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GABA signaling in peripheral sensory neurons: a potential role in inflammatory pain

말초 신경세포에서의 GABA신호: 염증 모델에서의 잠재적인 역할

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Abstract

GABA signaling in peripheral sensory neurons: a potential role in inflammatory pain

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Gamma-aminobutyric acid (GABA) depolarizes dorsal root ganglia (DRG) neurons through $GABA_A$ receptor activation.

In the present study, we tested whether $GABA_A$ receptors are involved in nociceptive signaling and pain behavior during formalin-induced acute inflammatory pain in adult mice. We found that peripheral administration of the $GABA_A$ receptor agonist, muscimol, restored spontaneous licking behavior after subsidence of formalin-induced pain behavior.

We performed extracellular single-unit recordings from spinal cord wide dynamic range neurons in vivo and showed that spike frequency was increased by muscmiol injection into hind paw after, but not before, formalin treatment.

Using Ca^{2+} imaging in vitro we show that formalin, as well as the major inflammatory mediator prostaglandin E2, can potentiate GABA-induced Ca^{2+} transients in cultured DRG neurons, an effect that is blocked by the prostaglandin EP4 receptor antagonist AH23848.

Taken together, these results demonstrated that $GABA_A$ receptors may contribute to excitation of peripheral sensory neurons in inflammation through PGE2-EP4 signaling.

Key words: GABA, $GABA_A$ receptor, Formalin, Prostaglandin E2, EP4 receptor, AH23848

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Contents

Introduction

γ-aminobutyric acid (GABA) acts on $GABA_A$ receptors (Bormann *et al.*, 1987; Kaila & Voipio, 1987; Kaila *et al.*, 1993) and induces membrane hyperpolarization in matured CNS neurons which are maintained intracellular Cl^- concentration by K^+-$2Cl^-$ co-transporter (KCC).

Interestingly, $GABA_A$ receptor can shift the role of itself dependent on intracellular Cl^- concentration (Rivera *et al.*, 1999; Payne *et al.*, 2003). For example, in the developmental status of CNS neurons, Na^+-K^+-$2Cl^-$ co-transporter (NKCC) is highly expressed so that keep its intracellular Cl^- concentration, thus activation of $GABA_A$ receptor can elicit membrane depolarization (Alvarez-Leefmans *et al.*, 1988).

Likewise, the shifting role of $GABA_A$ receptors also has been observed in pathological condition (Hammond & Drower, 1984; Roberts *et al.*, 1986). Spinal GABAergic neuron shows inhibition of synaptic input by membrane hyperpolarization in normal condition. On the contrary to this, spinal glia activation in neuropathic pain model which releases BDNF that up-regulate high intracellular Cl^- concentration of GABAergic neurons causes disinhibition of pain control (Jeffery *et al.*, 2003; Coull *et al.*, 2005).

$GABA_A$ receptors have also expressed in primary afferent sensory neurons (Morris *et al.*, 1983; Farrant & Nusser, 2005; Zeilhofer *et al.*, 2012). Especially, Doral root ganglion neurons which conduct sensory stimulus from the environment to spinal cord show membrane depolarization by activation of $GABA_A$ receptors, it is suggested that as Primary afferent depolarization mechanism which blocks primary afferent signal conductance through voltage-

gated Na^+ channel inactivation and thus, reduced neurotransmitter release in afferent terminal (Willis, 1999; Kullmann *et al.*, 2005).

Recent study shows that peripheral administration of $GABA_A$ receptor specific agonist, muscimol can facilitate formalin-induced biphasic pain behavior (Carlton *et al.*, 1999). However, underlying mechanism how peripheral $GABA_A$ contributes to inflammatory pain is still unknown. Previous study demonstrated that the formalin-induced inflammation produced inflammatory mediator, for example, Prostaglandin E2 (PGE_2) (Malmberg & Yaksh, 1995).

Furthermore, PGE_2 sensitizes peripheral nociceptive neurons through EP receptors present on the peripheral terminals of these high-threshold sensory neurons (Omote *et al.*, 2002). There are four subtypes of EP receptors, EP2 and EP4 are couple to Gs, EP3 receptor is coupled to Gs and Gi, whereas EP1 receptor is coupled to Gq/G11. Especially, EP4 receptor is expressed by primary sensory neurons and that EP4 levels increase in the DRG after peripheral inflammation (Lin *et al.*, 2006), however, little is known about how PGE2-EP4 signaling modulates peripheral $GABA_A$ depolarization.

In the present study, I hypothesized that the activation of peripheral $GABA_A$ receptor may be potent enough to act as a nociceptor through PGE_2-EP4 signaling in inflammatory pain conditions.

Materials and Methods

All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the School of Dentistry, Seoul National University. Animal treatments were performed according to the guidelines of the International Association for the Study of Pain. Adult C57BL/6J (wild type) male mice were purchased from DaehanBio (Korea) and Na^+-K^+-$2Cl^-$ co-transporter 1 (NKCC1) knockout mice provided by the department of physiology in Korea university. Animals were housed in a conventional facility with a 12:12 hr light cycle (lights on 8.00am) and ad libitum access to water and chow. Mice were acclimatized for at least one week prior to experiments.

Behavioral studies

All animals were placed in an observation chamber (60 X 100 X 60 mm each) and allowed to habituate. A mirror was positioned behind the observation chamber to provide an unobstructed view. Spontaneous pain behaviors were assessed by measuring the time each animal spent licking, its hind paws. The cumulative time spent licking hind paws during a 5 min period was recorded immediately before drug administration and then again up to 110min after drug administration. The mice were then left alone for at least 30 min to allow them to acclimate before testing. To assess mechanical sensitivities, the withdrawal threshold of the hind paw was measured using a series of von Frey filaments [0.20, 0.69, 1.57, 3.92, 5.88, 9.80, 19.60, and 39.20 mN, Stoelting; equivalent to (in grams) 0.02, 0.07, 0.16, 0.40, 0.60, 1.0, 2.0, and 4.0]. The 50% withdrawal threshold was determined using the up–down method as previously described (Chaplan *et al.*, 1994). A brisk hind paw lift in response to von Frey

filament stimulation was regarded as a withdrawal response. The 0.4 g filament was the first stimulus to be used, and, when a withdrawal response was obtained, the next-weakest filament was used. This process was repeated until no response was obtained, at which time the next-strongest filament was administered. Interpolation of the 50% threshold was then performed using the method of (Dixon, 1980). All behavior tests were performed by an investigator who was blind to the genetic background of the mice.

Dorsum of hind paw injection of drug

2% Formaldehyde and 1mM muscimol were dissolved in 0.9% saline using an ultrasonic washer and applied to dorsum of right hind paw, subcutaneously. Briefly, this injection needs two experimenters. The vertebral side of mouse skin was held using the thumb and fore finger of the left hand, and the right hind paw was held using right hand by one experimenter, the other experimenter performed injections. The drugs were injected into each mouse at approximately the most wide space area of dorsum hind paw by use of a 0.3ml insulin syringe fitted with a 31 gauge needle. The injection volume was 20µl. Before conducting experiments, the injection method was practiced until the success rate was consistently > 95%.

In vivo extracellular recording

Methods used for the present study were modifications of those used in preceding studies (Furue *et al.*, 1999; Narikawa *et al.*, 2000; Sonohata *et al.*, 2004; Kato *et al.*, 2006). Briefly, Adult C57BL/6J (wild type) male mice (25-30g) purchased from Japan SLC (Hamamatsu, Japan), were anaesthetized with urethane (1.5g/kg, i.p.). A dorsal laminectomy was performed to expose the

lumbar enlargement of the spinal cord. The mouse was placed in a stereotaxic apparatus (Model STS-A, Narishige, Tokyo, Japan). After the dura mater was opened, the pia-arachnoid membrane was cut to make a window to allow a tungsten electrode to enter into the spinal cord. The surface of the spinal cord was irrigated with 95% O2 and 5% CO2 equilibrated Krebs solution (in mmol/L: 117 NaCl, 3.6 KCl, 2.5 CaCl2, 1.2 MgCl2, 1.2 NaH2PO4, 11 glucose, and 25 NaHCO3) at a flow rate of 10 to 15 mL/min at 38°C ± 1°C. The tungsten electrode (impedance, 1 MX, A-M systems, Sequim, WA) was advanced into the spinal cord using a micromanipulator (Model SM-11, Narishige). The tungsten electrode was placed into the spinal cord dorsal horn and action potentials in spinal cord neurons were extracellularly recorded with an AC differential amplifier (DAM 80, World Precision Instruments, Sarasota, FL). Firing rate of spinal cord neurons was analyzed with Offline Sorter software (version 3, Plexon, Dallas, TX).

DRG preparation

DRG neurons obtained from 6-8week old mice were prepared. Animals were decapitated, and DRGs were rapidly removed under aseptic conditions and placed in HBSS (Gibco). DRGs were digested in 1 mg/ml collagenase A (Roche) and 2.4 U/ml dispase II (Roche) in HBSS for 60 min, respectively, followed by 8 min in 0.25% trypsin (Sigma), all at 37℃. The DRGs were then washed in DMEM (Gibco) three times and resuspended in DMEM medium supplemented with 10% FBS (Invitrogen) and 1% penicillin/streptomycin (Sigma). DRGs were then mechanically dissociated using fire-polished glass pipettes, centrifuged (800 rpm, 5 min), resuspended in DMEM medium supplemented with 5% FBS (Invitrogen), 20 ng/ml NGF (Invitrogen), 1× N-2

supplement (Invitrogen), and 1% penicillin/streptomycin (Invitrogen), and plated on 0.5 mg/ml poly-D-lysine (Sigma)-coated glass coverslips. Cells were maintained at 37℃ in a 5% CO2 incubator.

Perforated-patch whole-cell recordings

Electrophysiological responses were recorded using the gramicidin perforated-patch whole-cell recording technique with EPC-10 amplifier and Pulse 8.30 software (both from HEKA). For perforated-patch whole-cell recordings in DRG neurons, we used an external bath solution (normal Tyrode's solution) of the following composition (in mM): 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. Patch pipettes with resistances of 3–5 MΩ were made from borosilicate glass capillaries. The pipette tip was initially filled with gramicidin-free pipette solution by brief immersion, and the remainder of the pipette was back-filled with a 310 mOsm internal solution containing gramicidin plus 140 mM CsCl 5 mM EGTA, and 10 mM HEPES, pH 7.4. A 50 mg/ml stock solution of gramicidin (Calbiochem, La Jolla, CA) was prepared in dimethylsulfoxide (DMSO; Sigma). Gramicidin was diluted into the pipette solution to a final concentration of 100 mg/ml before use.. All drug solutions were applied to cells by local perfusion through a capillary tube (1.1 mm inner diameter) positioned near the cell of interest. The junction potential between the patch pipette and the bath solutions was nulled before the gigaohm seal was formed. After the formation of a tight seal, the progress of gramicidin perforation was evaluated by monitoring the capacitive current transient produced by a 10 m sec hyperpolarizing voltage step (5 mV) from the holding potential (250 mV) every 30sec. With 100 mg/ml gramicidin in the pipette, the access resistance dropped to 20 MΩ within 20 min after seal formation. The solution flow was driven by

gravity (flow rate, 4–5 ml/min) and controlled by miniature solenoid valves (The Lee Company). Drugs were dissolved in external solution and delivered from a linear array of micro capillary tubes (1.1 mm inner diameter).

Ca^{2+} imaging

We performed fura-2 AM-based (Molecular Probes) $Ca2^{+}$ imaging experiments. Briefly, DRG neurons prepared were loaded with fura-2 AM (2 μm) for 40 min at 37°C in a balanced salt solution [BSS; containing (in mm) 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), 10 glucose, adjusted to pH 7.3 with NaOH]. Then, the cells were rinsed with DMEM and incubated in DMEM for an additional 20 min to de-esterify the dye. Cells on slides were placed onto an inverted microscope and illuminated with a 175 W xenon arc lamp; excitation wavelengths (340/380 nm) were selected by a monochromatic wavelength changer. Intracellular calcium concentrations ($[Ca^{2+}]_i$) were measured by digital video microfluorometry with an intensified charge-coupled-device camera (CasCade, Roper Scientific) coupled to the microscope and a computer with Metafluor software (Universal Imaging). All drugs were applied via bath perfusion at a flow rate of 5 ml/min.

Drugs

Bicuculline was purchased from Tocris Bioscience. Capsaicin, Formaldehyde,prostaglandin E2, thapsigargin, picrotoxin, lidocaine, γ-Aminobutyric acid (GABA), 3-Hydroxy-5-aminomethyl-isoxazole, 5-Aminomethyl-3-hydroxy-isoxazole, 5-Aminomethyl-3-isoxazolol (Muscimol), 1,2,3,6-Tetrahydro-1,3-dimethyl-N-[4-(1-methylethyl)phenyl]-2,6-dioxo-7H-

purine-7-acetamide, 2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide (HC-030031), 3-(Aminosulfonyl)-5-(butylamino)-4-phenoxybenzoic acid (Bumetanide), (4Z)-7-[(rel-1S,2S,5R)-5-((1,1′-Biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl]- 4-heptenoic acid hemicalcium salt (AH23848), 6-Isopropoxy-9-oxoxanthene-2-carboxylic acid (AH6809) were purchased from Sigma.

Data analysis

Data are expressed as mean SEM. For behavioral testing, statistical analyses of the data obtained from the drug tests were conducted with one way and two-way repeated-measures ANOVA followed by a pairwise comparison of pain behaviors before and after the injection, using Tukey's test's method. Student's t test was used for the comparison between the knock-out and wild-type (WT) mice and $p<0.05$ was considered statistically significant. For other studies, results were compared using Student's t test and $p<0.05$ was considered statistically significant.

Results

Activation of $GABA_A$ receptors leads to membrane depolarization and voltage-gated Ca^{2+} channel activation.

It has been previously reported that GABA elicits membrane depolarization in dorsal root ganglion neurons (DRG). I first confirm whether GABA induces Ca^{2+} response in DRG neurons using fura2-AM ratio-metric based Ca^{2+} imaging method (Fig. 1). GABA induced Ca^{2+} responses in subpopulations of DRG neurons, acutely isolated from adult mice. DRG neurons were identified by their responsiveness to a 10 s application of 50 mM KCl at the end of each experiment. In a subpopulation of KCl-sensitive neurons (45.7%; n=157/342), a 10 s application of 300 μM GABA induced a transient increase in $[Ca^{2+}]_i$ that produced constant peaks during repetitive application of GABA (n=65) (Fig. 1 A, B). GABA induced Ca^{2+} transients were concentration-dependent with 1, 3, 10, 30, 100, 300 μM of GABA (n=37, Fig. 1 C, D).

GABA is a ligand of GABA receptors, which are expressed in DRG neurons (Bormann et al., 1987; Kaila & Voipio, 1987; Kaila et al., 1993). GABA can elicit membrane depolarization through $GABA_A$ receptors in primary afferent neurons. GABA-induced Ca^{2+} responses in DRG neurons were abolished by $GABA_A$ receptor competitive antagonist, bicuculline 10 μM (n=11) (Fig. 2 A). $GABA_A$ receptor selective agonist, muscimol 10 μM induced a Ca^{2+} response, it also blocked by non-competitive $GABA_A$ receptor antagonist, picrotoxin 100 μM (n=15) (Fig. 2 B). And then, GABA-induced Ca^{2+} transients were blocked by non-selective voltage-gated Ca^{2+} channel (VGCC) blocker, CdCl 100 μM (n=9) (Fig. 2 C). To confirm whether GABA-induced Ca^{2+} responses were

mediated by Ca^{2+} influx through VGCC, I depleted extracellular Ca^{2+} in bath solution, it abolished GABA-induced Ca^{2+} transients (n=11; ***p<0.05) (Fig. 2 D, E), but not in depletion of stored Ca^{2+} (n=18; ns=no significance) (Fig. 2 F, G). These data suggest that GABA can elicit depolarization through $GABA_A$ receptor expressed on DRG neurons and also activate VGCC.

GABA-induced Ca^{2+} transients are chloride-dependent and maintained by NKCC1 activity

Adult sensory neurons in PNS sustained high intracellular Cl- concentration which is enough to elicit membrane depolarization by activation of $GABA_A$ receptor. To check whether NKCC1 activity requires for GABA-induced DRG neurons activation, I controlled Cl^- concentration in extracellular bath solution, each application of Cl^- concentrations were 134, 60, 0 mM Cl^- (n=6; ***p>0.05) (Fig. 3 A, B). 0 mM extracellular Cl^- bath solution blocked 60% of fist GABA-induced Ca^{2+} response. Also NKCC1 inhibitor, bumetanide 10 μM gradually inhibited GABA-induced Ca^{2+} transients (n=16 ; p>0.05) (Fig. 3 C, D).

Direct measurement of GABA-induced Vm changes with perforated patch-clamp

Perforated whole-cell patch clamp was performed to check whether GABA directly induces membrane depolarization. 100 μM GABA for 10s evoked inward currents at a -60mV holding potentials in cultured DRG neurons (Fig. 4 A), and depolarizes cell membrane (Fig. 4 B). Further, the I–V relationship indicated that characteristics of GABA-induced currents with a reversal potential of -30 mV (Fig. 4 C, D)

No mechanical sensitivity after GABA and $GABA_A$ receptor agonist, muscimol injection into the hind paw.

Recent studies suggested that the activation of peripheral $GABA_A$ receptors contribute inflammatory pain. (Carlton et al., 1999). However, the activation of peripheral $GABA_A$ induced pain behavior is still elucidative. To check whether peripheral administration of GABA or muscimol raise pain behavior, mechanical sensitivity was measured after GABA and muscimol subcutaneous injection into hind paw. (each group; n=6) (Fig. 5) It did not show detectable changes in pain behavior compared with the pre-injection baseline, suggesting GABA or activation $GABA_A$ receptor does not elicit evoked pain.

Activation of peripheral $GABA_A$ receptors induces pain hypersensitivity after acute formalin inflammation but not in naïve mice.

It has been previously demonstrated that peripheral administration of muscimol facilitated formalin induced biphasic behaviour. Despite of these findings, however, the underlying mechanisms for the activation of peripheral $GABA_A$ under pathophysiological conditions remain unknown. In the present study, I hypothesized that $GABA_A$-induced membrane depolarization might convert to action potential during nociceptor sensitization by inflammatory mediators, thereby contributing to the pain transmission processing activity exerted by inflammation.

First, to discriminate contributions of peripheral $GABA_A$ receptor in inflammatory pain, after formalin-induced biphasic pain behaviour washed out, muscimol was injected into the same site of formalin. Interestingly, muscmiol injection after formalin behaviour restored spontaneous licking behaviour (each group; n=5, *p>0.05; ns=no significance, Fig. 6 A, B), but muscimol injection

after vehicle injection did not show pain behaviour (each group; n=5) (Fig. 6 C. D).These results indicated that peripheral $GABA_A$ signalling solely induces pain behaviour after inflammatory pain condition, but not in naïve condition.

Next, to support this behaviour data we addressed the peripheral $GABA_A$-induced neuronal activity after inflammation using in vivo extracellular single unit recording in a spinal wide dynamic range (WDR) neurons (Fig7. A, B). First muscimol injection into hind paw did not show significantly difference compared with spike frequency in baseline (20 min) (Fig. 7 C), and then, 2% formalin injection induced huge augmented spike frequency from WDR neurons. After activities of formalin-responsive neurons were washed out, the second muscimol injection into same site of formalin increased spike frequency (Fig. 7 D, E). But, WDR neurons, which did not respond to 2% formalin, also did not show second muscimol injection-induced increased spike frequency (Fig. 7 G). These results indicated that activation of peripheral $GABA_A$ involves nociceptive transmission after acute inflammatory pain conditions.

Formalin and Prostaglandin E2 potentiate GABA-induced Ca^{2+} transients in cultured DRG neurons

To determine the mechanisms underlying the peripheral $GABA_A$-induced nociception after inflammatory conditions, I examined whether formalin facilitated GABA-induced Ca^{2+} transients in cultured DRG neurons. Nociceptive DRG neurons were identified by their responsiveness to a 10 s application of 1 μM capsaicin at the end of each experiment. GABA induced Ca^{2+} response was facilitated after 0.003% formalin pre-treatment for 2 min (n=16; *$p<0.05$) (Fig. 8 A, B). Formalin only did not show Ca^{2+} transients, it suggested that 0.003% formalin is sup-threshold concentration for Ca^{2+}

response. It has been known that formalin directly activates nociceptor through TRPA1 (McNamara et al., 2007). To eliminate the possible role of TRPA1 for formalin-induced GABA response potentiation, TRPA1 selective antagonist, HC030031 10 μM was treated during recordings. Data showed that HC030031 did not block potentiation of GABA-induced Ca^{2+} response. (n=16; **p<0.05). These results indicated that GABA-induced responses are facilitated by formalin in TRPV1-positive DRG neurons.

One possible mechanism of this potentiation of GABA-induced Ca^{2+} transients would be sensitized nociceptive sensory neurons by inflammatory mediators. To investigate this possibility, I used prostaglandin E2 (PGE_2) which is a major inflammatory mediator and produced in local site of inflamed area (Fulton et al., 2006). In a subpopulation of capsaicin-sensitive neurons (11.5%; n=21/182) (Fig. 9 C), GABA-induced Ca^{2+} response was also facilitated by pre-treatment of PGE_2 10 μM for 3 min. (n=20; p<0.05) (Fig. 9 A). Then, to describe PGE_2-sensitization of nociceptive neurons affect to GABA-induced depolarization to convert the production of action potential, perforated-whole cell patch clamp was performed. Majority of DRG neurons were expressed GABA-induced depolarization (n=8) (Fig. 10 A). Interestingly, GABA-induced action potentials were observed during PGE_2 application (n=11) (Fig. 10 B). In addition, PGE_2-induced spontaneous licking behavior was also increased by co-injection of muscimol (n=6; * p<0.05) (Fig. 9d). These results suggest that PGE_2 increased the gain of GABA-induced depolarization in vivo and in vitro under the mechanism which might be GABA-induced action potential in nociception.

Formalin and PGE_2 potentiation of GABA responses are mediated by EP4 receptor.

PGE_2 is a group of EP receptors agonist, and the three subtypes of EP receptor, EP1, EP2, and EP4 are involved in inflammatory conditions (Fulton et al., 2006). Thus, I hypothesized that which EP receptor is major contributor to PGE_2 potentiation of GABA responses in DRG neurons. EP1-2 receptor antagonist, AH6809 50 μM did not block PGE_2 potentiation of GABA responses. (n=6; *** $p<0.05$; ns=no significance) (Fig. 11 C, D), whereas EP4 receptor antagonist, AH23848 10 μM abolished PGE_2 potentiation of GABA responses (n=8; *** $p<0.05$; ns=no significance) (Fig. 11 A, B).

Furthermore, some populations of capsaicin-positive DRG neurons show that co-expression of formalin potentiation of GABA responses with PGE_2 potentiation. To determine whether formalin and PGE_2 potentiation of GABA responses share the mechanism through EP4 signaling, I measured that formalin potentiation of GABA response blocked by EP4 receptor antagonist, AH23848 10 μM. These data indicated that formalin and PGE_2 induced potentiation of GABA response mediated by EP4 receptor.

NKCC1 is not required for nociceptive role of $GABA_A$ receptors in inflammation.

NKCC1 is a key molecule to maintain intracellular Cl^- concentration of DRG neurons leading to GABA-induced depolarization (Sung et al., 2000). It has been suggested that possible mechanism which NKCC1 would be up-regulated by cAMP-dependent protein kinase (PKA) and protein kinase C (PKC) EP4 receptor signaling pathway also has PKA downstream (Smith et al., 2008; Flemmer et al., 2010), thus, I hypothesized that NKCC1 activity might involve to potentiation of GABA induced sensitivity after inflammation. However, PGE_2 potentiation of GABA response was not abolished in $NKCC1^{-/-}$ DRG

neurons (n=5; **p<0.05) (Fig. 13A, B). Furthermore, administration of muscimol after formalin biphasic behavior washed out still restored spontaneous licking behavior. (Each group; n=3) (Fig. 13 C, D). These results suggest that NKCC1 activity is not involved to peripheral $GABA_A$ sensitivity after inflammation.

Prostaglandin potentiates $GABA_A$ signaling through a persistent Na^+ channel and protein kinase-dependent mechanism.

PGE_2 is an important mediator in the development of peripheral inflammation and peripheral sensitization (Vane, 1971; Julius & Basbaum, 2001). PGE_2 direct activate TTX-R sodium channel, for example, Nav1.8 expressed on peripheral nociceptive neurons. (England et al., 1996; Gold et al., 1998). To confirm whether PGE_2 activated Na^+ channel gains GABA-induced depolarization in inflammation, Na^+ channel blocker, lidocaine 300μM was applied to PGE_2 potentiation of GABA response (n=4; *p<0.05) (Fig. 14 A, B). Lidocaine inhibited PGE_2 potentiation of GABA response, and did not affect GABA-induced Ca^{2+} response (n=10; ns=no significance) (Fig. 14 C, D).

PGE_2 directly modulate persistent Na^+ channel in nociceptive sensory neurons through protein kinase signaling. To check whether persistent Na^+ channel involve to this PGE_2-induced peripheral GABA signaling potentiation, persistent Na^+ channel blocker, 100 μM riluzole was applied. Riluzole blocked PGE_2-indcued GABA Ca^{2+} transients, also Nav1.8 channel blocker, 100 nM A887826 also blocked GABA Ca^{2+} response potentiated by PGE_2. Downstream signaling of PGE_2 is protein kinase A (PKA) and protein kinase C (PKC). Staurosporine, broad protein kinase inhibitor, also blocked PGE_2-indcued GABA Ca^{2+} transients (n=8-17;p<0.05) (Fig 15 A). GABA-induced action

potentials during PGE2 treatment were also blocked by AH23848 and riluzole (n=8-10) (Fig. 16)

Figure 1. GABA induces Ca^{2+} transient in mouse dorsal root ganglion neurons

Fura-2 AM-based Ca^{2+} imaging in 1day acute dissociated DRG neurons **A,** Ca^{2+} transients evoked by sequential application of 300μM GABA (10sec, n=65). **B,** 45.7%(n=157) of total KCl respond dissociated DRG neurons(n=342) respond to 300μM GABA. **C,** Ca^{2+} transients were elicited by 1, 3, 10, 30 μM GABA in concentration-dependent manner. **D,** Concentration-response curve of GABA response under Ca^{2+} imaging condition. Data points represent the mean plus minus S.E.M. for at least 8-10 determinations from separate cells.

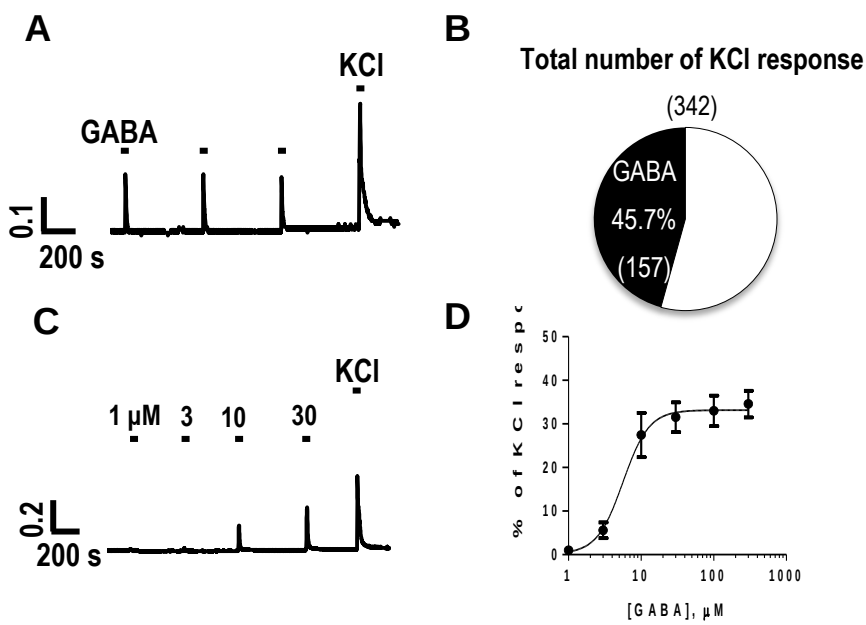
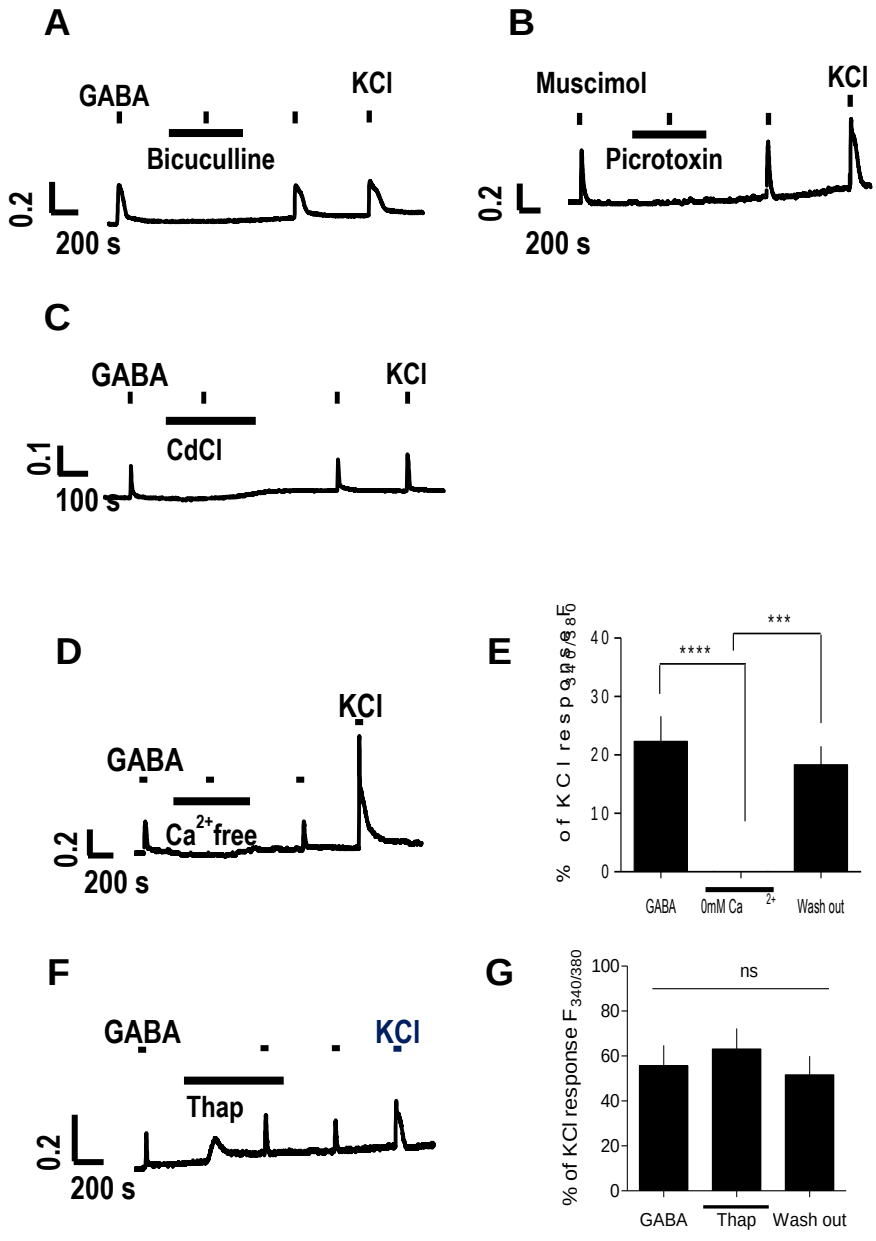


Figure 2. $GABA_A$ receptor-mediated Ca^{2+} transients depend on Ca^{2+} influx through voltage-gated Ca^{2+} channels

A, 300μM GABA-induced Ca^{2+} transients (10sec) are blocked by 10 μM bicuculline, a competitive $GABA_A$ receptor antagonist (n=11). **B,** Ca^{2+} transients evoked by 10 μM Muscimol, $GABA_A$ receptor agonist **C,** were abolished by 100 μM Picrotoxin, a non-competitive antagonist (n=15). **C,** 100 μM CdCl, non-selective VGCC blocker, inhibited GABA response to DRG neurons (n=9). **D,** GABA-induced Ca^{2+} response were blocked by extracellular Ca^{2+}-free condition (n=11;***p<0.05) **E,** but not by 1 μM Thapsigargin(n=18). **E, G,** Summary of Ca^{2+} response relative to peak amplitude of first GABA response (paired *t* test versus first GABA response) .Results are presented as the mean ± SEM.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GABA
KCl
Bicuculline
0.2
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KCl
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CdCl
0.1
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D
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Ca2+free
0.2
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% of KCl response F340/380
40
30
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0.2
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100
80
60
40
20
0
ns
GABA
Thap
Wash out

Figure 3. GABA-induced Ca^{2+} transients are chloride-dependent and maintained by NKCC1 activity

A, 300μM GABA-induced Ca^{2+} transients(10sec) were diminished by decreased extracellular Cl- concentration in 134, 60, 0 mM Cl- solutions each (n=6; ****p*<0.05) **C,** GABA were also gradually blocked by 10μM Bumetanide, NKCC1 co-transporter inhibitor (n=16) **B, D,** Summary of Ca^{2+} response relative to peak amplitude of first GABA response (****p*<0.05, ### *p<0.01*; paired *t* test versus first GABA response) .Results are presented as the mean ± SEM.

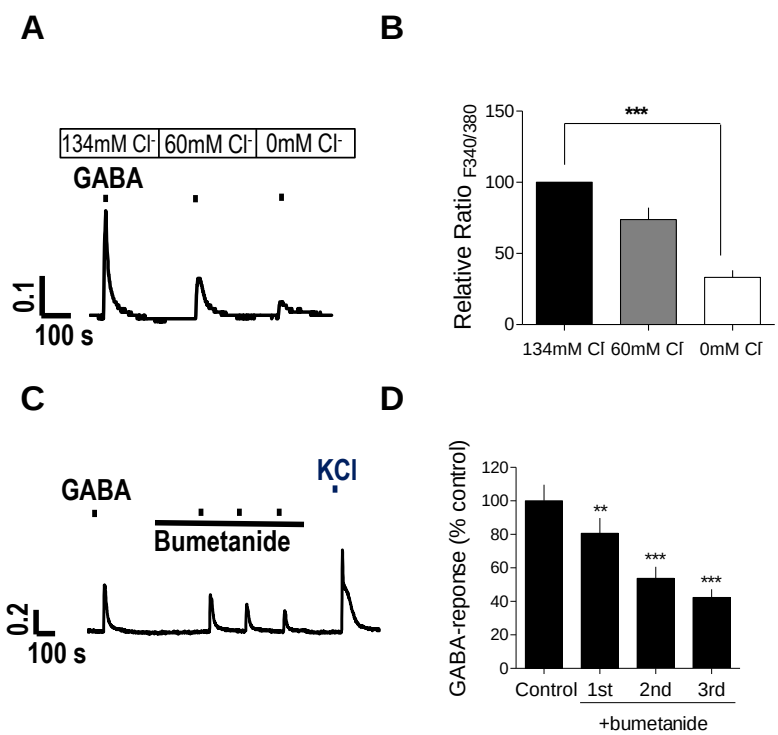


Figure 4. GABA elicits inward currents and depolarization in DRG neurons

A, Representative GABA-induced current traces in DRG neurons at a holding potential of -60mV. DRG neurons were repetitively exposed to 100μM GABA. **B,** DRG neurons show GABA-induced depolarization and action potential. **C,** GABA induced linear I-V relationships and **D,** a ramp protocol was used to determined E [GABA] , was -30mV in 1d dissociated DRG neurons in mouse.

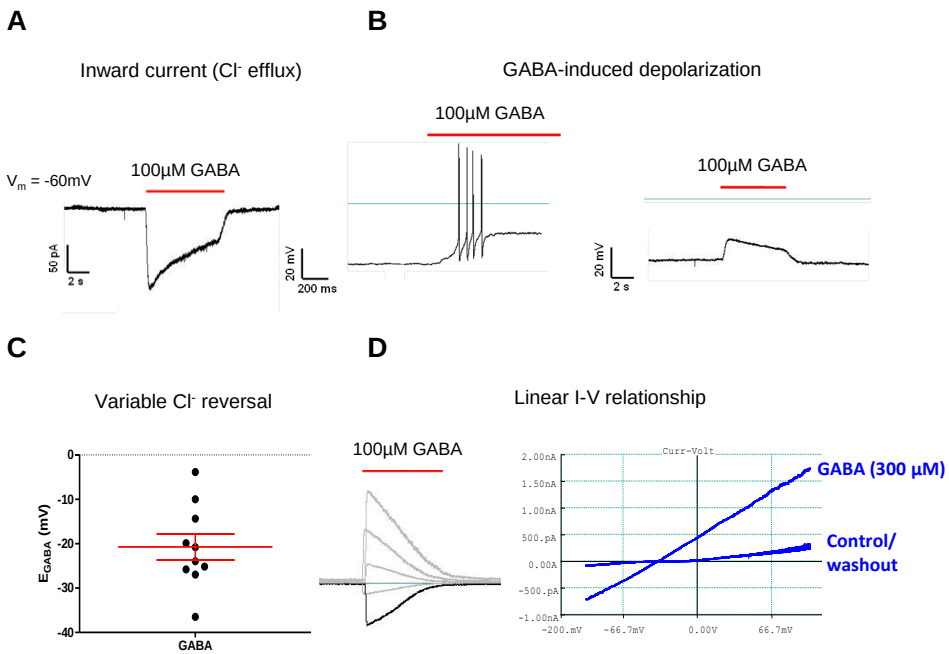


Figure 5. No mechanical sensitivity after GABA and $GABA_A$ receptor agonist, Muscimol injection.

A, Mechanical sensitivity to von Frey filaments after GABA(opened square), muscimol(closed triangle) and Saline(closed square) injection into right hind paw in ipsilateral **B**, and contralateral hind paw (n=6;each group)

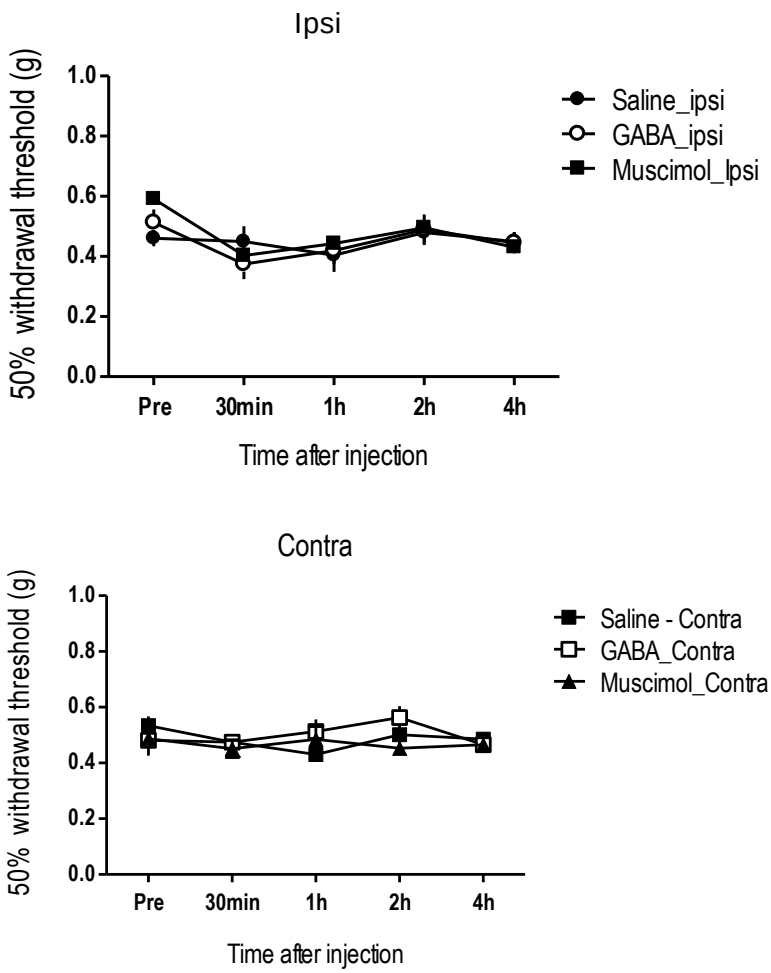


Figure 6. Activation of peripheral $GABA_A$ receptors induces pain hypersensitivity after acute formalin inflammation but not in naïve mice

A, After 2% formalin-induced biphasic behavior washed out, 1mM muscimol (red line) reversed spontaneous licking behavior but not in 0.9% saline injection (blue line; vehicle). **B,** The bar graph represented first phase (0-10min) and second phase (10-60min) of formalin-induced accumulative licking time, after 1mM muscmiol injection restored spontaneous licking behavior during 30min. **C,** 1mM muscimol did not elicit spontaneous licking behavior after 0.9% saline injection (grey line), also saline(70min)-saline(30min) injection group showed no pain response **D,** and the bar graph indicated no significantly difference in saline(70min)-muscimol(30min) and saline(70min)-saline(30min) group. Data are expressed as the mean time of licking behavior per min ±S.E.M. for five animals. *Significantly different (p<0.05) from the group treated with 0.2%formalin,as determined by repeated measures two-way analysis of variance followed by the Tukey's test. (n=5; each group).

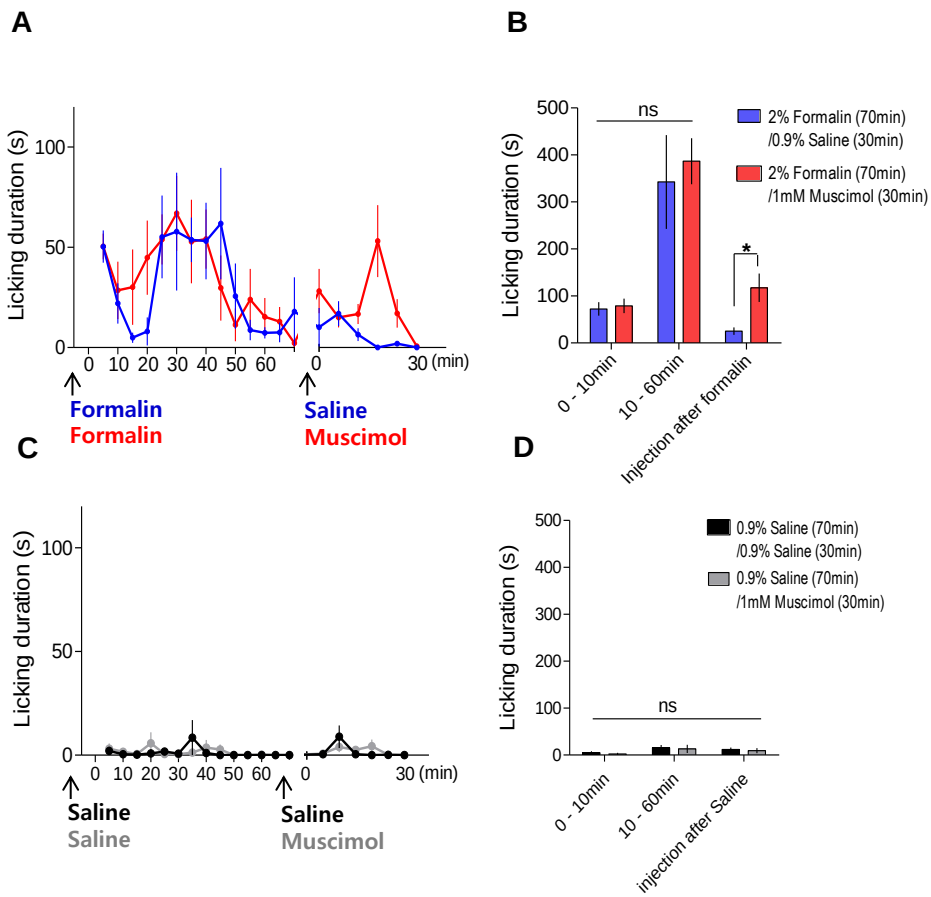
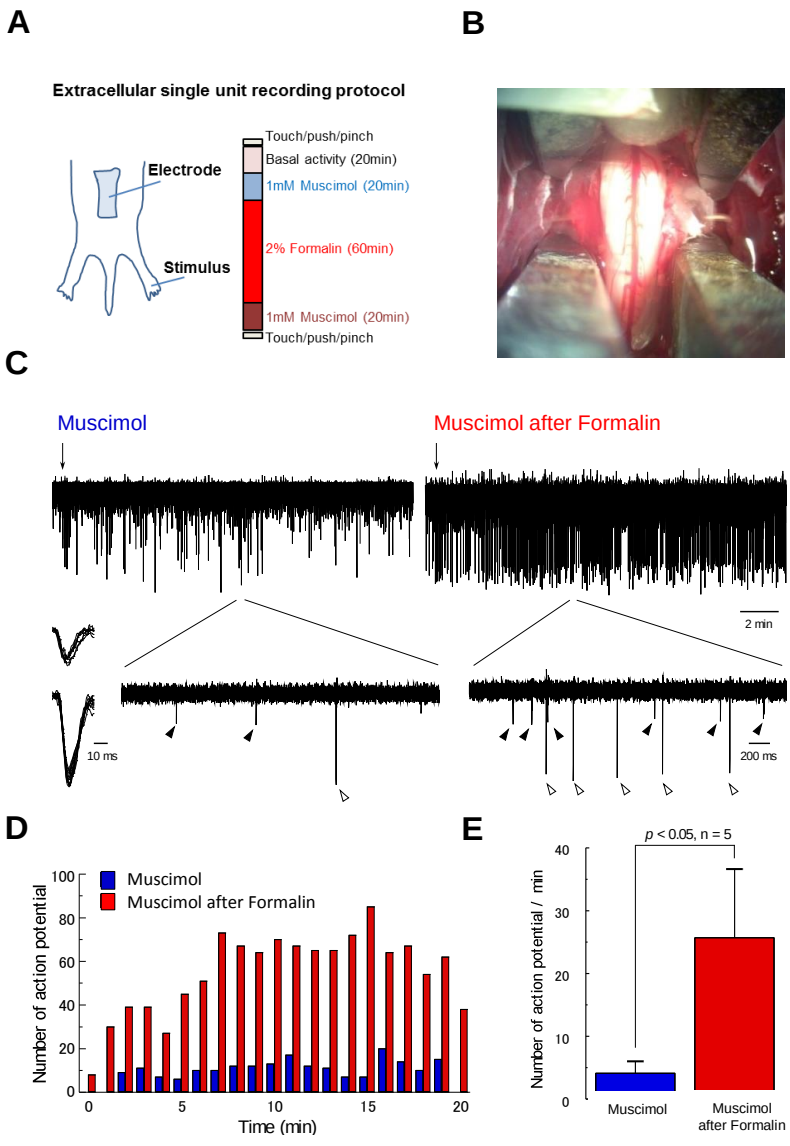

A
B
Licking duration (s)
100
50
0
0 10 20 30 40 50 60
0
30 (min)
Formalin
Formalin
Saline
Muscimol
500
400
300
200
100
0
ns
*
2% Formalin (70min)
/0.9% Saline (30min)
2% Formalin (70min)
/1mM Muscimol (30min)
0 - 10min
10 - 60min
Injection after formalin
C
D
Saline
Saline
Saline
Muscimol
0.9% Saline (70min)
/0.9% Saline (30min)
0.9% Saline (70min)
/1mM Muscimol (30min)
injection after Saline

Figure 7. Activation of peripheral $GABA_A$ receptors increases spike frequency in spinal wide dynamic range (WDR) neurons after acute formalin inflammation but not in naïve mice. *A*, Scheme of in vivo extracellular single unit recording

A, Experimental protocol to check activation of peripheral $GABA_A$-induced neural activity after formalin-induced spike frequency: 20min basal response- 20min 1st 1mM muscimol injection into dorsum of hindpaw - 70min 2% formalin injection - 20min 2nd muscimol injection **B,** After laminectomy, lumbar level of spinal cord fixed by stereotaxic apparatus **C,** 1st 1mM muscimol injection and 2nd muscimol injection after 2% formalin injection, 2nd 1mM muscimol augmented spike frequency in spinal WDR neurons(n=5). **D, F,** The blue bar was 1mM muscmol injection before 2% formalin, and red bar was 1mM muscimol after 2% formalin. *Significantly different (p<0.05) from the group treated with 0.2%formalin,as determined by repeated measures two-way analysis of variance followed by the Tukey's test. **G**, Unit a, b and c showed the pattern of spike frequency after drug administration into hind paw during extracellular single unit recording in spinal WDR neurons. Unit a and b responded to 2% formalin and increased spike frequency in 2nd muscmol injection, but unit c did not respond to 2% formalin and no augmented spike frequency in 2nd muscimol session.


A
B
Extracellular single unit recording protocol
Electrode
Stimulus
Touch/push/pinch
Basal activity (20min)
1mM Muscimol (20min)
2% Formalin (60min)
1mM Muscimol (20min)
Touch/push/pinch
C
Muscimol
Muscimol after Formalin
2 min
10 ms
200 ms
D
Muscimol
Muscimol after Formalin
Number of action potential
Time (min)
E
p < 0.05, n = 5
Number of action potential / min
Muscimol
Muscimol after Formalin

G

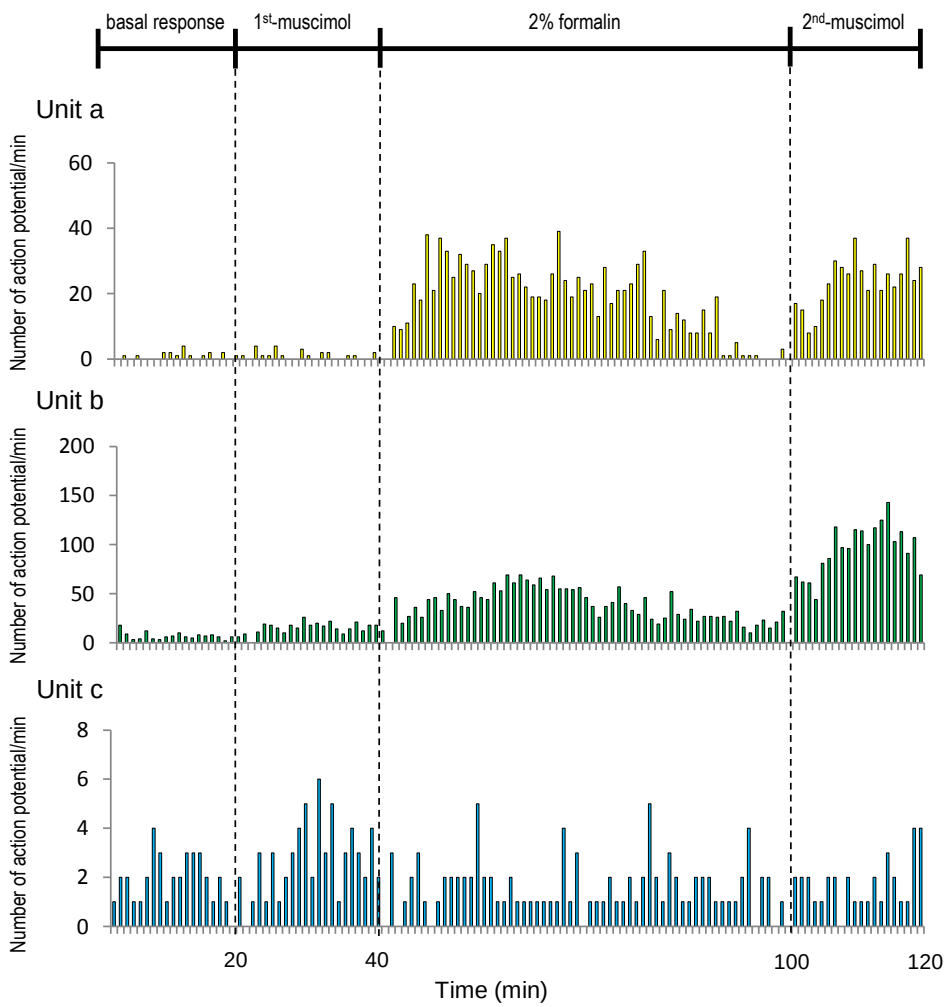

basal response
1st-muscimol
2% formalin
2nd-muscimol
Unit a
Number of action potential/min
60
40
20
0
Unit b
Number of action potential/min
200
150
100
50
0
Unit c
Number of action potential/min
8
6
4
2
0
20
40
100
120
Time (min)

Figure 8. Formalin potentiates GABA-induced Ca^{2+} transients in cultured DRG neurons

A, Ca^{2+} transient evoked by 300μM of GABA (10sec) was potentiated by 0.003% formalin pretreatment (120sec) in 1 μM capsaicin-positive DRG neurons. Acute treatment of 0.003% formalin did not elicit Ca^{2+} response (n=16). **C,** 0.003% formalin-induced GABA response potentiation did not blocked by 10μM HC030031, TRPA1 receptor selective antagonist (n=16). **B, D,** Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (**p<0.05; paired t test versus first GABA response) .Results are presented as the mean ± SEM.

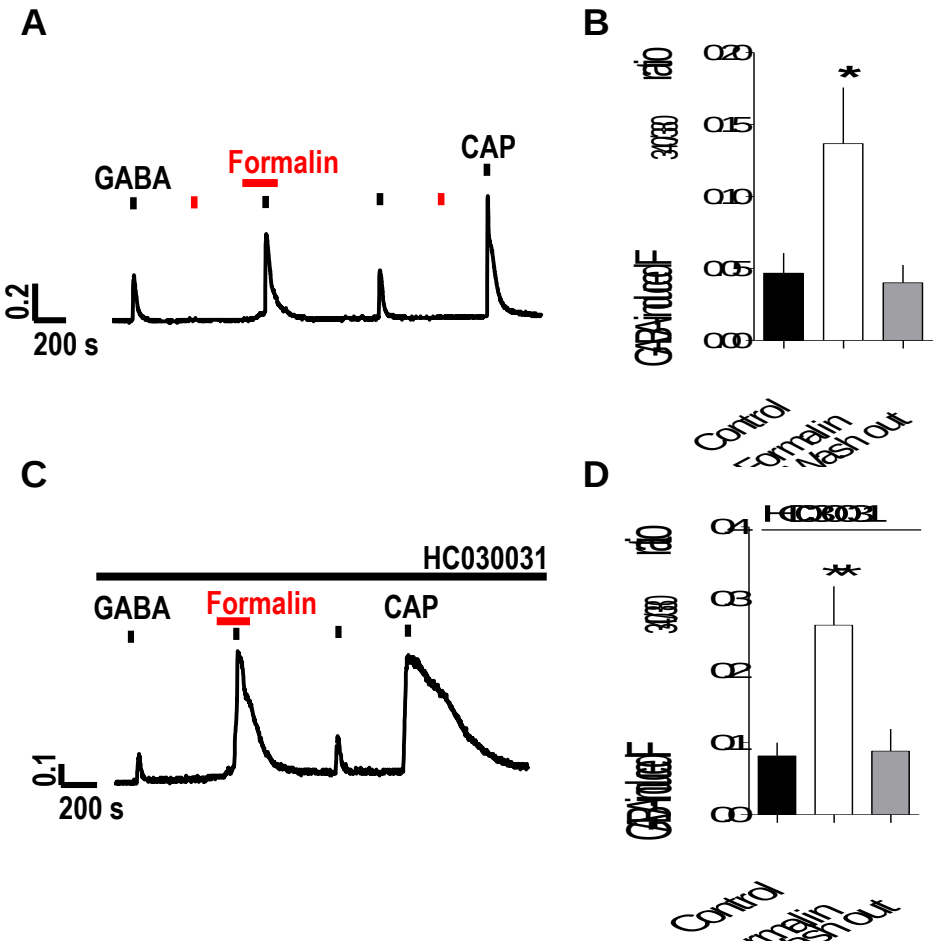

A
GABA
Formalin
CAP
0.2
200 s
B
*
Control
C
HC030031
GABA
Formalin
CAP
0.1
200 s
D
**
Control

Figure 9. Prostaglandin E2 potentiates GABA-induced Ca^{2+} transients in cultured DRG neurons

A, Ca^{2+} transient evoked by 300μM of GABA(10sec) was potentiated by 10μM PGE_2 pretreatment(180sec) in 1 μM capsaicin-positive DRG neurons. Acute treatment of 0.003% formalin did not elicit Ca^{2+} response (n=20). **B,** Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (*** p<0.01; paired t test versus first GABA response) (Among GABA-responded DRG neurons(n=132), about 11.5% neurons were potentiated by 10μM PGE_2 (n=21).(c) PGE_2-induced spontaneous licking behavior was facilitated by co-injection with 1mM muscimol.(each group; n=6, saline group; n=5) (d) Results are presented as the mean ± SEM.

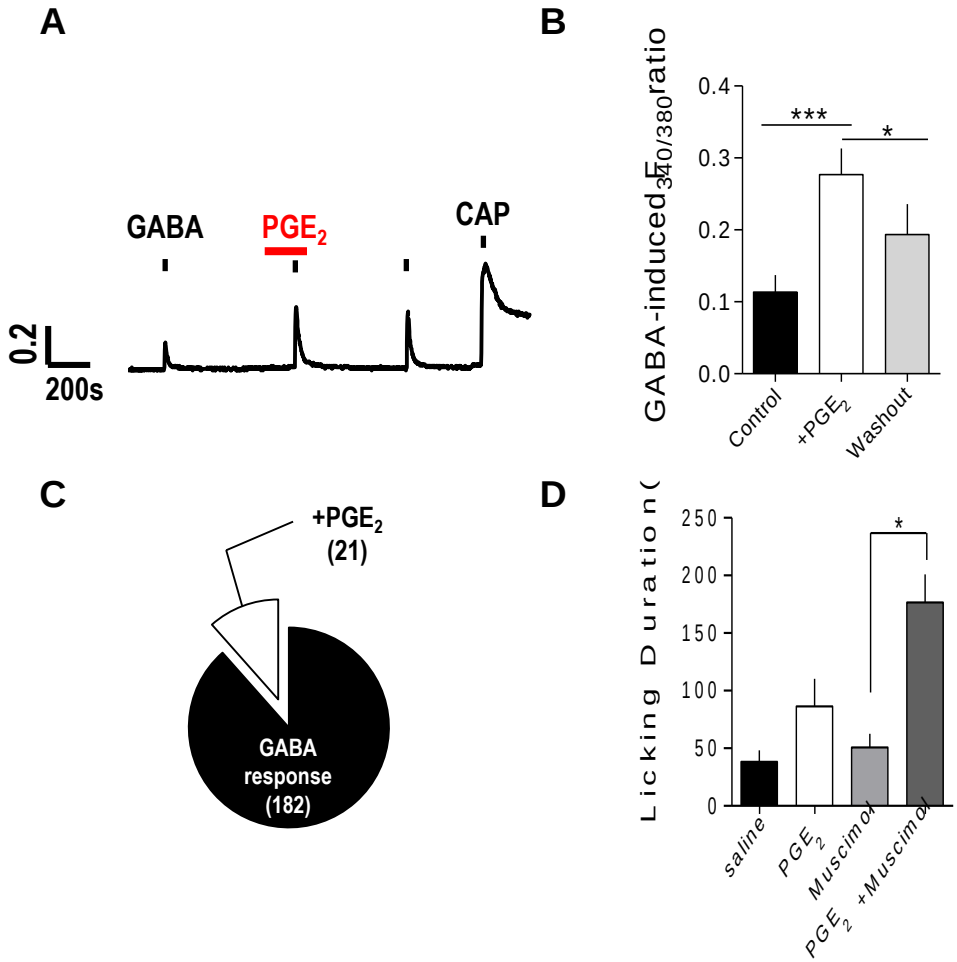

A
GABA
PGE2
CAP
0.2
200s
B
GABA-induced F340/380 ratio
0.4
0.3
0.2
0.1
0.0
***
*
Control
+PGE2
Washout
C
+PGE2
(21)
GABA
response
(182)
D
Licking Duration(
250
200
150
100
50
0
*
saline
PGE2
Muscimol
PGE2 +Muscimol

Figure 10. Summative depolarization of DRG membrane potential by prostaglandin E2 and GABA leads to action potential firing

A, 300 μM GABA-induced depolarization using perforated-patch whole cell clamp in DRG neurons. **B,** During PGE_2 10 μM treatment, GABA elicits action potentials. **C,** GABA induces quantitative changes of membrane potential after PGE_2 application (n=10) (40-50s) **D,** GABA induced depolarization is enough to elicit action potential during PGE_2 treatment.

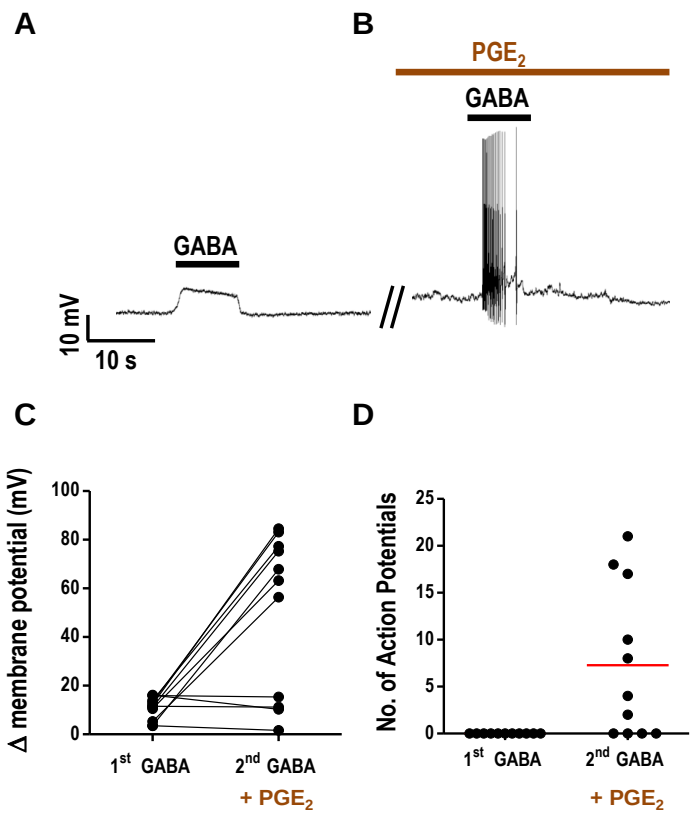


Figure 11. Prostaglandin E2 potentiation of GABA-induced Ca^{2+} transients mediated by EP4 receptor

A, 300μM GABA-induced Ca^{2+} transient potentiated by 10μM PGE_2 was inhibited by 10μM AH23848, EP4 receptor antagonist in 1μM CAP-positive DRG neurons (n=8), (a) but not by 50μM AH6809 (n=6), EP1-2 receptor antagonist. (c) Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (***p< 0.05; paired t test versus first GABA response) Results are presented as the mean ± SEM (b, d).

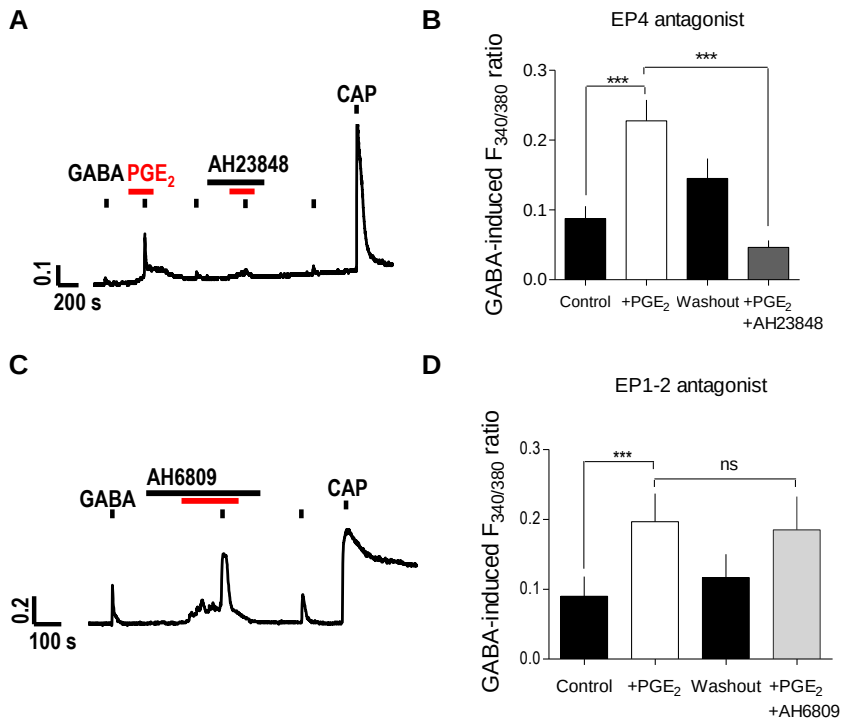


Figure 12. Formalin potentiation of GABA-induced Ca^{2+} transients also mediated by EP4 receptor

A, B, Co-expression of 2% formalin and 10μM PGE_2-induced GABA Ca^{2+} response potentiation in 1μM CAP-positive neurons **C,** and it was blocked by 10μM AH23848, EP4 receptor antagonist (n=10).

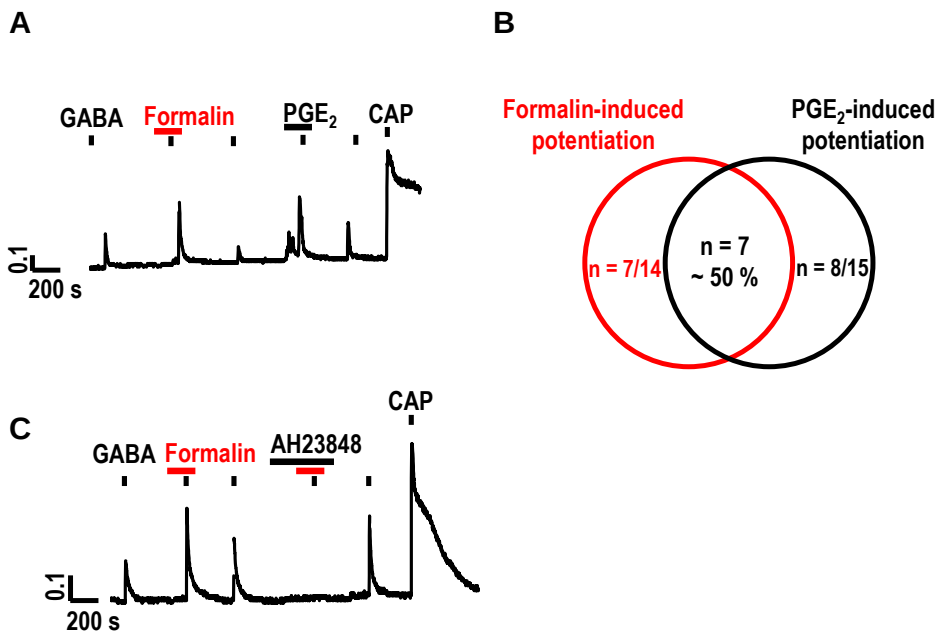


Figure 13. NKCC1 is not required for GABA-induced pain after acute formalin inflammation

A, PGE_2 treatment (120sec) potentiated GABA-induced Ca^{2+} transient in $NKCC1^{-/-}$ mouse DRG neurons, acutely dissociated (n=5) **B,** Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (**p<0.05; paired t test versus first GABA response) **C,** After 2% formalin-induced biphasic behavior washed out, 1mM muscimol restored spontaneous licking behavior.(a, red line: $NKCC1^{-/-}$ mice, blue line: wild type mice, each group n=3) **D,** The bar graph represented first phase (0-10min) and second phase (10-60min) of formalin-induced accumulative licking time, after 1mM muscmiol injection restored spontaneous licking behavior during 30min.

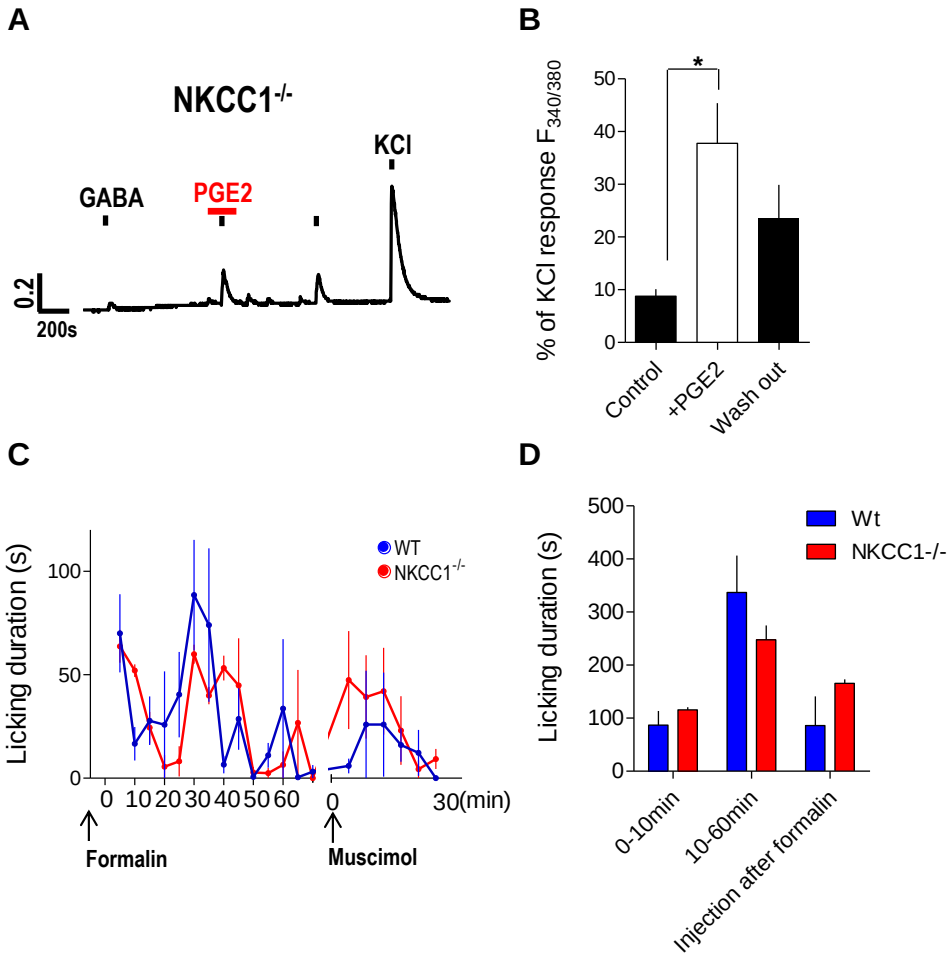

A
NKCC1-/-
KCl
GABA
PGE2
0.2
200s
B
% of KCl response F340/380
50
40
30
20
10
0
*
Control
+PGE2
Wash out
C
Licking duration (s)
100
50
0
WT
NKCC1-/-
0 10 20 30 40 50 60
0
30(min)
Formalin
Muscimol
D
Licking duration (s)
500
400
300
200
100
0
Wt
NKCC1-/-
0-10min
10-60min
Injection after formalin

Figure 14. . Prostaglandin E2 potentiates $GABA_A$ signaling through a Na^+ channel-dependent mechanism

A, GABA-induced Ca^{2+} transient potentiated by PGE2 was inhibited by 300μM lidocaine, Na^+ channel blocker, in 1μM CAP-positive DRG neurons (a) and 300μM lidocaine did not affect GABA-induced Ca^{2+} transient (b). Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (**p*< 0.05; paired *t* test versus first GABA response) Results are presented as the mean ± SEM.

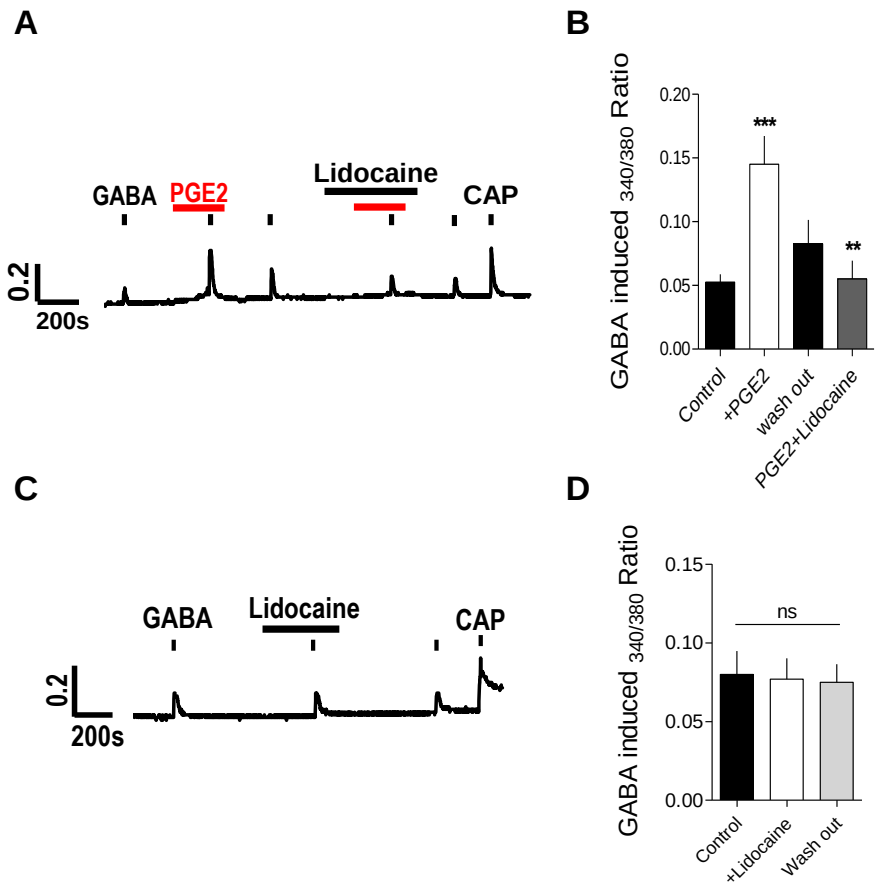


Figure 15. . Prostaglandin E2 potentiates $GABA_A$ signaling through a persistent Na^+ channel and protein kinase signaling-dependent mechanism

A, GABA-induced Ca^{2+} transient potentiated by PGE_2 was decreased by 10 μM EP4 receptor antagonist AH23848, persistent Na^+ channel blocker 100 μM riluzole, Nav1.8 channel blocker 100 nM A887826 and protein kinase inhibitor 500 nM staurosporine in 1μM CAP-positive DRG neurons. Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response ($p< 0.05$; paired *t* test versus first GABA response) Results are presented as the mean ± SEM

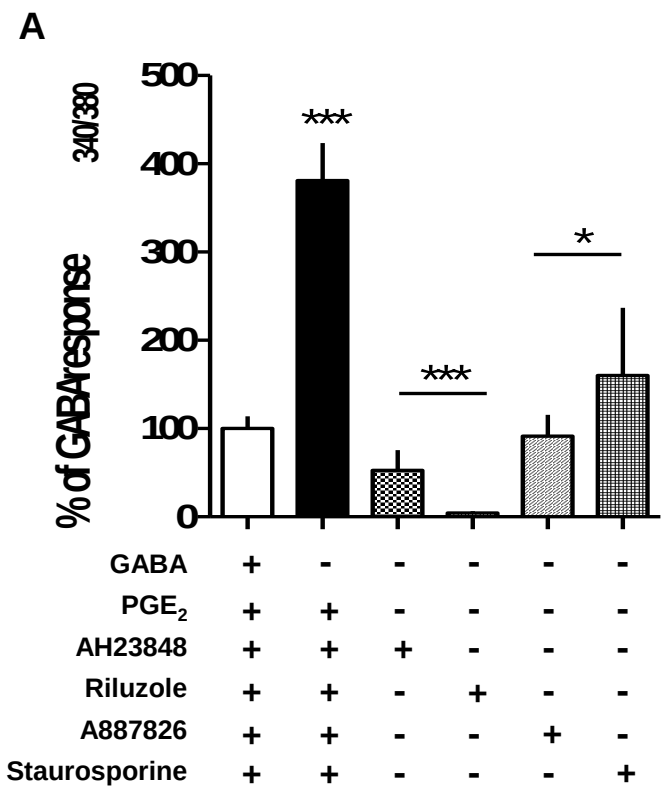


Figure 16. Prostaglandin potentiates $GABA_A$ signaling through a Na^+ channel-dependent mechanism

A, GABA-induced Ca^{2+} transient potentiated by PGE2 was inhibited by 300μM lidocaine, Na^+ channel blocker, in 1μM CAP-positive DRG neurons (a) and 300μM lidocaine did not affect GABA-induced Ca^{2+} transient (b). Bar graph represented summary of Ca^{2+} response relative to peak amplitude of first GABA response (*p< 0.05; paired t test versus first GABA response) Results are presented as the mean ± SEM

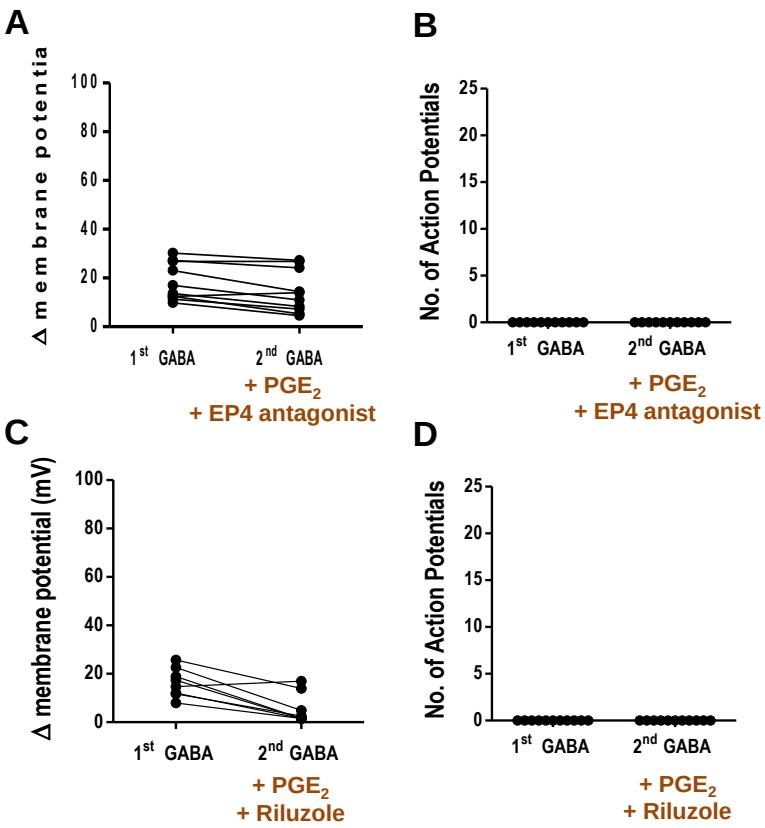
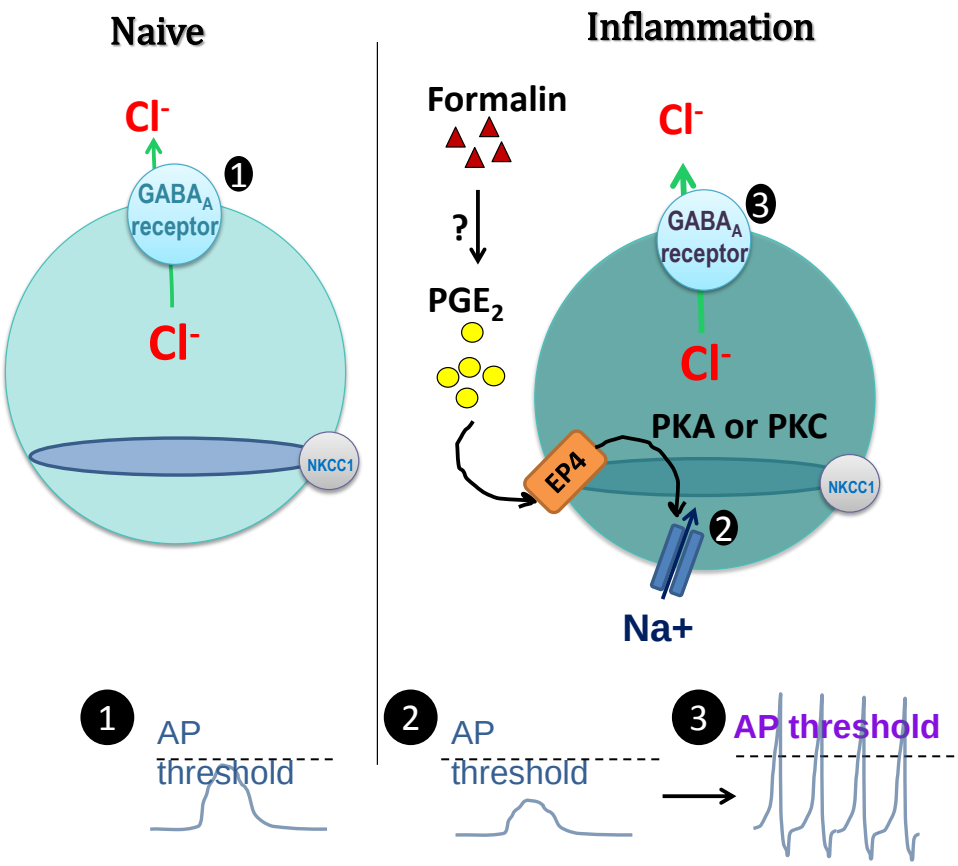


Figure 17. Scheme of peripheral $GABA_A$ signaling properties in PGE_2-EP4 responsive nociceptors in acute inflammatory pain condition

GABA induces cell membrane depolarization in adult DRG neurons. Majority of DRG neurons show GABA-induced membrane excitability under the action potential (AP) threshold. However, in acute inflammatory conditions, especially, administration of formalin into peripheral region mimics inflammatory conditions in mouse. It produces PGE_2, which also makes activation of nociceptive sensory neurons under the AP threshold through activation of persistent Na^+ channel. GABA-induced depolarization could possibly over the AP threshold in nociceptive transmission, thus, GABA-induced AP may contribute to pain signal transduction in acute inflammatory phase.


Naive
Inflammation
Cl-
GABAA receptor
1
Cl-
NKCC1
Formalin
?
PGE2
Cl-
3
GABAA receptor
Cl-
PKA or PKC
EP4
NKCC1
2
Na+
1
AP
threshold
2
AP
threshold
3
AP threshold

Discussion

Characterizations of GABA responses in primary afferent sensory neurons in mouse

I confirmed GABA-induced response in cultured dorsal root ganglion (DRG) neurons as primary afferent sensory neurons using Ca^{2+} imaging technique. Ca^{2+} imaging technique has a benefit, which does not disrupt intracellular ion concentrations of DRG neurons without any aggressiveness to the cell, thus it could suggest GABA response from the intact ion concentrations of the adult primary sensory neurons.

It has been known that GABA induces cell membrane depolarization in peripheral sensory neurons through higher intracellular Cl^- concentration than the adult CNS neurons. Data also showed that application of GABA to DRG neurons induced Ca^{2+} transients which was mediated by $GABA_A$ receptors, were blocked by CdCl, non-selective voltage gated Ca^{2+} channel blocker. It proposed GABA elicited membrane depolarization and Ca^{2+} influx through VGCC activations in DRG neurons. Moreover, modulations of intracellular Cl^- concentration using 134mM, 60mM, and 0mM extracellular Cl^- bath solution could affect GABA-induced Ca^{2+} transient. And I confirm that GABA response in DRG neurons was maintained by Na^+-K^+-$2Cl^-$ co-transporter1 (NKCC1).

However, I used Ca^{2+} imaging to check role of $GABA_A$ receptor, which is Cl^- permeable anion channel, it was difficult to explain direct evidence of GABA-induced depolarization. To support the evidence of GABA-induced depolarization in DRG neurons, gramicidin-perforated whole cell patch clamp was performed. Gramacidin is an antibiotic agent which diffuses into the cell membrane; it forms small perforations and gives electrical access without membrane rupture (Ebihara et al., 1995; Kyrozis & Reichling, 1995).

Application of GABA induced membrane depolarization in DRG neurons in perforated-whole cell patch mode, also some populations of DRG neuron showed action potential.

Acute formalin inflammation reveals nociceptive function of peripheral $GABA_A$ receptors in vivo

GABA depolarizes DRG neurons through $GABA_A$ receptors; however, activation of peripheral $GABA_A$ receptor does not elicit mechanical sensitivity and spontaneous pain behavior. Nevertheless, recent study suggests that activation of peripheral $GABA_A$ facilitates formalin-induced biphasic behavior and peripheral $GABA_A$ could convey nociceptive signals. Even though, to date, whether activation of peripheral $GABA_A$ solely conducts as a nociceptor is still unknown.

Thus, I hypothesized that what if peripheral $GABA_A$ signaling is gained by inflammation and contributes to inflammatory pain. Formalin test is a representative acute-inflammatory pain model (Hunskaar & Hole, 1987). It is well known that formalin-induced biphasic pain behavior is almost washed out after 60min. To prove peripheral $GABA_A$ signaling is a signal gainer in inflammatory pain, peripheral administration of muscimol injection was performed after formalin behavior. Muscimol injection into the hind paw after formalin solely elicits spontaneous licking behavior. Also spike frequency in spinal WDR neurons was augmented by muscimol injection after formalin-induced spike frequency. This Data suggest that formalin-induced inflammation uncovers the nociceptive role of peripheral $GABA_A$ receptors.

Formalin and PGE_2 potentiation of GABA responses are mediated by EP4 receptor.

Formalin induced pain behavior is separated by two phases, first acute phase is mediated by nociceptor transmissions which is TRPA1, second phase is known that mediated by central sensitizations. Thus, I confirmed whether formalin induced potentiation of GABA response is affected by activation of TRPA1 expressed in nociceptive sensory neurons. Despite of complex mechanism in formalin-induced inflammation, somehow in vitro culture system mimics formalin effect to DRG neurons for understanding the modulations of GABA signaling. In my Ca^{2+} imaging data, TRPA1 selective antagonist, HC030031 did not block formalin induced potentiation of GABA response, suggesting that there is TRPA1-independent mechanism in formalin induced potentiation of GABA response.

Interestingly, PGE_2 potentiates GABA-induced Ca^{2+} transients and generates GABA-induced action potential. PGE_2 is one of the inflammatory mediators produced by formalin-induced inflammation. Notably, I found that the PGE_2 also elevates muscimol-induced spontaneous licking behaviors. Next, I addressed which subtypes PGE_2 receptors are involved to this potentiation of GABA response in inflammatory conditions. Among the 4 types of EP receptors, I eliminated EP3 receptor due to its inhibitory downstream pathway which blocks activation of adenylyl cyclase (AC) and cAMP production. PGE_2 induced potentiation of GABA Ca^{2+} transient was blocked by EP4 receptor antagonist, AH23848, but not by EP1-2 receptor antagonist, AH6809.

Furthermore, Formalin induced facilitation of GABA Ca^{2+} transient was also abolished by AH23848. This in vitro data should further demonstrate that PGE_2 release from formalin application to DRG neurons is possible or not. Based on our observations, it was thought that cellular mechanism of GABA potentiation by formalin and PGE_2 might have same downstream pathway through EP4 receptor. In addition, it should be further investigated that whether EP4 receptor antagonist

blocks peripheral $GABA_A$-induced pain behavior after formalin-induced inflammation.

NKCC1 is not required for nociceptive role of $GABA_A$ receptors in inflammation.

It has been suggested that one of the inevitable evidence for role of peripheral $GABA_A$ receptor in inflammation is NKCC1. Recent study suggests that inflammatory mediators increased intracellular Cl^- concentration in DRG neurons after 1hr treatment (Funk *et al.*, 2008). Despite of this, in vivo data showed muscimol-induced spontaneous licking behavior after formalin was still observed in $NKCC1^{-/-}$ mice. Also PGE_2-induced potentiation of GABA Ca^{2+} transient was produced in $NKCC1^{-/-}$ DRG neurons. It is thought to be acute effect of PGE_2 application in Ca^{2+} imaging, which was not enough duration to up-regulate intracellular Cl^- concentrations. As well as in vitro data, acute inflammatory pain was generated by formalin injection. It might suggest that NKCC1 could involve to chronic inflammatory conditions and regulate intracellular concentration.

Prostaglandin increases gain of DRG GABA response through persistent Na^+ channel-dependent membrane depolarization.

PGE_2 is produced in DRG neurons in inflammatory conditions, also its receptor, EP4 expression is increased in inflamed DRG (Lin *et al.*, 2006). Previous study suggests that PGE_2 pathway can modulate TTX-resistant Na^+ channel so that sensitized sensory neurons. I found that lidocaine blocks PGE_2-induced GABA response potentiation, whereas GABA-induced Ca^{2+} response did not affected by lidocaine. These results suggest that PGE_2-induced GABA response potentiation may be associated with the PGE_2-induced sensitization of nociceptive neurons. To address the link between GABA signaling and PGE_2-induced sensitization in

primary sensory neurons, selective TTX-resistant Na^+ channel blocker could be applied to PGE_2-induced GABA response.

These Data suggest a nociceptive role of peripheral $GABA_A$ receptor in acute inflammatory pain. Activation of $GABA_A$ receptor after formalin-induced inflammation elicits spontaneous pain behavior. Also in vitro culture system shows formalin and PGE_2 induced potentiation of GABA response through EP4 receptor. Nociceptive effect of peripheral $GABA_A$ signaling is not mediated by NKCC1. This cellular mechanism implies a unknown significant role of peripheral $GABA_A$ as a gainer of pain transmission in acute inflammatory pain condition.

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국문초록

말초 신경세포에서의 GABA 신호:
염증 모델에서의 잠재적인 역할

GABA는 대표적인 억제성 신경 전달 물질로써 중추 신경에서 연구가 많이 되어 왔다. GABA는 GABA 수용체의 리간드 이며 그 중 $GABA_A$ 수용체는 Cl- 이온에 투과성을 가진다. 중추 신경의 발달 과정에서 $GABA_A$ 수용체의 역할은 세포의 막전압의 탈분극에서 저분극으로 변화하게 된다. 이러한 $GABA_A$ 수용체의 역할 변화는 통증상황에서 억제성 척수 신경에서도 나타나며 이는 막전압이 저분극에서 탈분극으로 변화하여 통증 신호에 기여한다는 것으로 알려져 있다.

한편, 말초 신경에서의 GABA는 $GABA_A$ 수용체를 통해서 척수 후근절 신경세포의 막전압을 탈분극 시킨다. 그러나, 말초 GABA에 대한 $GABA_A$ 수용체의 막전압 흥분성이 말초 통증 상황에서 어떻게 작용할

지는 잘 알려지지 않았다. 따라서 본 연구에서는 염증상황에서 말초 $GABA_A$ 수용체의 역할을 규명하기 위해 Formalin에 의한 급성 염증성 통증 모델을 통하여 $GABA_A$ 수용체의 활성에 따른 통증 행동 변화를 관찰하고 그 기전을 연구 하였다

Formalin 에 의한 염증성 통증 이후에 $GABA_A$ 수용체의 작용물질인 Muscimol을 처리 했을 때 통증 행동이 다시 생성되는 것을 확인 하였다. Formalin과 관련된 대표적 염증물질인 Prostaglandin E2에 의한 통증 행동 역시 Muscimol에 의해 증가되는 것을 관찰 하였다. 생체 내 단일 세포 기록 방법을 통해 척수 내 통증 자극에 반응하는 신경에서 Formalin에 의한 통증을 유도 했을 경우에 Muscimol에 의한 전기적 활성이 증가하는 것을 확인 하였다. 이에 대한 기전 연구로 Perforated-패치 클램프 결과에서는 척수 후근절 신경세포에서 Prostaglandin E2에 의해서 GABA에 의한 활동전위의 빈도가 증가함을 관찰 하였다. 칼슘 이미징 기법을 통해서도 Formalin과 대표적인 염증성 물질인 Prostaglandin E2가 척수 후근절 신경 세포에서의 GABA에 의한 칼슘 반응을 증가시키는 것을 확인 하였고, 이것은 Prostaglandin E2 수용체 중 EP4 수용체의 길항제인 AH23848에 의해서 차단되었다.

따라서 이 결과들을 통해 말초 $GABA_A$ 수용체는 Prostaglandin E2 - EP4 신호작용을 통해 염증성 통증에 기여 하고 있음을 알 수 있다.

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주요어: GABA, $GABA_A$ 수용체, Muscimol, Formalin, Prostaglandin E2, EP4 수용체

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