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Original Article

The role of Ca^{2+}/calmodulin-dependent protein kinase II-cyclic AMP-responsive element binding protein signaling pathway on sensory-discriminative and affective-motivational pain responses in a rat model of chronic constriction injury of sciatic nerve

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Abstract: Background: CaMKII-CREB signaling plays an important role in maintenance of chronic neuropathic pain. However, very little is known in relation to the contribution in emotional component of chronic pain. This study sought to study the effect of CaMKII-CREB signaling pathway on sensory-discriminative and affective-motivational pain responses in a rat model of chronic constriction injury of sciatic nerve. Methods: Paw withdrawal thermal latency (PWTL), mechanical paw withdrawal threshold (PWMT) and place escape/avoidance paradigm test were measured after CCI surgery and intrathecal injection of CaMKII inhibitor KN-93 and m-AIP. The expression of pCaMKII, pCREB and NR2B were detected by Western-Blot at both the spinal and ACC level. Results: CCI rats developed long-term thermal hyperalgesia, mechanical allodynia and place escape/avoidance behaviors with the upregulation of pCaMKII, pCREB and NR2B at both the spinal and ACC level. Intrathecal treatment with KN-93 and m-AIP on day 7 after CCI surgery markedly improved pain behavior and pain related affect and decreased the expression of pCaMKII and pCREB protein at the spinal and ACC level. Conclusion: These data suggest that CaMKII-CREB signaling pathway participate in the process of sensory-discriminative and affective-motivational pain response. It might be a potential strategy for chronic pain treatment.

Keywords: CaMKII, CREB, neuropathic pain, pain negative affect

Introduction

Pain regarded as the fifth vital sign affects millions of individuals' lives every day, and is the most common reason for physician consultation in contemporary society [1]. The sensory-discriminative and motivational-affective dimensions are considered to be the two basic components of pain [2]. The sensory dimension involves stimulus location and intensity and is encoded by the lateral nociceptive system mainly. The affective dimension involves the emotional component of pain and is encoded by the medial nociceptive system mainly. Increasing clinical evidence have shown that pain-related anxiety, fear and depression are important in the development and maintenance of pain behavior, and have severely effect on patients daily activities suffering chronic pain [3]. Given complex crosstalk between pain perception and related affect, little is known about the role and underlying mechanism of pain related negative effect in chronic pain.

Numerous molecules and receptors which formed a complex signal pathway network is involved in behavior resulting from chronic pain. Among these, the transcription factor cyclic AMP-responsive element binding protein (CREB) has been well characterized. It binds to *cAMP response elements* (CRE), thereby regulating the transcription of the downstream genes including *c-fos*, brain-derived neurotrophic fac-

tor (BDNF) which is closely linked to pain and central sensitization [4, 5]. CREB is activated rapidly by phosphorylation of the Ser-133 via mitogen-activated protein kinases (MAPKs), extracellular Protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase (CaMK) [6]. Of note, CaMK signaling which is critical for a variety of neuronal functions and participating in nociceptive signal pathway paid us much attention.

CaMKII is a serine/threonine protein distributed ubiquitously in both local and central nervous system [7]. Studies have observed that activated CaMKII (phosphorylated, pCaMKII) expression is significantly upregulated in neuropathic pain and bone cancer pain at the spinal level in rodents, while when treated with CaMKII inhibitors reversed pain behaviors [8, 9]. These suggest CaMKII plays an important role in maintenance of chronic neuropathic pain. However, very little is known in relation to the contribution of CaMKII in emotional (affective-motivational) component of chronic pain.

Therefore, the aim of our study was to examine whether activated CaMKII-CREB signal pathway result in chronic pain like behaviors and affective-motivational pain processing in CCI rats. Thus, we tested the injection of CaMKII inhibitors (KN-93 & m-AIP) following CCI attenuation of pain behaviors and negative effect, CaMKII/CREB phosphorylation up-regulation at both spinal cord and ACC level.

Material and methods

Animal models of chronic constriction injury

All experimental procedures were carried out in accordance with the United Kingdom (UK) Animals Scientific Procedures Act 1986 and approved by the Institutional Animal Care and Use Committee of Nanjing University. Every effort was made to minimize animal suffering and the necessary number of animals used to obtain valid results. Adult male Sprague Dawley rats weighing 180-220 g (provided by the Laboratory Animal Center of Drum Tower Hospital) were maintained in a light-controlled room (lights on from 8:00 A.M. to 8:00 P.M) at a constant temperature of 26±2°C. The rats were housed in individual cages with free access to water and food pellets. Aseptic techniques were used in all surgical procedures. Chronic constriction of the sciatic nerve was performed according to the method described by Bennett [10]. In brief, the surgical procedure was performed under sodium pentobarbital (50 mg/kg intraperitoneally). Right sciatic nerve was exposed at the level of the mid-thigh. Three ligatures (5-0 chromic gut, Ethicon, Rome, Italy) were tied loosely around the sciatic nerve with a distance of 1 mm between each ligature. The wound was then sutured with a 4-0 Ethicon silk suture in layers. In the following descriptions, the injured right hind paw will be designated as the ipsilateral paw, and the uninjured left hind paw will be designated as the contralateral paw. The same procedure was used for sham surgeries except that the sciatic nerve was exposed without ligation. All animals were treated with an intraperitoneal injection of cefuroxime at the prophylactic dose of 20 mg/kg at 2 h preoperatively and once daily for the next 2 days to prevent infection.

Drug preparation and intrathecal injection

Water-soluble m-AIP (Biomol, BML-P212) was dissolved in normal saline at a concentration of 0.5 nmol/20 μl and water-soluble KN-93 (Enzo, ALX-430-127) was dissolved in saline at a concentration of 20 nmol/20 μl. Intrathecal injections were conducted according to the method of Mestre [11]. The injection was performed in a volume of 20 μl at the level of the L5 or L6 lumbar vertebra in sevoflurane anesthetized male rats using a micro syringe. The accurate placement of the needle was ensured by a quick "flick" of the rat's tail.

Behavioral tests

Thermal hyperalgesia test: Thermal hyperalgesia to radiant heat was determined according to the method described by Hargreaves et al [12]. Briefly, the rats were placed in clear plastic cages on an elevated glass plate and allowed to acclimatize for 30 min before testing. A radiant thermal stimulator (Dynamic Plantar Analgesiometer, Model 37370, UgoBasile, Italy) was focused onto the plantar surface of the hindpaw through the glass plate. The nociceptive end-points in the radiant heat test were the characteristic lifting or licking of the hindpaw, and the time to the end-point was considered the paw withdrawal thermal latency (PWTL). A cutoff time of 20 s was used to avoid tissue damage. There were five trials per rat and 5 min

intervals between trials. The mean PWTL was obtained from the latter three stimuli.

Mechanical allodynia test: Mechanical allodynia was assessed using a Dynamic Plantar Analgesiometer (Model 37370, Ugo Basile, Italy) on each hind paw. The metal wire was placed on the center of the plantar surface with increased pressure applied, and the pressure of the wire stopped increasing when the rats' paws lifted, and the instrument automatically recorded a value that was regarded as the PWMT.

Place escape/avoidance paradigm testing: Place escape/avoidance testing was performed on CCI rats 1 week after surgery. The rats were placed within a novel Plexiglas chamber (60×30×30 cm). One half was white (light area) and the other half was black (dark area) Animals were allowed unrestricted movement throughout the test chamber for the duration of test period. At the beginning, the rats were placed over the midline of the chamber, and stimulation of the plantar surface of the hindpaw was initiated with a 60 g von Frey hair once every 15 s for 30 min. When residing within the dark area, the injured hindpaw was stimulated. Conversely, the non-injured hindpaw was stimulated when residing within the light area. Suprathreshold stimulation with the 60 g hair was chosen based on the observation that it initiates a reflex nociceptive response of both the injured and non-injured hindpaw. However, the nature of the stimulus is likely to be more aversive when presented to the injured hindpaw since the rats 'choose' to move from the dark to the light side of the chamber [13]. Time spent in the light area and in the dark area were recorded respectively. These data were binned into 5 min time intervals prior to statistical analysis.

Tissue collection

The rats were killed rapidly under pentobarbital sodium anesthesia. The ACC which were dissected according to the method of Franklin and Paxinos [14] and the L3-L5 lumbar spinal cord segments were removed and immediately frozen in liquid nitrogen and stored at -80°C.

Western blot

The tissues were homogenized in a sodium dodecyl sulfate sample buffer containing a mixture of proteinase inhibitors (Sigma, USA). The quantification of the protein contents was performed using the BSA method. The protein samples (40 μg) were separated on a SDS-PAGE gel (NR2B 6% gradient gel) and transferred to polyvinylidenedifluoride filters (Millipore, USA). The filters were blocked with 5% milk and immunoblotted using anti-phosphor-Ser133 CREB (Affbiotech, AF3189, 1:1000 dilution), anti-phosphor-T-286CaMKII (Abcam, ab32678, 1:1000 dilution) and anti-NR2B (Abcam, ab65783, 1:1000 dilution). The membrane was washed with TBST and incubated with a goat polyclonal secondary antibody to rabbit IgG (Abcam, UK, 1:5000 dilution). The blots were visualized in ECL solution (DuPont-NEN, USA) for 1 min and exposed to hyperfilms (Amersham Biosciences) for 1-10 min. The density of specific bands was measured using a computer-assisted imaging analysis system and normalized against the corresponding loading control bands. β-actin (Abcam, UK, 1:1000 dilution) was used as the loading control.

Statistical analysis

All of the data were expressed as the mean ± SD (standard deviation). The data from the behavioral tests were analyzed using repeated ANOVA measurements across testing time points to detect overall differences among the treatment groups. The data from the western blot were analyzed by One-way ANOVA to determine the differences among all of the experimental groups. When significant main effects were observed, the LSD post hoc tests were performed to determine the sources of the differences. Statistical analysis was performed using SPSS 16.0 software (IBM Corporation, Armonk, NY). The differences were considered statistically significant at the level of $P < 0.05$.

Results

CCI-induced long-term thermal hyperalgesia and mechanical allodynia

Before the CCI surgery (day 0), the baseline of PWTL and PWMT were similar in all groups. On day 3, the ipsilateral hindpaw of CCI rats showed decreased PWTL (10.25±2.61) when compared with the sham group (12.59±3.12, $P < 0.05$), and the results lasted up for at least 14 days (9.82±0.98 vs 14.18±1.35, $P < 0.05$);

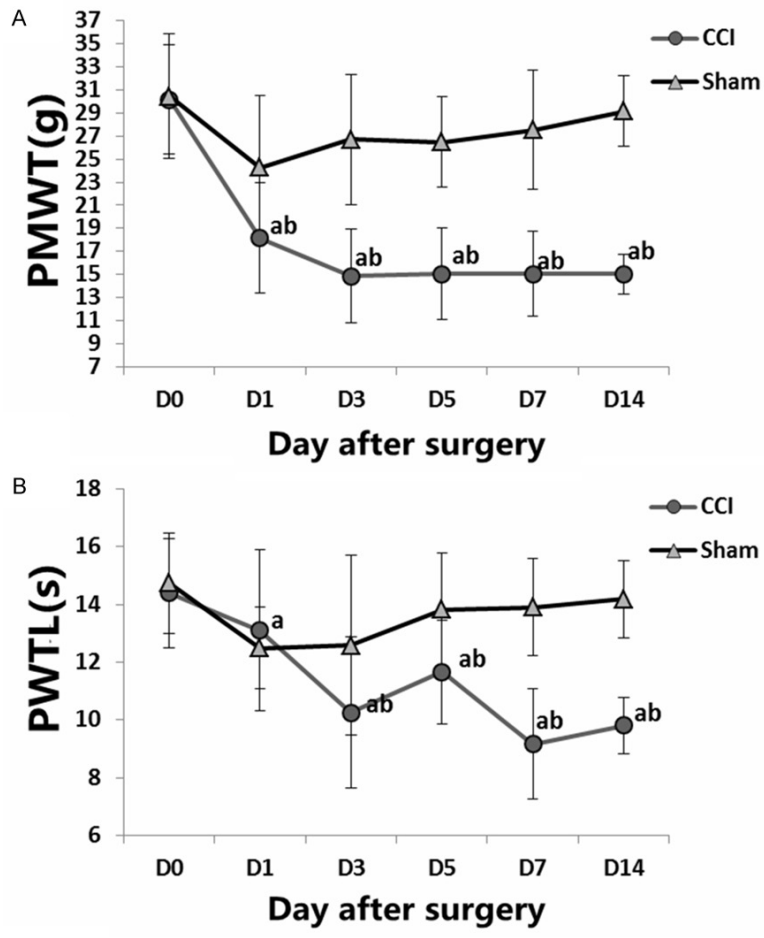

Figure 1. The time course of changes pain behaviors paw withdraw mechanical threshold (PWMT; A) and thermal latency (PWTL; B) on day 0 before CCI surgery and days 1, 3, 5, 7and 14 after CCI surgery (n=6 rats for each group). CCI group significantly decreased PWMT and PWTL compared with the sham group. All of the data points represent the means ± SD. [a]$P < 0.05$ as compared with the values before surgery [b]$P < 0.05$ as compared with the sham group; repeated measures analysis of variance.

On day 1, compared with the sham group (24.29±6.19), PWMT of CCI rats (18.16±4.81, $P < 0.05$) was significantly decreased, and this phenomenon lasted up for at least 14 days (15.03±1.74 vs 29.16±3.07, $P < 0.05$); No significant postoperative changes was seen on the contralateral side ($P > 0.05$; data not shown) (**Figure 1A** and **1B**).

CCI-induced place escape/avoidance

The place escape/avoidance behavior was used to measure the affective-emotional component of pain. When compared with sham group (26.71±6.29), the learning curve showed increased in the amount of time spent in the light area within the second 5 min in rats 7 days after CCI surgery (44.30±10.76, $P < 0.05$), and this phenomenon lasted to the end of the 30 min test period (**Figure 2**).

Changes in the expression of pCaMKII, pCREB and NR2B protein at the spinal and ACC level in response to CCI surgery

Compared with sham group, the level of pCaMKII, pCREB and NR2B protein of CCI rats gradually increased over time at the spinal level. At day 14, significant up-regulation of spinal pCaMKII (1.19±0.01), pCREB (1.26±0.05) and NR-2B (0.97±0.01) were observed in CCI group when compared with those in sham group (0.77±0.01, $P < 0.05$; 0.44±0.04, $P < 0.05$; 0.38±0.01, $P < 0.05$) (**Figure 3A** and **3B**).

Similar results with spinal level were observed in the ACC region. The levels of expression of pCaMKII, pCREB and NR2B protein in the ACC from CCI rats increased gradually over time. At day 14, pCaMKII (1.38±0.09), pCREB (1.09.±0.03) and NR2B (1.34±0.14) showed significantly increased in CCI group when compared with those in sham group (0.60±0.01, $P < 0.05$; 0.72±0.04, $P < 0.05$; 0.54±0.04, $P < 0.05$) (**Figure 3C** and **3D**).

Effect of intrathecal injection of CaMKII antagonist on CCI-induced nociceptive behaviors

To further investigate the effect of CaMKII in CCI rats, we used two kinds of CaMKII antagonist KN-93 and m-AIP to intervene CaMKII activity. Compared with the level before treatment and the same time point in the rats receiving vehicle, the pain behaviors were ameliorated after CaMKII inhibitor treatment. Two hours after drug administration, PWTL of KN-93 group (11.36±1.68, $P < 0.05$) and m-AIP group (11.45±2.04, $P < 0.05$) showed significant dif-

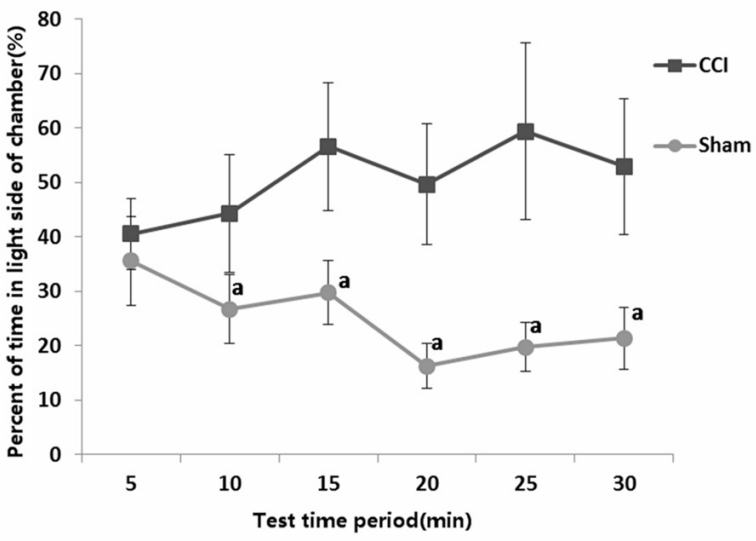

Figure 2. Percent of time spent within the light side of the chamber for CCI rats and Sham group. All of the data points represent the means ± SD. [a]*P* < 0.05 as compared with the CCI group repeated measures analysis of variance.

ferences compared to control group (9.63± 1.65). Similar analgesic effects were found 4 hours after KN-93 (11.06±2.68, *P* < *0.05*) and m-AIP (10.26±1.48, *P* < *0.05*) treatment. 8 hours after treatment, PWTLs recovered to (KN-93 group, 9.06±1.28, *P* > 0.05) and (m-AIP group, 9.71±1.30, *P* > 0.05) and did not show significant differences with control group (9.15±1.00).

Intrathecal treatment of CaMKII inhibitor also attenuated CCI-induced mechanical allodynia. Two hours after drug administration, PWMT of KN-93 group (23.87±4.40, *P* < *0.05*) and m-AIP group (21.15±4.32, *P* < *0.05*) showed significant increased compared to the level before treatment (13.87±2.36). Similar analgesic effects were found 4 hours after KN-93 (23.68±4.96, *P* < *0.05*) and m-AIP (20.45±4.09, *P* < *0.05*) treatment. 8 hours after treatment, PWTLs recovered to (KN-93 group, 14.25±3.95, *P* > 0.05) and (m-AIP group, 15.73±3.21, *P* > 0.05) and did not show significant differences with control group (**Figure 4A** and **4B**).

Effect of intrathecal injection of CaMKII antagonist on CCI-induced place escape/avoidance behaviors

A time course analysis of the PEAP test was used on CCI rats which were injected with CaMKII inhibitors 1.5 h before testing. The learning curve showed decreased in the amount of time spent in the light area already within the second 5 min in both KN-93 group (29.88± 8.15, *P* < *0.05*) and m-AIP group (31.43±11.13, *P* < *0.05*), compared with saline-treated CCI animals (45.13± 10.61), and this phenomenon lasted to the end of the test period (**Figure 5**).

Intrathecal injection of CaMKII antagonist changes the expression of CCI-induced pCaMKII, pCREB and NR2B protein at the spinal and ACC level

At the spinal level, the expression of pCaMKII were 0.91± 0.04 (2 hours after treatment), 0.90±0.06 (4 hours after treatment) in m-AIP group and 0.81±0.05 (2 hours after treatment), 0.93±0.01 (4 hours after treatment) in KN-93 group, and the levels were decreased significantly compared with the vehicle group (1.22±0.04, *P* < 0.05). 8 hours after treatment, the expression of pCaMKII in m-AIP group (1.17±0.01) and KN-93 (1.15±0.08) recovered, and no differences were observed with vehicle group. The expression of pCREB was 0.79±0.04 (2 hours after treatment), 0.75±0.07 (4 hours after treatment) in m-AIP group and 0.95±0.01 (2 hours after treatment), 1.04±0.04 (4 hours after treatment) in KN-93 group, and the levels were decreased significantly compared with the vehicle group (1.15±0.01, *P* < 0.05). 8 hours after treatment, the expression of pCREB in m-AIP group (1.07±0.05) and KN-93 (1.23±0.11) recovered, and no differences were observed with vehicle group. The levels of NR2B expression were of no differences at each time point after KN-93 and m-AIP treatment when compared with vehicle group (*P* > 0.05) (**Figure 6A** and **6B**).

Meanwhile, we detected the protein expression at the ACC level which is closely-related to emotional responses. Similar results were observed. 2 hours after treatment, the level of pCaMKII (0.76±0.09, m-AIP group; 0.86±0.01, KN-93 group) and pCREB (0.56±0.06, m-AIP gro-

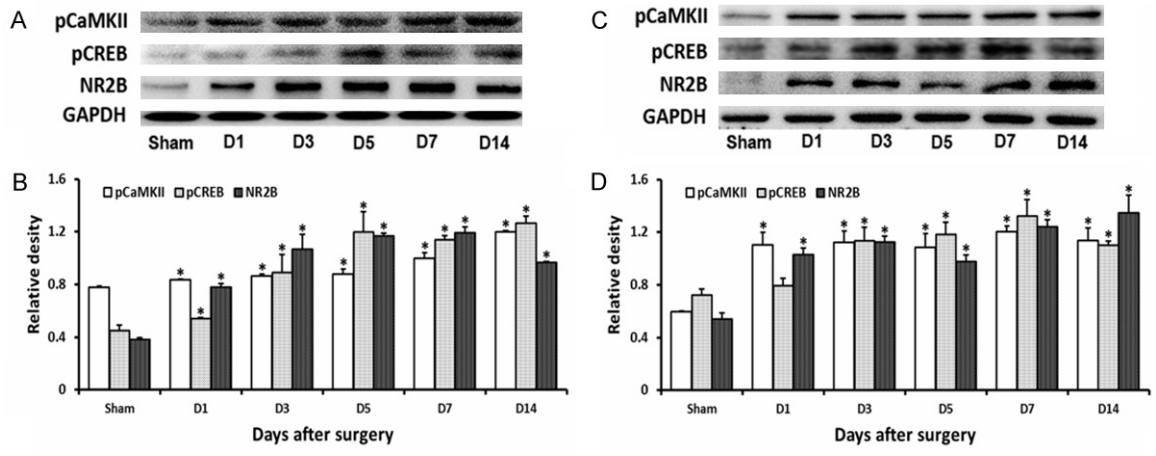

Figure 3. Representative blot showing CaMKII phosphorylation, CREB phosphorylation and NR2B at the spinal level (A) and the ACC level (C) in CCI rats. Statistical analysis of the relative protein expression levels of CaMKII phosphorylation, CREB phosphorylation and NR2B (n=3 in each group) at the spinal level (B) and the ACC level (D). Data are expressed as the means ± SD. *P < 0.05 compared with the sham group; one-way analysis of variance.

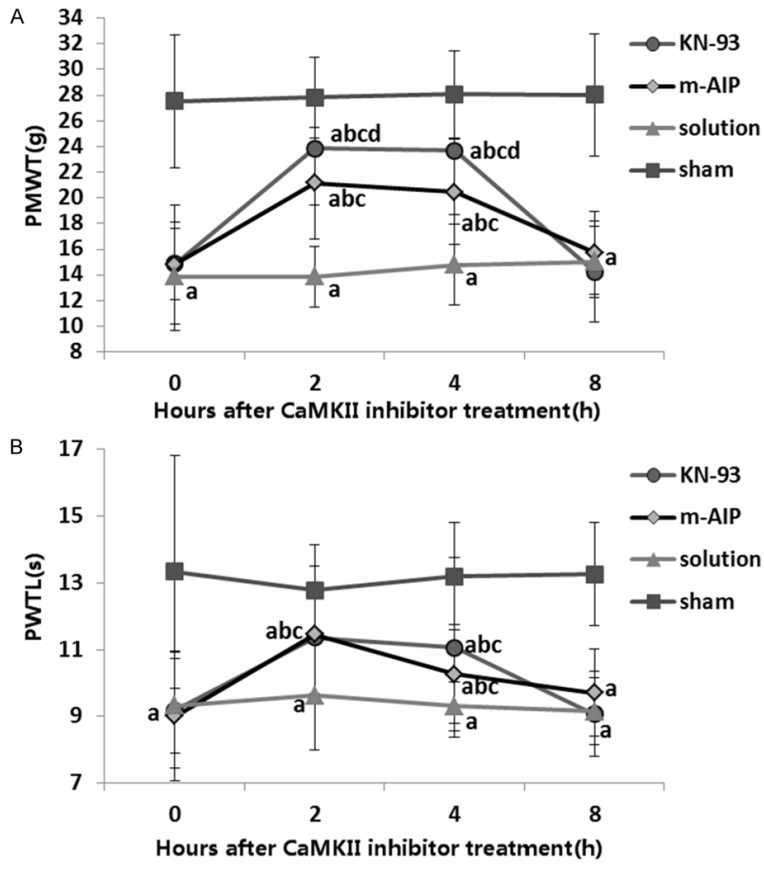

Figure 4. Effect of the intrathecal injection of m-AIP and KN-93on CCI-induced mechanical allodynia (A) and thermal hyperalgesia (B) in rats (n=6 rats for each group. All of the data points represent the mean ± SD. Repeated measures analysis of variance and LSD tests. [a]*P* < 0.05 compared with sham group, [b]*P* < 0.05 compared with control group, [c]*P* < 0.05 compared with baseline before treatment (0 h), [d]*P* < 0.05 compared with m-AIP group.

up; 0.74±0.14, KN-93 group) were significantly decreased compared with vehicle group (pCaMKII: 1.20±0.08, *P* < 0.05; pCREB: 1.40±0.18, *P* < 0.05). Similar results were observed. 4 hours after treatment. 8 hours after treatment, the level of pCaMKII (1.13±0.03, m-AIP group; 1.12±0.06, KN-93 group) and pCREB (1.11±0.06, m-AIP group; 1.39±0.11, KN-93 group) showed no significantly difference compared with vehicle group (*P* > 0.05). No differences were found of the levels of NR2B expression at each time point after KN-93 and m-AIP treatment compared with vehicle group (*P* > 0.05) (**Figure 6C** and **6D**).

Discussion

Chronic pain is often accompanied with negative emotions such as anxiety, depression and fear. Besides, it also interferes with patient's activities and overall quality of life [15]. The specific mechanism of pain formation and maintenance is complex and can be

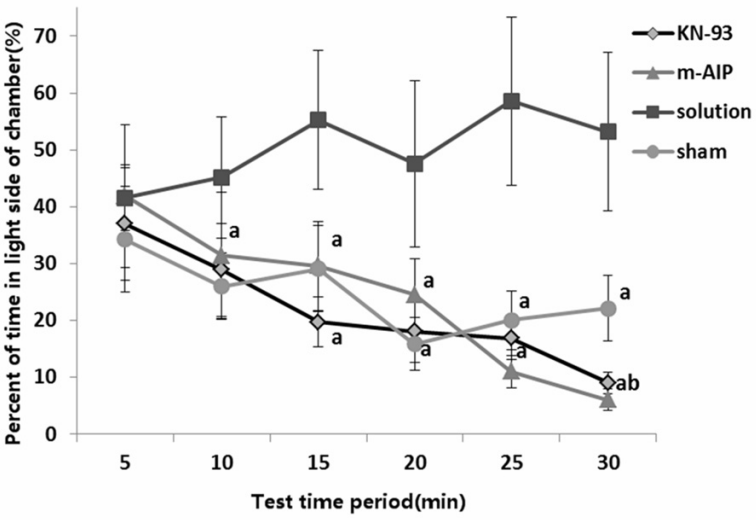

Figure 5. Effect of the intrathecal injection of m-AIP and KN-93 on CCI-induced pain negative effect. All of the data points represent the means ± SD. Repeated measures analysis of variance and LSD tests. [a]$P < 0.05$ compared with sham group, [b]$P < 0.05$ compared with control group, [c]$P < 0.05$ compared with baseline before treatment (0 h), [d]$P < 0.05$ compared with m-AIP group.

modulated by medial and lateral pain systems [16]. Spinal dorsal horn neurons present to be persistent hyperexcitable in response to periphery noxious stimulation and thus resulting in the formation of central sensitization. Studies have shown that negative affect like anxiety and depression are significantly correlated with chronic pain [17], and have an effect on the development of chronic pain and disability [18]. In the present study, we used the CCI model to mimic chronic neuropathic pain, and found that CaMKII-CREB signal pathway participate in sensory-discriminative and affective-motivational pain responses.

In our study, the behavioral data presented that significantly increased scores in the withdrawal threshold to thermal and mechanical stimulation after CCI surgery, and the increase state lasted for at least 14 days. According to LaBuda's research [13], PEAP test was conducted on day 7 after CCI to reflect the affective-emotional component of pain. Animals were faced with the conflict between natural preference of dark environment, accompanied by repeated painful stimulation, and natural aversive bright environment with the motivation of escape from such stimulation. Our result showed increased place escape/avoidance behaviors in rats after CCI, suggesting that CCI rat has developed significant pain-related negative effect. Similar results were reported in Pedersen and colleagues studies [19].

Meanwhile, we found that the level of pCaMKII, pCREB and NR2B expression were significantly increased after CCI surgery both at the spinal and ACC level, and lasted up to at least 14 days. This showed relatively synchronous with pain behaviors of CCI rats. These results suggested that CaMKII-CREB pathway may participate in CCI processing and interrelated negative emotions. We inhibited overexpressed CaMKII phosphorylation by intrathecal treatment of specific CaMKII inhibitor m-AIP and non-specific inhibitor KN-93. As a result, significant analgesic effect was observed 2 hours and 4 hours after two CaMKII inhibitor injections. Similar results were found in Chen's study [20]. In addition, marked reduction of place escape/avoidance behavior was found in CCI rats after inhibition of CaMKII activity in PEAP test. This provides us behavior evidence of the effect of CaMKII in pain and related negative affect. 2 hours and 4 hours after m-AIP and KN-93 treatment, the expression of pCaMKII and pCREB protein were significantly decreased at the spinal cord level. Similar results were also found at the ACC level. The agreement of the expression of target protein and pain behavioral and affect further confirmed the effect of CaMKII in sensory-discriminative and affective-motivational pain responses in chronic pain.

Herein, we focus on the Anterior Cingulate Cortex (ACC), a component of limbic system, plays an important role in pain, negative and cognitive control.

Animals' studies have found that increased Fos expression in the ACC is followed by formalin-induced conditioned place avoidance in rodents [21]. Neurons in the ACC that are affected by neuropathic pain have enhanced excitatory synaptic transmission and resulted long-term pain allodynia [22]. Clinical studies have revealed that increased activation in the ACC fol-

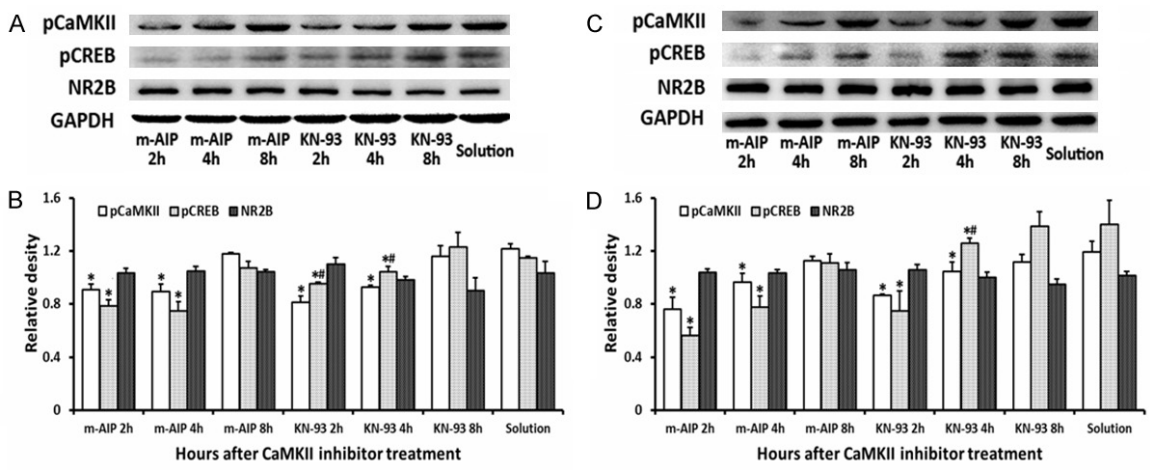

Figure 6. Representative blot showing the changes of CaMKII phosphorylation, CREB phosphorylation and NR2B at the spinal cord level (A) and the ACC level (C) in CCI rats following intrathecal KN-93 and m-AIP treatments. Statistical analysis of the relative protein expression levels of CaMKII phosphorylation, CREB phosphorylation and NR2B (n=3 in each group) at the spinal cord level (B) and the ACC level (D). Data are expressed as the means ± SD. One-way analysis of variance and LSD tests. [a]$P < 0.05$ compared with sham group, [b]$P < 0.05$ compared with control group, [c]$P < 0.05$ compared with baseline before treatment (0 h), [d]$P < 0.05$ compared with m-AIP group.

lowing recall-induced emotion and changed states of pain affect by hypnosis [23, 24]. We present data showing that the level of pCaMKII and pCREB protein expression changed in the ACC after CCI surgery and CaMKII inhibitor injection. The result is not only consistent with the changes of protein expression in the spinal cord, but also consistent with behavioral studies. This fully indicated that the sensory component and affective component of pain integrated in the ACC region and that this is expressed by a mechanism involves CaMKII-CREB signal pathway.

Numerous studies showed that NMDAR NR2B subunit, mainly distributed in the area involved in transmission and regulation of nociceptive signals including forebrain and spinal cord dorsal horn [25] is vital in chronic pain and central sensitization. Enhanced surface expression of NR2B in the spinal cord dorsal horn after noxious stimulation could be a mechanism in nociceptive processing [26-28]. Besides, studies have indicated that upregulation of NR2B subunits and enhancement of neurons NMDA-evoked currents in the ACC following peripheral noxious stimulation [29]. Our research also found that increased expression of NR2B protein in CCI rats and suggested that NR2B could be involved in pain affect.

It is widely accepted that a significant increase of Ca^{2+} in intracellular through opened NMDA receptor following nociceptive stimulation can trigger a series of protein kinases, such as PKA, PKC, as well as CaMKII. 7 Numerous articles showed that up regulation of CaMKII phosphorylation at the spinal cord in rats with neuropathic pain, and after treatment with CaMKII inhibitor KN-93 improved pain behaviors [30]. Analgesic effect also found in bone cancer mice when inhibit CaMKII activity [31]. Transcription factor CREB has been identified as one target for CaMKII during central sensitization. Studies have reported that phosphorylation of CREB was involved in the development of central sensation following peripheral nerve injury [32]. Furthermore, significantly reduction of increased pCREB level after CaMKII inhibitor KN93 injection [33]. In this article, we also found that the expression of pCREB was decreased in CCI rats following KN-93 and m-AIP treatment at both spinal cord and ACC area. These suggested that CaMKII take part in pain perception and pain related affect by regulating downstream transcription factor of activity CREB. However, no changes were found of the level of NR2B protein expression after CaMKII inhibitor treatment neither at the spinal cord nor ACC level. The changes of calcium ion level caused by CaMKII inhibitor intervention could not feedback to the upstream NMDA receptor might be the reason. Whether NR2B activity changed or not need further exploration.

Conclusions

In conclusion, our work suggests that increased pCaMK II and pCREB at the spinal cord and ACC level following pain hyperalgesia and pain affect in CCI processing, and intrathecal injection of CaMKII inhibitorm-AIP and KN-93 can effectively improve pain behaviors and attenuated negative effect. We can conclude that CaMKII-CREB signaling pathway participated in the process of sensory-discriminative and affective-motivational pain responses. This might provide us a new strategy for the treatment of chronic pain.

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Disclosure of conflict of interest

None.

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References

[1] Debono DJ, Hoeksema LJ, Hobbs RD. Caring for patients with chronic pain: pearls and pitfalls. J Am Osteopath Assoc 2013; 113: 620-627.

[2] Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science 2000; 288: 1769-1772.

[3] Crombez G, Vlaeyen JW, Heuts PH, Lysens R. Pain-related fear is more disabling than pain itself: evidence on the role of pain-related fear in chronic back pain disability. Pain 1999; 80: 329-339.

[4] Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. Nat Neurosci 2002; 5: 405-414.

[5] Barco A, Bailey CH, Kandel ER. Common molecular mechanisms in explicit and implicit memory. J Neurochem 2006; 97: 1520-1533.

[6] Lonze BE and Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron 2002; 35: 605-623.

[7] Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 2002; 3: 175-190.

[8] Kim Y, Cho H, Ahn YJ, Kim J, Yoon YW. Effect of NMDA NR2B antagonist on neuropathic pain in two spinal cord injury models. Pain 2012; 153: 1022-1029.

[9] Katano T, Nakazawa T, Nakatsuka T, Watanabe M, Yamamoto T, Ito S. Involvement of spinal phosphorylation cascade of Tyr1472-NR2B, Thr286-CaMKII, and Ser831-GluR1 in neuropathic pain. Neuropharmacology 2011; 60: 609-616.

[10] Bennett GJ and Xie Y. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87-107.

[11] Mestre C, Pélissier T, Fialip J, Wilcox G, Eschalier A. A method to perform direct transcutaneous intrathecal injection in rats. J Pharmacol Toxicol Methods 1994; 32: 197-200.

[12] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32: 77-88.

[13] LaBuda CJ and Fuchs PN. A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp Neurol 2000; 163: 490-494.

[14] Paxinos G and Franklin KB. The mouse brain in stereotaxic coordinates. Gulf Professional Publishing; 2004.

[15] Jensen MP, Hoffman AJ, Cardenas DD. Chronic pain in individuals with spinal cord injury: a survey and longitudinal study. Spinal Cord 2005; 43: 704-712.

[16] Apkarian AV, Hashmi JA, Baliki MN. Pain and the brain: specificity and plasticity of the brain in clinical chronic pain. Pain 2011; 152: S49-S64.

[17] Holzberg AD, Robinson ME, Geisser ME, Gremillion HA. The effects of depression and chronic pain on psychosocial and physical functioning. Clin J Pain 1996; 12: 118-125.

[18] Hasenbring MI, Chehadi O, Titze C, Kreddig N. Fear and anxiety in the transition from acute to chronic pain: there is evidence for endurance besides avoidance. Pain Manag 2014; 4: 363-374.

[19] Pedersen LH, Scheel-Krüger J, Blackburn-Munro G. Amygdala GABA-A receptor involve-

ment in mediating sensory-discriminative and affective-motivational pain responses in a rat model of peripheral nerve injury. Pain 2007; 127: 17-26.

[20] Chen Y, Luo F, Yang C, Kirkmire CM, Wang ZJ. Acute inhibition of Ca2+/calmodulin-dependent protein kinase II reverses experimental neuropathic pain in mice. J Pharmacol Exp Ther 2009; 330: 650-659.

[21] Lei L, Zhang Y, Zhao Z. Pain-related aversion and Fos expression in the central nervous system in rats. Neuroreport 2004; 15: 67-71.

[22] Bushnell MC, Čeko M, Low LA. Cognitive and emotional control of pain and its disruption in chronic pain. Nat Rev Neurosci 2013; 14: 502-511.

[23] Casey KL. Forebrain mechanisms of nociception and pain: analysis through imaging. P Natl Acad Sci U S A 1999; 96: 7668.

[24] Rainville P, Carrier B, Hofbauer RK, Bushnell MC, Duncan GH. Dissociation of sensory and affective dimensions of pain using hypnotic modulation. Pain 1999; 82: 159-171.

[25] Nagy GG, Watanabe M, Fukaya M, Todd AJ. Synaptic distribution of the NR1, NR2A and NR2B subunits of the N-methyl-d-aspartate receptor in the rat lumbar spinal cord revealed with an antigen-unmasking technique. Eur J Neurosci 2004; 20: 3301-3312.

[26] Gu X, Wu X, Liu Y, Cui S, Ma Z. Tyrosine phosphorylation of the N-Methyl-D-Aspartate receptor 2B subunit in spinal cord contributes to remifentanil-induced postoperative hyperalgesia: the preventive effect of ketamine. Mol Pain 2009; 5: 76-78.

[27] Zheng Y, Cui S, Liu Y, Zhang J, Zhang W, Zhang J, Gu X, Ma Z. Dexmedetomidine prevents remifentanil-induced postoperative hyperalgesia and decreases spinal tyrosine phosphorylation of N-methyl-d-aspartate receptor 2B subunit. Brain Res Bull 2012; 87: 427-431.

[28] Peng H, Chen G, Lai C, Tung K, Chang J, Lin T. Endogenous ephrinB2 mediates colon-urethra cross-organ sensitization via Src kinase-dependent tyrosine phosphorylation of NR2B. Am J Physiol-Renal 2010; 298: F109-F117.

[29] Li T, Ren W, Xiao X, Nan J, Cheng L, Zhang X, Zhao Z, Zhang Y. NMDA NR2A and NR2B receptors in the rostral anterior cingulate cortex contribute to pain-related aversion in male rats. Pain 2009; 146: 183-193.

[30] Crown ED, Gwak YS, Ye Z, Tan HY, Johnson KM, Xu G, McAdoo DJ, Hulsebosch CE. Calcium/calmodulin dependent kinase II contributes to persistent central neuropathic pain following spinal cord injury. Pain 2012; 153: 710-721.

[31] Ma Z, Ren B, Liang Y, Gu X. Inhibition of calcium/calmodulin-dependent protein kinase II (CaMKII) attenuates bone cancer pain via spinal KIF17/NR2B signal transduction in mice: 14AP7-3. European Journal of Anaesthesiology (EJA) 2012; 29: 208.

[32] Ma W and Quirion R. Increased phosphorylation of cyclic AMP response element-binding protein (CREB) in the superficial dorsal horn neurons following partial sciatic nerve ligation. Pain 2001; 93: 295-301.

[33] Fang L, Wu J, Zhang X, Lin Q, Willis WD. 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.