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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%),

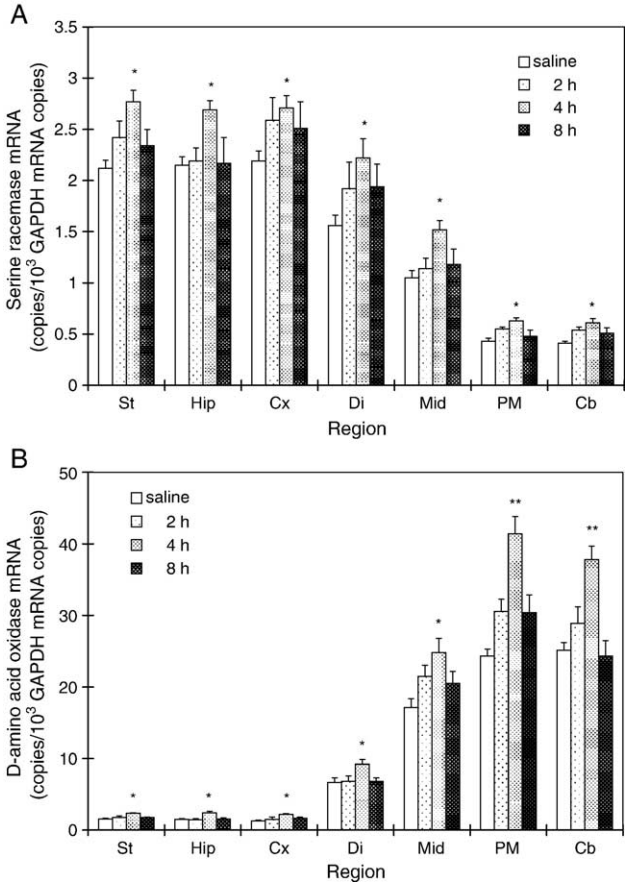

Fig. 1. Time course of changes in the gene expression of serine racemase (A) and D-amino acid oxidase (B) in several areas of the rat brain after acute treatment with morphine (20 mg/kg, i.p.). Results are means with S.E.M. of data obtained from four to five rats. *$P<0.05$; **$P<0.01$ as compared with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