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p38 MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IN AMYGDALA MEDIATES κ OPIOID RECEPTOR AGONIST U50,488H-INDUCED CONDITIONED PLACE AVERSION

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Abstract—κ opioid receptor agonists produce aversive effects in rodents, however the underlying mechanisms remain unclear. Activation of p38 mitogen-activated protein kinase (MAPK) has been discovered to play a critical role in the modulation of affective behaviors. The present study was undertaken to detect the possible involvement of p38 MAPK in the aversive effects induced by κ opioid receptor activation. We found that the κ opioid receptor agonist trans-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzenacetamide methanesulfonate salt (U50,488H) produced significant place aversion in mice as measured by the conditioned place preference procedure, accompanied with significant p38 MAPK activation in the amygdala, but not in the nucleus accumbens and hippocampus. Stereotaxic microinjection of the p38 MAPK inhibitor 4-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-5-(4-pyridyl)-1H-imidazole (SB203580) into amygdala significantly inhibited p38 MAPK activation and completely blocked the conditioned place aversion in mice. Thus, these results suggested that activation of p38 MAPK in the amygdala was required to mediate κ opioid receptor-induced aversive behavior.

Key words: κ opioid receptor, U50, 488H, conditioned place aversion, p38 MAPK.

INTRODUCTION

The three "classical" opioid receptor subtypes, mu (μ), kappa (κ), and delta (δ) are widely expressed throughout the central nervous system and sensory neurons (Pert and Snyder, 1973; Mansour et al., 1995). Among them, κ opioid receptors are important targets for the development of analgesics (Bhargava et al., 1994) without addictive potential, which attract attention in the field of drug discovery. However, κ opioid receptor agonist has been shown to cause dysphoria (Pfeiffer et al., 1986; Wang et al., 2010; Hang et al., 2015) in human and aversive effects in animals (Shippenberg and Herz, 1986; Suzuki et al., 1992; Barr et al., 1994; Land et al., 2008), which greatly restricts its clinical use. Understanding the mechanism responsible for κ-dependent aversion may help develop better analgesics.

In transfected cells and primary cultured neurons, κ agonists have been shown to activate p38 MAPK (Belcheva et al., 2005; Bruchas et al., 2006). *In vivo* studies further demonstrated that κ agonist-induced aversive emotion was accompanied with p38 MAPK activation in dorsal raphe nucleus (DRN) and ventral tegmental area (VTA) dopamine neurons (Land et al., 2009; Ehrich et al., 2015). The deletion of κ opioid receptor in VTA dopaminergic and DRN serotonergic neurons both blocked U50,488H-induced conditioned place aversion (Ehrich et al., 2015). Microinjection of κ antagonist into VTA and DRN impaired U50,488H-induced conditioned place aversion, and viral reexpression of κ opioid receptor in the DRN and VTA of κ opioid receptor knockout mice restored the conditioned place aversion (Land et al., 2009; Ehrich et al., 2015). These data implicate that p38 MAPK activation in the DRN and VTA are required to mediate κ opioid receptor-induced aversive behavior.

The amygdala is another key brain region that is associated with the aversive events in both human and rodents (Zald and Pardo, 1997; Janak and Tye, 2015). We previously demonstrated that amygdala played a critical role in aversive emotion (Hou et al., 2009; Liu et al., 2012), with evidence showing that excitotoxic lesions of the amygdala abolished the conditioned place aversion induced by morphine withdrawal. However, little is known about the role of amygdala in κ opioid receptor-induced aversive effects and the underlying mechanism. κ Opioid receptor activation of p38 MAPK pathway in amygdala is one possible mechanism for κ agonist-induced aversive emotion. Here, in the present study, we utilized a

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Abbreviations: DRN, dorsal raphe nucleus; LC, locus coeruleus; MAPK, mitogen-activated protein kinase; PB, phosphate buffer; P-p38, phospho-p38; SERT, serotonin transporter; VTA, ventral tegmental area.

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U50,488H conditioned place aversion mice model to study the mechanism of κ opioid receptor-mediated aversive behavior. We then quantified phospho-p38 (P-p38) MAPK protein levels in the amygdala after U50,488H pairing. Finally, the ability of intra-amygdala microinjection with p38 MAPK inhibitor SB203580 to block the acquisition of aversive behavior was detected.

EXPERIMENTAL PROCEDURES

Materials

U50,488H (Sigma, D5040 100 mg, St. Louis, MO, United States) was prepared in saline at concentrations of 0.25 mg/ml and 0.5 mg/ml, and the solution was given to mice at a dose of 0.1 ml/10 g by intraperitoneal administration (i.p.). SB203580 (Santa Cruz, sc-222296, CA, United States) was dissolved in DMSO and stored in −70° refrigerator at a concentration of 10 nmol/μl, before microinjection, the storage solution was diluted in PBS at a concentration of 1 nmol/μl. SB203580 was injected into amygdala (0.5 μl/side) 30 min before U50,488H pairing. Anti-p38 (Cell Signaling Technology, 9212S, Danvers, MA, United States) and anti-P-p38 antibodies (Cell Signaling Technology, 9211S, Danvers, MA, United States) were diluted in 5% milk/TBST at a concentration of 1:1000. HRP-goat anti-rabbit IgG antibody and HRP-goat anti-mouse IgG antibody were purchased from Santa Cruz Biotechnology.

Animals

Male C57BL/6 J mice (20–26 g, 8–9 weeks) were purchased from the Experimental Animal Center of Fudan University (Shanghai, China). Mice were raised eight per cage with freedom access to food and water in a temperature controlled room with 12-h light and dark (the light was on at 6:00 am). All tests were conducted as described in the National Institutes of Health Guide for the Care and Use of Laboratory. Mice were moved to the testing room 24 h before behavioral test.

Conditioned place aversion

Apparatus. The conditioned place aversion apparatus [32 cm (length) × 16 cm (width) × 38 cm (height)] made of plexiglas was divided into two equal-sized compartments by a removable partition with an opening (6 × 6 cm) at one end, which allowed mice free access to each compartment. Two compartments are distinguished by visual and tactile cues: one has a black wall and a smooth floor, whereas the other has a white wall and a textured floor. Another partition without an opening was used to confine mice to a given compartment in the conditioning phase of conditioned place aversion procedure.

Procedures. The method to establish conditioned place aversion was similar to that used by Suzuki et al. (1992). The conditioned place aversion procedure consisted of three phases: preconditioning, conditioning and testing. In the preconditioning phase, mice were allowed to freely explore the entire apparatus for 15 min, movement and time spent in each compartment were recorded with camera from above. The measurement and data were analyzed with the mouse conditioned place aversion software (Shanghai Jiliang Software Technology, Shanghai, China). The conditioning phase took place during the next 4 days. In the morning, mice were given saline (0.9% saline, 100 μl/10 g of body weight, i.p.) and assigned to the unfavored compartment. 4 h later, they were injected with U50,488H (2.5 mg/kg, 5 mg/kg, i.p.) and assigned to the preferred compartment for 45 min and this compartment would be referred as the "drug treatment-paired compartment". In the testing phase (24 h after the last conditioning trial), all mice were allowed to freely explore the entire apparatus for 15 min, and the amount of time spent in each compartment was recorded. Conditioned place aversion score represents the time in the drug treatment-paired compartment during the testing phase minus that during the preconditioning phase.

The drug (or vehicle control) was microinjected 30 min before the U50,488H (5 mg/kg) injection. Conditioned preference was assessed by allowing the mice to roam freely in both the compartments and recording the time spent respectively.

Cannulation and microinjections

Mice were anesthetized by use of sodium pentobarbital (70 mg/kg, i.p.) under aseptic conditions, and a stereotaxic instrument with nonpuncture ear bars (Narishige, Japan) was used to localize the accurate sites of cannulation. For amygdala infusion, guide cannulas (26 gauge) were placed in the amygdala (anteroposterior: −1.4 mm; mediolateral: ±3 mm; dorsoventral: −3 mm). The implanted cannulas were anchored with three screws on the skull. All mice were given 7 days for recovery in their home cages before any experiments. Mice were bilaterally injected in the guide cannula by a 33-gauge dummy cannula (Plastics One, USA) that extended 2 mm longer than the guide cannula to prevent blockage as described previously (Zan et al., 2015), and the cannulas were attached to a 10-μl microsyringe with a microinfusion pump (Harvard Apparatus, USA). 0.5 μl (each side) of vehicle or SB203580 with a rate of 200 nl/min was injected for the test of conditioned place aversion. Internal cannulas were removed at least 2 min after injection to allow diffusion. Then mice were returned to their home cages for 30 min rest before conditioned place aversion test.

Immunoblotting

Mice were decapitated, the amygdala were dissected and homogenized with a tissue homogenizer in protein lysis buffer (Beyotime Biotechnology, P0013, Wuhan, China), 1× phosphatase (Roche, 4906837001) and protease inhibitor (Roche, 11836170001, Indianapolis, IN, United States) were dissolved in the lysis buffer. Samples were homogenated and then centrifuged at 10,000*g* for 5 min at 4 °C. The lower sediments were discarded, the upper clarified liquids were mixed with 4× loading buffer, then boiled in 90 °C hot water for 10 min. Protein