

Received: 2012.06.06
Accepted: 2012.11.27
Published: 2013.04.02

Phosphorylated CaMKII levels increase in rat central nervous system after large-dose intravenous remifentanil

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCEF 1 **Qiang Wang***
BCEF 1 **Xin Zhao***
ADFG 1 **Shuren Li**
BC 2 **Song Han**
BC 2 **Zhifeng Peng**
ADEFG 2 **Junfa Li**

1 Department of Anesthesia, Capital Medical University-affiliated Beijing Friendship Hospital, Beijing, China
2 Department of Neurobiology, Beijing Institute for Brain Disorders, Capital Medical University, Beijing, China

* Q. Wang and X. Zhao contributed equally to this work

Corresponding Author:

Junfa Li, e-mail: junfali@ccmu.edu.cn and Shuren Li, e-mail: csa8@china.com

Source of support:

This work was supported by grants from the National Natural Science Foundation of China (Grant No. 31071048 and 31171147), the "973" Pre-program (Grant No. 2011CB512109), and the "973" Program (Grant No. 2012CB518200)

Background:

Postoperative remifentanil-induced pain sensitization is common, but its molecular mechanism remains unclear. Calcium/calmodulin-dependent protein kinase II (CaMKII) has been shown to have a critical role in morphine-induced hyperalgesia. This study was designed to determine how CaMKII phosphorylation and protein expression levels change in the central nervous system of rats with remifentanil-induced hyperalgesia.

Material/Methods:

Male Sprague-Dawley® rats were exposed to large-dose (bolus of 6.0 µg/kg and 2.5 µg/kg/min for 2 hours) intravenous remifentanil to induce post-transfusion hyperalgesia. Levels of phosphorylated CaMKII (P-CaMKII) and total protein of CaMKII (T-CaMKII) were determined at different post-transfusion times by Western blot and immunostaining and were compared with controls.

Results:

P-CaMKII increased significantly ($P<0.05$) at 0, 0.5, and 2 hours. However, P-CaMKII at 5 to 24 hours and T-CaMKII at 0 to 24 hours post-transfusion did not change significantly in rats' spinal dorsal horn, hippocampus, or primary somatosensory (S1) cortex ($n=6$ per group). Similarly, immunostaining showed stronger P-CaMKII immunoreactants ($P<0.05$) and more P-CaMKII-positive cells ($P<0.05$) in the spinal dorsal horn, CA1 region of the hippocampus, and S1 cortex of rats 0.5 hours post-transfusion compared with the control group treated with 0.9% sodium chloride ($n=3$ per group).

Conclusions:

These results suggest that a temporary rise in the P-CaMKII level in the central nervous system may correlate with remifentanil-induced pain sensitization in the postoperative period.

Key words:

remifentanil • hyperalgesia • central nervous system • calcium/calmodulin-dependent protein kinase II (CaMKII)

Full-text PDF:

<http://www.medscimonit.com/download/index/idArt/883866>



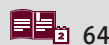
2969



1



5



64

Background

Remifentanyl, an ultra-short-acting opioid, is widely used in clinical practice. However, severe postoperative pain, or opioid-induced hyperalgesia, often occurs when remifentanyl is discontinued, most likely as the result of nociceptive sensitization [1,2]. Research has elucidated the factors responsible for the paradoxical effects of opioids, but neurobiological evidence of remifentanyl-induced hyperalgesia remains scarce.

The N-methyl-D-aspartate (NMDA) receptor is known to be critical in mediating calcium (Ca^{2+}) signaling and may contribute to the development and maintenance of hyperalgesia induced by discontinuation of remifentanyl or other opioids [3–5]. Direct phosphorylation of the NMDA receptor is crucial in regulating the channel function and localization of NMDA receptors at synapses [6]. Some serine/threonine phosphorylation sites that are substrates for protein kinase C (PKC) or Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) have been identified in NMDA receptors [7]. A positive feedback loop shows that the activation of NMDA receptors leads to increased intracellular Ca^{2+} levels and the subsequent activation of PKC and CaMKII. PKC and CaMKII can in turn activate NMDA receptors by phosphorylation, which is the proposed mechanism for neuropathic pain [8] and opioid tolerance [9]. In previous studies, we demonstrated that conventional PKC (cPKC) γ membrane translocation is involved in remifentanyl-induced hyperalgesia [10]. Moreover, PKC is often consistent with CaMKII in regulating neural plasticity [11–13]. Therefore, in this study, we explored how CaMKII levels change with remifentanyl exposure.

CaMKII, a multifunctional serine/threonine protein kinase diffused throughout the central nervous system, is encoded by 4 genes; its α subunit has an important role in regulating neuronal plasticity [14,15]. Elevated intracellular Ca^{2+} and calmodulin levels are essential in the activation of CaMKII. The activated CaMKII begins to phosphorylate itself and protein subunits such as neuronal membrane receptors and intracellular transcription factors [16,17]. Inhibition of CaMKII activity has been shown to reverse morphine tolerance and dependence in a dose-dependent manner [18]. Spinal CaMKII activity increased in subcutaneous injection of a morphine-induced hyperalgesia mouse model, whereas mechanical allodynia and thermal hyperalgesia were not detected in CaMKII α T286A mutant mice [19].

Upregulation of CaMKII has been implicated in neuropathic and inflammatory pain. CaMKII inhibitors reversed thermal hyperalgesia and mechanical allodynia in these experimental models in a dose-dependent manner [20–22]. In addition, patients have reported less pain after discontinuation of remifentanyl when the commonly used anesthetics propofol or ketamine were added to the treatment protocol [23,24]. Both propofol and ketamine have been demonstrated to inhibit CaMKII phosphorylation in a dose-dependent manner [25]; therefore,

determination of how CaMKII activity changes with remifentanyl administration will help identify the therapeutic target for opioid-induced hyperalgesia.

The spinal dorsal horn, rather than the hippocampus or cortex, is typically the focus of studies of opioid-induced hyperalgesia. However, intravenous administration of remifentanyl is a systemic event, and some research supports the involvement of the hippocampus and primary somatosensory (S1) cortex in hypersensitivity and hyperalgesia. For example, fentanyl exposure increases the susceptibility of CA1 pyramidal neurons to presynaptic stimulation [26]. Altered sensory processing related to hyperalgesia is reflected in laser C-fiber-evoked potentials in the forelimb and hind limb S1 cortex [27]. Induction of spinal long-term potentiation (LTP), which may contribute to hypersensitivity and hyperalgesia, first causes an acute metabolic response in the S1 cortex rather than in other regions of the brain [28]. Expression of the CaMKII gene in the rat hippocampus and frontal cortex has been found to be upregulated after repeated morphine administration [29]. Therefore, the objective of this study was to investigate how levels of CaMKII phosphorylation at threonine 286 (P-CaMKII) and total protein (T-CaMKII) change in the spinal dorsal horn, hippocampus, and S1 cortex of rats with remifentanyl-induced hyperalgesia.

Material and Methods

Experiments were carried out on 8- to 10-week-old male Sprague-Dawley® rats weighing 240 to 260 g. The rats were initially housed in a controlled room at 21°C to 24°C with a 12:12 hour light-dark cycle. Rats had ad libitum access to food and water. The animal protocol was approved by the Institutional Animal Care and Use Committee of Capital Medical University and is consistent with the ethical guidelines of the International Association for the Study of Pain for pain research in conscious animals.

The following materials were obtained from the indicated sources: phosphatase inhibitors (okadaic acid, sodium pyrophosphate, and potassium fluoride); proteinase inhibitors (leupeptin, aprotinin, pepstatin A, and chymostatin); mouse monoclonal antibodies against β -actin; ethylene diaminetetraacetic acid (EDTA); ethylene glycol tetraacetic acid (EGTA); sodium dodecyl sulfate (SDS); dithiothreitol (DTT); Nonidet™ P-40 (Sigma-Aldrich, St. Louis, MO, USA); horseradish peroxidase-conjugated goat anti-rabbit IgG and goat anti-mouse IgG (Bio-Rad, Hercules, CA, USA); and remifentanyl (Yichang Humanwell Pharmaceutical Co LTD, PR China).

Drug administration

Rats were assigned to a treatment group that received intravenous remifentanyl, or to a control group that received

intravenous 0.9% sodium chloride via a tail vein. Intravenous lines were established through a tail vein via a 24-G trocar (Suzhou Becton Dickinson Medical Devices Co. Ltd., PR China). After successful cannulation, infusion pumps (Graseby 3500 Anesthesia Syringe Pump, Graseby Medical Ltd., UK) were connected for transfusion. The treatment protocol was performed as follows: rats in the control group received 0.9% sodium chloride with a bolus of 0.3 mL/kg at 0.125 mL/kg/min; rats in the treatment group received remifentanyl with a bolus of 6.0 µg/kg at 2.5 µg/kg/min (a large dose with a 20 µg/mL concentration). The transfusion lasted 2 hours, and spontaneous breaths and eyelash reflexes were monitored to ensure the rats' well being.

Mechanical sensitivity test

Before the transfusion, rats in the control group (n=10) and treatment group (n=10) were separately placed in a Plexiglas® container (25×25×25 cm) with a wire mesh bottom and allowed to acclimate for 30 minutes. The basal value of the paw withdrawal threshold (PWT) of each rat was measured by applying a probe to the plantar surface of the hind paw with an electronic von Frey anesthesiometer until a withdrawal response occurred. A cut-off value for the PWT was set at 120 g to prevent mechanical injury. After the transfusion, the PWT was measured by the same method at 0, 0.5, 2, 5, and 24 hours post-transfusion. The PWT of each rat was tested 3 times at different post-transfusion time points. The mean value of the 3 measurements was used.

Sample preparation and Western blot analysis

After the transfusion, rats in the control (n=6) and treatment groups were sacrificed 0, 0.5, 2, 5, and 24 hours (n=6 per group) post-transfusion. The rats were anesthetized with 10% chloral hydrate, and a cannula was inserted into the left ventricle of each rat 0.5 hours post-transfusion [10]. After cannulation, 100 mL 0.9% NaCl and 250 mL 10% neutral buffered formalin 250 mL were perfused. Spinal cords and brains were quickly removed and placed into ice-cold artificial cerebrospinal fluid (NaCl 125 mM, KCl 2.5 mM, CaCl₂ 2.0 mM, NaHCO₃ 26 mM, NaH₂PO₄ 1.25 mM, MgCl₂ 1.0 mM, glucose 5 mM, pH 7.4) with 95% O₂ and 5% CO₂. The dorsal horn area of the lumbosacral enlargement, hippocampus, and S1 cortex were identified and collected as required, and then they were frozen in liquid nitrogen and kept at -80°C for analysis.

As previously reported [30], the frozen samples were rapidly thawed, homogenized at 4°C in Buffer C (50 mM Tris-Cl, pH 7.5) containing 2 mM DTT, 2 mM EDTA, 2 mM EGTA, 50 mM 4-[2-aminoethyl]-benzenesulfonylfluoride hydrochloride; 5 mg/mL each of leupeptin, aprotinin, pepstatin A, and chymostatin; 50 mM KF, 50 mM okadaic acid, 5 mM sodium

pyrophosphate, and 2% SDS, and sonicated to dissolve the tissue completely. Protein concentration was measured with a BCA Protein Assay Kit (Thermo Scientific, Pittsburgh, PA, USA).

SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot analysis were performed as in a previous study [25]. Briefly, 35 µg of total protein in each sample was loaded in the corresponding lane for a 10% SDS-PAGE gel. After electrophoresis and transfer of proteins onto polyvinylidene difluoride membrane (PVDF, GE Healthcare, USA) at 4°C, the PVDF membrane was blocked with 10% nonfat milk in TTBS (20 mM Tris-Cl, pH 7.5, containing 0.15 M NaCl and 0.05% Tween 20) for 1 hour. The blocked membrane was incubated with primary rabbit polyclonal antibody against P-CaMKII (T286, 1:1000) for 3 hours or for 1 hour in T-CaMKII (1:1000, Santa Cruz Biotechnology, Inc., USA). To ensure uniform loading of protein, the same PVDF membrane was reprobed with primary mouse monoclonal antibody against β-actin (Sigma-Aldrich, USA) at a 1:2000 dilution for 1 hour. The horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (GE Healthcare, USA) were used as second antibodies at a 1:4000 dilution and incubated for 1 hour. An enhanced chemiluminescence kit (PerkinElmer Life Science, USA) was used to identify the signals on radiographic film. The sequence to detect the target protein is P-CaMKII, T-CaMKII, β-actin. Before starting each new round of detection on the same PVDF membrane, stripping buffer containing 100 mM 2-mercaptoethanol, 2% SDS, and 62.5 mM Tris-HCl (pH 6.7) at 55°C was applied and the membrane incubated until no signals appeared in the radiographic film, indicating that the previously bonded antibodies had been stripped from the membrane.

Immunostaining

Post-fixed spinal cords and brains were embedded in paraffin, and serial 4-µm sections were prepared with a microtome. Immunohistochemistry was performed using a PV-6000 kit (Zymed Co., USA). To detect P-CaMKII expressions in the spinal cord, hippocampus, and cortex, the sections were dewaxed and hydrated in xylene and graded alcohol solution.

Sections underwent an antigen-retrieval treatment in a pressure cooker for 5 minutes and were treated with 0.3% hydrogen peroxide for 15 minutes at room temperature to inhibit endogenous peroxidase activity. Sections were incubated with primary rabbit polyclonal antibody against P-CaMKII (T286, Santa Cruz Biotechnology, Inc., USA) for 24 hours in a 1:100 dilution at 4°C. Incubated sections were kept in room temperature for 30 minutes and washed with phosphate buffer solution (PBS) 3 times. An immunoreaction was triggered with a PV-6000 kit and visualized with peroxidase-3,3'-diaminobenzidine. Finally, sections were counterstained with haematoxylin

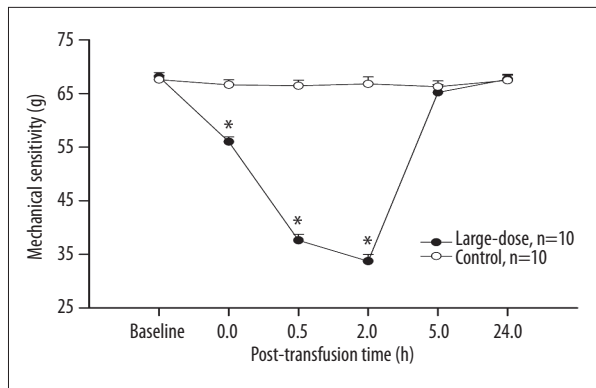


Figure 1. Changes in paw withdrawal threshold (PWT) of rats following intravenous remifentanyl. Quantitative analysis of a mechanical sensitivity test demonstrated that PWT decreased significantly ($P<0.05$, $n=10$ per group) at 0, 0.5, and 2 hours post-transfusion of large-dose remifentanyl, while it remained stable in control rats.

and mounted. Identical spinal cord and brain samples from controls were set with PBS, rather than the primary antibodies, and incubated. The images were captured with a Leica microscope imaging system (Leica, Wetzlar, Germany). Three selected fields in 3 serial sections were analyzed under a light microscope (10×40 magnification).

Statistical analysis

The within-group difference of rats' PWT before and after the transfusion was analyzed for mechanical sensitivity. Quantitative analysis for Western blot results was performed after scanning the radiographic film with Quantitative One software (Gel Doc-2000, Bio-Rad, USA). To determine the T-CaMKII and P-CaMKII levels, the relative optical density (ROD) of T-CaMKII or P-CaMKII bands were normalized against that of β -actin or T-CaMKII, respectively, and expressed as percentage of the control group (100%). For immunostaining, the P-CaMKII expression was determined as the mean integrated optical density (IOD, mean IOD=IOD/area) of captured images using Image-Pro Plus 6.0 software (Media Cybernetics, Inc, USA). The presented values are expressed as mean \pm SE. Statistical analysis was conducted by one-way analysis of variance (ANOVA) followed by pairwise multiple comparison procedures using the Bonferroni test. The significance level was set at $P<0.05$.

Results

PWT decreased significantly ($P<0.05$) at 0 hours (56.01 ± 0.89 g), 0.5 hours (37.61 ± 1.13 g), and 2 hours (33.73 ± 1.23 g) after transfusion of remifentanyl, and it remained stable in rats in the control group ($n=10$ per group) (Figure 1).

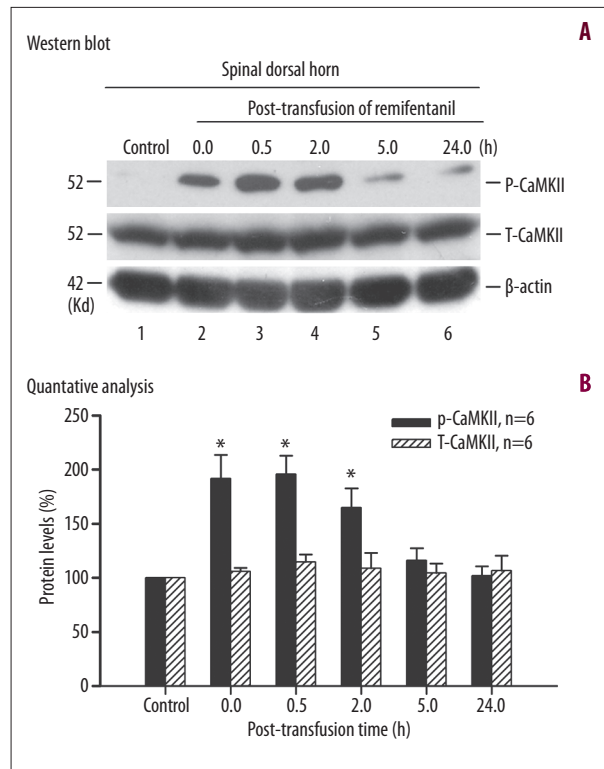


Figure 2. Changes in P-CaMKII and T-CaMKII levels in the spinal dorsal horn at 0 to 24 hours post-transfusion with large-dose intravenous remifentanyl. (A) Typical Western blot results showed changes in levels of P-CaMKII and T-CaMKII in the spinal dorsal horn of rats at 0 to 24 hours post-transfusion with remifentanyl; (B) Quantitative analysis demonstrated a significant increase in P-CaMKII levels at 0, 0.5, and 2 hours, but no significant changes at 5 to 24 hours or T-CaMKII at 0 to 24 hours post-transfusion with remifentanyl were observed in the spinal dorsal horn. * $P<0.05$ vs. control (0.9% sodium chloride, 100%), $n=6$ per group; large-dose remifentanyl transfusion, a bolus of 6 mg/kg at 2.5 mg/kg/min for 2 hours; P-CaMKII, phosphorylated CaMKII; T-CaMKII, total protein of CaMKII.

Changes in P-CaMKII and T-CaMKII levels in the spinal dorsal horn, hippocampus, and S1 cortex

P-CaMKII and T-CaMKII were detected at 52.0 KD with Western blot (Figure 2A). Quantitative analysis (Figure 2B) showed that P-CaMKII increased significantly ($P<0.05$, $n=6$ per group) at 0 hours (192 ± 22), 0.5 hours (196 ± 17), and 2 hours (165 ± 18) after transfusion with remifentanyl in the spinal dorsal horn, compared with that of the control group (100%).

Similarly, the P-CaMKII levels increased significantly ($P<0.05$, $n=6$ per group) in the hippocampus (Figure 3A and 3B) and S1 cortex (Figure 4A and 4B) of rats at 0 hours (hippocampus: 232 ± 22 ; S1 cortex: 231 ± 37), 0.5 hours (hippocampus: 225 ± 11 ; S1 cortex: 239 ± 68), and 2 hours (hippocampus: 281 ± 26 ; S1

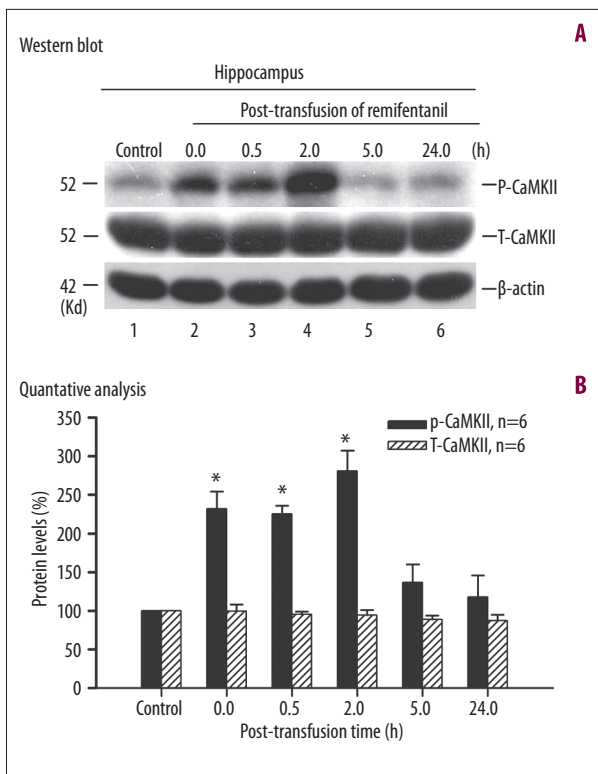


Figure 3. Changes in P-CaMKII and T-CaMKII levels in the hippocampus at 0 to 24 hours post-transfusion of large-dose intravenous remifentanyl. (A) Typical Western blot showed changes in P-CaMKII and T-CaMKII in the hippocampus of rats at 0 to 24 hours post-transfusion with remifentanyl; (B) Quantitative analysis demonstrated a significant increase of P-CaMKII at 0, 0.5, and 2 hours, but no significant changes in P-CaMKII at 5 to 24 hours or T-CaMKII at 0 to 24 hours post-transfusion with remifentanyl were observed in the hippocampus. * $P < 0.05$ vs. control (100%), $n = 6$ per group.

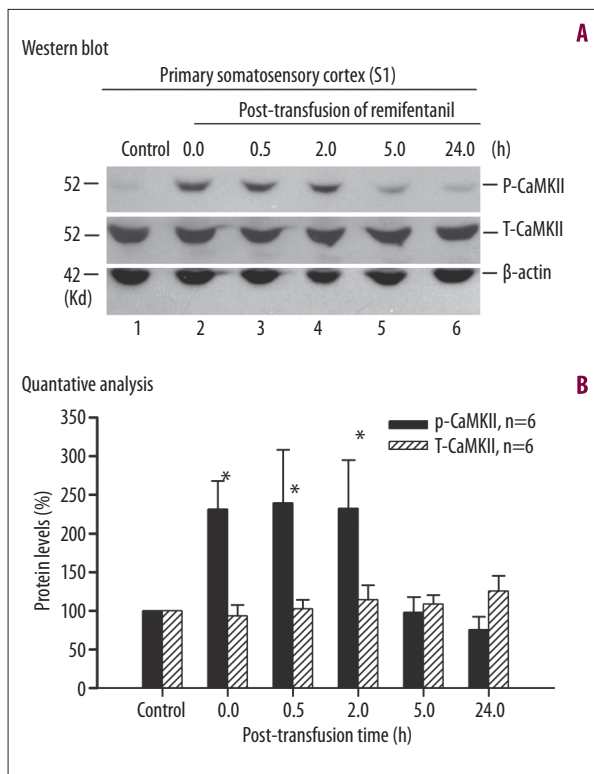


Figure 4. Changes in P-CaMKII and T-CaMKII levels in the S1 cortex at 0 to 24 hours post-transfusion of large-dose intravenous remifentanyl. (A) Typical results of Western blot showed changes in P-CaMKII and T-CaMKII levels in the S1 cortex of rats at 0 to 24 hours post-transfusion with remifentanyl; (B) Quantitative analysis demonstrated a significant increase in P-CaMKII at 0, 0.5, and 2 hours, but no significant changes in levels of P-CaMKII at 5 to 24 hours or T-CaMKII at 0 to 24 hours post-transfusion with remifentanyl were observed in the S1 cortex. * $P < 0.05$ vs. control (100%), $n = 6$ per group.

cortex: 232 ± 62) after transfusion with remifentanyl. However, no significant changes in levels of P-CaMKII at 5 to 24 hours or T-CaMKII at 0 to 24 hours after transfusion with remifentanyl were observed in the spinal dorsal horn, hippocampus, or S1 cortex of rats (Figures 2–4).

Distribution of P-CaMKII-positive cells in the spinal cord, hippocampus and S1 cortex

To determine the distribution of P-CaMKII in the spinal cord, hippocampus, and S1 cortex of rats after intravenous administration of remifentanyl, we performed immunostaining in 3 rats each in the treatment group and control group at 0.5 hours post-transfusion. Similar to the results of Western blot analysis, stronger P-CaMKII immunoreactants (mean IOD, $P < 0.01$) and more P-CaMKII-positive cells ($P < 0.05$) were observed in spinal dorsal horn (Figure 5B1 and 5B2), the CA1 region of the

hippocampus (Figure 5D1 and 5D2), and S1 cortex (Figure 5F1 and 5F2) at 0.5 hours after transfusion with remifentanyl (Figure 5G and 5H). Significantly increased P-CaMKII levels were found mainly in the cytoplasm.

Discussion

Some investigators have reported that Remifentanyl-induced hyperalgesia is easier to induce with high doses than with low doses [31–33]. Continuous intravenous transfusion of remifentanyl at $0.4 \mu\text{g/kg/min}$, with or without a bolus of $1.0 \mu\text{g/kg}$, is regarded as a large dose in clinical practice and has been demonstrated to increase postoperative pain sensitivity [34–36]. The equivalent dose used in rats for Western blot and immunohistochemistry analysis in this study was converted according to the experimental formula in *FDA (2005) Guidance for*

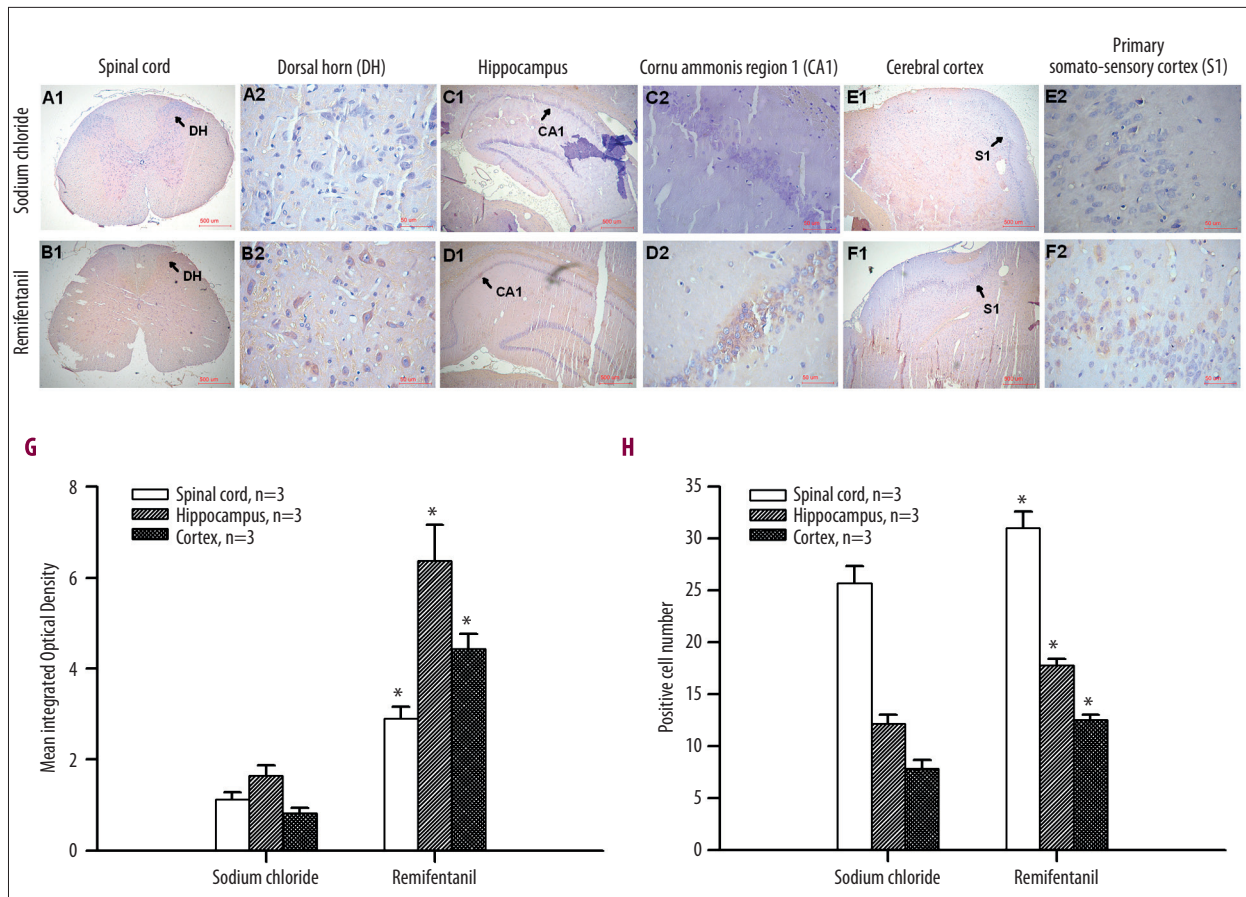


Figure 5. Immunostaining of P-CaMKII in the spinal cord, hippocampus and S1 cortex of rats at 0.5 hours post-transfusion of large-dose intravenous remifentanyl. Fixed spinal cord and brain were embedded in paraffin after the preparation of 4.0- μ m serial sections and then stained with polyclonal antibody against P-CaMKII (A1 to F2). Stronger P-CaMKII immunoreactants (G, * $P < 0.01$, n=3 per group) and more positive cells (H, * $P < 0.05$, n=3 per group) were observed in the spinal dorsal horn (B2 vs. A2), CA1 of the hippocampus (D2 vs. C2) and S1 cortex (F2 vs. E2) of rats at 0.5 hours post-transfusion with remifentanyl. Scale bar=500 μ m in images in A1 to F1; Scale bar=50 μ m in images in A2 to F2; CA1, cornu ammonis region 1; S1, primary somatosensory cortex.

Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (rat dose = human equivalent dose $\times 6.2$). The finding that half a clinical large dose (bolus of 6.0 μ g/kg and a transfusion at 1.2 μ g/kg/min) had triggered hyperalgesia [10], taken with the results of the mechanical sensitivity test in this study, increased levels of P-CaMKII after a large-dose intravenous transfusion with remifentanyl may reflect a correlation between CaMKII activity and hyperalgesia.

A rat model of incision pain is often used in the investigation on postoperative pain in humans [37]. Because remifentanyl-induced hyperalgesia occurred primarily in postoperative patients, the plantar incision pain model is commonly used to reproduce hyperalgesia in clinical practice [10;38]. However, a surgical procedure itself could also induce thermal hyperalgesia and mechanical allodynia [39,40], and incision-evoked

hyperalgesia was found to be closely related with spinal CaMKII activation [41]. Based on this evidence, the non-incision model further reduces confounding factors and better reflects the relationship between remifentanyl exposure and changes in CaMKII levels in the central nervous system. Therefore, a non-incision model was chosen for this study.

Through the detection of changes in P-CaMKII and T-CaMKII levels in the spinal cord, hippocampus, and S1 cortex through Western blot and immunohistochemical analysis, we have formed 3 conclusions. First, short-term exposure to remifentanyl at the dose that tends to induce postoperative hyperalgesia in clinical practice could enhance CaMKII activity by increasing P-CaMKII rather than T-CaMKII levels. Second, the spinal dorsal horn is critical but may not be the only important site involved in remifentanyl-induced hyperalgesia; supraspinal structures such as the hippocampus and S1 cortex may

also be responsible. Third, the effect of remifentanyl on CaMKII activity must be temporary (less than 5 hours) to maintain the P-CaMKII at a higher level.

A series of studies have demonstrated the importance of supraspinal structures in hyperalgesia [42–46]. As an important regulator, levels of P-CaMKII increased in both the spinal dorsal horn and the hippocampus during the nociceptive processes of inflammatory pain [47,48]. In a rodent model of morphine antinociceptive tolerance and physical dependence, tissues from the frontal cortex and lumbar spinal sections show increased activity, but not expression, of CaMKII [49]. A clinical observation has also revealed that the visual analogue scale at rest was significantly increased from only 0 to 2 hours postoperation in the remifentanyl group [35]. In addition, our finding of increased levels of P-CaMKII but not T-CaMKII in the spinal dorsal horn, hippocampus, and S1 cortex 2 hours after transfusion of remifentanyl is consistent with findings of a report of morphine antinociceptive tolerance [50].

Among all possible causes of remifentanyl-induced hyperalgesia, the NMDA receptor is most often mentioned [3–5,51]. A large-dose transfusion of remifentanyl has been shown to enhance the function of NMDA receptors via activation of the δ -opioid receptor, resulting in remifentanyl-induced hyperalgesia [4]. Although no findings have elucidated the mechanism by which CaMKII interacts with the NMDA receptor in remifentanyl-induced hyperalgesia, some reports support the interaction between CaMKII and the NMDA receptor in neuropathic pain. For example, the disruption of CaMKII docking on the NMDA receptor did not induce neuropathic behavioral reflex sensitization [52]; lack of phosphorylation of NR2B subunits

of the NMDA receptor at Tyr1472 in the dorsal horn impaired CaMKII activation and attenuate neuropathic pain [53]. A non-selective NMDA receptor antagonist significantly decreased CaMKII levels in a chronic constriction injury model [20]. In addition, it was reported that neuroprotection against ischemic injury could be induced by diminishing the translocation of CaMKII from cytoplasm to cell membrane [54]. Whether inhibition of the membrane translocation of P-CaMKII is a therapeutic target for opioid-induced hyperalgesia should be the subject of future research.

As indicated in the results that increased P-CaMKII restored to the baseline 5 hours after surgery, an optimal drug to inhibit the increased P-CaMKII should be short-lasting, otherwise the state of “decreased P-CaMKII” may occur. As previously described, propofol, a short-lasting anesthetic, has been demonstrated to inhibit CaMKII phosphorylation in an anesthetic depth-dependent way and to provide better postoperative analgesia when it was added to the treatment protocol in patients after remifentanyl-based anesthesia. Therefore, it may be an optimal drug in remifentanyl-induced hyperalgesia.

Conclusions

This study demonstrated that large-dose intravenous transfusion of remifentanyl can temporarily increase P-CaMKII levels in the central nervous system, which may correlate with postoperative pain sensitization. These results also suggest that drugs chosen for treatment of remifentanyl-induced hyperalgesia should be short-acting rather than long-acting.

References:

- Konopka KH, van WM: Opioid-induced hyperalgesia: pain hurts? *Br J Anaesth*, 2010; 105: 555–57
- Colvin LA, Fallon MT: Opioid-induced hyperalgesia: a clinical challenge. *Br J Anaesth*, 2010; 104: 125–27
- Hahnenkamp K, Nollet J, Van Aken HK et al: Remifentanyl directly activates human N-methyl-D-aspartate receptors expressed in *Xenopus laevis* oocytes. *Anesthesiology*, 2004; 100: 1531–37
- Zhao M, Joo DT: Enhancement of spinal N-methyl-D-aspartate receptor function by remifentanyl action at delta-opioid receptors as a mechanism for acute opioid-induced hyperalgesia or tolerance. *Anesthesiology*, 2008; 109: 308–17
- Angst MS, Koppert W, Pahl I et al: Short-term infusion of the mu-opioid agonist remifentanyl in humans causes hyperalgesia during withdrawal. *Pain*, 2003; 106: 49–57
- Lee HK: Synaptic plasticity and phosphorylation. *Pharmacol Ther*, 2006; 112: 810–32
- Chen BS, Roche KW: Regulation of NMDA receptors by phosphorylation. *Neuropharmacology*, 2007; 53: 362–68
- Wang ZJ, Wilkie DJ, Molokie R: Neurobiological mechanisms of pain in sickle cell disease. *Hematology Am Soc Hematol Educ Program*, 2010; 2010: 403–8
- Wang ZJ, Wang LX: Phosphorylation: a molecular switch in opioid tolerance. *Life Sci*, 2006; 79: 1681–91
- Cui W, Li Y, Li S et al: Systemic lidocaine inhibits remifentanyl-induced hyperalgesia via the inhibition of cPKC γ membrane translocation in spinal dorsal horn of rats. *J Neurosurg Anesthesiol*, 2009; 21: 318–25
- Moriguchi S, Shioda N, Yamamoto Y, Fukunaga K: Platelet-activating factor-induced synaptic facilitation is associated with increased calcium/calmodulin-dependent protein kinase II, protein kinase C and extracellular signal-regulated kinase activities in the rat hippocampal CA1 region. *Neuroscience*, 2010; 166: 1158–66
- Moriguchi S, Oomura Y, Shioda N et al: Ca²⁺/calmodulin-dependent protein kinase II and protein kinase C activities mediate extracellular glucose-regulated hippocampal synaptic efficacy. *Mol Cell Neurosci*, 2011; 46: 101–7
- Yan JZ, Xu Z, Ren SQ et al: Protein kinase C promotes N-methyl-D-aspartate (NMDA) receptor trafficking by indirectly triggering calcium/calmodulin-dependent protein kinase II (CaMKII) autophosphorylation. *J Biol Chem*, 2011; 286: 25187–200
- Colbran RJ, Brown AM: Calcium/calmodulin-dependent protein kinase II and synaptic plasticity. *Curr Opin Neurobiol*, 2004; 14: 318–27
- Okabe S: Molecular anatomy of the postsynaptic density. *Mol Cell Neurosci*, 2007; 34: 503–18
- Fang L, Wu J, Zhang X et al: Calcium/calmodulin dependent protein kinase II regulates the phosphorylation of cyclic AMP-responsive element-binding protein of spinal cord in rats following noxious stimulation. *Neurosci Lett*, 2005; 374: 1–4
- Lu W, Isozaki K, Roche KW, Nicoll RA: Synaptic targeting of AMPA receptors is regulated by a CaMKII site in the first intracellular loop of GluA1. *Proc Natl Acad Sci USA*, 2010; 107: 22266–71

18. Tang L, Shukla PK, Wang LX, Wang ZJ: Reversal of morphine antinociceptive tolerance and dependence by the acute supraspinal inhibition of Ca(2+)/calmodulin-dependent protein kinase II. *J Pharmacol Exp Ther*, 2006; 317: 901–9
19. Chen Y, Yang C, Wang ZJ: Ca2+/calmodulin-dependent protein kinase II alpha is required for the initiation and maintenance of opioid-induced hyperalgesia. *J Neurosci*, 2010; 30: 38–46
20. Dai Y, Wang H, Ogawa A et al: Ca2+/calmodulin-dependent protein kinase II in the spinal cord contributes to neuropathic pain in a rat model of mono-neuropathy. *Eur J Neurosci*, 2005; 21: 2467–74
21. Ogawa A, Dai Y, Yamanaka H et al: Ca(2+)/calmodulin-protein kinase IIalpha in the trigeminal subnucleus caudalis contributes to neuropathic pain following inferior alveolar nerve transection. *Exp Neurol*, 2005; 192: 310–19
22. Luo F, Yang C, Chen Y et al: Reversal of chronic inflammatory pain by acute inhibition of Ca2+/calmodulin-dependent protein kinase II. *J Pharmacol Exp Ther*, 2008; 325: 267–75
23. Singler B, Troster A, Manering N et al: Modulation of remifentanyl-induced postinfusion hyperalgesia by propofol. *Anesth Analg*, 2007; 104: 1397–403
24. Hadi BA, Al RR, Daas R, Naylor I, Zelko R: Remifentanyl in combination with ketamine versus remifentanyl in spinal fusion surgery – a double blind study. *Int J Clin Pharmacol Ther*, 2010; 48: 542–48
25. Cui X, Li J, Li T et al: Propofol and ketamine-induced anesthetic depth-dependent decrease of CaMKII phosphorylation levels in rat hippocampus and cortex. *J Neurosurg Anesthesiol*, 2009; 21: 145–54
26. Kouvaras E, Asprodini EK, Asouchidou I et al: Fentanyl treatment reduces GABAergic inhibition in the CA1 area of the hippocampus 24 h after acute exposure to the drug. *Neuropharmacology*, 2008; 55: 1172–82
27. Jensen T, Granmo M, Schouenborg J: Altered nociceptive C fibre input to primary somatosensory cortex in an animal model of hyperalgesia. *Eur J Pain*, 2011; 15: 368–75
28. Hjernevik T, Jacobsen LM, Qu H et al: Metabolic plasticity in the supraspinal pain modulating circuitry after noxious stimulus-induced spinal cord LTP. *Pain*, 2008; 140: 456–64
29. Chen Y, Jiang Y, Yue W et al: Chronic, but not acute morphine treatment, up-regulates alpha-Ca2+/calmodulin dependent protein kinase II gene expression in rat brain. *Neurochem Res*, 2008; 33: 2092–98
30. Shi Y, Wang C, Han S et al: Determination of PKC isoform-specific protein expression in pulmonary arteries of rats with chronic hypoxia-induced pulmonary hypertension. *Med Sci Monit*, 2012; 18(2): BR69–75
31. Shin SW, Cho AR, Lee HJ et al: Maintenance anaesthetics during remifentanyl-based anaesthesia might affect postoperative pain control after breast cancer surgery. *Br J Anaesth*, 2010; 105: 661–67
32. Nakasuji M, Nakamura M, Imanaka N et al: Intraoperative high-dose remifentanyl increases post-anaesthetic shivering. *Br J Anaesth*, 2010; 105: 162–67
33. Song JW, Lee YW, Yoon KB et al: Magnesium Sulfate Prevents Remifentanyl-Induced Postoperative Hyperalgesia in Patients Undergoing Thyroidectomy. *Anesth Analg*, 2011; 113: 390–97
34. Joly V, Richebe P, Guignard B et al: Remifentanyl-induced postoperative hyperalgesia and its prevention with small-dose ketamine. *Anesthesiology*, 2005; 103: 147–55
35. Hansen EG, Duedahl TH, Romsing J et al: Intra-operative remifentanyl might influence pain levels in the immediate post-operative period after major abdominal surgery. *Acta Anaesthesiol Scand*, 2005; 49: 1464–70
36. Schmidt S, Bethge C, Forster MH, Schafer M: Enhanced postoperative sensitivity to painful pressure stimulation after intraoperative high dose remifentanyl in patients without significant surgical site pain. *Clin J Pain*, 2007; 23: 605–11
37. Brennan TJ, Vandermeulen EP, Gebhart GF: Characterization of a rat model of incisional pain. *Pain*, 1996; 64: 493–501
38. Rivat C, Vera-Portocarrero LP, Ibrahim MM et al: Spinal NK-1 receptor-expressing neurons and descending pathways support fentanyl-induced pain hypersensitivity in a rat model of postoperative pain. *Eur J Neurosci*, 2009; 29: 727–37
39. Pogatzki EM, Raja SN: A mouse model of incisional pain. *Anesthesiology*, 2003; 99: 1023–27
40. Cabanero D, Celerier E, Garcia-Nogales P et al: The pro-nociceptive effects of remifentanyl or surgical injury in mice are associated with a decrease in delta-opioid receptor mRNA levels: Prevention of the nociceptive response by on-site delivery of enkephalins. *Pain*, 2009; 141: 88–96
41. Jones TL, Lustig AC, Sorkin LS: Secondary hyperalgesia in the postoperative pain model is dependent on spinal calcium/calmodulin-dependent protein kinase II alpha activation. *Anesth Analg*, 2007; 105: 1650–56
42. Kim SK, Nabekura J: Rapid synaptic remodeling in the adult somatosensory cortex following peripheral nerve injury and its association with neuropathic pain. *J Neurosci*, 2011; 31: 5477–82
43. Kodama D, Ono H, Tanabe M: Increased hippocampal glycine uptake and cognitive dysfunction after peripheral nerve injury. *Pain*, 2011; 152: 809–17
44. Martins I, Costa-Araujo S, Fadel J et al: Reversal of neuropathic pain by HSV-1-mediated decrease of noradrenaline in a pain facilitatory area of the brain. *Pain*, 2010; 151: 137–45
45. Olesen SS, Brock C, Krarup AL et al: Descending inhibitory pain modulation is impaired in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol*, 2010; 8: 724–30
46. Da Silva LF, Desantana JM, Sluka KA: Activation of NMDA receptors in the brainstem, rostral ventromedial medulla, and nucleus reticularis gigantocellularis mediates mechanical hyperalgesia produced by repeated intramuscular injections of acidic saline in rats. *J Pain*, 2010; 11: 378–87
47. Choi SS, Seo YJ, Kwon MS et al: Increase of phosphorylation of calcium/calmodulin-dependent protein kinase-II in several brain regions by substance P administered intrathecally in mice. *Brain Res Bull*, 2005; 65: 375–81
48. Choi SS, Seo YJ, Shim EJ et al: Involvement of phosphorylated Ca2+/calmodulin-dependent protein kinase II and phosphorylated extracellular signal-regulated protein in the mouse formalin pain model. *Brain Res*, 2006; 1108: 28–38
49. Yang C, Chen Y, Tang L, Wang ZJ: Haloperidol disrupts opioid antinociceptive tolerance and physical dependence. *J Pharmacol Exp Ther*, 2011; 338: 164–72
50. Tang L, Shukla PK, Wang ZJ: Trifluoperazine, an orally available clinically used drug, disrupts opioid antinociceptive tolerance. *Neurosci Lett*, 2006; 397: 1–4
51. Gu X, Wu X, Liu Y et al: Tyrosine phosphorylation of the N-Methyl-D-Aspartate receptor 2B subunit in spinal cord contributes to remifentanyl-induced postoperative hyperalgesia: the preventive effect of ketamine. *Mol Pain*, 2009; 5: 76
52. Garry EM, Moss A, Delaney A et al: Neuropathic sensitization of behavioral reflexes and spinal NMDA receptor/CaM kinase II interactions are disrupted in PSD-95 mutant mice. *Curr Biol*, 2003; 13: 321–28
53. Matsumura S, Kunori S, Mabuchi T et al: Impairment of CaMKII activation and attenuation of neuropathic pain in mice lacking NR2B phosphorylated at Tyr1472. *Eur J Neurosci*, 2010; 32: 798–810
54. Matsumoto S, Murozono M, Nagaoka D et al: Isoflurane inhibits protein kinase Cgamma and calcium/calmodulin dependent protein kinase II-alpha translocation to synaptic membranes in ischemic mice brains. *Neurochem Res*, 2008; 33: 2302–9