RESEARCH ARTICLE

CaMKIIα may modulate fentanyl-induced hyperalgesia via a CeLC-PAG-RVM-spinal cord descending facilitative pain pathway in rats

Zhen Li¹, Pingping Yin¹, Jian Chen², Shenglan Jin³, Jieqiong Liu¹, Fang Luo¹*

¹ Department of Anesthesiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, ² The Laboratory of Membrane Ion Channels and Medicine, Key Laboratory of Cognitive Science, State Ethnic Affairs Commission, College of Biomedical Engineering, South-Central University for Nationalities, Wuhan, China, ³ Department of Anesthesiology, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

* luofang0909@hotmail.com

Abstract

Each of the lateral capsular division of central nucleus of amygdala (CeLC), periaqueductal gray (PAG), rostral ventromedial medulla (RVM) and spinal cord has been proved to contribute to the development of opioid-induced hyperalgesia (OIH). Especially, Ca²⁺/calmodulin-dependent protein kinase IIα (CaMKIIα) in CeLC and spinal cord seems to play a key role in OIH modulation. However, the pain pathway through which CaMKIIα modulates OIH is not clear. The pathway from CeLC to spinal cord for this modulation was explored in the present study. Mechanical and thermal hyperalgesia were tested by von Frey test or Hargreaves test, respectively. CaMKIIα activity (phospho-CaMKIIα, p-CaMKIIα) was evaluated by western blot analysis. CaMKIIα antagonist (KN93) was micro-injected into CeLC, spinal cord or PAG, respectively, to evaluate its effect on behavioral hyperalgesia and p-CaMKIIα expression in CeLC, PAG, RVM and spinal cord. Then the underlying synaptic mechanism was explored by recording miniature excitatory postsynaptic currents (mEPSCs) on PAG slices using whole-cell voltage-clamp methods. Results showed that inhibition of CeLC, PAG or spinal CaMKIIα activity respectively by KN93, reversed both mechanical and thermal hyperalgesia. Microinjection of KN93 into CeLC decreased p-CaMKIIα expression in CeLC, spinal cord or PAG, respectively, to evaluate its effect on behavioral hyperalgesia and p-CaMKIIα expression in CeLC, PAG, RVM and spinal cord. Then the underlying synaptic mechanism was explored by recording miniature excitatory postsynaptic currents (mEPSCs) on PAG slices using whole-cell voltage-clamp methods. Results showed that inhibition of CeLC, PAG or spinal CaMKIIα activity respectively by KN93, reversed both mechanical and thermal hyperalgesia. Microinjection of KN93 into CeLC decreased p-CaMKIIα expression in CeLC, PAG, RVM and spinal cord; while intrathecal KN93 can only block spinal but not CeLC CaMKIIα activity. KN93 injected into PAG just decreased p-CaMKIIα expression in PAG, RVM and spinal cord, but not in the CeLC. Similarly, whole-cell voltage-clamp recording found the frequency and amplitude of mEPSCs in PAG cells were decreased by KN93 added in PAG slice or micro-infused into CeLC in vivo. These results together with previous findings suggest that CaMKIIα may modulate OIH via a CeLC-PAG-RVM-spinal cord descending facilitative pain pathway.
Introduction

Opioids can provide effective pain relief but their use is limited because of a clinical paradoxical syndrome in which patients on long-term opioids exposure become more sensitive to pain. This paradoxical phenomenon has been named as opioid-induced hyperalgesia (OIH) [1–3]. OIH has been reliably demonstrated not only in humans suffered from different types of pain but also in healthy volunteers and opioid addicts [4–6]. Several rodent models have also been setup to facilitate the study of this phenomenon. Morphine and remifentanil induced hyperalgesia have been largely explored at spinal level as well as supraspinal level. Recently, we have explored the mechanism of fentanyl-induced hyperalgesia at both spinal cord and the lateral capsular division of central nucleus of amygdala (CeLC). In addition, we have found a pivotal role of \( \text{Ca}^{2+}/\text{calmodulin-dependent protein kinase II} \alpha \) (CaMKII\( \alpha \)) in the modulation of OIH.

CaMKII\( \alpha \), contributing to hyperalgesia priming[7], is a multifunctional serine/threonine protein kinase that has an outstanding effect on glutamate neurotransmission and behavior-related synaptic plasticity [8, 9], which makes it a very important target to explore the neural mechanisms of OIH. Although the underlying mechanism of OIH is not yet completely understood[1, 3, 4, 10], previous reports from our groups have shown that CaMKII\( \alpha \), which is highly expressed in the CeLC and superficial spinal dorsal horn, plays an important role in modulation of OIH[11, 12]. Either spinal or CeLC CaMKII\( \alpha \) inhibition can attenuate OIH [11, 12].

The descending pain modulation from the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) has clearly been involved in the development of hyperalgesia and analgesic tolerance induced by opiates [13–16]. Local administration of lidocaine into RVM abolished OIH in rat[17]. In the PAG also, Toll Like Receptor-4 signaling, phosphorylation of mitogen-activated protein kinase and c-Fos have been shown to participate in OIH [18–21]. CeLC, a target of the spino-parabrachio-amygdaloid pain pathway that has been considered as “the nociceptive amygdala” [22–24], is also the upstream of PAG-RVM-spinal cord descending pain pathway[22, 25–29]. Anatomical studies have demonstrated direct reciprocal neurons projections between the amygdala and the PAG [30, 31]. Given the close anatomical connectivity between these regions and their role in OIH pain modulation, we speculate that there may exist a CeLC-PAG-RVM-spinal cord descending pathway to modulate OIH.

The purpose of this study was to explore whether CaMKII\( \alpha \) modulates OIH via a CeLC-PAG-RVM-spinal cord descending pathway. In our previously experiment, we have found that detectable mechanical and thermal behavioral hyperalgesia peaked at 6.5 h post fentanyl injection, and lasted for 3 d-4 d [11]. Therefore, 6.5 h post fentanyl injection, the CaMKII\( \alpha \) inhibitor KN93 was micro-infused into CeLC, spinal cord and PAG respectively to evaluate its effect on behavioral hyperalgesia and the activity of CaMKII\( \alpha \) at different level of the CeLC-PAG-RVM-spinal cord descending pathway. Finally, the underlying synaptic mechanism was explored by recording miniature excitatory postsynaptic currents (mEPSCs), which are frequently used as a parameter to reflect altered synaptic transmission [32, 33], on the PAG slices using whole-cell voltage-clamp methods.

Materials and methods

Ethics statement

All procedures were approved by and implemented in accordance with the Institutional Animal Care and Use Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Permit Number: TJ- A20141217), and performed in the light of the guides and policies by the International Association for the Study of Pain[34]. Rats were
sacrificed by subsequent decapitation after sodium pentobarbital (50 mg/kg, i.p.) and all available efforts were for minimizing animal suffering.

Animal care and utilization
Male Sprague-Dawley rats (80-120g, obtained from animal laboratory of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) were maintained on a 12:12 h light-dark cycle in a climate-controlled room (24˚C) with food and water available ad libitum. We monitored the physical condition of the experiment animals every day and none of the experiment animals became severely ill or died at any time. Intraperitoneal administration of sodium pentobarbital (50 mg/kg) was used to anaesthetize the experiment animals.

Experimental protocol and drug delivery
The detailed experimental design was illustrated in Fig 1. Chemicals other indicated were obtained from Beyotime and Boster (Shanghai, China). Four times injections of fentanyl (60 μg/kg per injection, s.c.) at 15 min intervals, resulting in a cumulative dose of 240 μg/kg [10, 11] were conducted to induce OIH in this study. The control animals received an equal volume (1.2 ml/kg) of physiological saline. KN93(N-[2-[[3-(4’-Chlorophenyl)-2-propenyl] methylamino|methyl|phenyl]-N-(2-hydroxyethyl)-4’-methoxybenzenesulfonylamide phosphate salt) and KN92 (2-[N-(4-methoxybenzenesulfonyl)]amino-N-(4-chlorocinnamyl)-N-methyl-benzylamine, monohydrochloride) were purchased from Cayman (Ann Arbor, MI) and were dissolved in 50% dimethyl sulfoxide (DMSO). 50% DMSO was used as a vehicle control. Intrathecal (i.t.) injections were performed manually between the L5 and L6 inter-vertebral space in conscious rats as previously described [12]. The injection was performed using a glass microsyringe (RWD Life Science, Shenzhen, China). Each rat was injected with a volume of 0.45 μl. Success of the i.t. injection was verified by a lateral tail-flick.

Behavior testing
Mechanical hyperalgesia. Mechanical sensitivity was tested using the von Frey filaments (North Coast, San Jose, CA, USA) according to the Dixon method[35, 36]. Before the test, rats were placed into separate Plexiglas containers over mesh platforms and allowed to adapt about 30 min to achieve immobility. Beginning with 1.0 g, fibers of sequentially increasing stiffness were perpendicularly applied to the mid-plantar surface according to the up-down paradigm. Positive response was defined as paw flinching shook, or licking its paw or brisk withdrawal. The interval between adjacent tests was more than 5 minutes. The 50% probability of paw withdrawal threshold was calculated using the up and down paradigm [11, 36, 37].

Thermal hyperalgesia. Rats were habituated to the test environment for 30 min before assessment. Thermal sensitivity was assayed with a radiant thermal stimulator (BME-410C, Biomedical Engineering, Boerni science and technology limited company, Guangzhou, China) according to the Hargreaves’ method [35, 36, 38]. The time appearing positive response, which was defined as a clear paw withdrawal, was recorded as the paw withdrawal latency after the application of radiant thermal stimulator to the mid-plantar surface of the left hind paw through the glass floor. The cutoff was 15 s to prevent damaging of tissues. The test was repeated three trials with a 5-minute interval, and then the thermal latency was defined as the mean of three trials [11].
Fig 1. Illustration of the experimental design. Experiment 1: To investigate the effect of CeLC CaMKIIα antagonism on the level of CaMKII activity in CeLC, PAG, RVM and spinal cord regions and the behavioral hyperalgesia induced by fentanyl, rats were first implanted with CeLC cannulas before the induction of OIH by fentanyl. Then the Control group (n = 6) and OIH group (n = 6) received 50% DMSO (vehicle) 0.3 μl. OIH+KN92 10 nmol group (n = 6) and OIH+KN93 10 nmol group (n = 6) received equal volume (0.3 μl) of KN92 10 nmol and KN93 10 nmol respectively 6.5 h after the last injection of fentanyl. Experiment 2: To test whether spinal cord has a reverse effect on amygdala CaMKIIα signal, animals were randomly divided into four groups.
Western blotting

The proteins (see flowchart illustrated in Fig 1) from each group after the behavioral test were extracted using the radio-immuno-precipitation assay buffer and protein concentration was determined by bicinchoninic acid assay [11, 12]. Samples (20 μg of total protein) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred electrophoretically onto polyvinylidene fluoride membrane. After blocking with 5% skim milk for 2 h at room temperature, the membranes were incubated in primary rabbit anti-(T286) p-CaMKII α antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) or a mouse anti-GAPDH antibody (1:400; Boster, Shanghai, China) at 4˚C overnight. After having been washed, the membranes were incubated with HRP conjugated anti-rabbit (for p-CaMKII α) or anti-mouse (for GAPDH) secondary antibody IgG (1:10,000, Boster, Shanghai, China) at room temperature for 90 min. The p-CaMKII α antibody detected double bands in the experiments, both of which correspond to p-CaMKII α[39]. The immunoblots were visualized by chemiluminescence or enhanced chemiluminescence signals (Thermo Fisher, Shanghai, China) detection using a Bio-Rad ChemiDoc (Shanghai, China) system and normalized to GAPDH bands.

Cannulation and microinjection

Briefly, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and mounted in a stereotaxic frame (Zenda, Austin, Texas, USA. As reported, the guide cannula (RWD Life Science, Shenzhen, China) was implanted into the right CeLC—2.0 mm rostrally, 4.2 mm laterally, and 7 mm ventrally[11, 24, 40, 41] or the right ventrolateral periaqueductal grey (vlPAG) -7.6 mm rostrally, -0.5 mm laterally, and 5 mm ventrally [42–45] toward the bregma [46] and fixed to the skull with dental acrylic. A dummy cannula (RWD Life Science, Shenzhen, China) that inserts into the guide cannula, served to reduce the incidence of occlusion. Then animals were returned to their cages and housed individually to recover for one week prior to the next experiment.

For drug infusion, the various solutions were injected into the CeLC or the vlPAG through an injector (RWD Life Science, Shenzhen, China), which extended 0.5 mm beyond the guide cannula to target CeLC or vlPAG. The injector was attached to a 10 μl Hamilton syringe via polyethylene tubing (PE-10), and the solution was infused with a pump at 0.15 μl/min for 2 min. Waiting for another 2 min, the injection cannula was gently removed.

Electrophysiology experiment

Slice preparation. Sprague Dawley male rats anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) were decapitated, and coronal midbrain slices (350 μm) containing PAG tissue

https://doi.org/10.1371/journal.pone.0177412.g001
were prepared by a vibrating microtome (VT1000S, Leica Microsystems, Nussloch, Germany) in cutting solution at 4°C. The dissection solution contained (in mM) 213 sucrose, 3 KCl, 1 NaH2PO4, 0.5 CaCl2, 5 MgCl2, 26 NaHCO3 and 10 glucose. The slices were incubated in the artificial cerebrospinal (ACSF) at 34°C for at least 1h. A single brain slice was then placed onto the recording chamber and perfused with 37°C running ACSF that was equilibrated with 95%O2 and 5%CO2 at a rate of 2 ml/min. The ACSF perfusion solution contained (in mM) 125 NaCl, 5 KCl, 1.2 NaH2PO4, 2.6 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 glucose [47, 48]. Only 2–3 brain slices per animal were used, and only 1 neuron was recorded in each slice.

**Whole-cell patch-clamp recording.** A whole cell voltage-clamp technique was used to record mEPSCs in the vl-PAG neurons as previously described [11, 47–49]. Borosilicate glass capillaries (1.5 mm outer diameter, 1.0 mm inner diameter; WPI, USA) were pulled to make the recording pipettes (impedance was 4 MΩ- 6 MΩ) filled with the following internal solution (in mM):145 KCl, 5 NaCl, 10 HEPES, 5 EGTA, 4 Mg-ATP, and 0.3 Na3-GTP, pH adjusted to 7.3 with KOH. A 5 min equilibration period was allowed to reach a steady state after whole cell access was established. Cells that exhibited any obvious (> 15%) change during the recording period were abandoned. A HEKA EPC-10 amplifier (HEKA, Lambrecht, Germany) and patchmaster software (Molecular Devices, Sunnyvale, Calif, USA) were used for data acquisition and analysis. mEPSCs were obtained at a holding potential of -70 mV (in the presence of 50 μM picrotoxin and 1 μM tetrodotoxin) and measured 10 min before and 15 min after drug application[50]. A 5min fixed length of traces was analyzed for frequency and amplitude changes using Mini Analysis program 6.0 (Synaptosoft Inc, Fort Lee, NJ, USA) and pCLAMP10 software (Molecular Devices, Sunnyvale, Calif, USA).

**Statistics**

The sample size used in experiments was based on our previous studies [11]. Based on this calculation, we have chosen to increase the sample size to ensure accurate data. Data are presented as Mean ± SD. Using the D’Agostino and Pearson omnibus normality test, data were normal distribution and parametric statistics were applied. Homogeneity of variance was proved by the Bartlett’s test. Western blot analysis was assessed using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc comparisons. Behavioral test in all experiments were analyzed by two-way repeated measures ANOVA (time and drug as variables) followed by Bonferroni multiple post-hoc comparisons. The effect of KN93 on the change of frequency and amplitude of mEPSCs in vl-PAG cells (added in the PAG slices) were analyzed by Student’s t-tests (paired), and in PAG cells (micro-infused into CeLC) were analyzed by one-way ANOVA followed by the Bonferroni post hoc test. All graphs and statistical analysis were performed using GraphPad Prism6.0 (GraphPad Software, San Diego, CA). Significant differences were defined as P < 0.05.

**Results**

**Intra-CeLC KN93 micro-injection reversed fentanyl-induced hyperalgesia and inhibited the CaMKIIα activation in CeLC, vlPAG, RVM and spinal cord in OIH rats (Experiment 1)**

To investigate the effect of intra-CeLC KN93 (CaMKIIα antagonist) micro-injection on the CaMKIIα activity in the proposed ‘CeLC-PAG-RVM-spinal cord’ pathway and the behavioral hyperalgesia induced by fentanyl, rats were first implanted with CeLC cannulas before the
induction of OIH. Then the Control group (n = 6) and OIH group (n = 6) received 50% DMSO (vehicle) 0.3 μl. OIH+KN92 10 nmol group (n = 6) and OIH+KN93 10 nmol group (n = 6) received equal volume (0.3 μl) of KN92 10 nmol and KN93 10 nmol respectively 6.5 h after the last injection of fentanyl. Confirming previous reports [11], our data revealed that the established fentanyl-induced hyperalgesia was rapidly attenuated by KN93, while the same treatment with KN92 (kinase-inactive chemical analogue) or vehicle did not alter the pain threshold (F = 34.63, p < 0.001, two-way repeated measures ANOVA followed by Bonferroni multiple post-hoc comparisons; Fig 2A and 2B). Correlated with the behavioral effects, intra-CeLC KN93(10 nmol) application not only attenuated the increased p-CaMKIIα expression in the CeLC area, but also the PAG, RVM and the spinal cord regions induced by fentanyl. While KN92 or vehicle had little effect (one-way ANOVA followed by Bonferroni post-hoc comparisons; Fig 2C–2J). It implied that CeLC should be the upstream of the PAG, RVM and spinal cord in modulating fentanyl-induced hyperalgesia.

**Intrathecal KN93 injection attenuated fentanyl-induced hyperalgesia and decreased the level of p-CaMKIIα in spinal cord but not CeLC in OIH rats (Experiment 2)**

According to our former results that CeLC might be the upstream of the PAG, RVM and spinal cord in modulating OIH, the next experiment was performed to test whether spinal cord has a reverse effect on amygdala CaMKIIα signal. In line with the previous studies [12, 51], inhibition of spinal CaMKIIα by an i.t. injection of KN93 (45 nmol) attenuated behavioral hyperalgesia induced by fentanyl (n = 6 for each group, p < 0.001, two-way repeated measures ANOVA followed by Bonferroni multiple post-hoc comparisons; Fig 3A and 3B). While KN92 and vehicle both had little effect on nociceptive behaviors. After behavioral test, the spinal cord and CeLC tissue is dissected to perform Western blot analysis to explore whether i.t. KN93 could reverse the CaMKIIα activation in CeLC in OIH rats. Results shown that inhibition of spinal CaMKIIα by KN93 (45 nmol) could not attenuate the increased level of CeLC p-CaMKIIα in OIH rats (one-way ANOVA followed by Bonferroni post-hoc comparisons; Fig 3C–3F), demonstrating that amygdala can facilitate downstream spinal CaMKIIα activation during the maintenance of OIH, on the contrary, the spinal cord exhibit little reverse activation on amygdala CaMKIIα signal.

**Intra-vlPAG KN93 micro-injection reversed fentanyl-induced hyperalgesia and inhibited CaMKIIα activation in PAG, RVM and spinal cord but not in CeLC (Experiment 3)**

Since amygdala can facilitate downstream spinal CaMKIIα activation during the maintenance of OIH, we then explored if this effect was exerted via a ‘CeLC-PAG-RVM-spinal cord’ descending pathway. To confirm whether p-CaMKIIα in vlPAG is involved in fentanyl-induced hyperalgesia and whether there is connection between PAG and other regions including CeLC, RVM and spinal cord in OIH, intra-vlPAG injection of KN93 was performed in OIH rats. Control group (n = 6) and OIH group (n = 6) received 50% DMSO (vehicle) 0.3 μl. OIH+KN92 10 nmol group (n = 6) and OIH+KN93 10 nmol group (n = 6) received KN92 10 nmol and KN93 10 nmol [52, 53] respectively through an intra-vlPAG cannula 6.5 h after the last injection of fentanyl. As shown in Fig 4, the established behavioral hyperalgesia induced by fentanyl was rapidly reversed by Intra-vlPAG injection of KN93, while KN92 and vehicle did not alter the pain threshold (p < 0.001, two-way repeated measures ANOVA followed by Bonferroni multiple post-hoc comparisons; Fig 4A and 4B). Correlated with the behavioral changes, intra-vlPAG KN93 (not KN92 and vehicle) injection decreased p-CaMKIIα.
Fig 2. Intra-CeLC KN93 micro-injection reversed fentanyl-induced hyperalgesia and inhibited the CaMKIIα activation in CeLC, vPAG, RVM and spinal cord in OIH rats. (A-B) Bar graphs show the measurements of the mechanical threshold of paw withdrawal (A) and thermal paw withdrawal latency (±SD) collected at baseline (before Cannulation), pre-drug (6h after the last injection of fentanyl or saline) and then post-drug (0.5 h after microinjection). (C-J) Bar graphs show the mean relative density of p-CaMKIIα to GAPDH in CeLC (C-D), vPAG (E-F), RVM (G-H) and spinal cord (I-J). Control, OIH, OIH+KN92 45 nmol, and OIH+KN93 45 nmol group received 50% DMSO (vehicle), 50%DMSO (vehicle), KN92 10 nmol and KN93 10 nmol respectively through the CeLC cannula. *, compared with Control group; &, compared with OIH group; +, compared with OIH+KN92 45 nmol group, n = 6 for each group; One symbol: p < 0.05, Two symbols: p < 0.01, Three symbols: p < 0.001.

https://doi.org/10.1371/journal.pone.0177412.g002
expression in the PAG, RVM and the spinal cord but not in CeLC compared with control (one-way ANOVA followed by Bonferroni post-hoc comparisons; Fig 4C–4J). These results proved that PAG might be the upstream of RVM and spinal cord and the downstream of CeLC in modulating fentanyl-induced hyperalgesia.

Fig 3. Intrathecal KN93 injection attenuated fentanyl-induced hyperalgesia and decreased the level of $p$-CaMKII$\alpha$ in spinal cord but not CeLC in OIH rats. (A, B) Graphical display of the mechanical threshold of paw withdrawal (A) and thermal paw withdrawal latency (B) collected at baseline, pre-drug (6 h after the last injection of fentanyl or saline), and post-drug (0.5 h after Intrathecal injection). (C-F) Representative Immunoblots of activated CaMKII$\alpha$ ($p$-CaMKII$\alpha$) and histogram of relative density of $p$-CaMKII$\alpha$ to GAPDH in the spinal cord (C-D) and CeLC (E-F). Control, OIH, OIH+KN92 45 nmol, and OIH+KN93 45 nmol group received i.t. injection with 50% DMSO (vehicle), 50% DMSO (vehicle), KN92 10 nmol and KN93 10 nmol respectively. Results are expressed as mean ± SD; *, compared with Control group; &, compared with OIH group; +, compared with OIH+KN92 45 nmol group, n = 6 for each group; One symbol: $p<0.05$, Two symbols: $p<0.01$, Three symbols: $p<0.001$.

https://doi.org/10.1371/journal.pone.0177412.g003
Fig 4. Intra-viPAG KN93 micro-injection reversed fentanyl-induced hyperalgesia and inhibited CaMKIIα activation in PAG, RVM and spinal cord but not in CeLC. (A–B) Graphical display of the mechanical threshold of paw withdrawal (A) and thermal paw withdrawal latency collected at baseline (before Cannulation), pre-drug (6 h after the last injection of fentanyl or saline) and then post-drug (0.5 h after microinjection). (C–J) Representative immunoblots of activated CaMKIIα (p-CaMKIIα) and histogram of relative density of p-CaMKIIα to GAPDH in the CeLC (C–D), viPAG (E–F), RVM (G–H) and spinal cord (I–J). Control group (n = 6) and OIH group (n = 6) received 50% DMSO (vehicle) 0.3 μl. OIH+ KN92 10 nmol group.
Reversion of increased mEPSCs in vlPAG neurons from the OIH rats by KN93 (added in the ACSF) (Experiment 4)

The analysis of mEPSCs is a well-established electrophysiological approach to determine presynaptic versus postsynaptic mechanisms. Presynaptic changes at the transmitter release site affect mEPSCs frequency, whereas changes at the postsynaptic membrane alter mEPSCs amplitude (quantal size) [41, 54]. Previous studies from our laboratory have shown that KN93 significantly decreased both the frequency and amplitude of mEPSCs in CeLC neurons from the OIH rats, suggesting that CaMKIIα regulates synaptic transmission in CeLC neurons through pre- and postsynaptic mechanisms [11]. In addition, anatomical studies have demonstrated direct reciprocal neurons projections between the amygdala and the PAG [30, 31]. Therefore, mEPSCs were recorded in the PAG neurons to determine whether there were differences between the control rats and OIH rats in the synaptic transmission, and whether CaMKIIα modulates synaptic transmission in PAG neurons in OIH rats. Results shown that KN93 (10 μM, added in the ACSF) [55–57] significantly reversed both the increased frequency (Fig 5C) and amplitude (Fig 5D) of mEPSCs recorded from the vlPAG neurons in slices from OIH rats (12 h post OIH induction), but not those from the control rats (paired t-tests; n = 6).

Inhibition of mEPSCs in vlPAG neurons from the OIH rats by intro-CeLC injection of KN93 (Experiment 5)

To further confirm whether in vivo inhibition of CeLC CaMKIIα activity has a direct effect on the enhanced synaptic transmission of vlPAG neurons in OIH rats, mEPSCs was recorded in vlPAG neurons after intro-CeLC injection of KN93 (10 nmol) [11]. It was shown that, compared with control (non-OIH rats), intro-CeLC injection of KN93, but not KN92, significantly decreased the frequency (Fig 6C) and amplitude (Fig 6D) of mEPSCs recorded from the vlPAG neurons in slices from OIH rats (12 h post OIH induction) (one-way ANOVA followed by Bonferroni post-hoc comparisons; n = 8–11).

Discussion

To our knowledge, this is the first report addressing the role of CaMKIIα in the CeLC, PAG, RVM and spinal cord as a whole in the modulation of fentanyl-induced hyperalgesia. Our data reveal that, CaMKIIα may modulate OIH via a CeLC-PAG-RVM-spinal cord descending facilitative pain pathway. These findings may provide novel insight into the mechanisms underlying fentanyl-induced hyperalgesia.

In this study, results shown that inhibition of CaMKIIα activity at any level of the ‘CeLC--PAG-RVM-spinal cord’ pathway can reverse the behavioral hyperalgesia induced by fentanyl. However, only intervention at upstream of this pathway can modulate the downstream molecule (CaMKIIα) activation and synaptic potentiation, but not vice versa. A possible explanation of this phenomenon is that there is a pronociceptive facilitative cascade from CeLC to PAG, RVM, and spinal cord. And CaMKIIα is the key molecule for this facilitative cascade.

PAG, one of the core components of the descending pain-modulatory system, has been proved to project to the rostral ventromedial medulla (RVM) and the spinal cord. The PAG also receives inputs from the CeLC, which is involved in mediating top-down analgesia.
Fig 5. KN93 (added in the ACSF) reversed the enhanced mEPSCs in vPAG neurons from the OIH rats. (A) mEPSCs recordings in control and OIH group at baseline and after the application of KN93. (B) Individual traces (average) of mEPSCs obtained from respective recordings. Calibration: 1 s, 20 pA. (C-D). Bar graphs showing the frequency (C) and amplitude (D) of mEPSCs in vPAG neurons in control and OIH group (12 h post induction). Results are expressed as mean ± SD; n = 6 for each group; *, p < 0.05.

https://doi.org/10.1371/journal.pone.0177412.g005
Fig 6. Inhibition of mEPSCs in vPAG neurons from the OIH rats by intro-CeLC injection of KN93. (A) mEPSCs recordings in control and OIH group after the application of KN92 or KN93. (B) Individual traces (average) of mEPSCs obtained from respective recordings. Calibration: 1 s, 20 pA. (C-D) Bar graphs showing the frequency (C) and amplitude (D) of mEPSCs from vPAG neurons in slices from control and OIH group (12 h post induction). Results are expressed as mean ± SD; n = 8–11 for each group; *, p < 0.05, **, p < 0.01.

https://doi.org/10.1371/journal.pone.0177412.g006
It is now believed that the CeLC produces descending modulation of pain through peptidergic projections to the PAG and RVM [28, 58, 61]. Reports have shown that the protein kinase C-δ-positive neurons and somatostatin-expressing neurons are the major population of the lateral subdivision of the central amygdala (CeLC) neurons [31, 62]. Whether CeLC neurons that project to vlPAG have the same cell types will be further studied. The modulatory facilitatory or inhibitory effect of PAG on pain is generated concomitantly with the changes of the RVM neurons that related the facilitative or inhibitory modulation of pain [63, 64]. The RVM can both facilitate and inhibit pain through recruitment of RVM on- or off-cells, respectively [65–69]. The pronociceptive on-cells and antinociceptive off-cells in RVM region that project to spinal cord offer a neuronal condition for facilitation and inhibition pain regulation from the descending pain pathway [66]. It is reported that pain threshold might change with the balance between the on-cells and off-cells populations in RVM [65] and sustained exposure to morphine resulted in an increased proportion of on-cells, which might contribute to morphine-induced paradoxical pain [70]. So we speculate that synaptic facilitation of PAG manifested by the enhancement of mEPSCs, likely acting through the RVM on-cells activity, contributes to the OIH modulation derived from CeLC, driving the exaggerated behavioral responses and increased spinal neuronal excitability to non-noxious stimulations observed in OIH rats. Of course, the activity and the proportion of on- or off-cells recorded from the RVM under OIH condition need to be identified in future studies.

mEPSCs, a well-established electrophysiological approach to determine pre- or postsynaptic mechanisms, is assumed to represent the spontaneous release of individual vesicles or quanta of neurotransmitter from the presynaptic membrane [71]. Changes at the postsynaptic membrane are known to alter quantal size (mEPSCs amplitude), whereas presynaptic changes at the transmitter release site affect the frequency of mEPSCs [72]. Consistent with previous report that both pre- and postsynaptic CaMKIIα are necessary for the induction of synaptic plasticity [73], our results shown that CaMKIIα inhibitor added in ACSF or injected into CeLC significantly reversed or inhibited both the increased frequency and amplitude of mEPSCs recorded on vlPAG neurons in slices from OIH rats. So, we can safely speculate that CaMKIIα modulated the synaptic plasticity in vlPAG neurons involved both pre- and postsynaptic mechanisms.

CaMKIIα is expressed at presynaptic nerve terminal as well as the postsynaptic density [74]. At presynaptic location, transient receptor potential vanilloid type 1 channel (TRPV1) has been proved plays an important role in OIH [75–78] and it can interact with CaMKIIα physiologically and pharmacologically [79, 80], thus forming possible feed-forward loops [81] to enhance vesicle motility and facilitates presynaptic spontaneous neurotransmitter release [74]. This mechanism may underlie the increased frequency observed in vlPAG neurons. At postsynaptic location, N-methyl-D-aspartate receptor (NMDAR), which plays an important role in the development of OIH [10, 82], can also interact with CaMKIIα to form a positive feed-forward to facilitate synaptic potentiation. This mechanism may contribute to the amplitude increase of the mEPSC recorded in the present study. Actually, it has reported that opioids promote the activation of CaMKIIα in PAG synaptosomes probably via activation of NMDA receptors [83–86].

It is shown that only right amygdala develops pain-related plasticity that is coupled to pain facilitation in the arthritis pain model [87]. And only ventral lateral part of PAG (vlPAG) has been proved functionally connected to pain circuit in the RVM [43, 88]. In addition, there is a strong contralateral projection of pain pathway [22], so behavioral tests in all the experiments in the present study were performed in the left plantar of animal, while analyzing the level of phosphorylation of CaMKIIα (p-CaMKIIα) and recording synaptic transmission in the right CeLC and vlPAG.
The dose of fentanyl used in pediatric patients is usually about (2–3) μg/kg during surgery, and in this experiment the dosage of fentanyl (60 μg/kg, 4 times, 15 min intervals, s.c.) was based on our previous study [11]. According to equivalent dose conversion between the species, this dose (240μg/kg) was used to mimic the high dose used in pediatric patient’s surgeries. In line with the observation that up to postnatal day 21 in rats, the RVM solely has the facilitatory effect on spinal pain transmission but that after this age (postnatal day 28 to adult), the facilitatory effect of the RVM turns to biphasic facilitation and inhibition [89]. Moreover, most relevant studies have only focused on the mechanisms of OIH in adult rats, but we cannot deny the increased morbidity rate and opioid exposure in the adolescents. Therefore, we used adolescent rats (about 4 weeks) in all the experiments. In this study, all the experiments are conducted on rats without obvious peripheral nerve injury or inflammation. Nevertheless, in clinics, opioids are mostly administered to patients suffering from chronic or acute pain disorders, further studies of an animal model of surgical pain are needed to test.

Although the mechanisms by which CaMKIIα regulates OIH are unknown, we suppose CaMKIIα may modulate OIH via a CeLC-PAG-RVM-spinal cord descending pain pathway. However, in addition to the PAG-RVM system, the dorsal reticular nucleus (DRt) and caudal lateral ventrolateral medulla (VLM) have also been reported to be involved in modulation of descending pain[65]. Neurons that project along the prefrontal cortex (PFC)–amygdala–PAG pathway have been implicated in mediating descending pain[46] and the PAG also receives inputs from the hypothalamus, PFC, and anterior cingulate cortex (ACC), which plays a major role in mediating pain[60]. Thus, we cannot deny the role of hypothalamus, PFC, ACC, DRt and VLM on this descending pathway. In a word, we hope that our study provides novel insight into the mechanisms underlying fentanyl-induced hyperalgesia.

Author Contributions

Conceptualization: ZL FL.
Data curation: ZL PY JC.
Formal analysis: ZL SJ JL.
Funding acquisition: FL.
Investigation: ZL PY JC.
Methodology: ZL.
Project administration: ZL FL.
Resources: FL.
Software: ZL.
Supervision: FL.
Validation: PY JC.
Visualization: ZL PY JC FL.
Writing – original draft: ZL.
Writing – review & editing: FL SJ JL.
References

1. Ossipov MH, Lai J, King T, Vanderah TW, Porreca F. Underlying mechanisms of pronociceptive consequences of prolonged morphine exposure. Biopolymers. 2005; 80(2–3):319–24. https://doi.org/10.1002/bip.20254 PMID: 15795927

2. Mao J. Opioid-induced abnormal pain sensitivity: implications in clinical opioid therapy. Pain. 2002; 100(3):213–7. PMID: 12467992

3. Vanderah TW, Suenaga NM, Ossipov MH, Malan TP Jr., Lai J, Porreca F. Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced abnormal pain and antinociceptive tolerance. J Neurosci. 2001; 21(1):279–86. PMID: 1150345

4. Lee HJ, Yeomans DC. Opioid induced hyperalgesia in anesthetic settings. Korean J Anesthesiol. 2014; 67(5):299–304. https://doi.org/10.4097/kjae.2014.67.5.299 PMID: 25475457

5. Stoicea N, Russell D, Weidner G, Durda M, Joseph NC, Yu J, et al. Opioid-induced hyperalgesia in chronic pain patients and the mitigating effects of gabapentin. Front Pharmacol. 2015; 6:104–9. https://doi.org/10.3389/fphar.2015.00104 PMID: 26074817

6. Mauermann E, Fillitz J, Dolder P, Rentsch KM, Bandschapp O, Ruppen W. Does Fentanyl Lead to Opioid-induced Hyperalgesia in Healthy Volunteers? A Double-blind, Randomized, Crossover Trial. Anesthesiology. 2016; 124(2):453–63. https://doi.org/10.1097/ALN.0000000000000976 PMID: 26654993

7. Ferrari LF, Bogen O, Levine JD. Role of nociceptor alphaCaMK II in transition from acute to chronic pain (hyperalgesic priming) in male and female rats. J Neurosci. 2013; 33(27):11002–11. https://doi.org/10.1523/JNEUROS CI.1785-13.2013 PMID: 23825405

8. Salling MC, Faccidom o SP, Li C, Psilos K, Galunas C, Spanos M, et al. Moderate Alcohol Drinking and the Amygdala Proteome: Identification and Validation of Calcium/Calmodulin Dependent Kinase II and AMPA Receptor Activity as Novel Molecular Mechanisms of the Positive Reinforcing Effects of Alcohol. Biological psychiatry. 2016; 79(6):430–42. https://doi.org/10.1016/j.biops ych.2014.10.020 PMID: 25579851

9. Lisman J, Schulman H, Cline H. The molecular basis of CaMKII function in synaptic and behavioural memory. Nature reviews Neuroscience. 2002; 3(3):175–90. https://doi.org/10.1038/nrn753 PMID: 11994750

10. Celieri E, Rivat C, Jun Y, Laulin JP, Larcher A, Reynier P, et al. Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine. Anesthesiology. 2000; 92(2):465–72. PMID: 10691234

11. Li Z, Li C, Yin P, Wang ZJ, Luo F. Inhibition of CaMKIIalpha in the Central Nucleus of Amygdala Attenuates Fentanyl-Induced Hyperalgesia in Rats. J Pharmacol Exp Ther. 2016; 359(1):82–9. https://doi.org/10.1124/jpet.116.233817 PMID: 25819118

12. Vanderah TW, Suenaga NM, Ossipov MH, Jr MT, Lai J, Porreca F. Tonic descending facilitation from the rostral ventromedial medulla mediates opioid-induced hyperalgiesia and antinociceptive tolerance. Journal of Neuroscience the Official Journal of the Society for Neuroscience. 2001; 21(1):279–86.

13. Lu CE, Shi L, Zhang J, Kong M, Yue L, Yu Z, et al. Neuron-restrictive silencer factor in periaqueductal gray contributes to remifentanil-induced postoperative hyperalgesia via repression of the mu-opioid receptor. J Neurol Sci. 2015; 352(1–2):48–52. https://doi.org/10.1016/j.jns.2015.03.018 PMID: 25819118

14. Xie JY, Herman DS Stiller CO, Gardell LR, Ossipov MH, Lai J, Porreca F, et al. Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance. Journal of Neuroscience the Official Journal of the Society for Neuroscience. 2005; 25(2):409–16.

15. Roeckel LA, Le Coz GM, Gaveriaux-Ruff C, Simonin F. Opioid-induced hyperalgesia: Cellular and molecular mechanisms. Neuroscienc e. 2016.

16. R C, Vera-Portocarrero LP, Ibrahim MM, Mata HP, Stagg NJ, Felice MD, 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(4):727–37. https://doi.org/10.1111/j.1460-9568.2009.06616.x PMID: 19200067

17. Eidson LN, Murphy AZ. Persistent Peripheral Inflammation Attenuates Morphine-Induced Periaqueductal Gray Glial Cell Activation and Analgesic Tolerance in the Male Rat. The Journal of Pain. 2013; 14(4):393–404. https://doi.org/10.1016/j.jpain.2012.12.010 PMID: 23395474

18. Sanna MD, Mello T, Ghezzi C, Ghezzi C, Galeotti N. Inhibition of spinal ERK1/2–c-JUN signaling pathway counteracts the development of low doses morphine-induced hyperalgesia. Eur J Pharmacol. 2015; 764:271–7. https://doi.org/10.1016/j.ejphar.2015.07.022 PMID: 26165762
20. Arout CA, Caldwell M, Mccloskey DP, Kest B. C-Fos activation in the periaqueductal gray following acute morphine-3β-D-glucuronide or morphine administration. Physiol Behav. 2014; 130:28–33. https://doi.org/10.1016/j.physbeh.2014.02.056 PMID: 24631297

21. Sanna MD, Ghelardini C, Galeotti N. Regionally selective activation of ERK and JNK in morphine paradoxical hyperalgesia: A step toward improving opioid pain therapy. Neuropharmacology. 2014; 86:67–77. https://doi.org/10.1016/j.neuropharm.2014.06.007 PMID: 24950452

22. Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. Neuroscientist. 2004; 10(3):221–34. https://doi.org/10.1177/1073858403261077 PMID: 15155061

23. Sarhan M, Freund-Mercier MJ, Veinante P. Branching patterns of parabrachial neurons projecting to the central extended amygdala: single axonal reconstructions. J Comp Neurol. 2005; 491(4):418–42. https://doi.org/10.1002/cne.20697 PMID: 16175547

24. Sarhan M, Pawlowski SA, Barthas F, Yalcin I, Kauffling J, Dardente H, et al. BDNF parabrachial-amygdaloïd pathway in morphine-induced analgesia. Int J Neuropsychopharmacol. 2013; 16(7):1649–60. https://doi.org/10.1017/S146114571200168X PMID: 23425507

25. Fabry ME, Bouhassira EE, Suzuka SM, Nagel RL. Transgenic mice and hemoglobinopathies. Methods Mol Med. 2003; 82:213–41. https://doi.org/10.1385/1-59259-373-9:213 PMID: 12669646

26. Carrasquillo Y, Gereau RWt. Activation of the extracellular signal-regulated kinase in the amygdala modulates pain perception. J Neurosci. 2007; 27(7):1543–51. https://doi.org/10.1523/JNEUROSCI.3536-06.2007 PMID: 17301163

27. Tracey I, Mantyh PW. The cerebral signature for pain perception and its modulation. Neuron. 2007; 55(3):377–91. https://doi.org/10.1016/j.neuron.2007.07.012 PMID: 17678852

28. Martin TJ, Ewan E. Chronic pain alters drug self-administration: implications for addiction and pain mechanisms. Exp Clin Psychopharmacol. 2008; 16(5):357–66. https://doi.org/10.1037/a0013597 PMID: 18837632

29. Hamlin AS, McNally GP, Osborne PB. Induction of c-Fos and zif268 in the nociceptive amygdala parallel abstinence hyperalgesia in rats briefly exposed to morphine. Neuropharmacology. 2007; 53(2):330–43. https://doi.org/10.1016/j.neuropharm.2007.05.017 PMID: 17631915

30. Oliveira MA, Prado WA. Role of PAG in the antinociception evoked from the medial or central amygdala in rats. Brain Res Bull. 2001; 54(1):55–63. PMID: 11226714

31. Penzo MA, Robert V, Li B. Fear conditioning potentiates synaptic transmission onto long-range projection neurons in the lateral subdivision of central amygdala. J Neurosci. 2014; 34(7):2432–7. https://doi.org/10.1523/JNEUROSCI.1461-13.2014 PMID: 24523533

32. Xu H, Wu Lj, Wang H, Zhang X, Vadakkan KI, Kim SS, et al. Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. J Neurosci. 2008; 28(29):7445–53. https://doi.org/10.1523/JNEUROSCI.1812-08.2008 PMID: 18632948

33. Wang XL, Zhang HM, Chen SR, Pan HL. Altered synaptic input and GABAB receptor function in spinal superficial dorsal horn neurons in rats with diabetic neuropathy. J Physiol. 2007; 579(Pt 3):267–75. https://doi.org/10.1124/jpet.107.132167 PMID: 18178903

34. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16(2):109–10. PMID: 6877845

35. 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(2):650–9. https://doi.org/10.1124/jpet.109.152165 PMID: 19478130

36. Luo F, Yang C, Chen Y, Shukla P, Tang L, Wang LX, et al. Reversal of chronic inflammatory pain by acute inhibition of Ca2+/calmodulin-dependent protein kinase II. J Pharmacol Exp Ther. 2008; 325(1):267–75. https://doi.org/10.1124/jpet.107.132167 PMID: 18178903

37. Dixon WJ, Mood AM. A method for obtaining and analyzing sensitivity data. Journal of the American Statistical Association. 1948; 43(241):109–26.

38. 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(1):77–88. PMID: 3340425

39. Hu X, Huang F, Szymusiak M, Tian X, Liu Y, Wang ZJ. PLGA-Curcumin Attenuates Opioid-Induced Hyperalgesia and Inhibits Spinal CaMKIIα. PLoS One. 2016; 11(1):e0146393. https://doi.org/10.1371/journal.pone.0146393 PMID: 27648442

40. Zhang RX, Zhang M, Li A, Pan L, Berman BM, Ren K, et al. DAMGO in the central amygdala alleviates the affective dimension of pain in a rat model of inflammatory hyperalgesia. Neuroscience. 2013; 222:359–66. https://doi.org/10.1016/j.neuroscience.2013.08.039 PMID: 23994597

41. Han JS, Adwanikar H, Li Z, Ji G, Neugebauer V. Facilitation of synaptic transmission and pain responses by CGRP in the amygdala of normal rats. Mol Pain. 2010; 6:10–23. https://doi.org/10.1186/1744-8069-6-10 PMID: 20144185
42. Lei J, Sun T, Lumb BM, You HJ. Roles of the periaqueductal gray in descending facilitatory and inhibitory controls of intramuscular hypertonic saline induced muscle nociception. Exp Neurol. 2014; 257:88–94. https://doi.org/10.1016/j.expneurol.2014.04.019 PMID: 24792920

43. de Novellis V, Luongo L, Guida F, Cristino L, Palazzo E, Russo R, et al. Effects of intra-ventrolateral periaqueductal grey palmitoylethanolamide on thermoceptive threshold and rostral ventromedial medulla cell activity. Eur J Pharmacol. 2012; 676(1–3):41–50. https://doi.org/10.1016/j.ejphar.2011.11.034 PMID: 22178921

44. Palazzo E, Guida F, Gatta L, Luongo L, Boccella S, Bellini G, et al. EP1 receptor within the ventrolateral periaqueductal gray controls thermoception and rostral ventromedial medulla cell activity in healthy and neuropathic rat. Mol Pain. 2011; 7:82. https://doi.org/10.1186/1744-8069-7-82 PMID: 22023852

45. Rosen A, Zhang YX, Lund I, Lundeberg T, Yu LC. Substance P microinjected into the periaqueductal gray matter induces antinoceception and is released following morphine administration. Brain Res. 2004; 1001(1–2):87–94. https://doi.org/10.1016/j.brainres.2003.11.060 PMID: 14972657

46. Butler RK, Nilsson-Todd L, Cleren C, Lena I, Garcia R, Finn DP. Molecular and electrophysiological changes in the prefrontal cortex-amygdala-dorsal periaqueductal grey pathway during persistent pain state and fear-conditioned analgesia. Physiol Behav. 2011; 104(5):1075–81. https://doi.org/10.1016/j.physbeh.2011.05.028 PMID: 21683728

47. Yang K, Furue H, Kumamoto E, Dong Y-X, Yoshimura M. Pre- and postsynaptic inhibition mediated by GABAB receptors in rat ventrolateral periaqueductal gray neuronal. Biochem Biophys Res Commun. 2003; 302(2):233–7. PMID: 12604336

48. Xing J, Li J. TRPV1 Receptor Mediates Glutamatergic Synaptic Input to Dorsolateral Periaqueductal Gray (dl-PAG) Neurons. J Neurophysiol. 2007; 97(1):503–11. https://doi.org/10.1152/jn.01023.2006 PMID: 17065246

49. Hu J, Wang Z, Guo YY, Zhang XN, Xu ZH, Liu SB, et al. A role of periaqueductal grey NR2B-containing NMDA receptor in mediating persistent inflammatory pain. Mol Pain. 2009; 5:71. https://doi.org/10.1186/1744-8069-5-71 PMID: 20003379

50. Kiritoshi T, Sun H, Ren W, Stauffer SR, Lindsley CW, Conn PJ, et al. Modulation of pyramidal cell output in the medial prefrontal cortex by mGluR5 interacting with CB1. Neuropharmacology. 2013; 66:170–8. https://doi.org/10.1016/j.neuropharm.2012.03.024 PMID: 22521499

51. Jiang M, Zhang W, Cheng C, Ma Z, Gu X. Intrathecal injection of KN93 attenuates paradoxical remifentanil-induced postoperative hyperalgesia by inhibiting spinal CaMKII phosphorylation in rats. Biochimie. 2015; 134:35–41. https://doi.org/10.1016/j.bchm.2015.04.015 PMID: 25937575

52. Garzon J, Rodriguez-Munoz M, Vicente-Sanchez A, Baijon C, Martinez-Murillo R, Sanchez-Blazquez P. RGSZ2 binds to the neural nitric oxide synthase PDZ domain to regulate mu-opioid receptor-mediated potentiation of the N-methyl-D-aspartate receptor-calmodulin-dependent protein kinase II pathway. Antioxid Redox Signal. 2011; 15(4):873–87. https://doi.org/10.1089/ars.2010.3767 PMID: 21348811

53. Sanchez-Blazquez P, Rodriguez-Munoz M, Montero C, de la Torre-Madrid E, Garzon J. Calcium/calmodulin-dependent protein kinase II supports morphine antinociceptive tolerance by phosphorylation of glycylated phosphducin-like protein. Neuropharmacology. 2008; 54(2):319–30. https://doi.org/10.1016/j.neuropharm.2007.10.002 PMID: 18060204

54. Toyoda H, Zhao MG, Zhuo M. Enhanced quantal release of excitatory transmitter in anterior cingulate cortex of adult mice with chronic pain. Mol Pain. 2009; 5:4–12. https://doi.org/10.1186/1744-8069-5-4 PMID: 19171071

55. Sai Wang S, Au ALS, Poon CCW, Qian Z, Li RWS, Yeung JHK, et al. Acute simvastatin inhibits k ATP channels of porcine coronary artery myocytes. PLoS One. 2013; 8(6):10454–61.

56. Goforth PB, Ellis EF, Satin LS. Mechanical injury modulates AMPA receptor kinetics via an NMDA receptor-dependent pathway. J Neurotrauma. 2004; 21(6):719–32. https://doi.org/10.1089/jn.2004.05.015 PMID: 15253800

57. Guofu S, Mohamed MS, Paromita D, Tietz EI. Positive allosteric activation of GABAA receptors bi-directionally modulates hippocampal glutamate plasticity and behaviour. Biochem Soc Trans. 2009; 37(Pt 6):1394–8. https://doi.org/10.1042/BST0371394 PMID: 19909283

58. Chen YL, Li AH, Yeh TH, Chou AH, Wang HL. Nocistatin and nociceptin exert opposite effects on the excitability of central amygdala nucleus-periaqueductal gray projection neurons. Mol Cell Neurosci. 2009; 40(1):76–88. https://doi.org/10.1016/j.mcn.2008.09.003 PMID: 18930828

59. Ni HD, Yao M, Huang B, Xu LS, Zheng Y, Chu YX, et al. Glial activation in the periaqueductal gray promotes descending facilitation of neuropathic pain through the p38 MAPK signaling pathway. J Neurosci Res. 2016; 94(1):50–61. https://doi.org/10.1002/jnr.23672 PMID: 26423029
60. Cheng JC, Erpelding N, Kucyi A, DeSouza DD, Davis KD. Individual Differences in Temporal Summation of Pain Reflect Pronociceptive and Antinociceptive Brain Structure and Function. J Neurosci. 2015; 35(26):9689–700. https://doi.org/10.1523/JNEUROSCI.5039-14.2015 PMID: 26134651

61. Yu R, Gollub RL, Spaeth R, Napadow V, Wasan A, Kong J. Disrupted functional connectivity of the periaqueductal gray in chronic low back pain. Neuroimage Clin. 2014; 6:100–8. https://doi.org/10.1016/j.nic.2014.08.019 PMID: 25379421

62. Hou WH, Kuo N, Fang GW, Huang HS, Wu KP, Zimmer A, et al. Wiring Specificity and Synaptic Diversity in the Mouse Lateral Central Amygdala. J Neurosci. 2016; 36(16):4549–63. https://doi.org/10.1523/JNEUROSCI.3309-15.2016 PMID: 27098697

63. Vanegas H, Schaible HG. Descending control of persistent pain: inhibitory or facilitatory? Brain Res Brain Res Rev. 2004; 46(3):295–309. https://doi.org/10.1016/j.brainresrev.2004.07.004 PMID: 15571771

64. Bee LA, Dickenson AH. Rostral ventromedial medulla control of spinal sensory processing in normal and pathophysiological states. Neuroscience. 2007; 147(3):786–93. https://doi.org/10.1016/j.neuroscience.2007.05.004 PMID: 17570596

65. Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. Brain Res Rev. 2009; 60(1):214–25. https://doi.org/10.1016/j.brainresrev.2008.12.009 PMID: 19146877

66. Ossipov MH, Morimura K, Porreca F. Descending pain modulation and chronic pain. Curr Opin Support Palliat Care. 2014; 8(2):143–51. https://doi.org/10.1097/SPC.000000000000055 PMID: 24725199

67. Palazzo E, Marabese I, Luongo L, Boccella S, Bellini G, Giordano M, et al. Effects of a metabotropic glutamate receptor subtype 7 negative allosteric modulator in the periaqueductal grey on pain responses and rostral ventromedial medulla cell activity in rat. Molecular Pain. 2013; 9(1):44.

68. Kincaid W, Neubert MJ, Mei X, Chang JK, Heinricher MM. Role for medullary pain facilitating neurons in secondary thermal hyperalgesia. J Neurophysiol. 2006; 95(1):33–41. https://doi.org/10.1152/jn.00449.2005 PMID: 16192337

69. Neubert MJ, Kincaid W, Heinricher MM. Nociceptive facilitating neurons in the rostral ventromedial medulla. Pain. 2004; 110(1–2):158–65. https://doi.org/10.1016/j.pain.2004.03.017 PMID: 15275763

70. Meng ID, Harasawa I. Chronic morphine exposure increases the proportion of on-cells in the rostral ventromedial medulla in rats. Life Sci. 2007; 80(20):1915–20. https://doi.org/10.1016/j.lfs.2007.02.022 PMID: 17400254

71. Kaelser PS, Regehr WG. Molecular mechanisms for synchronous, asynchronous, and spontaneous neurotransmitter release. Annu Rev Physiol. 2014; 76:333–63. https://doi.org/10.1146/annurev-physiol-021113-170338 PMID: 24274737

72. Wyllie DJ, Manabe T, Nicoll RA. A rise in postsynaptic Ca2+ potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. Neuron. 1994; 12(1):127–38. PMID: 7507335

73. Ninan I, Arancio O. Presynaptic CaMKII Is Necessary for Synaptic Plasticity in Cultured Hippocampal Neurons. Neuron. 2004; 42(1):129–41. PMID: 15066270

74. Wang ZW. Regulation of synaptic transmission by presynaptic CaMKII and BK channels. Mol Neurobiol. 2008; 38(2):153–66. https://doi.org/10.1007/s12035-008-0309-7 PMID: 18759010

75. Shoudai K, Peters JH, McDougall SJ, Fawley JA, Andresen MC. Thermally active TRPV1 tonically drives central spontaneous glutamate release. J Neurosci. 2010; 30(43):14470–5. https://doi.org/10.1523/JNEUROSCI.2557-10.2010 PMID: 20980604

76. Sikand P, Premkumar LS. Potentiation of glutamatergic synaptic transmission by protein kinase C-mediated sensitization of TRPV1 at the first sensory synapse. J Physiol. 2007; 581(Pt 2):631–47. https://doi.org/10.1113/jphysiol.2006.118620 PMID: 17363391

77. Vardanyan A, Wang R, Vanderah TW, Ossipov MH, Lai J, Porreca F, et al. TRPV1 receptor in expression of opioid-induced hyperalgesia. J Pain. 2009; 10(3):243–52. https://doi.org/10.1016/j.jpain.2008.07.004 PMID: 18774343

78. Zhou HY, Chen SR, Chen H, Pan HL. Opioid-induced long-term potentiation in the spinal cord is a presynaptic event. Journal of Neuroscience the Official Journal of the Society for Neuroscience. 2010; 30 (12):4460–6.

79. Price TJ, Jeske NA, Flores CM, Hargreaves KM. Pharmacological interactions between calcium/calmodulin-dependent kinase II alpha and TRPV1 receptors in rat trigeminal sensory neurons. Neurosci Lett. 2005; 389(2):94–8. https://doi.org/10.1016/j.neulet.2005.07.029 PMID: 16095822

80. Nakashima M, Hata K, Nagayama T, Sakurai T, Nishisho T, Wakabayashi H, et al. Acid activation of TRPV1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion...
neurons via the CaMK-CREB cascade: a potential mechanism of inflammatory pain. Mol Biol Cell. 2010; 21(15):2568–77. https://doi.org/10.1091/mbc.E10-01-0049 PMID: 20534813

81. Wang ZJ, Wilkie DJ, Molokie R. Neurobiological mechanisms of pain in sickle cell disease. Hematology Am Soc Hematol Educ Program. 2010; 2010:403–8. https://doi.org/10.1182/asheducation-2010.1.403 PMID: 21239826

82. Rivat C, Laulin JP, Corcuff JB, Celerier E, Pain L, Simonnet G. Fentanyl enhancement of carrageenan-induced long-lasting hyperalgesia in rats: prevention by the N-methyl-D-aspartate receptor antagonist ketamine. Anesthesiology. 2002; 96(2):381–91. PMID: 11818772

83. Rodriguez-Munoz M, de la Torre-Madrid E, Gaitan G, Sanchez-Blazquez P, Garzon J. RGS14 prevents morphine from internalizing Mu-opioid receptors in periaqueductal gray neurons. Cell Signal. 2007; 19(12):2558–71. https://doi.org/10.1016/j.cellsig.2007.08.003 PMID: 17825524

84. Wilkie DJ, Molokie R, Boyd-Seal D, Suarez ML, Kim YO, Zong S, et al. Patient-reported outcomes: descriptors of nociceptive and neuropathic pain and barriers to effective pain management in adult outpatients with sickle cell disease. J Natl Med Assoc. 2010; 102(1):18–27. PMID: 20158132

85. Xin L, Wang ZJ. Animal and cellular models of chronic pain. Advanced Drug Delivery Review. 2003; 55(8):949–65.

86. Sanchez-Blazquez P, Rodriguez-Munoz M, Garzon J. Mu-opioid receptors transiently activate the Akt-nNOS pathway to produce sustained potentiation of PKC-mediated NMDAR-CaMKII signaling. PLoS One. 2010; 5(6):e11278. https://doi.org/10.1371/journal.pone.0011278 PMID: 20585660

87. Han JS, Neugebauer V. mGluR1 and mGluR5 antagonists in the amygdala inhibit different components of audible and ultrasonic vocalizations in a model of arthritic pain. Pain. 2005; 113(1–2):211–22. https://doi.org/10.1016/j.pain.2004.10.022 PMID: 15621382

88. Coulombe MA, Erpelding N, Kucyi A, Davis KD. Intrinsic functional connectivity of periaqueductal gray subregions in humans. Hum Brain Mapp. 2016; 37(4):1514–30. https://doi.org/10.1002/hbm.23117 PMID: 26821847

89. Hathway GJ, Koch S, Low L, Fitzgerald M. The changing balance of brainstem-spinal cord modulation of pain processing over the first weeks of rat postnatal life. J Physiol. 2009; 587(Pt 12):2927–35. https://doi.org/10.1113/jphysiol.2008.168013 PMID: 19403624