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Short communication

Acute effects of morphine on the expression of mRNAs for NMDA receptor subunits in the rat hippocampus, hypothalamus and spinal cord

Pierre Le Grevès *, Wan Huang, Qin Zhou, Madeleine Thörnwall, Fred Nyberg

Department of Pharmaceutical Biosciences, Division of Biological Research on Drug Dependence, Box 591, Uppsala University, S-751 24 Uppsala, Sweden

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Abstract

The acute effects of subcutaneously injected morphine on transcripts of the NMDA receptor subunits NR1, NR2A and NR2B in certain areas of the central nervous system of male rats were examined by Northern blot analysis. The result clearly indicated that a single dose (10 mg/kg) of the opioid alters the expression of the mRNA for receptor subunits in the hippocampus and hypothalamus 4 h after drug injection. No change in the mRNA levels was observed 30 min following injection, and after 24 h most of the levels were restored to control values. The observation suggests that morphine affects this type of glutamate receptor already in the acute phase of its administration. © 1998 Elsevier Science B.V.

Keywords: Morphine, acute; NMDA receptor subunits NR1, NR2A and NR2B; Hippocampus; Hypothalamus; Spinal cord; (Rat)

1. Introduction

The NMDA subtype of glutamate receptors has been implicated in the development of tolerance to and dependence of several drugs of abuse, such as amphetamine, cocaine and morphine. The evidence for this is mainly based on the pharmacological effects of different NMDA receptor antagonists. For example, the non-competitive NMDA receptor antagonist dizocilpine maleate (MK-801) has been shown to inhibit the development of tolerance to and physical dependence on morphine (Trujillo and Akil, 1991). The majority of the reports on this issue involve studies on long-term neuronal changes subsequent to chronic exposure to drugs. However, it has become evident that NMDA receptor antagonists also can attenuate acute responses to cocaine (Karler and Calder, 1992) and amphetamine (Kelley and Delfs, 1994). It seems that both behavioral and neuroadaptive effects of morphine and psychostimulants depend on activation of NMDA receptors (Jerziorsky et al., 1994; Ohno et al., 1994), which in turn are proposed to regulate dopaminergic activity (Krebs et al., 1991). Furthermore, MK-801 has been shown to inhibit the development of morphine tolerance at spinal sites (Gutstein and Trujillo, 1993), and both chronic (Bhargava et al., 1995) and acute (Kreeger et al., 1995) administration of morphine alter the NMDA receptor in rat and mouse spinal cord, respectively.

Although the influence of chronic administration of drugs of abuse on the regulation of the NMDA receptor are well documented (Tokuyama et al., 1996), there are fewer data on the acute effects. Since a recent study in rats indicated that naloxone can precipitate withdrawal signs after a single dose of morphine (Schulteis et al., 1997), the question arises whether the glutamate system is involved in this mechanism as well. To examine this issue further, in the present study we investigated the effect of a single dose of morphine on the expression of mRNAs for NMDA receptor subunits in the rat hippocampus, hypothalamus and spinal cord.

2. Materials and methods

Male Sprague–Dawley rats (200–220 g) (Alab, Sweden) were housed under controlled environmental conditions in an air-conditioned room with controlled humidity (60%) at a temperature of 21 ± 1°C. Commercial food pellets (R36; Lactamine, Södertälje) and tap water were supplied ad libitum. Rats were injected subcutaneously

* Corresponding author. Tel.: +46-18-174169; fax: +46-18-501920; e-mail: pierre.legreves@farmbio.uu.se.

with morphine HCl (10 mg/kg) and control rats were similarly injected with saline. The rats were divided into three groups, with respect to survival time, with 6 morphine- and 6 saline-treated rats in each group. The animals were decapitated 0.5 h, 4 h or 24 h after the administration of morphine.

Brain tissues were rapidly removed on ice, using a rat brain matrix (Activational System, Mortella Drive Warren, MI), and placed on dry ice. Tissues were kept at −80°C until further analysis. Total RNA was extracted according to the method of Chomczynski and Sacchi (1987). The Northern blot procedure has been described elsewhere (Le Grevès et al., 1997a). The plasmids containing the cDNA for the NR1, NR2A and NR2B subunits were generous gifts from Professor S. Nakanishi, (Kyoto University, Kyoto). The hybridization signals from the NMDA receptor subunit mRNAs were quantified by a digital image analysis system, using NIH Image software (W. Rasband, NIMH, Bethesda, MD), and normalized to that of GAPDH (glyceraldehyde 3-phosphate dehydrogenase) mRNA. The values were mathematically transformed so that the mRNA mean values for the control group were set to 100%.

Data were analyzed by using the unpaired Student *t*-test, where $P < 0.05$ was considered significant.

3. Results

Fig. 1 shows a representative Northern blot of mRNAs for NR1, NR2A, NR2B and GAPDH (used as internal control) in the hippocampus. There were no significant changes in the NMDA receptor subunit mRNAs 30 min after a single dose of morphine (10 mg/kg) in any of the areas analyzed (Fig. 2). However, in the hippocampus the levels of mRNAs for all three NMDA receptor subunits decreased significantly after 4 h (NR1; $P < 0.01$, NR2A; $P < 0.05$, NR2B; $P < 0.01$). The levels were restored to control values 24 h after drug administration, except for that of the NR1 mRNA, which increased to a significantly higher level compared to that of the control group ($P < 0.05$). In the hypothalamus the mRNA levels for the NR1 and NR2B subunits were significantly decreased 4 h after administration of the drug ($P < 0.01$ and $P < 0.001$, respectively). The level of NR2B mRNA was restored after 24 h while the NR1 mRNA level increased compared to that observed at 4 h, but remained significantly lower than that of the control ($P < 0.05$). The NR2A transcript was unaffected in the hypothalamus. In the spinal cord, mRNA for the NR1 subunit remained unchanged up to 24 h after a single dose of morphine (Fig. 2).

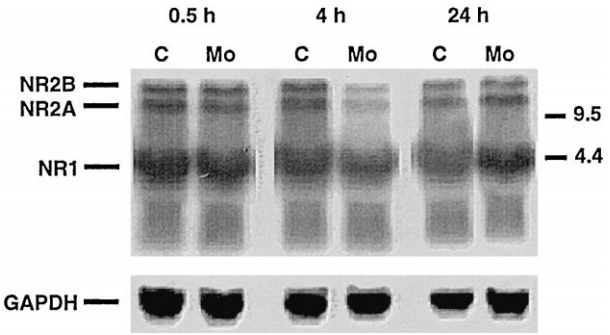

Fig. 1. Representative Northern blot analysis of mRNAs for NR1, NR2A and NR2B subunits isolated from the hippocampus of rats injected with a single dose of saline (control) or morphine (10 mg/kg). The animals were decapitated 0.5 h, 4 h or 24 h after the administration of the drug. Each well was loaded with 20 μg total RNA. The hybridization signal for GAPDH was used for correction of interlane variability in the amount of total RNA. The position of RNA molecular size (kb) standards is indicated.

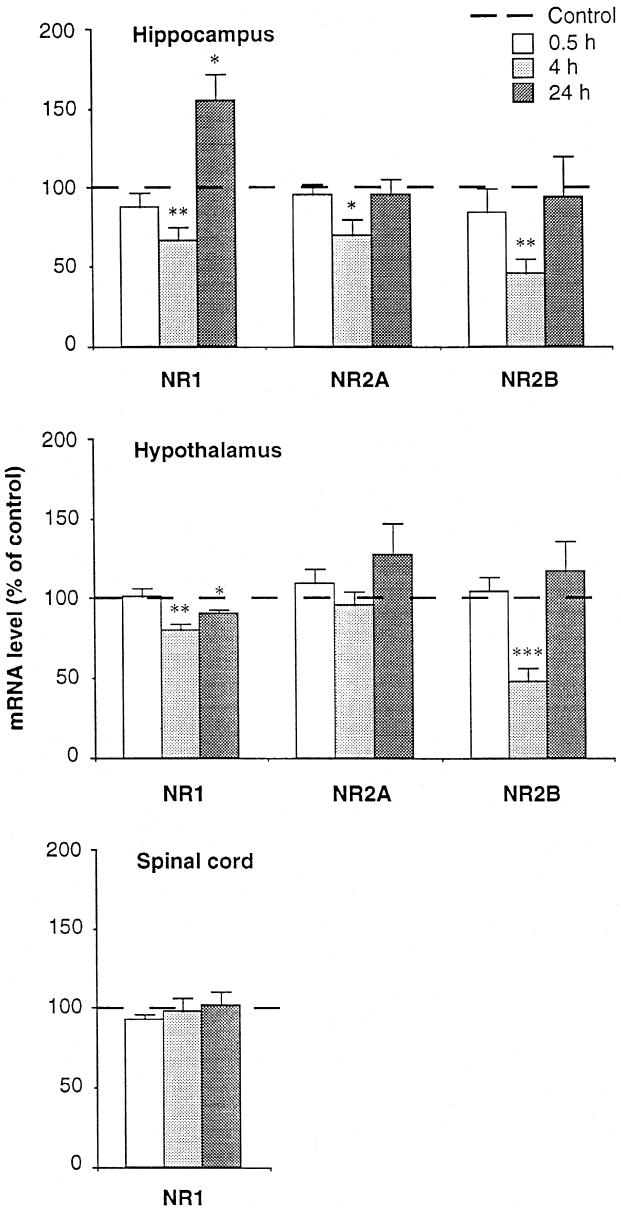

Fig. 2. Effects of saline (control) or morphine (10 mg/kg) on mRNAs for NMDA receptor subunits in rat hippocampus, hypothalamus and spinal cord at 0.5 h, 4 h or 24 h after a single injection. Each group consisted of 6 animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Student's *t*-test).

4. Discussion

Chronic opiate administration leads to physical dependence, as revealed by well-defined withdrawal signs (Schulteis et al., 1994). It has been shown that the NMDA receptor antagonist MK-801 can inhibit this action of opiates, which suggests the possible involvement of this glutamate receptor subtype (Trujillo and Akil, 1991). Withdrawal responses can also be precipitated in rats by administration of an opioid receptor antagonist following exposure to a single dose of morphine (Schulteis et al., 1997). This state, referred to as acute dependence, has been suggested to reflect the beginning of an adaptational change that leads to chronic opiate dependence. The results of the present study clearly show that a single dose of morphine alters the expression of the NMDA receptor subunit NR1, NR2A and NR2B mRNAs in the rat hippocampus and hypothalamus. The mRNAs for almost all NMDA receptor subunits studied in the brain were decreased at a time point, 4 h after the administration of the drug, which coincides with that when naloxone-precipitated withdrawal signs can be detected (Schulteis et al., 1997).

The down-regulation of the NMDA receptor transcripts would be consistent with an enhanced glutaminergic activity stimulated by opiates. Such increased activity has been proposed in both behavioral and biochemical studies in recent years (Huang et al., 1997; Pulvirenti et al., 1991). However, the effect of opiates on the release of glutamate is controversial, and it has been suggested that opioid receptor agonists, specifically κ-receptor agonists, acutely inhibit glutamate release in rat striatum (Hill and Brotchie, 1995) and in primary cultures of rat cortex (Vlaskovska et al., 1997). Nevertheless, the NMDA receptor has previously been shown to be affected by opiate administration. Bhargava et al. (1995) demonstrated a down-regulation of [^{3}H]MK-801 binding in the midbrain and spinal cord of rats chronically treated with morphine, and others have shown that repeated exposure to morphine is accompanied by a small increase in the NR1 protein level in the ventral tegmental area in rats (Fitzgerald et al., 1996). Acutely, morphine has been shown to alter NMDA receptor activity in mouse spinal cord (Kreeger et al., 1995) and in isolated guinea pig ileum (Yukhananov and Larson, 1994), while there are fewer data on the acute regulation of receptor biosynthesis. In the present study we did not see any effect of a single dose of morphine on the transcript for NR1 in the spinal cord. The treatment could of course induce changes in other NR2 mRNAs present in this area, but which are not detected by our method.

Activation of the NMDA receptor has been shown to mediate the activation of nitric oxide (NO) synthase and the formation of NO (Bredt and Snyder, 1992). Interestingly, the development of tolerance to and physical dependence on morphine can also be attenuated by NO synthase inhibitors (Kolesnikov et al., 1993), indicating a role for NO in these processes. We have in a recent study shown that 4 h of heat stress in rats results in increased levels of NO synthase and, in addition, in down-regulation of the expression of mRNAs for the NR2A and NR2B subunits in the rat hippocampus (Le Grevès et al., 1997b). Accordingly, it seems that the activation of the NMDA receptor is linked to an increase in NO synthase activity and to a compensatory down-regulation of the mRNAs encoding the NR1, NR2A and NR2B subunits.

It is notable that the NMDA receptor is affected as early as 4 h after a single dose of morphine. Schulteis et al. (1997) found a 5-fold increase in the potency of naloxone to precipitate withdrawal signs 4 h after a daily dose of morphine on the second day compared to that of the dose on the first day. This indicates that the adaptive changes leading to chronic dependence occur during the period when morphine exerts its acute effect each time it is administered. Our findings support this hypothesis, which is different from the suggestion that the NMDA receptor is mainly involved in long-term neuronal changes subsequent to the initial behavior activating effects of morphine or stimulants.

In summary, we observed an alteration in the levels of the transcripts coding for NMDA receptor subunits after a single dose of morphine. Since this glutamate receptor subtype is believed to be involved in the development of morphine dependency, the results indicate that this process may begin already after the first administration of the drug.

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