Research report

TRPV1 modulates morphine-induced conditioned place preference via p38 MAPK in the nucleus accumbens

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ABSTRACT

Emerging evidence suggests that the transient receptor potential vanilloid type 1 channel (TRPV1) is a novel target for the treatment of drug addiction, such as cocaine and morphine. Previously we reported that TRPV1 inhibition reduced morphine reward in the dorsal striatum (DSt) of mice and morphine self-administration through a decrease in accumbal activity in rats. However, the role of TRPV1 on morphine-conditioned reward in addiction-related brain regions, such as the nucleus accumbens (NAc), has not been previously established. Here, we investigated the effects of TRPV1 on morphine conditioned place preference (CPP) and intracellular mechanisms of TRPV1 using Western blot analysis and immunohistochemistry (IHC) in morphine-administered mice. TRPV1 knockout mice did not exhibit morphine reward responses, and both i.p. and intra-NAc injections of SB366791, a selective TRPV1 antagonist, reduced morphine-induced CPP in wild-type mice. Furthermore, i.p. injection of SB203580, a selective p38 MAPK inhibitor, also dampened morphine-induced CPP. To determine the molecular mechanisms of the TRPV1/p38 MAPK pathway in morphine CPP, we investigated the expression of adenylyl cyclase type 1 (AC1) and phospho-p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) in the NAc. Either SB366791 or SB203580 decreased the protein expression levels of phospho-p38 MAPK, phosphor-NF-κB, and AC1 in the NAc of morphine CPP mice. Taken together, our findings suggest that TRPV1 may modulate morphine-induced conditioned reward effects via the p38 MAPK signaling pathway in the NAc. Therefore, blockade of TRPV1 may provide a novel therapeutic approach for the prevention and treatment of opioid addiction.

1. Introduction

The transient receptor potential vanilloid type 1 channel (TRPV1), a member of the TRP family, is a cation channel that is activated by capsaicin, heat, and low pH [1]. Although TRPV1 is well-known as a mediator of pain-related neuronal signaling [2,3], emerging evidence suggests that TRPV1 is a novel target that modulates many mental illnesses, such as anxiety, depression, and drug addiction [4–6]. TRPV1 is expressed and activated in the brain regions that are closely involved in drug addiction, such as the frontal cortex, hippocampus, ventral tegmental area, and striatum [5,7,8]. Although the role of TRPV1 is not well understood, several studies recently reported that the TRPV1 antagonist SB366791 inhibited the reinstatement of cocaine-seeking behavior in rats and reduced morphine-induced addictive behaviors in mice and rats, while the TRPV1 agonist capsaicin promoted morphine reward [5,9,10]. In addition, the withdrawal duration of morphine-conditioned place preference (CPP) in rats was shortened by intra-NAc injections of SB366791. In rats, a selective TRPV1 antagonist, reduced morphine-induced CPP in wild-type mice. Furthermore, i.p. injection of SB203580, a selective p38 MAPK inhibitor, also dampened morphine-induced CPP. To determine the molecular mechanisms of the TRPV1/p38 MAPK pathway in morphine CPP, we investigated the expression of adenylyl cyclase type 1 (AC1) and phospho-p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) in the NAc. Either SB366791 or SB203580 decreased the protein expression levels of phospho-p38 MAPK, phosphor-NF-κB, and AC1 in the NAc of morphine CPP mice. Taken together, our findings suggest that TRPV1 may modulate morphine-induced conditioned reward effects via the p38 MAPK signaling pathway in the NAc.

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reward signal in the brain. However, the mechanisms of this morphine reward signal are not fully understood. In our previous study, we showed that morphine reward is modulated by TRPV1 in the dorsal striatum and that p38 mitogen-activated protein kinase (MAPK) is a crucial modulator of morphine place preference [5].

The relationship between TRPV1 and p38 MAPK is well known. p38 MAPK phosphorylation in the NAc mediated the development and maintenance of morphine-induced conditioned place preference in rats [14]. In addition, a recent report showed that expression changes in TRPV1 were mediated by the phosphorylation of p38 MAPK in an inflammatory pain rodent model [15]. Furthermore, TRPV1 increased the induction of inflammatory cytokines through a p38 MAPK pathway related to oxidative stress [16]. These results support our present hypothesis that morphine reward is modulated via the TRPV1-p38 MAPK pathway in the NAc. However, the involvement of TRPV1 in morphine-conditioned reward behaviors is not well understood.

In this study, we first determined if TRPV1 knockout mice were resistant to developing morphine-induced place preference. Then, we evaluated mice behaviors in morphine place preference tests when TRPV1 was pharmacologically blocked, either systemically or specifically in the NAc. Finally, the relationship between p38 MAPK and TRPV1 in the NAc of morphine CPP mice were investigated with either SB366791 or SB203580 treatments.

2. Materials and methods

2.1. Animals

We used C57BL/6J background male mice to investigate the effects of TRPV1 in WT and knockout (KO) mice and utilized male CD-1 mice for estimating other behaviors and biochemical experiments. Male CD-1 mice (4-week-old, 18–20 g) were purchased from Koatech CO., Ltd. (Pyongtaek, Republic of Korea) and TRPV1 knockout (KO) (B6.129 × 1-Trpv1tm1jul/J) and wild-type (WT) (C57BL/6J) mice were obtained from the Orient Bio Co., Ltd (a branch of the Jackson Laboratory, Seongnam, Republic of Korea). The CD-1 mice were housed 10 per cage [(Width) 260x (Height) 420x (Depth) 180 mm] and both TRPV1 KO mice and WT mice were housed 5 per cage [(Width) 200x (Height) 260x (Depth) 130 mm]. All animals were allowed access to water and food ad libitum, and maintained at a constant temperature (23 ± 1 °C) and humidity (55% ± 5%) with a 12 h light/dark cycle (lights on 07:00–19:00 h). Mice weighed 22–26 g at the time of testing. All experiments were conducted in accordance with the NIH guidelines for the Care and Use of Laboratory Animals and with the approval of the Institutional Animal Care and Use Committee of Sungkyunkwan University.

2.2. Drugs and chemicals

Mouse anti-β-actin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rabbit anti-TRPV1 antibody was purchased from Abcam.
(Cambridge, MA, USA). Rabbit anti-total-p38 MAPK and anti-phospho-p38 MAPK (Thr180/Tyr182) antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). Rabbit anti-adenylyl cyclase type 1 (AC1) antibody was purchased from Novus Biologicals (Minneapolis, MN, USA). Goat anti-adenylyl cyclase type 8 (AC8) antibody was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Rabbit anti-phospho-nuclear factor-kappa B p65 (p-NF-κB p65) and anti-nuclear factor-kappa B p65 (NF-κB p65) antibodies were purchased from Abcam (Cambridge, UK). Anti-rabbit and anti-mouse horseradish peroxidase-linked IgG antibodies were purchased from Jackson Immunoresearch Laboratories, Inc., (West Grove, PA, USA). Morphine hydrochloride (Macfarlan Smith, Edinburgh, UK) was dissolved in physiological saline. Capsaicin, SB366791, and SB203580 (Tocris Cookson, Bristol, UK) were dissolved in physiological saline containing 5% dimethyl sulfoxide (DMSO) and 5% Tween 80 (Sigma-Aldrich). All other chemicals were of analytical grade.

2.3. Guide cannula implantation and intra-NAc injection

Mice were anesthetized with pentobarbital (50 mg/kg, i.p.) and a guide cannula (9 mm, 24-gauge) was unilaterally implanted into the NAc area. The coordinates were as follows: +1.42 mm A/P + 1.0 mm M/L and −3.7 mm D/V (Fig. 1D). Infusions were performed at a rate of 1 μL/min/site for 2 min.

2.4. Conditioned place preference (CPP) test

The CPP apparatus consisted of two compartments (15 × 15 × 15 cm) separated by closable guillotine doors. One compartment was white with a smooth floor and the other was black with a striped floor. The compartments were illuminated by dim lighting (12–13 Lux). The procedure for CPP consisted of pre-conditioning (days 1–3), conditioning (days 4–11), and post-conditioning (day 12). On days 1–3 and day 12, mice were allowed to explore both compartments. On day 3, the amount of time spent and distance moved on each side were recorded for 1200 s using a video tracking system (NeuroVision, Pusan, Korea). Subsequently, one compartment was paired to drug injection and the other compartment to saline during the conditioning phase. The conditioning phase (50 min per session) was conducted using a biased procedure and carried out daily over 8 consecutive days with alternates injections of drug or saline. We performed the following four types of conditioning phases to ascertain the role of TRPV1 on morphine CPP. 1) To estimate the effects of genetic deletion of TRPV1 on morphine CPP, both WT and TRPV1 KO mice were exposed to either morphine (5 mg/kg, i.p.) or saline on days 4, 6, 8, and 10 and saline on days 5, 7, 9, and 11. 2) To examine the effects of systemic injection of the TRPV1 antagonist SB366791, mice were pre-injected with either SB366791 (0.15, 0.3, and 0.6 mg/kg, i.p.) or saline/DMSO/Tween 80 solution 30 min before the administration of morphine (5 mg/kg, s.c.) on days 4, 6, 8, 10. They received an injection of saline in the opposite compartment on days 5, 7, 9, and 11. 3. To determine the effects of intra-NAc injections of SB366791, mice were pre-administered either SB366791 (0.2 mg/2 μL/site, unilateral) or saline/DMSO/Tween 80 solution 30 min before the injection of morphine (5 mg/kg, s.c.) on days 4, 6, 8, 10. They were treated with saline on days 5, 7, 9, and 11. 4. Lastly, to investigate the effects of p38 MAPK on morphine CPP, conditioning-phase type 2 was replicated using the p38 MAPK inhibitor SB203580 in place of SB366791. For all conditioning-phase types, on day 12, the mice were given free access to both compartments for 1200 s and the time spent and distance moved in each side was recorded using a video tracking system (NeuroVision, Pusan, Korea). We recorded 6 mice tracking behaviors per one session. CPP apparatuses were cleaned with distilled water between each session. A CPP was determined by comparing the preferences for the less-preferred side (i.e., drug-paired side) during the post-conditioning and pre-conditioning sessions.

2.5. Western blot analysis

After the CPP post-conditioning, mouse brains were removed immediately after decapitation and frozen on dry ice. An approximately 400 μm long section of the NAc region including both core and shell (start: bregma 1.54 mm) was punched from the frozen brain using a cryostat (Leica, Wetzlar, Germany) and a 17-gauge stainless steel stylet. Mouse brain tissues were homogenized using a rotary homogenizer in ice-cold lysis T-PER tissue protein extraction buffer (Thermo Fisher Scientific, Rockford, IL, USA) containing protease and phosphatase inhibitor cocktails (Roche Diagnostics, GmbH, Mannheim, Germany) and incubated on ice for 30 min. After centrifugation at 13,000 × g for 15 min, the supernatant was separated and stored at 70 °C. Protein concentration was determined using Pierce™ BCA protein assay kit (Thermo Fisher Scientific). Proteins were subjected to 8%–10% SDS-polyacrylamide gel separation under reducing conditions, transferred onto a polyvinylidene fluoride transfer membrane (Pall Corporation, Pensacola, FL, USA) in transfer buffer [25 mM Tri-HCl buffer (pH 7.4) containing 192 mM glycine and 20% v/v methanol] for 1 h at 4 °C, and blocked with 5% non-fat milk in 0.5 mM Tris-HCL (pH 7.5), 150 mM NaCl, and 0.1% Tween-20 for 1 h at room temperature. The membrane was subsequently incubated with one of the primary antibodies at a 1:1000 dilution (except β-actin, which was used at a 1:10000 dilution) overnight at 4 °C. After three washes with TBST (Tris-buffered saline with 0.1% Tween-20), the blots were incubated with a 1:10000 dilution of horseradish-peroxidase-conjugated secondary antibodies in TBST with 5% non-fat milk for 1 h at room temperature. The blots were then washed five times in TBST buffer. Blots were developed using the enhanced chemiluminescence detection method by immersing them for 5 min in a mixture of ECL reagents A and B (Anigen, Hwaseong, Korea) at a 1:1 ratio and exposing them to photographic film (FujiFilm, Stamford, CT, USA). Protein bands were quantified by densitometric analysis using ImageJ software (NIH, Bethesda, MD, USA).

2.6. Immunohistochemistry (IHC)

Immediately after the CPP post-conditioning test, mice were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused with a pre-perfusion solution containing 10 units/mL heparin, 0.9% NaCl, and 0.5% NaNO2 followed by a perfusion solution containing 4% paraformaldehyde in PBS. Brains were post-fixed in 4% paraformaldehyde and then placed in 30% sucrose, after which they were frozen and sectioned coronally at 4 μm using a cryostat (Leica, Wetzlar, Germany). Brain slices were stored at −20 °C in a cryoprotectant solution containing 80 mM K2HPO4, 20 mM KH2PO4, 0.9% NaCl, 30% sucrose, 30% ethylene glycol, and 1% polyvinyl pyrrolidone. Brain sections were incubated in 0.3% hydrogen peroxide in PBS for 15 min and then in a blocking solution containing 10% horse serum, 1% BSA in Tris-buffered saline (TBS) for 1 h at room temperature followed by incubation with the primary antibodies [anti-p-38 MAPK (1:50), anti-AC1 (1:200), anti-AC8 (1:50), anti-p-NF-κB p65 (1:100)]. For immunoperoxidase staining, biotinylated horse, anti-goat or goat anti-rabbit antibody (5 μg/mL; Vector Laboratories, Burlingame, CA, USA) was applied for 60 min at room temperature. The peroxidase label was detected by incubating in 3,3′-diaminobenzidine (DAB, Sigma). The sections were rinsed twice with TBS for 15 min, mounted onto a glass slide coated with gelatin and cover-slipped with mounting solution. Images were taken with a DP digital microscope camera (Olympus Optical Co., LTD., Tokyo, Japan) connected to a microscope (BX 51, Olympus, Tokyo, Japan). All immunohistochemical quantifications were conducted by a blinded observer.

2.7. Statistics

Data were expressed as the mean ± S.E.M. and analyzed with Prism 6.0 software (GraphPad Software, Inc.). In the TRPV1 KO mice
CPP study, the data were analyzed via two-way analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) test. The remaining data were analyzed via one-way analysis of variance (ANOVA) followed by Fisher’s LSD post-hoc test. Statistical significance was set at \( p < 0.05 \).

3. Results

3.1. Effects of TRPV1 gene deletion on morphine-conditioned reward

Place-preference changes following morphine conditioning were evaluated to determine whether the TRPV1 gene KO decreased morphine reward in mice (Fig. 1A, interaction, \( F_{(3,44)} = 4.104, p < 0.05 \); gene factor, \( F_{(1,44)} = 4.786, p < 0.05 \); drug factor, \( F_{(1,44)} = 7.598, p < 0.01 \); two-way ANOVA followed by the Fisher’s LSD test, \( n = 12 \)). Morphine (5 mg/kg, s.c.) increased CPP scores in WT mice \( (p < 0.01) \), whereas it did not produce significant CPP in TRPV1 KO mice \( (p < 0.01) \). In addition, TRPV1 KO decreased the response to morphine compared to that of WT mice treated with morphine \( (p < 0.01) \).

3.2. Effects of systemic and intra-NAc SB366791 injections on morphine CPP

Our previous study reported that systemic injection of the TRPV1 antagonist SB366791 reduced morphine-induced place preference and SB366791 alone had no significant effects on the CPP test in mice [5], which was confirmed in this study. SB366791 at 0.15 and 0.3 mg/kg (i.p.) decreased morphine-induced CPP in CD-1 mice (Fig. 1B, \( F_{(4,55)} = 4.186, p < 0.01, n = 12 \), one-way ANOVA followed by the Fisher’s LSD test), while a dose of 0.6 mg/kg did not show a significant reduction of morphine CPP \( (p < 0.05) \). Previously we found SB366791 injection into the dorsal striatum dampened morphine CPP in CD-1 mice. In this study, intra-NAc administered SB366791 (0.5 ng/site, unilateral) decreased morphine CPP (Fig. 1C, \( F_{(3,42)} = 5.559, p < 0.01, n = 10-12 \), one-way ANOVA followed by the Fisher’s LSD test), however SB366791 at a dose of 2 ng/site did not alter morphine’s action \( (p > 0.05) \).

3.3. The relationship between p38 MAPK and TRPV1 on morphine CPP in CD-1 mice

The effects of the p38 MAPK inhibitor SB203580 and the phosphorylation of p38 MAPK in morphine CPP mice were tested to explore the involvement of p38 MAPK and TRPV1 in morphine-conditioned reward. SB203580 (0.5 mg/kg, i.p.) dramatically decreased morphine-induced CPP (Fig. 2A, \( F_{(2,23)} = 5.079, p < 0.05, n = 12 \), one-way ANOVA followed by the Fisher’s LSD test). This result is consistent with our previous results and we previously observed SB203580 0.5 mg/kg (i.p.) did not significantly affect place preference. Moreover, a reduction of phospho-p38 MAPK was observed using Western blot analysis of the NAc from morphine CPP mice treated with either SB366791 or SB203580 (Fig. 2B, \( F_{(3,20)} = 6.162, p < 0.01, n = 6 \), one-way ANOVA followed by the Fisher’s LSD test). In addition, we observed a decrease in the phosphorylation of p38 MAPK in the NAc of morphine CPP mice treated with SB366791 using IHC staining (Fig. 2C, \( F_{(2,15)} = 21.95, p < 0.001, n = 6 \), one-way ANOVA followed by the Fisher’s LSD test). Phospho-p38 MAPK-stained cells in the NAc are shown in Fig. 2D-F.

3.4. Effects of SB366791 and SB203580 on the expression of AC1 and AC8 in the NAc of CD-1 mice that were conditioned to morphine

We examined the change in expression of AC1 and AC8 using Western blot analysis and IHC staining of the NAc of morphine CPP mice to verify the involvement of AC1 and AC8 in mediating morphine-conditioned reward. As shown in Fig. 3A, both SB366791 and SB203580 decreased AC1 protein expression levels in the NAc of morphine-conditioned mice (Fig. 3A, \( F_{(3,8)} = 76.91, p < 0.001, n = 3 \), one-way ANOVA followed by the Fisher’s LSD test). In addition, in IHC experiments, the number of AC1-expressing cells in the NAc was decreased after SB366791 administration (Fig. 3B, \( F_{(2,15)} = 97.28, p < 0.001, n = 6 \), one-way ANOVA followed by the Fisher’s LSD test). However, the number of AC8-immunoreactive (IR) cells in the NAc did not change after SB366791 treatment in morphine CPP mice (Fig. 3G, \( F_{(2,15)} = 0.818, p > 0.05, n = 3 \), one-way ANOVA followed by the Fisher’s LSD test). AC1- or AC8-stained cells in the NAc are shown in Fig. 3C-F and H-K, respectively.

3.5. Effects of SB366791 and SB203580 on the phosphorylation of NF-κB p65 in the NAc of CD-1 mice that conditioned to morphine

In order to further evaluate whether the NF-κB signaling pathway through p38 MAPK and TRPV1 are required for morphine-induced CPP, we investigated the protein expression of p-NF-κB p65 in the NAc of morphine-induced CPP mice. Blockade of either p38 MAPK or TRPV1 decreased the phosphorylation of NF-κB p65 that was produced by morphine CPP in the NAc (Fig. 4A, \( F_{(3,40)} = 46.42, p < 0.001, n = 3 \), one-way ANOVA followed by the Fisher’s LSD test). In addition, our present IHC data show that both SB366791 and SB203580 decreased the phosphorylation of NF-κB p65 in the NAc (Fig. 4B, \( F_{(3,20)} = 4.321, p < 0.05, n = 6 \), one-way ANOVA followed by the Fisher’s LSD test). Phospho-NF-κB p65-stained cells in the NAc are shown in Fig. 4C-F.

4. Discussion

In this study, we present the effects of either genetic or pharmacological inhibition of TRPV1 on morphine conditioned reward in mice. And it is the first finding that TRPV1 KO abolished morphine CPP. Although the effects and mechanisms of TRPV1 KO on morphine-conditioned preference are not well understood, TRPV1 KO dampens the intake of and preference for ethanol [17,18] which increases the function and expression of μ-opioid receptors in the dorsal and ventral striatum [19,20]. Actually pharmacological inhibition of TRPV1 decreased the protein expression level of dorsal striatal μ-opioid receptors in our previous study [5]. Furthermore, morphine increased intracellular calcium level in C57BL/6 background WT mice, while TRPV1 KO decreased in this influx [21]. Additionally, the sensitivity of TRPV1 KO mice could be altered at higher doses of morphine [22]. Therefore, we suggest that TRPV1 KO may reduce the expression and function of μ-opioid receptors, which may abolish the rewarding effects of morphine.

We further examined the effects of TRPV1 inhibition on morphine-conditioned reward in mice. Interestingly, the two lowest doses of SB366791 treatment significantly decreased morphine-induced CPP, whereas the highest dose did not reduce morphine-induced rewarding effects. Additionally SB366791 (0.5 ng/site) administered into the NAc core dampened morphine CPP, but a high dose of SB366791 (2 ng/site) did not have significant effects. This is presumably because neurons are inactivated following membrane depolarization without the generation of action potentials through Na+ channels [23]. A low dose of the TRPV1 agonist capsaicin increased neural activity in the midbrain, whereas a higher dose of capsaicin treatment induced a decrease in both neural currents and firing frequency [24]. Moreover, capsazepine, a TRPV1 antagonist and an activator of Na+ channels, induced an inward current in the same manner as capsaicin [25], and interestingly, the selective TRPV1 antagonist SB366791 decreased capsaicin-induced Na+ channel currents in neurons [26]. These studies suggest that high doses of SB366791 did not reduce morphine CPP due to modulated sensitivity of neural activity through Na+ channels.

In addition to studying the effects on morphine CPP from TRPV1 knock-out/inhibition, we evaluated the interaction between morphine CPP and the inhibition of p38 MAPK. Little has been reported about how morphine-induced rewarding effects are mediated via the p38 MAPK.
MAPK pathway. One study reported that morphine CPP was not decreased by the administration of SB203580 (1, 3, 10 nmol/mouse) into the cerebral ventricle [27], while a recent study revealed that SB203580 (500 ng/0.5 μL/side) infused into the NAc blocked the production of morphine-conditioned reward in rats [28]. In our present study, systemic administration of SB203580 (0.5 mg/kg) dampened morphine-driven place preference in mice. These differential findings may be due to different drug doses and administration paradigms.

Previously we published the role of TRPV1 in the NAc core of morphine-addicted rats [10]. In our previous paper, morphine self-administration increased c-fos levels, the marker of neuronal activity, whereas a TRPV1 selective antagonist, AMG9810, dampened this effect in the NAc core blocked the production of morphine-conditioned reward in rats [28]. In our present study, systemic administration of SB203580 (0.5 mg/kg) dampened morphine-driven place preference in mice. These differential findings may be due to different drug doses and administration paradigms.

Fig. 2. Effects of SB203580 on morphine-induced behavior and p38MAPK protein phosphorylation changes in the NAc of CD-1 mice. (A) The p38 MAPK inhibitor SB203580 (0.5 mg/kg, i.p.) decreased morphine-induced CPP in mice. (B) Both SB366791 (0.15 mg/kg, i.p.) and SB203580 (0.5 mg/kg, i.p.) reduced the phosphorylation of p38 MAPK in the NAc of morphine CPP mice. (C) SB366791 reduced morphine-increased phospho-p38 MAPK-positive cells in the NAc. (D-F) Brown stained cells are phospho-p38 MAPK-immunoreactive cells in the NAc; scale bar, 40 μm. Data are expressed as the mean ± SEM. Significant differences were identified by one-way ANOVA followed by Fisher’s LSD post hoc test (**p < 0.01, ***p < 0.001 compared with saline-treated group; #p < 0.05, ##p < 0.01 compared to morphine-treated group).
pathway in the NAc. Elevated intracellular Ca\(^{2+}\) levels via Ca\(^{2+}\)/calmodulin stimulated both AC1 and AC8. Knocking out both AC1 and AC8 significantly reduced morphine-induced CPP effects, implicating that AC1 and AC8 contributed to the development of morphine rewarding effects [36]. According to our results, the rewarding effects of morphine are possibly more closely related to AC1 than AC8, since AC1 but not AC8 was upregulated in the reward systems after morphine treatment. AC1 stimulated by low Ca\(^{2+}\) concentrations was approximately five times more sensitive to Ca\(^{2+}\) than AC8 [37]. The TRPV1 channel is highly permeable to Ca\(^{2+}\) [38] therefore activating TRPV1 may trigger Ca\(^{2+}\) stimulation of AC1. Subsequently, stimulated AC1 may activate the MAPK/NF-κB pathway.

Taken together, we found that blockade of TRPV1, either through genetic deletion or systemic or intra-NAc pharmacological means, inhibited morphine-induced CPP in mice. In addition, p38 MAPK inhibition blocked the development of morphine CPP as well. Moreover, blockade of either NAc p38 MAPK or TRPV1 dampened protein expression levels of p-p38 MAPK, AC1, and p-NF-κB which are normally induced by morphine CPP.

**Conflicts of interest**

The authors have declared that no competing interests exist.
Author contributions

C.G.J., S.I.H., and T.N.L. participated in research design. T.N.L. and S.X.M., conducted experiments and performed data analysis. S.I.H., T.N.L., and C.G.J. wrote the manuscript. H.C.K. and S.Y.L. contributed to the discussion.

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References


Fig. 4. Effects of SB203580 and SB366791 on morphine-induced phospho-NF-κB expression in the NAc of CD-1 mice. (A) Both SB366791 (0.15 mg/kg, i.p.) and SB203580 (0.5 mg/kg, i.p.) reduced the phosphorylation of NF-κB in the NAc. (B) Both SB366791 (0.15 mg/kg, i.p.) and SB203580 (0.5 mg/kg, i.p.) decreased the number of phospho-NF-κB-immunoreactive cells in the NAc. (C-F) Brown stained cells are phospho-NF-κB-positive cells; scale bar, 40 μm. Data are expressed as the mean ± SEM. Significant differences were identified by one-way ANOVA followed by the Fisher’s LSD post hoc test (*p < 0.05, ***p < 0.001 compared with the saline-treated group; #p < 0.01, ##p < 0.001 compared to the morphine-treated group).