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The spinal nitric oxide involved in the inhibitory effect of midazolam on morphine-induced analgesia tolerance

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Abstract

Previous studies had shown that pretreatment with midazolam inhibited morphine-induced tolerance and dependence. The present study was to investigate the role of spinal nitric oxide (NO) in the inhibitory effect of midazolam on the development of morphine-induced analgesia tolerance. Subcutaneous injection of 100 mg/kg morphine to mice caused an acute morphine-induced analgesia tolerance model. To develop chronic morphine tolerance in mice, morphine was injected for three consecutive days (10, 20, 50 mg/kg sc on Day 1, 2, 3, respectively). In order to develop chronic tolerance model in rats, 10 mg/kg of morphine was given twice daily at 12 h intervals for 10 days. Midazolam was intraperitoneally injected 30 min prior to administration of morphine. Tail-flick test, hot-plate and formalin test were conducted to assess the nociceptive response. Immunocytochemistry, histochemistry and western blot were performed to determine the effect of midazolam on formalin-induced expression of Fos protein, nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) and nitric oxide synthase (NOS) in chronic morphine-tolerant rats, respectively. The results showed that pretreatment with midazolam significantly inhibited the development of acute and chronic morphine tolerance in mice, which could be partially reversed by intrathecal injection of NO precursor L-arginine (L-Arg). In chronic morphine-tolerant rats, pretreatment with midazolam significantly decreased the formalin-induced expression of Fos and Fos/NADPH-d double-labeled neurons in the contralateral spinal cord and NADPH-d positive neurons in the bilateral spinal cord. Both inducible NOS (iNOS) and neuronal NOS (nNOS) protein levels in the spinal cord were significantly increased after injection of formalin, which could be inhibited by pretreatment with midazolam. The above results suggested that the decrease of the activity and expression of NOS contributed to the inhibitory effect of midazolam on the development of morphine tolerance.

Keywords: Midazolam; Morphine tolerance; Spinal cord; Nitric oxide

1. Introduction

The development of tolerance to the antinociceptive effects of morphine and other opioids continues to be a significant clinical problem in the treatment of conditions associated with chronic, severe pain. Likewise, the biochemical mechanisms underlying the development of opioid tolerance have been elusive (McNally, 1999; Law et al., 2004; Waldhoer et al., 2004). Elucidation of the various mechanisms involved in this phenomenon is requisite to the development of treatment strategies to attenuate or circumvent tolerance. Previous studies have shown that coadministration of midazolam inhibited morphine-induced tolerance and dependence (Tejwani et al., 1993; Rattan and Tejwani, 1996, 1997; Tejwani and Rattan, 1997; Cao et al., 2002), but its mechanism remains unclear.

Midazolam, a benzodiazepine-receptor agonist, has been widely used for inducing and maintaining anesthesia state by

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coadministration with opioids or inhaled anesthetics in clinics. Midazolam can occupy the benzodiazepine-receptor on a benzodiazepine-gamma amino butyric acid (GABA)-Cl^- channel complex and therefore facilitate the inhibitory action of GABA on neuronal transmission (Ticku, 1983; Saano, 1987). Thomas et al demonstrated that intrathecal administration of midazolam not only potentiated the antinociceptive effect of opioids in the spinal cord, but also produced segmental antinociceptive effect, which were mediated by $GABA_A$ receptor in the spinal dorsal cord (Thomas et al., 1995; Nadeson et al., 1996). Rattan et al reported that coadministration with midazolam abolished chronic treatment with morphine-induced the increase of β-endorphin level in the spinal cord of rats (Rattan and Tejwani, 1996). Our recent study also showed that midazolam suppressed morphine withdrawal response by inhibiting the hypersensitization of the spinal cord neurons (Cao et al., 2002). In clinic, long-term intrathecal infusion of morphine combined with midazolam achieved sufficient analgesia without major adverse effects and without rapid development of tolerance towards morphine in patients with chronic nonmalignant back and leg pain due to degenerative spinal disease and multiple spinal surgeries (Rainov et al., 2001). These studies indicate that spinal cord is an important site for inhibiting morphine tolerance and dependence by midazolam.

Nitric oxide (NO), which produces from the conversion of the L-arginine (L-Arg) catalyzed by nitric oxide synthase (NOS), is an important intra- and inter-cellular messenger and plays a crucial role in a number of physiological and pathological processes within the nervous system. A growing body of evidences suggests that the spinal NO participates in the initiation and development of morphine tolerance and dependence (Kumar and Bhargava, 1997; Machelska et al., 1997; Ozek et al., 2003; Cao et al., 2000, 2001). Three different types of NOS, called neuronal, endothelial and inducible NOS, have been identified in the brain and spinal cord. Moreover, histochemical mapping of NOS revealed that NOS-positive neurons are co-localized with GABA or GABA receptor in lamina I-II of rat spinal cord (Valthschanoff et al., 1992, 1993; Spike et al., 1993; Laing et al., 1994; Heinke et al., 2004). In vivo and in vitro studies suggest that NO modulates either release or uptake of GABA and the activity of $GABA_A$ receptor or acts directly on $GABA_A$ receptor (Lonart et al., 1992; Lipton et al., 1993; Guevara-Guzman et al., 1994; Segovia et al., 1994; Zarri et al., 1994; Kano et al., 1998). Furthermore, there are evidences that the interaction between NO and GABA or GABA receptor is involved in nociceptive information modulation of the spinal cord level (Lin et al., 1999a,b; Bie and Zhao, 2001). Galley et al reported that midazolam could inhibit the activity of nNOS in rat brain (Galley and Webster, 1996). The benzodiazepine-induced antinociception is intensified by coadministration with N-nitro-L-arginine methyl ester hydrochloride (L-NAME), a non-selective NOS inhibitor, as well as 7-nitroindazole (7-NI), a neuronal NOS inhibitor, and is decreased by L-Arg in mice (Talarek and Fidecka, 2002).

Taken together, these data suggest that NO may be involved in the inhibitory effect of midazolam on the development of morphine tolerance and dependence. The aim of this study was to investigate the role of the spinal NO in the effect of midazolam on acute and chronic morphine tolerance in mice by intrathecal administration of L-Arg and the effects of midazolam on formalin-induced sensitization of the spinal cord neurons and the activity and protein expression of NOS in chronic morphine tolerance rats.

2. Materials and methods

2.1. Animals

Male Kunming mice (20–24 g) and male adult Sprague–Dawley rats (200–250 g) were provided by Experimental Animal Center of Xuzhou Medical College. The animals were housed at room temperature (22±2 °C) under a natural light–dark cycle conditions (12 h: 12 h) with food and water available ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of Xuzhou Medical College and were in accordance with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Nociceptive response and motor function assessment

The nociceptive thresholds of mice were assessed by tail-flick test. The nociceptive endpoint in tail-flick test was the characteristic withdrawal the tail from warm water (52±0.1 °C). To avoid tissue damage, a cut-off time was established at 10 s. Basal tail flick latency (TFL) was measured prior to any treatment. Mice were excluded from study if the basal TFL exceeded 5 s. TFL for each mouse was expressed as the percentage of maximum possible effect (MPE %). MPE%= (latency after medication - baseline latency)/(10 - baseline latency)×100%.

Hot-plate test was conducted to assess the nociceptive response of rats. The metal plate surface was maintained at 54±0.1 °C. Licking the hind paw was considered as nociceptive endpoint and a cut-off time was established at 30 s.

In the formalin test, 5% formalin 100 μl was injected into the plantar surface of the left hind paw of rat. Within 1 h after formalin injection, the amount of time that each rat spent displaying one of the three following behaviors was continuously recorded according to the remodified method of Dubuisson and Dennis (1977). Behavior was recorded as a '2' if the rat licked or bit the injected paw, as a '1' if the rat elevated the paw from the floor, and as a '0' if any part of the paw other than the