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Short communication

Potential role of spinal TRPA1 channels in antinociceptive tolerance to spinally administered morphine

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ABSTRACT

Background: Prolonged morphine treatment leads to antinociceptive tolerance. Suppression of spinal astrocytes or D-amino acid oxidase (DAAO), an astroglial enzyme catalyzing oxidation of D-amino acids, has reversed morphine antinociceptive tolerance. Since the astrocyte-DAAO pathway generates hydrogen peroxide, an agonist of the TRPA1 channel expressed spinally on nociceptive nerve terminals and astrocytes, we tested a hypothesis that the spinal TRPA1 contributes to antinociceptive tolerance to prolonged spinal morphine treatment.

Methods: Nociception was assessed using hot-plate test in rats with an intrathecal (*it*) catheter. Drugs were administered *it* twice daily from day one to seven in five treatment groups: (i) Saline, (ii) Chembridge-5861528 (a TRPA1 antagonist; 10 μg), (iii) morphine (10 μg), (iv) Chembridge-5861528 (10 μg) + morphine (10 μg), (v) DMSO. Antinociceptive action of morphine was assessed at day one and eight. Additionally, mRNA for DAAO and TRPA1 in the spinal cord was determined on day 8.

Results: Morphine treatment produced antinociceptive tolerance, which was attenuated by co-administration of Chembridge-5861528 that alone had no effect on hot-plate latencies. In animals treated with morphine only, spinal mRNA for DAAO but not TRPA1 was increased. DAAO increase was prevented by co-administration of Chembridge-5861528.

Conclusions: Antinociceptive morphine tolerance and up-regulation of spinal DAAO were attenuated in morphine-treated animals by blocking the spinal TRPA1. This finding suggests that spinal TRPA1 may contribute, at least partly, to facilitation of morphine antinociceptive tolerance through mechanisms that possibly involve TRPA1-mediated up-regulation of the astroglial DAAO, a generator of hydrogen peroxide, a pronociceptive compound acting also on TRPA1.

Introduction

Prolonged application of morphine or other μ-opioid receptor agonists leads to antinociceptive tolerance, which reduces efficacy of analgesic treatment and thereby provides a clinical problem. Previous studies indicate that multiple mechanisms may contribute to the development and maintenance of morphine antinociceptive tolerance (see for review [1]). There is accumulating evidence indicating that among mechanisms of opioid antinociceptive tolerance is activation of glia that through multiple mechanisms may reduce and counteract opioid-induced antinociception (see for reviews [2,3]).

Astrocytes are a subgroup of glial cells. It has been shown that the development of antinociceptive tolerance to spinal morphine is accompanied by increased immunostaining of spinal astrocytes and that both of these changes can be attenuated by an inhibitor of glial cells [4]. In line with this, it has been shown that morphine antinociceptive tolerance is associated with up-regulation of spinal D-amino acid oxidase (DAAO), an astroglial enzyme catalyzing oxidation of D-amino acids to hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), and a consequent elevation of spinal hydrogen peroxide level [5,6]. Genetic ablation or pharmacological inhibition of DAAO as well as hydrogen peroxide scavenger or hydrogen peroxide catalyst catalase reversed the morphine

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antinociceptive tolerance [5,6]. Together, these findings indicate that activation of spinal astroglial DAAO-hydrogen peroxide pathway contributes to antinociceptive morphine tolerance.

Here we studied a specific hypothesis that at least partly, the antinociceptive morphine tolerance induced by the spinal astroglial DAAO-hydrogen peroxide pathway is due to activation of the spinal transient potential ankyrin 1 (TRPA1) channel, a calcium-permeable non-selective cation channel [7] that is known to be activated by hydrogen peroxide [8]. In the spinal cord, TRPA1 is expressed on central terminals of nociceptive nerve fibers where it amplifies transmission [9]. Additionally, TRPA1 is expressed on astrocytes [10] that have a role in prolonged pain [11]. To test the proposed hypothesis, we attempted to prevent the development of antinociceptive tolerance to spinal administration of morphine by blocking pharmacologically the spinal TRPA1 channel. Additionally, to assess whether TRPA1 regulates astroglial DAAO in tolerance development, we determined whether block of spinal TRPA1 prevents morphine-induced increase in the expression of spinal DAAO mRNA.

Materials and methods

Animals

The experiments were performed with adult male Hannover-Wistar rats (weight: 150–200 g; CAS, Shanghai, China). All experiments were approved by the institutional ethics committees and all experimental procedures are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (publication no. 85-23, revised 1985). Rats were housed in a 12-h light/dark cycle with food and water access *ad libitum*.

Preparation and procedure for intrathecal drug injections

For spinal drug delivery, a chronic intrathecal (*it*) catheter (Intramedic PE-10, Becton Dickinson and Company, Sparks, MD, USA) was installed using a catheter-through-needle technique at the lumbar spinal cord level under sodium pentobarbital anesthesia (60 mg/kg *ip*) one week before the experiments as described in detail elsewhere [12]. Only those rats that had no motor impairment and whose hind limbs were bilaterally paralyzed following *it* administration of lidocaine (4%, 7–10 μl) were studied further. The studied drugs were administered *it* at the volume of 5 μl followed by a flush with 15 μl of saline.

Assessment of pain behavior

Animals were habituated to the experimental conditions at least two hours per day for two days before assessing heat nociception using the Intelligence Hot Plate Analgesia Meter (model YLS-6B, Shandong Academy of Medical Sciences Device Co., Shangdong, China). The surface of the hot-plate was 55 ± 0.1 °C. The latency to either hind paw licking or jumping (whichever came first) was measured to the nearest 0.1 s. Cut-off latency was 30 s.

Real-time quantitative polymerase chain reaction (RT qPCR)

Rat spinal lumbar enlargements (L3–L5) were collected and homogenized using electronic microhomogenizer at 4000 rmp for 10 s in TRIzol (Invitrogen, Grand Island, NY, USA) on ice. Total RNA of the spinal lumbar enlargements was extracted and purified by use of TRIzol reagent. The cDNA was prepared from 1 μg of total RNA by using ReverTra Ace qPCR RT-Kit (Toyobo Co. Ltd., Osaka, Japan). Real-time quantitative PCR was performed on a Mastercycler ep realplex (Eppendorf, Hamburg, Germany) using Realmaster Mix (SYBR Green I) and synthetic primers. The sequences of primers were following: DAAO: 5′-CCC TTT CTG GAA AAG CAC AG-3′ (forward), 5′-CTC CTC TCA CCA CCT CTT CG-3′ (reverse) [13]; TRPA 1: 5′-CTC AGG TTC AAT GTG TCC GTTC-3′ (forward), 5′-GTG CTG TGT TCC CTT CTT CATC-3′ (reverse) [14]; GAPDH: 5′-CCA AGG TCA TCCATG ACA AC-3′ (forward), 5′-TCC ACA GTC TTC TGA GTGGC-3′ (reverse) [15]. The 2-ΔΔCt method was used to calculate the relative expression after normalization to the internal control gene GAPDH [13].

Drugs

Morphine hydrochloride was obtained from Sinopharm (Shanghai, China) and Chembridge-5861528 (a highly selective TRPA1 channel antagonist [16]) was synthetized by ChemBridge Corporation (San Diego, CA, USA). Chembridge-5861528 was dissolved in DMSO (100%) and morphine in saline.

Course of the study

There were five *it* treatment groups: (i) morphine 10 μg twice daily ($n = 6$), (ii) Chembridge-5861528 10 μg + morphine 10 μg twice daily ($n = 6$), (iii) Chembridge-5861528 10 μg twice daily ($n = 6$), (iv) saline twice daily ($n = 6$). (v) DMSO (5 μl, 100%) twice daily *it* ($n = 6$). In each group, hot-plate latency was assessed only on the first and the last (eighth) treatment day before and 30 min after drug administrations. Each latency measurement was performed twice at about a 2 min interval; the mean of these was used in calculations. At the end of experiment, the animal was given a lethal dose of sodium pentobarbital and except for group v, the lumbar spinal cord was removed for RT qPRC analyses as described above.

Statistics

Behavioral data were analyzed using two-way mixed-ANOVA (with time as a within subjects factor and treatment as between subjects factor) followed by Bonferroni-corrected *t*-test. RT qPCR data were analyzed by one-way ANOVA followed by Student–Newman–Keuls test, $p < 0.05$ was considered to represent significant difference.

Results

Hot-plate test in morphine-treated groups

Before drug treatments, the mean baseline response latency in the hot-plate test was 3.8 s (95% confidence interval [CI] from 3.6 s to 4.0 s). On the first treatment day, morphine alone at the dose of 10 μg intrathecally (*it*) increased the hot-plate response latency by 10.6 s (95% CI of the latency increase from 7.1 s to 14.1 s). On the eight treatment day, a significant attenuation of the antinociceptive effect was observed in morphine-treated groups (main effect of treatment duration: $F_{1,10} = 55.61$, $p < 0.0001$; Fig. 1A). The reduction in the antinociceptive effect from the first to the last treatment day varied depending on whether or not morphine was accompanied by Chembridge-5861528 (interaction between drug treatment and treatment duration: $F_{1,10} = 5.80$, $p = 0.037$). *Post hoc* tests indicated that the reduction of the antinociceptive effect from the first to the last treatment day was significant between the group treated with a combination of morphine and Chembridge-5861528 and the group treated with morphine alone. Importantly, while morphine-induced antinociceptive effects in these two treatments groups were not significantly different on the first treatment day, on the last treatment day the antinociceptive effect induced by a combination of Chembridge-5861528 and morphine was stronger than that induced by morphine alone (Fig. 1A).