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Involvement of NCAM and FGF receptor signaling in the development of analgesic tolerance to morphine

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ABSTRACT

This study examined the involvement of neural cell adhesion molecule (NCAM), a member of the immunoglobulin superfamily, in the development of tolerance to morphine. Furthermore, we focused on fibroblast growth factor (FGF) receptor and protein kinase C (PKC)-α as part of the intracellular signal transduction pathways underlying NCAM stimulation. The development of analgesic tolerance to morphine was gradually observed during daily treatment of morphine (10 mg/kg, s.c.) for 5 days. Morphine treatment gradually and significantly decreased the NCAM expression levels. However it returned to normal levels immediately after re-treatment of morphine. Treatment of AS-ODN against NCAM completely inhibited analgesic tolerance to morphine. Protein expression levels of PKC-α were significantly increased by repeated morphine treatment in a NCAM-AS-ODN-reversible manner. Interestingly, alterations of protein interactions between NCAM and FGF receptor were observed under repeated morphine treatment. In addition, SU5402 (2 μg/mouse, i.c.v.), an inhibitor of FGF receptor, completely abolished the development of analgesic tolerance to morphine. Furthermore, κ-opioid receptor stimulation using U-50,488 H, a κ-opioid receptor agonist, or establishment of formalin-induced chronic pain can completely suppress these changes in protein expression levels of NCAM and PKC-α and inhibit development of analgesic tolerance to morphine. These findings suggest that NCAM and its interaction with FGF receptor in the mechanism of up-regulation of PKC-α may contribute to the development of analgesic tolerance to morphine. Chronic pain or κ-opioid receptor stimulation could modulate these phenomena and suppress the development of analgesic tolerance to morphine.

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1. Introduction

Morphine is a common and potent analgesic agent for cancer pain in palliative care. However, the chronic use of morphine is limited by the analgesic tolerance to morphine (Kissin, 2009). It is well known that morphine tolerance is mediated not only by μ-opioid receptor desensitization (Zhang et al., 1998) but also by neuronal plasticity (Ronn et al., 1998) including the change of organization of neuronal networks and their function via alterations of intracellular signaling molecules, receptors, and channels (Ueda and Ueda, 2009). We have hitherto revealed that activation of κ-opioid receptor signaling and PKC-α up-regulation in the midbrain is involved in the development of analgesic tolerance to morphine (Fujita-Hamabe et al., 2010; Hamabe et al., 2008; Tokuyama et al., 2007). Although there are several reports about the mechanism of development of morphine tolerance (Chen et al., 2008; Yang et al., 2008), the key molecule involved is still unknown.

Neural cell adhesion molecule (NCAM), a member of the immunoglobulin superfamily of cell recognition molecules, is abundantly expressed in the nervous system, and plays an important roles in neuronal development and function (Crossin and Krushel, 2000; Maness and Schachner, 2007). The alterations of function of NCAM has been known to be linked to brain disorders including schizophrenia, bipolar disorder and Alzheimer's disease in humans and to learning and memory deficits in mice (Brennaman and Maness, 2010; Todaro et al., 2004; Vawter et al., 2001). Recent evidence suggests that NCAM also participates in neurite outgrowth, activation of signal transduction cascades, and synapse formation and elimination via homophilic or heterophilic interactions with other adhesion molecules and extracellular matrix molecules (Fields and Itoh, 1996; Kleene and Schachner, 2004; Maness and Schachner, 2007). For example, NCAM interacts with fibroblast growth factor (FGF) receptor, glial cell line-derived neurotrophic factor family receptor-α, prion protein, spectrin, Fyn, and focal adhesion kinase (Kiryushko et al., 2004; Maness and Schachner, 2007; Sakai et al., 2008). The heterophilic binding of NCAM facilitates the activation of intracellular signaling molecules such as phospholipase C (PLC), protein kinase C (PKC), mitogen-activated protein kinase (MAPK) resulting in promotion of neurite outgrowth, fasciculation and synaptic plasticity (Kolkova et al., 2000). Specifically, the direct interaction between

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NCAM and FGF receptor has recently been demonstrated to increase the intracellular Ca^{2+} entry into the cytoplasm and trigger neuritogenesis (Kiryushko et al., 2004; Sanchez-Heras et al., 2006). As described previously, calcium influx and neuronal plasticity may be involved in the mechanisms of development of analgesic tolerance to morphine (Ueda and Ueda, 2009).

In this study, we determined the involvement of NCAM in the mechanisms of analgesic tolerance to morphine induced by repeated morphine treatment. In particular, we focused on the interaction between NCAM and FGF receptor and the involvement of PKC activation in the mechanism. Furthermore, the influences of κ-opioid receptor signaling and inflammatory chronic pain on the alterations of NCAM under repeated morphine treatment were also analyzed.

2. Materials and methods

2.1. Animals

Male ddY mouse (Nihon SLC, Shizuoka, Japan) weighing 28–30 g at the beginning of the experiments were used in the study. They had access to food and tap water. All experimental procedures conformed to the Guiding Principles of the Care and Use of Laboratory Animals adopted by the Japanese Pharmacological Society. In addition, all experiments were approved by the ethical committee for animals of Kobe Gakuin University (approval number: A 090130-1).

2.2. Administration of drugs

The following drugs and substances were used: morphine hydrochloride (Takeda, Osaka, Japan), antisense oligodeoxynucleotide (AS-ODN) and scramble oligodeoxynucleotide (SC-ODN) for NCAM (AS-ODN: 5′-EZEGTGACAGAAGGCAGCATEZOC-3′, SC-ODN: 5′-ZEEAAG-GAGCTCAAGGACTAOZEC-3′) (Invitrogen, CA, USA), trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methanesulfonatehydrate (U-50,488 H) (Sigma, MO, USA), nor-binaltorphimine (nor-BNI) (Sigma) formalin (Wako, Osaka, Japan) and 3-[3-(2-carboxyethyl)-4-methylpyrrol-2-methylidenyl]-2-indorinone (SU5402) (Calbiochem, CA, USA). Morphine hydrochloride, U-50,488 H and nor-BNI were dissolved in saline. Morphine hydrochloride, U-50,488 H and nor-BNI were administered s.c. or i.p., in a volume equivalent to 0.1 mL per 10 g body weight. AS-ODN and SC-ODN were dissolved in saline at 10 μg/10 μL and administered i.c.v. for 3 days before and on the first day of repeated morphine treatment (after the tail-flick test on the first day). SU5402 was dissolved in 99.9% dimethylsulfoxide (DMSO), the solution was diluted by saline before use, and 2 μg was administered by i.c.v. (4% DMSO in saline) 10 min before morphine administration. As a vehicle treatment, 4% DMSO in saline (10 μL/mouse) was administered by i.c.v. I.c.v. administrations were performed as previously described (Haley and McCormick, 1957). Briefly, a microsyringe with a 27-gauge stainless-steel needle was used for all experiments. Tubing covered all but the terminal 2.5 to 3.0 mm of the needle so as to make a track through the brain and into the lateral ventricle but not through the floor of the lateral ventricle. The needle was inserted unilaterally into the lateral ventricle of the brain (1.0 mm lateral and 1.0 mm posterior to the bregma) as previously described (Franklin and Paxinos, 2008). Verification of the needle position in the lateral cerebroventricle was made by i.c.v. dye injection and subsequent post-mortem brain section verification of dye placement (Haley and McCormick, 1957).

2.3. Inflammatory chronic pain model mice

Mice were treated with 2% formalin into the dorsal part of left hind-paw, and used for experiments 24 h after treatment as we described previously (Fujita-Hamabe et al., 2010; Hamabe et al., 2008; Tokuyama et al., 2007).

2.4. Measurement of analgesic effect

The analgesic effect of morphine was measured by using a tail-flick analgesia meter (ML330B, Muromachi Kikai, Tokyo, Japan). To avoid tissue damage, thermal stimulation was never applied for longer than 10 s. We confirmed that the tail-flick reaction time was in a normal range (2–4 s) before drugs were administered. Then measurements were taken every 30 min for 90 min after administration of morphine and using the AUC as the analgesic effect of a day. To test the development of tolerance to morphine analgesia, the analgesic effect of morphine (10 mg/kg, s.c.) was measured once a day for 5 days.

2.5. Western blot analysis

The preparation of tissue homogenate was performed as previously described (Harada et al., 2010). The protein was resolved by 12% SDS-PAGE, and analyzed by Western blot using polyclonal rabbit anti-NCAM (sc-10735, Santa Cruz, CA, USA), monoclonal mouse anti-PKC-α (sc-8393, Santa Cruz), and monoclonal mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (MAB374, Chemicon, MA, USA). Horseradish peroxidase (HRP)-labeled anti-rabbit or HRP-labeled anti-mouse (MD, USA) was used for the second antibody. Visualization was performed with ECL™ western blotting analysis system. (GE Health Care, Tokyo, Japan) The band intensities of the film were analyzed by CS-Analyzer ver. 2.0 (Atto, Tokyo, Japan).

2.6. Immunoprecipitation

Mouse midbrain lysates were immunoprecipitated at 4 °C for 4 h using rabbit anti-FGF receptor 1 antibody (sc-121, Santa Cruz) Protein A-Sepharose® 4B (Sigma) was added to the samples. For immunoprecipitation, 250 μL of cell lysates was incubated overnight at 4 °C with Protein A-Sepharose® 4B. The samples were incubated with 3 mg of anti-FGF receptor 1 antibody for 4 h. The samples were washed three-times with homogenization buffer containing 1 mg/mL of BSA. The immunocomplex was solubilized by SDS sample buffer. The proteins were resolved by 10% SDS-PAGE and analyzed by Western blot using polyclonal rabbit anti-NCAM, polyclonal rabbit anti-FGF receptor 1 antibody or monoclonal mouse anti-GAPDH antibody.

2.7. Statistics

Data are presented as mean ± S.E.M. Significant differences were evaluated by one-way analysis of variance followed by Bonferroni/Dunnett's multiple comparison tests for the comparison between more than three groups or by Student's *t*-test for the comparison between 2 groups. A *P* value below 0.05 was regarded as significant.

3. Results

3.1. Effect of NCAM AS-ODN on the development of analgesic tolerance to morphine

On day 1 of the repeated morphine treatment, a prominent analgesic effect was observed 30 min after morphine treatment and lasted for at least 90 min in both the NCAM AS-ODN and NCAM SC-ODN treated groups (Fig. 1A). However, the analgesic effect of morphine gradually decreased from day 3 of repeated morphine treatment, and was completely diminished by day 5 in the SC-ODN treated group. On the contrary, pretreatment of AS-ODN against NCAM significantly inhibited the development of analgesic tolerance to morphine (Fig. 1B).

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