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Short communication

Acute treatment with morphine augments the expression of serine racemase and D-amino acid oxidase mRNAs in rat brain

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Abstract

To obtain further insight into the interactions between the *N*-methyl-D-aspartate receptor and opioid receptor systems, we have investigated the effects of the acute treatment of morphine on the expression of serine racemase and D-amino acid oxidase mRNAs in several brain areas of rats. The morphine administration produced a dose-dependent and transient elevation in the levels of serine racemase and D-amino acid oxidase mRNAs in all the brain areas. The present results are the first to suggest an interaction between the expression of the mRNAs for the D-serine-related enzymes and the opioid receptor activation.

Keywords: D-Amino acid oxidase; Morphine; NMDA (*N*-methyl-D-aspartate) receptor; D-Serine; Serine racemase

1. Introduction

A large body of evidence has demonstrated that activation of the *N*-methyl-D-aspartate (NMDA) receptor plays an important role in the opioid receptor-mediated events, such as the antinociception, tolerance, and dependence (Mao, 1999; Nestler and Aghajanian, 1997; Noda and Nabeshima, 2004; Przewlocki, 2004; Trujillo, 2002; Trujillo and Akil, 1991). The intrathecal (i.t.) administration of the NMDA receptor channel blocker, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d] cyclohepten-5,10-imine (MK-801), and competitive glutamate site antagonist, D-2-amino-5-phosphonopentanoic acid (D-AP5), has been shown to enhance antinociception by the i.t. administration of morphine using the tail-flick test (Wong et al., 1996). In contrast to the influence of the NMDA receptor antagonists at the spinal level, the intracerebroventricular (i.c.v.) administration of MK-801 attenuates the antinociception by the i.c.v. administration of morphine using the tail-flick and hot-plate tests, suggesting interactions between the NMDA and μ-opioid receptors at the supraspinal level (Suh et al., 1994). Interestingly, the i.c.v. administration of D-serine, an agonist to the glycine site of the NMDA receptor, enhances the antinociceptive effects of the subcutaneous (s.c.) administration of morphine in the rat formalin test (Hunter et al., 1994).

The NMDA-glycine receptor has a number of regulatory sites including a binding site for L-glutamate and a binding site for glycine or D-serine (Hashimoto and Oka, 1997). Especially, the occupation of the glycine site on the NMDA receptor by glycine or D-serine is absolutely required for the activation of the NMDA receptor (Matsui et al., 1995). A wide variety of evidence has indicated that a high level of D-serine occurs in the mammalian brain (Hashimoto and Oka, 1997; Hashimoto et al., 1993; Schell et al., 1995). D-serine is predominantly concentrated in the forebrain, where the NMDA receptors are enriched (Hashimoto and Oka, 1997; Hashimoto et al., 1993; Schell et al., 1995). Because D-serine potentiates the NMDA receptor-mediated transmission by selective stimulation of the glycine site of the NMDA receptor (Matsui et al., 1995), D-

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serine has been proposed as an endogenous coagonist for the NMDA receptor-associated glycine site in the mammalian brain (Hashimoto and Oka, 1997; Hashimoto et al., 1993).

Serine racemase that catalyzes the direct formation of D-serine from L-serine has been cloned from the mammalian brain (Konno, 2003; Wolosker et al., 1999). Several lines of evidence have demonstrated that the distribution of serine racemase and those of the endogenous D-serine and NMDA receptors share a similar regional pattern, with a higher level in the forebrain and a lower level in the hindbrain (Hashimoto et al., 1993; Wolosker et al., 1999; Yoshikawa et al., 2004a). In contrast, D-amino acid oxidase (DAO), which catalyzes the oxidative deamination of neutral D-amino acids, is confined to the hindbrain (Horiike et al., 1994; Schell et al., 1995; Yoshikawa et al., 2004b). Because D-serine is predominantly concentrated in the forebrain, the regional distribution of DAO in the brain inversely correlates with both those of D-serine and serine racemase (Hashimoto and Oka, 1997; Hashimoto et al., 1993; Horiike et al., 1994; Schell et al., 1995; Yoshikawa et al., 2004a,b). We have recently revealed that the acute administration of MK-801 (0.4 mg/kg) enhances the expression of serine racemase and DAO mRNAs in the rat brain, suggesting a link between the gene expression of the D-serine-metabolizing enzymes and the antagonism of the NMDA receptors (Yoshikawa et al., 2004a,b).

Despite a large number of studies indicating the involvement of the NMDA receptors in the behavioral evidence, such as antinociception, tolerance, and dependence associated with morphine (Mao, 1999; Nestler and Aghajanian, 1997; Noda and Nabeshima, 2004; Trujillo, 2002; Trujillo and Akil, 1991), it remains unclear how morphine modulates the NMDA receptor activity within the brain. To gain further insight into the interactions between the NMDA and opioid receptor systems, we investigated the acute effects of morphine on the expression of serine racemase and DAO mRNAs in the discrete brain areas of rats.

2. Materials and methods

The present animal experiments were performed in strict accordance with the guidelines of Tokai University, and were approved by the Animal Investigation Committee of the university. Male Wistar rats at postnatal week 7 were used in this study. Morphine was dissolved in physiological saline and then intraperitoneally injected. In the time-dependency experiment of morphine (20 mg/kg), the rats were stunned and decapitated 2, 4 or 8 h after the administration. In the dose-dependency experiment of morphine (10, 20 or 40 mg/kg), the rats were stunned and decapitated 4 h after the administration. The gene expression of the serine racemase and DAO was determined by the real-time polymerase chain reaction using the glyceraldehyde 3-phosphate dehydrogenase gene as an internal control and primers specific for serine racemase and DAO mRNAs as previously described (Yoshikawa et al., 2004a,b). These results are given as means with S.E.M. of the data. A statistical evaluation was carried out using the one-way analysis of variance followed by Dunnett's test. A P-value < 0.05 was considered as reaching statistical significance.

3. Results

Fig. 1A shows the time course of the changes in the levels of the serine racemase mRNA after the acute treatment with morphine (20 mg/kg). Following the administration of morphine, the levels of the serine racemase mRNA in all the brain areas significantly increased, peaked at 4 h, and then decreased. The levels increased by 23–46% in the seven brain areas examined 4 h after the administration: striatum (30% increase), hippocampus (25%), cortex (23%), diencephalon (42%), midbrain (44%), pons–medulla (46%), and cerebellum (41%). Fig. 1B shows the time course of the changes in the levels of the DAO mRNA after the acute treatment with morphine (20 mg/kg). Following the administration, the levels of the DAO mRNA in all the brain areas significantly increased and peaked at 4 h, and then decreased. The levels increased by 38–71% in the seven brain areas examined 4 h after the administration: striatum (52% increase), hippocampus (64%),

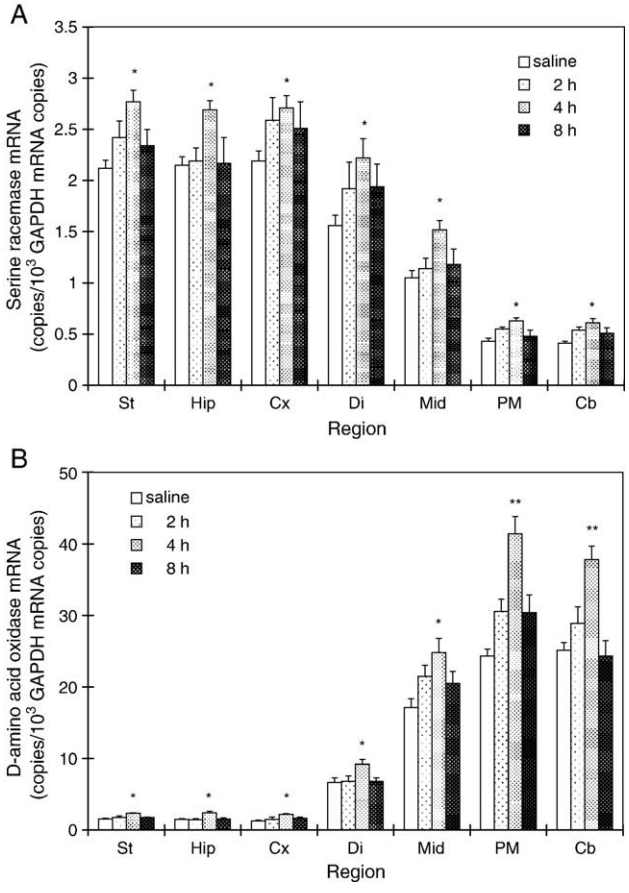

Fig. 1. Time course of changes in the gene expression of serine racemase (A) and D-amino acid oxidase (B) in several areas of the rat brain after acute treatment with morphine (20 mg/kg, i.p.). Results are means with S.E.M. of data obtained from four to five rats. *$P<0.05$; **$P<0.01$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons–medulla; Cb, cerebellum.

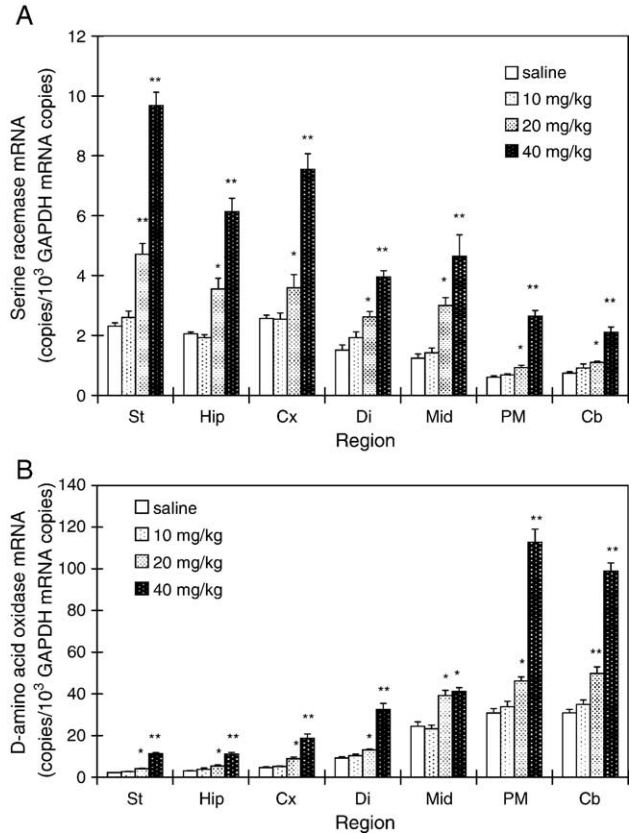

Fig. 2. Effects of increasing doses of morphine (10, 20 or 40 mg/kg, i.p.) on the gene expression of serine racemase (A) and D-amino acid oxidase (B) in several areas of the rat brain. Results are means with S.E.M. of data obtained from five rats. *$P<0.05$; **$P<0.01$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons–medulla; Cb, cerebellum.

cortex (71%), diencephalon (38%), midbrain (45%), pons–medulla (70%), and cerebellum (50%).

As shown in Fig. 2A, the acute administration of morphine (10, 20 or 40 mg/kg) caused a dose-dependent increase in the expression of serine racemase mRNA in all the brain areas. Following the administration of morphine (40 mg/kg), the levels increased by 162–340% in the seven brain areas examined 4 h after the administration: striatum (319% increase), hippocampus (199%), cortex (194%), diencephalon (162%), midbrain (274%), pons–medulla (340%), and cerebellum (184%). As shown in Fig. 2B, the acute treatment of morphine (10, 20 or 40 mg/kg) produced a dose-dependent elevation in the expression of DAO mRNA in all the brain areas. Following the administration of morphine (40 mg/kg), the levels increased by 69–417% in the seven brain areas examined 4 h after the administration: striatum (417% increase), hippocampus (264%), cortex (310%), diencephalon (251%), midbrain (69%), pons–medulla (266%), and cerebellum (210%).

4. Discussion

The present study demonstrated that the acute administration of morphine caused a transient and dose-dependent elevation in the expression of serine racemase and DAO mRNAs in most parts of the brain. The present findings are the first to suggest an association between the gene expression of the D-serine-related enzymes and the opioid receptor activation.

A transient elevation in the levels of serine racemase mRNA was found in all the brain areas after the administration. Because the acute administration of morphine induces immediate early genes, c-*fos* and *junB*, and activator protein-1 (*AP-1*) binding in the striatum and nucleus accumbens via dopamine D1 and NMDA receptors (Liu et al., 1994), and because the acute morphine administration elicits the AP-1 DNA binding activity that was accompanied by the increase in the AP-1-dependent transcriptional activation in Neuro2A MOR cells (Bilecki et al., 2004; Przewlocki, 2004), the AP-1 transcription factor could be involved in the upregulation of serine racemase by morphine. This possibility is further supported by the fact that the induction of serine racemase by inflammatory stimuli has recently been demonstrated to be dependent on AP-1 (Wu and Barger, 2004).

The acute morphine treatment caused a transient elevation in the gene expression of DAO in most parts of the brain. Because DAO is a potential down-stream target of the transcription factor CREB (cAMP response element binding protein) due to the presence of CRE-like binding sites in the promoter region of the human DAO gene (Fukui and Miyake, 1992), and because several transcription factors, such as c-Fos, FosB and CREB, have been identified to be regulated by morphine (Bilecki et al., 2004; Guitart et al., 1992; Przewlocki, 2004), the cAMP signaling pathway could play an important role in the regulation of DAO mRNA. Indeed, the acute morphine treatment regulates the levels of the phosphorylated (activated) CREB (Bilecki et al., 2004; Guitart et al., 1992; Przewlocki, 2004).

A moderate augmentation in the level of serine racemase mRNA and a slight increase in the level of DAO mRNA were observed in the forebrain after the morphine administration (20, 40 mg/kg), whereas the morphine administration produced a moderate enhancement in the level of DAO mRNA and a slight elevation in the level of serine racemase mRNA in the hindbrain such as the cerebellum and pons–medulla. Because serine racemase synthesizes D-serine from L-serine (Konno, 2003; Wolosker et al., 1999) and DAO catalyzes the oxidative deamination of D-serine (Hashimoto and Oka, 1997; Horiike et al., 1994), the acute morphine treatment might lead to the synthesis of D-serine by serine racemase in the forebrain and the degradation of D-serine by DAO in the hindbrain. Further investigations such as Western blot analysis, measurement of the D-serine concentration and chronic morphine administration are required to elucidate the mechanism underlying the induction of serine racemase and DAO mRNAs by morphine.

The NMDA receptors have been implicated in the development of tolerance to and dependence on morphine (Mao, 1999; Nestler and Aghajanian, 1997; Noda and Nabeshima, 2004; Trujillo, 2002; Trujillo and Akil, 1991). Because the acute and chronic administration of the NMDA-glycine site antagonists increases the antinociceptive potency of morphine and blocks morphine tolerance in the tail flick test (Lufty et al., 1995), and because the i.c.v. administration of D-serine potentiates the antinociception produced by morphine

against both the acute and tonic phases at the supraspinal level (Hunter et al., 1994), drugs affecting serine racemase and DAO might be beneficial for the management of pain and prevention of morphine tolerance.

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