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Short communication

A complete analysis of NMDA receptor subunits in periaqueductal grey and ventromedial medulla of morphine tolerant mice

Ewa Kozela *, Piotr Popik

Behavioral Neuroscience, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

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Abstract

Behavioral studies indicate that the inhibition of glutamatergic system with antagonists of ionotropic glutamate NMDA receptors (NMDA-Rs) during long term morphine administration inhibits the development of morphine tolerance and dependence. In the present study we investigated whether chronic morphine treatment leading to the development of morphine antinociceptive tolerance as observed in tail-flick test in mice, may affect the expression of NMDA-R subunits. The expression of NMDA-R subunits was examined in brain areas mediating nociceptive signaling and responsible for antinociceptive activity of morphine. These included periaqueductal grey matter (PAG) and rostral ventromedial medulla (RVM). The expression of NR1 and all subunits of NR2 family (NR2A, NR2B, NR2C, NR2D) of NMDA receptor complex was examined using immunoblotting method.

Although behavioral test indicated that morphine antinociceptive tolerance developed, changes in the expression of NMDA-R subunits were not observed, either in the PAG or in the RVM.

Keywords: Morphine; Tolerance; NMDA receptor subunits; Periaqueductal grey; Rostral ventromedial medulla

1. Introduction

Chronic administration of opiates, including morphine, leads to the development of antinociceptive tolerance. Although this effect is constantly reported across literature, the neurochemical changes and mechanisms associated with this phenomenon remain unknown. Diminished activity of NMDA glutamate receptors (NMDA-Rs) during chronic morphine administration results in the inhibition of morphine tolerance and dependence (Trujillo and Akil, 1991), for review see Mao (1999). Thus, the possibility investigated in the present report was whether NMDA-Rs undergo adaptive changes due to repeated opiate administration. NMDA-R complex is comprised with NR1 subunit (in rats, in mice represented by NMDAζ1) combined with subunits of NR2 family: NR2A, NR2B, NR2C and NR2D. In mice NR2 subunits are represented by NMDAε1, NMDAε2, NMDAε3 and NMDAε4, respectively. The NR1 subunit is a component of every functional form of the NMDA-R, while the presence of particular NR2 subunits determines the properties of the NMDA-R complex including various Ca++ permeability, sensitivity to Mg++ blockade or receptor ligands (Laurie and Seeburg, 1994; Monyer et al., 1992). Subtypes of NMDA-Rs have various distributions in the central nervous system. The NR2A and NR2B are the most abundant in forebrain and limbic areas, NR2C in the cerebellum while midbrain and medullary neurons as well as spinal cord highly express NR2D subunits (Laurie et al., 1997; Wenzel et al., 1995). Previous studies concerning NMDA-R subunit expression in opioid tolerant rodents in limbic areas focused on NR1, NR2A and NR2B subunits (Fitzgerald et al., 1996; Narita et al., 2000; Zhu et al., 1999). However, the expression of NR2C and NR2D subunits has not been addressed so far.

In the present study we investigated if the development of morphine antinociceptive tolerance is accompanied by changes in the expression of NMDA-R subunits in mice in midbrain periaqueductal grey (PAG) and rostral ventromedial medulla (RVM). The PAG and RVM constitute descending pain inhibitory system and have been implicated in morphine antinociception (Basbaum and Fields, 1984) and tolerance (Lane et al., 2004; Tortorici et al., 1999). Moreover, the PAG is densely

* Correspondence to: Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, 31-343 Krakow, Poland. Tel.: +48 12 66 23 374; fax: +48 12 63 74 500.
E-mail address: kozela@rabbit.if-pan.krakow.pl (E. Kozela).

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innervated by glutamatergic projections from the forebrain and opioid receptors are co-localized with NMDA-Rs on PAG neurons (Commons et al., 1999).

2. Materials and methods

2.1. Animals

Male C57/Bl mice (22–25 g) at the beginning of experiments were housed in the standard laboratory conditions in the animal room with controlled light–dark cycle (lights on 7:00; off: 19:00) with food and tap water provided *ad libitum*. All mice were tested only once.

2.2. The tail-flick test

A tail-flick apparatus (*Columbus Instruments*) with radiant heat source was used to assess antinociceptive responses. The intensity of heat stimulus was adjusted so that the baseline latency was ~3 s. A maximum latency of 10 s (i.e., cut-off) was used to minimize damage to the tail. Two tail-flick responses were recorded for each mouse and the latencies were then averaged.

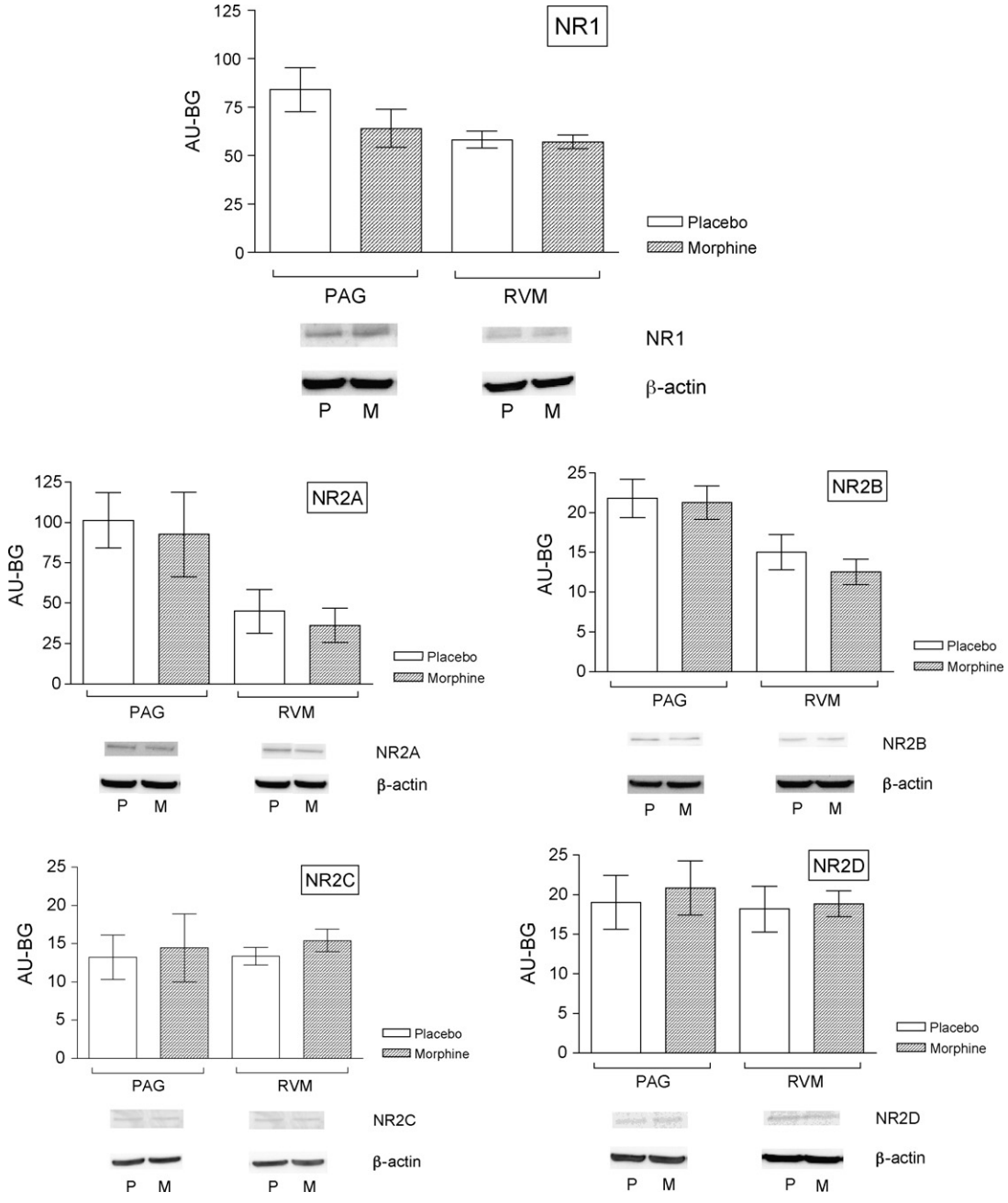

Fig. 1. The expression of protein for NR1, NR2A, NR2B, NR2C and NR2D subunits of NMDA-R in the mouse periaqueductal grey matter (PAG) and rostral ventromedial medulla (RVM) after 7-day twice daily administration with placebo or morphine (10 mg/kg). On day 8 all mice were challenged with morphine (10 mg/kg) and tissue was collected 60 min later. Data are expressed as mean arbitral luminescence units minus background (AU − BG) values ± S.E.M. Representative immunoblotting bands for all NMDA-R subunits were presented below the bars. β-Actin was used as a loading control. 'P' and 'M' below representative bands state for placebo or morphine-treated mice, respectively.