Essential role of protein kinase C in morphine-induced rewarding memory

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ABSTRACT

Protein kinase C (PKC) is involved in intra-cellular signal transduction in various physiological and pathological processes including substance abuse. In the present study, the role of PKC in morphine-induced rewarding memory was investigated using the conditioned place preference (CPP) model. We found a significant translocation of PKCs from cytosol to membrane component in nucleus accumbens (NAc) of morphine-conditioned rats in a dose-dependent manner. The translocation was reduced gradually with the maintenance of morphine-induced CPP. Specifically, the protein level of PKCγ in membrane of the NAc was increased in morphine CPP rats, and decreased during the attenuation of morphine-induced CPP, while the protein level of PKCγ in cytosol of the NAc showed an opposite change. Furthermore, the PKC translocation inhibitor γV5-3 impaired the morphine-induced CPP when micro-injected into the NAc. These findings indicated that PKC, especially the γ isofrom, is essential for the acquisition and maintenance of morphine-associated reward memory.

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1. Introduction

The protein kinase C (PKC) family, which includes at least 10 isoforms, is divided into 3 groups: conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ε, η, ζ) and atypical PKCs (ε, η, ζ) (Newton, 2003). The conventional PKCs are diacylglycerol (DAG) sensitive and Ca2⁺ responsive, the novel PKCs are sensitive to DAG but not to Ca2⁺, while the atypical PKCs are insensitive to both DAG and Ca2⁺ (Rykx et al., 2003). One of the major signal transduction cascades that lead to PKC activation is the phosphoinositide (PI) signaling pathway (Fisher et al., 1992). Stimulation of certain G protein-coupled receptors activates phospholipase C, which hydrolyzes phosphatidylinositol-4, 5-bis-phosphate to form inositol triphosphate (IP3) and DAG. IP3 binds to intra-cellular receptors causing release of Ca2⁺ from the endoplasmic reticulum, whereas DAG binds to and activates most PKC isoyzmes (Nishizuka, 1995).

Chen and Huang (1991) demonstrated that PKC is involved in μ-opioid receptor-induced signals that promote sustained potentiation of N-methyl-D-aspartate receptor (NMDAR)-mediated glutamate responses. In addition, our previous work demonstrated that NR2B-containing NMDA receptors in the NAc are essential in morphine-induced reinforcing effects (Ma et al., 2006, 2007). In the present study, using the conditioned place preference (CPP), we investigated the importance of PKC in the NAc in morphine-related associative memory paradigm, and also by intra-NAc microinjection of γV5-3, a PKCγ translocation inhibitor, we explored if the translocation of PKCγ from cytosol to membrane is necessary for acquisition and maintenance of morphine-induced CPP.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, 200–230 g at the beginning of the experiment, were housed 4 per cage in a 12:12 h light/dark cycle (lights on at 07:00 a.m.) with food and water supplied ad libitum except during behavioral training sessions. The room temperature was maintained at 22 ± 1 °C and relative humidity at 45–55%. Rats were handled twice a day during the first 5 days after arrival. All experimental procedures were approved by the Animal Use Committee of Peking University Health Science Center.

2.2. Apparatus

A three-chambered CPP apparatus (795 × 230 × 250 mm³) with distinct visual and tactile cues was used to conduct the conditioning (Shi et al., 2004). The two large black conditioning chambers (A and C, 280 × 220 × 225 mm³) were separated by a small gray center choice chamber B (135 × 220 × 225 mm³). Chamber A had 4...
were counterbalanced within each group. The data from pre-conditioning tests were recorded. CPP was not expressed any more on the 64th day.

2.3. Morphine CPP conditioning procedure

2.3.1. Pre-conditioning test

On day 0, rats were placed in the center choice chamber B with the guillotine doors removed to allow access to the entire apparatus for 15 min and time spent in each chamber was recorded. The pre-conditioning test showed that animals spent almost an equal amount of time in the two end chambers (A: 315 ± 2.83 s, C: 308 ± 2.51 s) and less time in the small center choice chamber (B: 275 ± 10.01 s). There were no significant differences in the time spent in the two end chambers (p > 0.05). Thus, the CPP apparatus was considered as unbiased in terms of chamber preferences of untreated rats. The chambers selected for pairing with morphine were counterbalanced within each group. The data from pre-conditioning tests were used to separate animals into groups with approximately equal biases for each chamber. Rats with a bias for either of the lateral chambers were excluded (about 5%) from the experiments.

2.3.2. Conditioning

The animals were allowed for training sessions twice a day (08:30 and 15:30) for 4 days (day 1–4). Before being confined into one lateral chamber for 45 min, rats were received morphine (i.p., 0.01, 0.03, 0.1, 0.3, 1.0, 10 mg/kg for CPP expression studies and 3 mg/kg for CPP retention studies), and physical saline in the other lateral chamber. Animals in control groups received saline injections before both training sessions in alternate lateral chambers. In the morphine treated groups, half of the animals were received morphine training in compartment A and saline training in compartment C, while the rest were received morphine and saline training in compartment C and A respectively for counterbalance. Moreover, half of the rats were conditioned with morphine in the morning session and saline in the afternoon, while the other half were received control sessions.

2.3.3. Post-conditioning test

On day 5, all animals were placed in chamber B with the guillotine doors removed to allow access to the entire apparatus for 15 min and time spent in each chamber was recorded.

2.3.4. Retention of morphine CPP

After the establishment of CPP, rats were randomly divided into 10 groups. At every test point of 1st, 8th, 16th, 32nd, 64th days after the last day of conditioning training, two corresponding groups of rats, conditioned with morphine and saline respectively, were tested in CPP chambers. The time spent in each chamber was recorded. CPP was not expressed any more on the 64th day.

The locomotor activity during all of the above mentioned CPP tests was estimated by counting the total number of crossings between any two adjacent compartments.

2.4. Implantation of microinjection cannulae

Rats were anesthetized with pentobarbital sodium solution (40 mg/kg, i.p.) and mounted on a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA). The scalp was incised and retracted, and the head position was adjusted to place bregma and lambda in the same horizontal plane. Small holes (1 mm in diameter) were drilled on the skull for bilateral placement of stainless-steel guide cannulae (0.8 mm in outer diameter) into the NAc shell (NAcS) (anteroposterior (AP) = 1.5 mm, lateral (L) = 0.9 mm, dorsoventral (DV) = 6.5 mm), 2.0 mm above the intended side of injection. Guide cannulae were fixed on the skull with sterile stainless-steel screws and the dental acrylic cement.

Stainless-steel obturators (0.4 mm in outer diameter) were inserted into the guide cannulae to avoid occlusion. The obturators were removed and reinserted every day during the recovery period after cannulation surgery to prevent them from adhering to the cannulae. Every animal was injected with penicillin (15 × 10^6 IU/rat, s.c.) after surgery and the following 3 days.

2.5. Microinjections

Animals were microinjected 30 min before conditioning. Obturators were removed and bilateral infusion cannulae (0.4 mm in outer diameter) were inserted, extending 1.0 mm beyond the tip of guide cannulae in the NAcS. Bilateral microinjections of V5-3 (1 μg or 10 μg/rat) or Tat (30 μg/rat) or vehicle (5 μl normal saline/rat) were administered into the NAcS using a 10-μl syringe (0.5 μl/min). The microinjections were left in place for an additional minute to allow for drug diffusion. The obturators were then reinserted, and rats were placed back to their home cages.

2.6. Histology and histochemistry

Histological verification of cannulae location was performed after behavioral tests. Rats were anesthetized with chloral hydrate (40 mg/kg, i.p.) and perfused intracardially with 0.9% saline (300 ml) followed by 4% paraformaldehyde solution (350 ml). The brains were removed and post-fixed in 4% paraformaldehyde solution for 24 h, then immersed in 20% sucrose solution followed by 30% sucrose solution until sectioning. Coronal sections (30 μm thick) were cut on a cryostat (−22 °C) and wet-mounted on glass microscope slides (Fig. 6). Four animals whose cannulae were placed incorrectly were excluded from data analysis.

To verify proper diffusion of V5-3, naïve rats were microinjected with biontin-labeled Tat–v5-3 (10 μg/rat) and perfused with 0.9% saline followed by 4% paraformaldehyde solution 30 min after microinjection and coronal slices were made as described above. Sections containing the NAc shell were processed using a StreptAvidin Peroxidase-DAB reaction to visualize the extent of the distribution and cellular uptake of the Tat–v5-3. For immunohistochemistry detection, slides were washed with phosphate-buffered saline (PBS) containing 0.5% Triton X-100 for 30 min, and incubated with peroxidase-labeled StreptAvidin (25 μg/ml) (Zhongshan Biotechnology, Beijing, China) for 2 h at room temperature. The sections were then washed with 0.5% Triton X-100 in PBS for 30 min again, and stained using DAB (Zhongshan Biotechnology, Beijing, China) reaction for 5 min. Slides were then dehydrated for 5 min in 80%, 5 min in 90%, 2 × 5 min in 100% ethanol and 2 × 5 min in xylol, and embedded in Neutral Balsam. Stained slides were viewed and photographed with a CCD camera attached to a microscope (DMIRE, Leica, Germany). As shown in Fig. 7, biontin-labeled Tat–v5-3 diffused into the cytoplasm of cells surrounding the cannula track.

2.7. Tissue dissection and preparation

Rats were decapitated immediately after testing. The brains were removed and placed on an ice-cold plate for dissection of the NAc. The samples were frozen in...
liquid nitrogen and stored at −80°C until analysis. The tissue was homogenized in 250 μl ice-cold buffer containing 20 mM Tris–HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM phenylmethylsulfonyluoride, 25 mg/ml of leupeptin, 0.1 mg/ml of aprotinin and 0.32 M sucrose using a Potter-Elvehjem tissue grinder with Teflon pestle. The homogenate was then centrifuged at 1000 x g for 10 min and the supernatant was centrifuged at 26,000 x g for 1 h at 4°C. The supernatant was used as the cytosol fraction. The pellet was then homogenized in homogenizing buffer containing 0.2% (w/v) Triton X-100. The homogenate was kept at 4°C for 1 h with

![Image](image1.png)

**Fig. 2.** Temporal-dependent change of the NAc PKC activity during CPP maintenance. A, C, PKC activity in NAc membrane and cytosol fractions during the maintenance of CPP conditioned by saline and morphine (3 mg/kg), respectively. Data were expressed as mean ± S.E.M and analyzed using two-way repeated measures ANOVA followed by Bonferroni post-hoc test. ***, p < 0.001, compared with day 1 (n = 4 in each group). B, D, Correlation of PKC activity in the NAc cytosol and membrane fractions with the CPP score during the maintenance of CPP conditioned by saline and morphine (3 mg/kg), respectively. Data were expressed as the means of PKC activity and CPP scores, and analyzed by linear regression.

![Image](image2.png)

**Fig. 3.** Effects of morphine dose on the protein levels of PKCy in CPP expression. A, Representative bands from Western blots. B, PKCy activity in the NAc membrane and cytosol fractions from rats conditioned by morphine (0, 0.3, 3.0 mg/kg). Data were expressed as mean ± S.E.M of the normalized PKCy content and analyzed using two-way repeated measures ANOVA followed by Bonferroni post-hoc test. *, p < 0.05, compared with saline (i.e. 0 mg/kg morphine) group (n = 4 in each group). C, Correlation of PKCy content in the NAc cytosol and membrane fractions with the CPP score from CPP expression tests. Data were expressed as the means of PKC activity and CPP scores, and analyzed by linear regression.

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Fractions from rats during the CPP retention tests. Data were expressed as mean ± S.E.M. of the normalized PKCγ content and analyzed using two-way repeated measures ANOVA followed by Bonferroni post-hoc test (*p < 0.05, compared with day 1 (n = 4 in each group). C. Correlation of PKCγ content in the NAc cytosol and membrane fractions with the CPP score from CPP expression tests. Data were expressed as the means of PKC activity and CPP scores, and analyzed by linear regression.

Fig. 4. Temporal-dependent changes of PKCγ protein levels during CPP maintenance. A, Representative bands from Western blots. B, PKCγ activity in the NAc membrane and cytosol fractions from rats during the CPP retention tests. Data were expressed as mean ± S.E.M. of the normalized PKCγ content and analyzed using two-way repeated measures ANOVA followed by Bonferroni post-hoc test (*p < 0.05, compared with day 1 (n = 4 in each group). C, Correlation of PKCγ content in the NAc cytosol and membrane fractions with the CPP score from CPP expression tests. Data were expressed as the means of PKC activity and CPP scores, and analyzed by linear regression.

2.8. Determination of PKC activity in membrane and cytosol fractions of rat brain

PKC activity in subcellular tissue fractions was measured following the procedure described by Dwivedi and Pandey (Dwivedi and Pandey, 1999). PKC activity was determined using the Amersham enzyme assay system, in which a PKC-specific targeting peptide as well as all the necessary cofactors was provided. Assay tubes (with a final incubation volume of 75 ml) contained 25 ml of a component mixture [3 mM Ca (C6H12O7)2, 75 g/ml l-α-phosphatidyl-l-serine, 6 g/ml PMA, 225 mM substrate peptide, and 75 mM diethiothreitol in 50 mM Tris–HCl containing 0.05% sodium azide, pH 7.5] and 25 ml of membrane or cytosol fraction. The reaction was initiated by addition of 25 ml of Mg-ATP buffer (10 mM Tris, 1 mM MgCl2, 1.2 mM ATP, 72 mM MgCl2, and 30 mM HEPES, pH 7.4) to each tube. The subcellular samples were incubated for 15 min at 37 °C, and the reaction was terminated by addition of 100 ml of the “stop” reagent (300 mM orthophosphoric acid containing carnosine red) to each tube. An aliquot of the solution from each tube (35 ml) was blotted onto individual peptide-binding papers. Papers were washed with 75 ml phosphoric acid twice for 5 min. Papers were dried, and the retained radioactivity was counted by a liquid scintillation counter. The result was expressed as nanomoles per minute per milligram of protein (Dwivedi and Pandey, 1999).

2.9. Immunoblotting of PKCγ

Equivalent amounts of membrane or cytosol fractions (50 ml) for each sample were resolved in 8% SDS-PAGE. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes after electrophoresis. The membranes were incubated in Tris-buffered saline (TBS) with 0.1% Tween 20 (TBST) containing 5% nonfat dried milk. After this incubation, the membranes were washed thrice for 5 min in TBST, followed by 1 h of incubation with primary antibody diluted in TBST containing 5% nonfat dried milk (PKCγ; 1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C. The resulting supernatant was used as the membrane fraction. Protein concentrations were determined using a BCA assay (Pierce, Rockford, IL).

2.10. Drugs

Morphine hydrochloride was purchased from the First Pharmaceutical Factory of Shenyang, China. γ-V5-3 [amino acids 659–664 (CRVYLVAS) conjugated to Tat transmembrane carrier peptide (amino acid 47–57) via a cysteine–cysteine bond at its N-terminus] (Chen et al., 2001) and Tat ([(CYGRKKRRQRRR)] (Wu et al., 2010), as well as biotin-labeled Tat-γ-V5-3 were synthesized by Chinese Peptide Company (Hangzhou, China). All drugs were dissolved in normal saline.

2.11. Statistical analysis

CPP score represents the index of place preference for each rat, calculated by dividing the time spent in the drug-paired compartment by the time spent in both conditioning compartments (Shi et al., 2004). Data were analyzed by commercially available software Graph Pad Prism 4.0. Results were presented as mean ± SEM. Results from Figs. 1–5 were analyzed with two-way repeated measures analysis of variance (ANOVA) followed by Bonferroni post-hoc tests, results from Tables 1 and 2 were analyzed with one-way ANOVA followed by Newman–Keuls post-hoc tests. The accepted level of statistical significance was p < 0.05.

3. Results

3.1. PKC activity was up-regulated in membrane fractions of the NAc in morphine-conditioned rats whereas that in cytosol was reduced

Different doses of morphine (0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10 mg/kg) were administered by intraperitoneal (i.p.) injection in the four sessions of conditioning as described by Ma et al. (2009). CPP test was performed in the followed day. Significant place preference was observed in the groups treated with morphine at doses from 0.3 to 10 mg/kg (CPP scores), with maximum response obtained at 3.0 mg/kg of morphine (Table 2). Using [32P] labeling substrate method, we determined the activity of PKC in the NAc of

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rats conditioned with morphine at doses of 0, 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10.0 mg/kg (Fig. 1A). Two-way repeated measures ANOVA showed significant effects of the dose of morphine ($F_{7, 24} = 2.55$, $p = 0.0413$), the membrane vs. cytosol fractions ($F_{1, 24} = 307.31$, $p < 0.0001$) and the interaction of these two factors ($F_{7, 24} = 26.06$, $p < 0.0001$). Bonferroni post-hoc test showed significant increases of PKC activity in the membrane protein (at least $p < 0.01$, between the rats conditioned by morphine of $>0.1$ mg/kg vs. saline) and significant decreases in the cytosol protein (at least $p < 0.01$, between the rats conditioned by morphine at $>0.03$ mg/kg vs. saline). Moreover, PKC activities in protein extracts from the membrane fraction presented strong positive correlations with CPP expression score ($r^2 = 0.8086$, $p = 0.0024$, Fig. 1B). Our data suggested that more PKC was transferred from cytosol to membrane (as an activated state) as the dose of morphine increased and, more importantly, PKC activity was positively correlated to the time spent in the morphine-paired chambers.

3.2. The PKC activity was decreased in membrane fractions but increased in cytosol of the NAc during the retention of morphine CPP

Lower panel in Table 2 shows the behavioral scores during CPP retention induced by 3 mg/kg morphine. Bonferroni post-test after significant two-way ANOVA indicated that the CPP induced by 3 mg/kg morphine lasted until 64 days after the last conditioning. Two-way repeated measures ANOVA (fractions of membrane vs. cytosol, $F_{1, 15} = 665.47$, $p < 0.0001$; days after last conditioning, $F_{4, 15} = 15.87$, $p < 0.0001$; interaction of these two factors, $F_{4, 15} = 30.89$, $p < 0.001$), followed by Bonferroni post-hoc test showed the activity of PKC in the NAc membrane of morphine-conditioned rats decreased gradually during the abstinence from morphine ($p < 0.01$, day 64 vs. day 1), while the PKC activity in the NAc cytosol displayed an opposite change, i.e. increasing since 8 days after the last CPP conditioning ($p < 0.001$, day8, 16, 32, 64 vs. days 1) (Fig. 2C). No significant differences were shown for the activity of PKC in the NAc of normal saline-conditioned rats (among days, $F_{4, 15} = 1.88$, $p = 0.1664$; interaction $F_{4, 15} = 1.27$, $p = 0.3241$), although there were differences between fractions of membrane vs. cytosol ($F_{1, 15} = 637.99$, $p < 0.0001$) (Fig. 2A). Moreover, PKC activities in protein extracts from the cytosol fraction presented strong positive correlations with CPP retention score ($r^2 = 0.8396$, $p = 0.0287$, Fig. 2D), suggesting that the translocation of PKC from cytosol to membrane decreased in parallel with the decrease of place preference to the morphine-paired chamber.

3.3. The translocation of PKCγ from cytosol to membrane in the NAc was increased when morphine CPP was established, but decreased during the retention of morphine CPP

We also determined the protein levels of PKCγ in the NAc of conditioned rats after the CPP expression test (representative bands of Western blots in Fig. 3A). Two-way repeated measures ANOVA showed a significant interaction between morphine doses and fractions ($F_{2, 9} = 15.6$, $p = 0.0012$) (Fig. 3B), although no significant effect was shown in the conditioning dose of morphine ($F_{2, 9} = 0.01$, $p = 0.9928$). Bonferroni post-hoc test showed a significant increase of PKCγ in the membrane fraction and a decrease in the cytosol fraction ($p < 0.05$, compared with saline-conditioned group). Moreover, protein level of PKCγ in extracts from the membrane and cytosol fractions presented strong positive and negative correlations, respectively, with CPP expression score ($r^2 > 0.9$, Fig. 3C), suggesting that the translocation of PKCγ from cytosol to...
membrane increased in parallel with the increase of place preference to the morphine-paired chamber after conditioning with high dose of morphine.

During the retention of morphine-conditioned CPP, the levels of PKC\(\gamma\) in membrane and cytosol fractions were also examined by Western blots (representative bands shown in Fig. 4A). Two-way repeated measures ANOVA showed a significant interaction between the two factors of day and fraction (\(F_2, 9 = 5.68, p = 0.0254\)), although no significant effect was shown among the days after the last conditioning (\(F_2, 9 = 1.09, p = 0.3761\), Fig. 4B). Bonferroni post-hoc test showed a significant decrease of PKC\(\gamma\) in the membrane fraction 64 days after the last conditioning (\(p < 0.05\), compared with 1 day after last conditioning). Moreover, protein level of PKC\(\gamma\) in extracts from the membrane fraction presented a strong positive correlation with CPP score in CPP retention (\(r^2 = 0.9954, p = 0.0430\), Fig. 4C), suggesting a significant decrease of PKC\(\gamma\) activity in parallel with the decrease of place preference to the morphine-paired chamber.

3.4. Intra-NAcS infusion of PKC\(\gamma\) inhibitor γV5-3 blocked the acquisition of morphine CPP

Since there was an increase of PKC\(\gamma\) translocation after the acquisition of morphine-induced CPP, we next used the PKC\(\gamma\) translocation inhibitor, γV5-3 to investigate if translocation of PKC\(\gamma\) was necessary in the establishment of morphine-induced CPP. In order to confirm if γV5-3 could induce any preference or aversion by itself, intra-NAcS microinjection of saline or 10 μg γV5-3 30 min before saline conditioning was performed in saline control rats. Two-way repeated measures ANOVA showed no differences of the CPP scores between pre- vs. post-conditionings (\(F_{1, 17} = 0.16, p = 0.6950\)) and intra-NAcS microinjection between saline vs. γV5-3 (\(F_{1, 17} = 0.01, p = 0.9418\)) as well as no interaction effect of these two factors (\(F_{1, 17} = 0.00, p = 0.9593\)) (Fig. 5A), suggesting no influence of γV5-3 on place preference tests.

Fig. 5B showed the effect of intra-NAcS injection of 5 μl saline, 10 μg Tat, 1 μg γV5-3 and 10 μg γV5-3 on morphine-induced place preference. Two-way repeated measures ANOVA displayed that there was an obvious change in preference score between pre- and post-conditionings (\(F_{1, 41} = 14.4, p = 0.0005\)), though the four treatments didn't show a significant effect (\(F_{3, 41} = 2.33, p = 0.0888\)), there's an interaction effect of morphine conditioning × intra-NAcS treatment (\(F_{3, 41} = 3.81, p = 0.0169\)). The followed Bonferroni post-hoc tests showed 3 mg/kg morphine-induced CPP was only observed in rats treated by microinjections of saline or Tat before each morphine conditioning (\(p < 0.001, 0.05, \text{pretest vs. test, respectively}\), but not by microinjections of γV5-3 (1, 10 μg). Furthermore, significant differences of test scores were demonstrated between groups treated by saline vs. 10 μg γV5-3 and Tat vs. 10 μg γV5-3 (\(p < 0.01\) respectively, two-way repeated measures ANOVA followed by Bonferroni post-hoc tests). These data indicated that inhibition of PKC\(\gamma\) translocation by γV5-3
The NAc is well-known as a pivotal region involved in rewarding effect induced by drug abuse. In our previous work, we demonstrated the level of NR2B-containing NMDA receptors in the NAc were elevated in morphine CPP rats, while inhibition of NR2B-containing NMDA receptors in the NAc blocked morphine CPP. However, the change of NR2B subunit levels in rats with morphine injection following the same schedule as in the CPP experiment but without the behavioral component of place preference was not significantly different from that in control rats with alternative saline injection only, suggesting that the changes in the concentration of the NR2B subunit were due to the combination of the activation of the reward circuit by morphine and the CPP paradigm but not by pharmacological actions of morphine alone (Ma et al., 2006, 2007). It has been showed that PKC can affect NR2B/N111 and NMDA currents by direct phosphorylation of the NR2B tail at residues S1303 and S1323 (Liao et al., 2001). Chen and Huang (1991) demonstrated that a selective μ-opioid receptor agonist DAMGO
caused a sustained increase in glutamate-activated currents that were mediated by NMDARs, and this increase was PKC dependent (Chen and Huang, 1991), so it is reasonable to speculate that NR2B may be one of the downstream substrates of PKC when it is activated via morphine, and the change of PKC activity may be result from the morphine-associated learning but not the only pharmacological effect of morphine.

It has been reported that inhibition of PKC activity decreases morphine-induced c-Fos expression in the striatum (Harlan et al., 2004), and the c-Fos expression in the striatum is also dependent on AMPA receptors (Arai et al., 1994) and mGluR1 (Moroni et al., 1997). Interestingly, both AMPA receptors and mGluR1 mediated, morphine-induced c-Fos expression is PKC activity-mediated (Garcia et al., 2003). These could be a way by which PKC may take part in morphine-related reinforcing effects, which suggests a prominent role of receptors coupled to PKC activation in the actions of morphine in the striatum. That might be one of the mechanisms by which PKC contributed to morphine dependence.

5. Conclusion

The present studies indicate that the translocation and activation of PKC may be a critical step in the development and maintenance of morphine rewarding memory. Modulation of PKC, especially PKCγ translocation and activation in the NAc may prove useful for the management of morphine dependence.

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