Epilepsy Research (2010) 91, 39—48

journal homepage: www.elsevier.com/locate/epilepsyres

Inhibition of NMDA receptor/NO signaling blocked tolerance to the anticonvulsant effect of morphine on pentylenetetrazole-induced seizures in mice

Mehdi Ghasemi[a,1], Hamed Shafaroodi[b], Saeed Nazarbeiki[c], Hossein Meskar[c], Abbas Ghasemi[a,d], Arash Bahremand[a], Pouya Ziai[a], Ahmad Reza Dehpour[a,*]

[a] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*
[b] *Pharmacology and Toxicology Department, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran*
[c] *School of Medicine, Shahed University of Medical Sciences, Tehran, Iran*
[d] *School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

Received 5 December 2009; received in revised form 31 May 2010; accepted 23 June 2010

KEYWORDS
Morphine;
Opioid tolerance;
N-Methyl-D-aspartate (NMDA) receptor;
Nitric oxide;
Seizure

Summary Although morphine has anticonvulsant effect in several animal models of seizure, its potential clinical application in epilepsy may be hindered by its adverse effects like the phenomenon of opioid tolerance. The present study evaluated the development of tolerance to the anticonvulsant effect of morphine in a model of clonic seizure induced by pentylenetetrazole (PTZ) in male Swiss mice. We also examined whether *N*-methyl-D-aspartate (NMDA) receptor/nitrergic system blockage was able to prevent the probable tolerance. Our data demonstrated that anticonvulsant effects of a potent dose of morphine (1 mg/kg) was abolished in chronic morphine-treated mice (with the same dose of morphine twice daily, 4 days, i.p.). Chronic pretreatment with low and non-effective doses of different NMDA antagonists ifenprodil (0.5 mg/kg), MK-801 (0.05 mg/kg) and ketamine (0.5 mg/kg) as well as the non-selective nitric oxide (NO) synthase inhibitor L-NAME (2 mg/kg) inhibited the development of tolerance to the anticonvulsant effect of morphine (1 mg/kg). Moreover, a single acute injection of the above mentioned agents at the same doses reversed the expression of tolerance to the anticonvulsant effects of morphine (1 mg/kg). These results demonstrate that anticonvulsant effect of morphine can be subject to tolerance after repeated administration. Both development and expression of tolerance are inhibited by NMDA receptor/nitrergic system blockage, suggesting a role for NMDA receptor/NO signaling in the development of tolerance to the anticonvulsant effect of morphine.

* Corresponding author at: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Poursina Ave., Keshavarz Boulvd., P.O. Box 13145-784, Tehran, Iran. Tel.: +98 21 6611 2802; fax: +98 21 6640 2569.
E-mail addresses: mghasem2@jhmi.edu, m82_ghasemi@yahoo.com (M. Ghasemi), dehpour@yahoo.com (A.R. Dehpour).
[1] Present address: Institute for Cell Engineering, Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

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doi:10.1016/j.eplepsyres.2010.06.010

Introduction

Opioids have been used by humans for pleasure and for treating several conditions especially pain for almost 6 millennia (Rosenblum et al., 2008; Snyder, 1979). However, one important and less known field of investigation is the modulatory effects of opioids on seizure susceptibility. Previous reports have indicated a heterogeneity of regulation by opioids of convulsive phenomena. Opioid receptor agonists such as morphine could modulate seizure susceptibility in a biphasic manner causing dose-dependent anti- and proconvulsant effects (Homayoun et al., 2002; Honar et al., 2004b; Lauretti et al., 1994; Massotti et al., 1984; Shafaroodi et al., 2007; Yajima et al., 2000; Zhang and Ko, 2009), while high dose treatment with exogenous opioids may lead to convulsions (Wikler and Altschul, 1950). Although the anticonvulsant effect of morphine is observed with doses as low as 0.5—5 mg/kg, its potential clinical application in epilepsy is hindered by side effects at this dose range like the phenomenon of opioid tolerance. This phenomenon is defined as a decrease in a subject's reaction to a repeated dose of opioids so that larger doses are required to achieve the same effect (Tso and Wong, 2003). In our recent study we for the first time demonstrated that the anticonvulsant effect of morphine in the pentylenetetrazole (PTZ) model of clonic seizure in mice undergoes tolerance due to chronic morphine administration (Roshanpour et al., 2009). However, the exact underlying mechanism has not been completely elucidated as yet.

N-Methyl-D-aspartate (NMDA) receptors are the most complex of the ionotropic receptors and play a pivotal role in excitatory neurotransmission. These ligand-gated cation channels modulates Ca^{2+} transfer from extracellular medium into the receptive neurons, resulting in the activation of several signaling pathways such as activation of nitric oxide synthase (NOS). NMDA receptors are considered as an important modulator of NOS activity within the central nervous system (Dawson et al., 1993; Dawson and Dawson, 1996a,b; Esplugues, 2002). Studies from several laboratories have implicated the NMDA/NO signaling in several different forms of drug-induced neural and behavioral plasticity, including the development of tolerance, sensitization or physical dependence to a variety of psychoactive drugs, including amphetamine, cocaine, opiates, nicotine, ethanol, benzodiazepines, barbiturates and cannabinoids (Abdel-Zaher et al., 2006; Inturrisi, 1994; Stephens, 1995; Trujillo, 2000). Marek et al. (1991a) and Trujillo and Akil (1991) provided the first evidence that blocking NMDA/NOS signaling interfere with the development of opiate tolerance and physical dependence. Using a variety of different drugs and approaches, a large number of studies have shown that NMDA receptor antagonists could prevent the development of tolerance to the analgesic effects of opiates since then (for review see Trujillo, 2000). Other studies also showed that NOS inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) are able to inhibit this phenomenon in a variety of animal pain models (Abdel-Zaher et al., 2006; Elliott et al., 1994; Kolesnikov et al., 1992). However, the issue that whether inhibition of NMDA receptor/NOS signaling could play a role in the possible tolerance to the anticonvulsant effects of opiates such as morphine has not been examined as yet. Therefore, the aims of the present study were to assess whether or not co-treatment with various NMDA receptor antagonists and a NOS inhibitor could attenuate the development of opioid tolerance. The seizure paradigm that we used to test these hypotheses was the assessment of the clonic seizure threshold induced by i.v. administration of the GABA receptor antagonist pentylenetetrazole (PTZ). This paradigm represents an animal model of seizures and is very sensitive to changes in seizure susceptibility (Löscher et al., 1991; Mandhane et al., 2007).

Experimental procedures

Chemicals

The following drugs were used throughout the study: pentylenetetrazole (PTZ), morphine chloride, N^G-nitro-L-arginine methyl ester (L-NAME), ketamine hydrochloride, dizolcipine (MK-801), ifenprodil tartrate (Sigma, Bristol, UK). All drugs were dissolved in saline (as a vehicle). All solutions were prepared immediately before the experiments. PTZ was administered intravenously (i.v.) (0.5%). All drugs were administered intraperitoneally (i.p.) in a constant volume of 5 ml/kg body weight. The doses were chosen based on previously published studies (Bahremand et al., 2009; Folbergrová, 1997; Ghasemi et al., 2009, 2010; Shafaroodi et al., 2007) and pilot experiments.

Animals

Male Swiss mice weighing 22—28 g (Pasteur Institute) were used throughout the study. Animals were housed in groups of 4—5 and were allowed free access to food and water except for the short time that animals were removed from their cages for testing. All behavioral experiments were conducted during the period between 10:00 and 13:00 with normal room light (12-h regular light/dark cycle) and temperature ($22 \pm 1\,^{\circ}C$). All procedures were carried out in accordance with the institutional guidelines for animal care and use. Each mouse was used only once, and each treatment group consisted of at least eight animals.

Determination of clonic seizure threshold

The infusion pump was adjusted to pump PTZ (0.5%) with constant rate (1 ml/min) in all the experiments (NE 1000, New Era Pump System, Inc.). A 30-gauge butterfly needle allowing access to the tail vein of mice was connected to a pump by a flexible tube which made it possible to infuse PTZ (0.5%) at a constant rate of 1 ml/min to unrestrained freely moving animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed and the dose of PTZ administered (mg/kg of mice weight) was measured as an index of clonic seizure threshold (Löscher et al., 1991; Niaki et al., 2008; Roshanpour et al., 2009; Shafaroodi et al., 2007, 2008). As a result, seizure threshold is dependent on PTZ dose administered and time-related (Bahremand et al., 2008, 2009; Shafaroodi et al., 2007, 2008).

Experiments

In experiment 1, mice in separate groups received a single injection of different doses of morphine (0 (saline), 0.5, 1, 3, 5, 15, 30 and 60 mg/kg, i.p.) 45 min before evaluating the PTZ-induced seizure threshold. In this step, the potent anticonvulsant dose of morphine was assessed for further experiments (Fig. 1).

In experiment 2, mice in separate groups received chronic injections of a potent anticonvulsant dose of morphine (1 mg/kg, twice