$)	175.6 ± 4.1 ($n = 6$)
Brainstem		
Locus coeruleus	105.1 ± 4.2 ($n = 7$)	132.7 ± 6.0 ($n = 9$)[a]
Central grey	70.4 ± 4.6 ($n = 6$)	61.1 ± 6.2 ($n = 6$)

Data are expressed as mean values ± S.E.M. (pCi/mg) of four consecutive 14 μm sections each from at least five animals.
Numbers in parentheses refer to the number of animals from which the indicated brain areas were evaluated.
[a] $P < 0.05$, compared to saline-treated values (two-way ANOVA and Newman–Keuls test). PVN, hypothalamic paraventricular nucleus.

then rinsed three times in PBS for 3 min, rinsed once in 2 × SSC for 3 min, rinsed briefly in deionized water and dried in a stream of room air. Each brain slice was hybridized with 30 μl hybridization buffer under a coverslip to prevent tissue drying and incubated overnight at 40°C in a high-humidity environment. After hybridization, the coverslips were carefully removed in 1 × SSC and the slides were washed in 1 × SSC three times for 3 min each to remove the excess hybridization buffer. Slides were then washed four times, 15 min each, in 2 × SSC + 50% formamide at 55°C, following by washing in 1 × SSC at room temperature twice for 15 min each. Finally, slides were briefly washed in deionized water and dried in a stream of room air. Competition hybridization was performed by adding an 80-fold excess of unlabeled probe, which showed negligible non-specific hybridization in the final image.

Dried tissue sections were apposed to Hyperfilm-βmax (Amersham, Arlington Heights, IL), along with ^{14}C-plastic standard slide (ARC-146, ARC, St. Louis, MO) for 2 to 3 weeks. Films were developed in D-19 developer and Rapid-Fix fixer (Eastman Kodak, Rochester, NY). The sections were stained with Cresyl violet after exposure. The optical densities of the hybridization signals were quantified using a digital scanning densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA). Tissue equivalent hybridization values (pCi/mg wet brain tissue) were derived from optical density values using a standard curve obtained from readings of ^{14}C-plastic standard sets.

2.5. Statistics

Data were expressed as mean values ± S.E.M., in pCi/mg wet brain tissue. The mean values were determined from five to nine rats for each group. Two-way analysis of variance (ANOVA), followed by the Newman–Keuls test, was performed to statistically differentiate mean values among groups. Significance was set at $P < 0.05$.

3. Results

Representative autoradiograms of the distribution of the NMDA receptor NR1 subunit mRNAs in coronal sections from control and morphine-infused rats are shown in Fig. 1. The distribution of the NR1, NR2A, and NR2B subunit mRNAs in both groups agreed with those reported previously (Kutsuwada et al., 1992; Meguro et al., 1992; Monyer et al., 1992). Thus, the NR1 mRNAs in the present study were expressed widely throughout the rat brain with high levels in the cortex, hippocampus, and cerebellar granule cell layer. The distribution of NR2A and NR2B subunit mRNAs was more regionally restricted than that of NR1.

Continuous i.c.v. infusion with morphine sulfate (26 nmol μl^{-1} h^{-1}) resulted in significant increases ($P < 0.05$)

Table 2
The effects of chronic morphine treatment on the expression of NMDA receptor NR2A subunit mRNA

Regions	mRNA levels (pCi/mg tissue)	
	Saline	Chronic morphine
Forebrain		
Frontal cortex	21.6 ± 1.1 ($n = 6$)	21.9 ± 2.1 ($n = 6$)
Caudate–putamen	14.4 ± 1.2 ($n = 6$)	14.4 ± 1.3 ($n = 6$)
Nucleus accumbens	13.6 ± 1.3 ($n = 6$)	15.0 ± 1.4 ($n = 6$)
Diencephalon		
Amygdala	20.8 ± 1.0 ($n = 5$)	22.4 ± 1.4 ($n = 5$)
PVN	17.7 ± 1.8 ($n = 5$)	18.3 ± 1.2 ($n = 5$)
Hippocampus		
CA1	57.6 ± 4.4 ($n = 6$)	59.1 ± 5.9 ($n = 6$)
CA2	71.5 ± 4.3 ($n = 6$)	66.2 ± 7.6 ($n = 6$)
Dentate gyrus	65.6 ± 5.5 ($n = 6$)	66.5 ± 2.4 ($n = 6$)
Brainstem		
Locus coeruleus	10.9 ± 0.5 ($n = 7$)	12.0 ± 0.8 ($n = 9$)
Central grey	11.0 ± 1.3 ($n = 6$)	11.8 ± 1.1 ($n = 6$)

Data are expressed as mean values ± S.E.M. (pCi/mg) of four consecutive 14 μm sections each from at least five animals.
Numbers in parentheses refer to the number of animals from which the indicated brain areas were evaluated. There is no significant difference among the groups. PVN, hypothalamic paraventricular nucleus.

Table 3
The effects of chronic morphine treatment on the expression of NMDA receptor NR2B subunit mRNA

Regions	mRNA levels (pCi/mg tissue)	
	Saline	Chronic morphine
Forebrain		
Frontal cortex	42.9 ± 3.1 ($n = 6$)	44.0 ± 1.2 ($n = 6$)
Caudate–putamen	41.3 ± 2.2 ($n = 6$)	41.5 ± 1.4 ($n = 6$)
Nucleus accumbens	39.2 ± 2.9 ($n = 6$)	43.1 ± 2.2 ($n = 6$)
Diencephalon		
Amygdala	42.5 ± 3.8 ($n = 5$)	46.0 ± 3.2 ($n = 5$)
PVN	32.3 ± 1.8 ($n = 5$)	36.7 ± 3.5 ($n = 5$)
Hippocampus		
CA1	172.5 ± 10.2 ($n = 6$)	195.8 ± 14.3 ($n = 6$)
CA2	171.0 ± 6.9 ($n = 6$)	182.2 ± 6.8 ($n = 6$)
Dentate gyrus	156.5 ± 9.1 ($n = 6$)	167.5 ± 8.4 ($n = 6$)
Brainstem		
Locus coeruleus	45.1 ± 4.6 ($n = 7$)	53.0 ± 2.8 ($n = 9$)
Central grey	45.3 ± 2.2 ($n = 6$)	49.8 ± 2.8 ($n = 6$)

Data are expressed as mean values ± S.E.M. (pCi/mg) of four consecutive 14 μm sections each from at least five animals.
Numbers in parentheses refer to the number of animals from which the indicated brain areas were evaluated. There is no significant difference among the groups. PVN, hypothalamic paraventricular nucleus.

in the hybridization signals of NR1 subunit mRNA in the locus coeruleus (126% of control values) and in the hypothalamic paraventricular nucleus (140% of control values) (Table 1). No changes in NR1 mRNA expression were detected in the frontal cortex, caudate–putamen, nucleus accumbens, amygdala, hippocampal regions (CA1, CA2, and the dentate gyrus), and in the central grey after morphine treatment. There were no statistically significant alternation in the expression of NR2A and NR2B subunit mRNAs in any brain region examined after chronic morphine treatment (Tables 2 and 3).

4. Discussion

The NMDA receptor has been proposed to play a role in the tolerance to and physical dependence on opioids. Systemic administration of NMDA receptor antagonists can inhibit the development of tolerance to and physical dependence on morphine (Marek et al., 1991a,b; Trujillo and Akil, 1991; Fundytus and Coderre, 1994). Recently, using an antisense technique to down-regulate the NMDA receptor, that is, with 6 days of i.c.v. treatment with an antisense oligonucleotide to the NMDA-R1 subunit mRNA, such down-regulation has been noted to attenuate certain withdrawal signs in morphine-dependent rats (Zhu and Ho, 1998).

The present study shows that chronic treatment with morphine induces changes in mRNA levels of the NMDA receptor NR1 subunit selectively in brain nuclei known to be involved in the development of tolerance to and dependence on morphine. A significant increase in levels of the mRNA for the NR1 subunit was found in the locus coeruleus and the hypothalamic paraventricular nucleus. However, the expression of NR2A and NR2B subunit mRNAs did not change after morphine treatment. These results suggest that regulation of NMDA receptor mRNA expression is involved in the development of morphine tolerance and dependence and that this regulation is highly regionally specific.

The pontine locus coeruleus plays an important role in opioid dependence. During morphine withdrawal, there is an increase in firing of locus coeruleus neurons associated with the behavioral aspects of the withdrawal syndrome (Aghajanian, 1978; Rasmussen et al., 1990). Recently, several lines of evidence suggest that activation of excitatory amino acid inputs to the locus coeruleus contributes to the hyperactivity of locus coeruleus neurons and the behavioral symptoms associated with opioid withdrawal (for review, see Rasmussen, 1991; Nestler et al., 1994; Zhu et al., 1998). Excitatory amino acid antagonists have been shown to suppress the activation of locus coeruleus neurons induced by naloxone-precipitated withdrawal (Rasmussen and Aghajanian, 1989; Tung et al., 1990; Akaoka and Aston-Jones, 1991) and lesions of the medullary nucleus paragigantocellularis (PGi), a major excitatory input to the locus coeruleus, have also been shown to attenuate the withdrawal-induced increased firing of locus coeruleus neurons (Rasmussen and Aghajanian, 1989). Microdialysis studies have shown a significant increase in the extracellular concentration of glutamate within the locus coeruleus during withdrawal from morphine (Aghajanian et al., 1994; Zhang et al., 1994). Direct evidence from our laboratory has shown that i.c.v. or intracoerulear injection of glutamate dose-dependently induced withdrawal signs in opioid-dependent, but not in opioid naive, animals (Tokuyama et al., 1996, 1998). Administration of the selective NMDA receptor antagonist, APH (2-amino-7-phosphonoheptanoic acid), has been shown to attenuate the naloxone-induced increase in the catechol oxidation current signal, which reflects neuronal activity in the locus coeruleus (Hong et al., 1993). Direct injection of NMDA into the locus coeruleus region of morphine-dependent rats can dose-dependently induce withdrawal signs (Tokuyama et al., personal communication). Such results are consistent with the hypothesis that at the presynaptic level within the locus coeruleus, a rapid release of glutamate is a key factor for the expression of withdrawal from dependence on opioids. In the present study, an increase in levels of NR1 mRNA in the locus coeruleus area was found after 3 days of continuous i.c.v. morphine treatment, a regimen known to induce both tolerance and dependence. It has been reported that locus coeruleus neurons express mRNAs encoding several NR1 subunit isoforms (4a, 2a > 2b, 4b) as well as NR2 subunits (2B > 2D), indicating that NMDA receptors in the locus

coeruleus are composed of unique combinations of the subunits, e.g., 4a–2B (Luque et al., 1995). However, the changes in the level of the NR2B subunit mRNA in the locus coeruleus following morphine treatment did not reach the level of significance. The observed increase in the level of NMDA receptor NR1 subunit mRNA indicates either an increase in the rate of translation of NMDA receptor proteins or an increased stability of levels of existing mRNA for the receptor.

The mechanism underlying this observation is not clear. It has been reported that acute morphine treatment inhibits glutamate release (Coutinho-Netto et al., 1980; Crowder et al., 1986). Therefore, the glutamate-mediated excitation of postsynaptic NMDA receptors within the locus coeruleus may be lessened, resulting in a reduction in the firing of locus coeruleus neurons. During chronic morphine administration, the opioid-induced decline in presynaptic glutamate release may trigger an adaptive increase in the expression of postsynaptic NMDA receptors to maintain synaptic homeostasis. The fact that a normal firing rate of locus coeruleus neurons is observed during chronic opioid treatment (i.e., in the tolerant/dependent state) may be due to the achievement of an altered balance between a reduction of glutamate release and an increase of number and/or affinity of postsynaptic NMDA receptors. Opioid antagonist-precipitated withdrawal abruptly eliminates any inhibitory influence exerted by an opioid in this system and elicits an exaggerated presynaptic release of glutamate. The presynaptic overflow of glutamate release and the up-regulation of postsynaptic NMDA receptors may act together to result in hyperactivity of locus coeruleus neurons and the expression of withdrawal behaviors. The above hypothesis focuses on the excitatory amino acid inputs to locus coeruleus neurons. However, intrinsic changes are also involved in withdrawal-induced hyperactivity of locus coeruleus neurons (for review see Guitart and Nestler, 1993; Nestler et al., 1994). Acutely, opioids hyperpolarize locus coeruleus neurons by increasing the conductance of a K^+ channel via pertussis toxin-sensitive G proteins (North and Williams, 1985; Aghajanian and Wang, 1986), and decreasing a Na^+-dependent inward current (Alreja and Aghajanian, 1993). Chronic opioid treatment results in increased levels of G proteins (Nestler et al., 1989), adenylate cyclase (Duman et al., 1988), cAMP-dependence protein kinase (Nestler and Tallman, 1988), and several phosphoproteins (Guitart and Nestler, 1989; Guitart et al., 1990) in the locus coeruleus. This up-regulation of G-protein/cAMP system contributes to the locus coeruleus hyperactivity during morphine withdrawal (Rasmussen et al., 1990).

The role of the locus coeruleus NMDA receptors in the opioid dependence/withdrawal has been questioned by Rasmussen et al. (1991). These authors suggested that ionotropic non-NMDA receptors mediate the withdrawal-induced hyperactivity of locus coeruleus neurons since systemic (s.c. or i.p.) administration of the NMDA antagonists did not suppress the withdrawal-induced activation of locus coeruleus neurons or the withdrawal-induced increase in norepinephrine turnover, although the behavioral responses to withdrawal were effectively inhibited. In contrast, central (either i.c.v. or intracoerulear) injection of three separate types of excitatory amino acid antagonists, i.e., non-selective, NMDA-selective and non-NMDA-selective, have been found to decrease morphine withdrawal-induced hyperactivity of locus coeruleus neurons, with the best effects produced by the non-selective excitatory amino acid antagonist (Akaoka and Aston-Jones, 1991). The disparities between these studies may be due to the route of administration of the NMDA antagonists. The results of Akaoka and Aston-Jones (1991) supported the involvement of the NMDA receptor in opioid dependence and withdrawal, and are consistent with the present findings.

The paraventricular nucleus of the hypothalamus is also involved in opioid addiction. It has been reported that the frequency of spontaneous action potentials, both in vivo and in brain slices, and expression of c-*fos* (a nuclear transcription factor that provides a biochemical measurement of neuronal activity) in the hypothalamic paraventricular nucleus are increased during withdrawal from morphine (Christie et al., 1997). Naloxone-induced morphine withdrawal leads to increased secretion of adrenocorticotropic hormone (ACTH, Zimmerman et al., 1975) and levels of both preproenkephalin A and corticotrophin releasing hormone (CRH) mRNAs in the hypothalamic paraventricular nucleus (Lightman and Young, 1987, 1988; Harbuz et al., 1991). Whether glutamatergic systems are involved in these changes is still unclear. However, direct injection of NMDA into the hypothalamic paraventricular nucleus induces several behaviors, including grooming, penile erection, and yawning (Roeling et al., 1991; Melis et al., 1994) that are frequently observed during withdrawal from dependence on morphine. In the present study, the expression of NMDA receptor NR1 subunit mRNA in the hypothalamic paraventricular nucleus was found to be increased in rats receiving a continuous morphine treatment that has been shown to induce tolerance/dependence. Similar changes in NR1 gene expression (Bartanusz et al., 1995) and in the content of preproenkephalin A and CRH mRNAs (Lightman and Young, 1987, 1988; Harbuz and Lightman, 1989) in this brain area have been noted following stress. This suggests a hypothesis that the responses to chronic morphine treatment and stress may share a common pathway through hypothalamic paraventricular nucleus neurons.

There are other brain regions that play a role in opioid tolerance/dependence, such as central grey and spinal cord (Christie et al., 1997). Although glutamatergic transmission is inhibited in the central grey by acute administration of μ-opioid receptor agonists (Chieng and Christie, 1994), the opioid withdrawal-induced depolarization of central grey neurons is not inhibited by the administration

of NMDA or non-NMDA receptor antagonists, indicating no involvement of glutamatergic system in opioid dependence in this brain area (Chieng and Christie, 1996). It has been found that the expression of NMDA-R1 subunit mRNA in the dorsal horn of the lumber enlargement of spinal cord was also increased after chronic intrathecal administration of morphine (Dr. Zhi He, personal communication). The spinal cord was not examined in the present study. However, these data, when considered in the light of studies using central vs. peripheral routes of opioid administration, may indicate different patterns of neural involvement in opioid dependent/withdrawal, depending on the route of opioid administration.

In conclusion, chronic i.c.v. treatment with morphine resulted in increased expression of NMDA-R1 mRNAs only within the pontine locus coeruleus and hypothalamic paraventricular nucleus. These results indicate that highly selective modulation of NMDA receptor subunit gene expression may be involved in the development of morphine tolerance and dependence.

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