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Increased Phosphorylation of extracellular signal-regulated kinase in trigeminal nociceptive neurons following propofol administration in rats

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Abstract

Although propofol (PRO) is widely used in clinic as a hypnotic agent, the underlying mechanisms of its action on pain pathways is still unknown. Sprague-Dawley rats were assigned to receive PRO or pentobarbital (PEN) and were divided into two groups as LIGHT and DEEP hypnotic levels based on the EEG analysis. Rats in each hypnotic level received capsaicin injection into the face and phosphorylated extracellular regulated-kinase (pERK) immunohistochemistry were performed in subnucleus caudalis (Vc) and upper cervical spinal cord. A large number of pERK-like immunoreactive (LI) cells was observed in the trigeminal spinal subnuclei interpolaris and caudalis transition zone (Vi/Vc), middle Vc and transition zone between Vc and upper cervical spinal cord (Vc/C2) in the rats with PEN or PRO administration following capsaicin injection into the whisker pad region. The number of pERK-LI cells in Vi/Vc, middle Vc and Vc/C2 was significantly larger in rats with PRO injection than those with PEN injection. The number of pERK-LI cells was increased following an increase in the dose of PRO but not in PEN. The pERK-LI cells were dominantly distributed in the Vi/Vc, middle Vc and Vc/C2 after the bolus injections of PRO. The expression of pERK-LI cells was depressed after the intravenous lidocaine application before PRO injection. The present findings suggested that PRO induced an enhancement of the activity of trigeminal nociceptive pathways through nociceptors innervating the venous structure, as indicated by a lidocaine-sensitive increase in pERK. This may explain deep pain around the injection regions during intravenous bolus injection of PRO.

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Perspective: The effect of propofol administration on ERK phosphorylation in the subregions of the spinal trigeminal complex and upper cervical spinal cord neurons were precisely analyzed in rats with PRO injection. A large number of pERK-LI cells was observed following intravenous PRO administration, suggesting an enhancement of trigeminal nociceptive activity and that PRO may produce pain through nociceptors innervating the venous structures during infusion.

Keywords

propofol; trigeminal nerve; caudalis; Mapkinase; nociception

Introduction

Propofol (PRO) is frequently used as a reliable intravenous hypnotic agent in clinic because it is safe and the depth of hypnotic level is easy to control.^{7,11,19,25,30,38} Intravenous administration of PRO causes depression of neuronal activity in wide areas of the central nervous system (CSN), reflected by the depression of EEG activity.^{33,46,47} It has been reported that the depression of EEG activity by PRO is dose dependent.^{33,46,47}

The action of PRO is induced via GABAergic system in the CNS based on *in vitro* electrophysiological experiments.^{9,12} PRO administration caused an increase in the duration of GABAA receptor activation.^{12,41} This led to the hypothesis that PRO has a modulatory effect on pain pathways through the central GABAergic system. However, some previous clinical studies have reported that PRO causes burnt pain around the injection region and an increase in pain intensity following an increase in PRO dosage.^{32,34} In contrast, other studies reported that PRO had antinociceptive as well as hypnotic action.^{1,19} It is, therefore, still controversial whether PRO has antinociceptive action, based on previous clinical and basic researches.^{1,19} For the clinical use of hypnotic agents, the invasive action such as pain should be relieved. It is very important to understand the neuronal mechanisms underlying the action of propofol on CNS pain pathways in order to improve its clinical use.

The extracellular signal-regulated kinase (ERK) is known as one of the mitogen-activated protein kinases (MAPKs).^{20,21,24} The Ca^{2+} influx is known to be involved in the ERK phosphorylation as neurons are activated.^{20,21,24} The ERK in dorsal root ganglion, spinal dorsal horn and trigeminal spinal subnucleus caudalis neurons was phosphorylated within 10 min following peripheral noxious stimulation.^{8,17,20,42} The number of phosphorylated ERK-(pERK) immunoreactive neurons was increased in the DRG and spinal DH as there was an increase in the noxious stimulus intensity.^{8,20} These results suggested that the ERK phosphorylation is a reliable indicator of neurons that are excited by peripheral noxious stimulation.^{20,21,29} Recently, it has also been reported that capsaicin stimulation of the tooth pulp induces pERK positive neurons in the Vc and upper cervical spinal cord.⁴² The tooth pulp-induced pERK positive neurons were arranged in the Vc and consistent with somatotopic organization. The distribution pattern of pERK positive neurons observed following tooth pulp stimulation was similar to that reported in electrophysiological studies.^{14,36} These suggest that the activation of neurons following noxious stimulation of the trigeminal structures is reflected by the phosphorylation of ERK in Vc and upper cervical spinal cord neurons. These results also indicate that the ERK phosphorylation is a good indicator of nociceptive neurons activated by peripheral noxious stimulation in the trigeminal system.

We have shown recently that propofol modulates neuronal activation in CNS pain pathways, as indicated by Fos protein expression, after noxious thermal stimulation of the face.²⁶ In the present study, we have used pERK as a marker to systematically analyze the effect of propofol on neuronal activation in the spinal trigeminal complex and upper cervical spinal cord in response to capsaicin, a commonly used chemical irritant that produces central sensitization

and hyperalgesia.⁵² The results show differential patterns of neuronal activation in the subregions of the spinal trigeminal complex and suggest a pronociceptive action of propofol.

Materials and Methods

Experiments were performed on a total of 108 Male Sprague Dawley rats weighing 200–300g. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a cannula was chronically implanted into the left jugular vein for drug administration. Stainless-steel-screw electrodes were implanted chronically in the skull for EEG recording. Two insulated stainless-steel-wires were also placed in the splenius capitis muscle for EMG recording. This study was approved by the Animal Experimentation Committee at Nihon University School of Dentistry and the animal treatments were performed according to the guidelines of the International Association for the Study of Pain.⁵¹

Drug administration and the measurement of depth of hypnosis

Two days after the surgery, rats were administered continuously with propofol (PRO) or sodium pentobarbital (PEN) intravenously. We defined the depth of hypnosis according to the neck electromyogram (EMG) activity, and EEG amplitude during intravenous infusion of PRO or PEN as illustrated in Fig. 1. The neck EMG activity was recorded during the application of drugs and the LIGHT hypnotic level was defined as when the spontaneous neck EMG activity could not be recorded during infusion (PRO: 1.72 ± 0.04 mg/kg/min, PEN: 1.03 ± 0.04 mg/kg/min, approximately 15–20 min in each drug) as illustrated in Fig. 1A and B. Studies show that the depth of hypnosis can be evaluated in animals by i-EEG analysis in which the EEG amplitude was measured and the duration of amplitude depression was calculated.^{4,10,15,26,31,33,46,47} Thus, we introduced the i-EEG analysis to measure the depth of hypnosis during intravenous administration of PRO or PEN. The neck EMG activity has also been shown as a good indicator or measure of the wakefulness level.^{18,28} We, therefore, also analyzed the neck EMG activity to define the levels of hypnosis.

The EEG activity was rectified and the time points with peak EEG amplitudes were detected using spike 2 software and the i-EEG was calculated according to the previous study by Kubota et al.²⁶ When more than 40 % reduction of the EEG amplitude was obtained during drug infusion, the hypnotic level was defined DEEP (PRO: 2.52 ± 0.21 mg/kg/min, PEN: 2.25 ± 0.15 mg/kg/min, approximately 15–20 min in each drug) as illustrated in Fig. 1C, D and E.

Experimental protocol

In order to define the peak time point of pERK-LI cell expression in the spinal trigeminal complex and upper cervical spinal cord after subcutaneous capsaicin injection into the right whisker pad, pERK immunohistochemical study was carried out in rats after different survival times following capsaicin injection. Rats (n=25) were infused PRO (2.52 ± 0.21 mg/kg/min) through the jugular vein and the hypnotic level was defined DEEP. Then, capsaicin (10 mM, 50 μ l) was injected to the whisker pad subcutaneously 30 min after PRO infusion. Rats were perfused at 2, 5, 10, 30 and 60 min after capsaicin injection. As the results of this time-course study, the peak time point of pERK-LI cell expression turned out 5 min after capsaicin injection. Therefore, rats were perfused at 5 min after capsaicin in the following experiment.

Eight groups of rats (n=5 for each group) were used in the main experiment: 2 (PRO or PEN) $\times$ 2 (DEEP or LIGHT) $\times$ 2 (capsaicin or saline). Capsaicin (10 mM, 50 μ l) or saline was injected into the right whisker pad area subcutaneously 30 min after PRO or PEN infusion. Then, rats were perfused at 5 min after capsaicin or saline injection. In another experiments without capsaicin treatment, rats were divided into 4 groups according to the following dose of propofol and whether lidocaine was injected (PRO: 16 mg/kg, 22 mg/kg or 28 mg/kg, 2% lidocaine 10

mg/kg+PRO28 mg/kg). A schematic illustration of the time-course of the present experimental protocol is illustrated in Fig. 2.

Furthermore, additional experiments were performed to evaluate the correlation between hypnotic levels and blood pressure (BP) or eye blink reflex. Rats (n=10) chronically implanted with EEG and EMG electrodes 2 days before BP and eye blink reflex recordings were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a cannula was inserted into the left jugular vein for drug administration. The polyethylene catheter (internal diameter: 0.58 mm) filled with heparinized saline was also inserted into the right femoral artery for recording systemic arterial blood pressure. Two hours after cannulation, PRO or PEN was infused through the left jugular vein. Eye blink reflex was analyzed after definition of the hypnotic level. The left eyelash was brushed with soft camel brush 10 times/5 min. This trial was applied 4 times and total number of eye blink reflexes by 40 times eyelash stimuli was counted and the occurrence of eye blink reflexes was shown as the total number of reflexes.

pERK and NeuN immunohistochemistries

Two to 60 min after capsaicin or 5 min after bolus injection of PRO, rats were perfused through the aorta with 500 ml 0.9% saline followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The whole brain including medulla, upper cervical spinal cord and L5 spinal cord (n=2) was removed and postfixed in the same fixative for 3 days at 4°C. The tissues were then transferred to 20% sucrose (w/v) in phosphate buffered saline (PBS) for several days for cryoprotection. Fifty-micron-thick sections were cut with a freezing microtome and every fourth section was collected in PBS. Free-floating tissue sections were rinsed in PBS, 10% normal goat serum in PBS for 1 hour, and then incubated in rabbit anti-Phospho-p44/42 MAP Kinase Antibody (1:1000, Cell Signaling Technology) for 72 hours at 4°C. Next, the sections were incubated in biotinylated goat anti-rabbit IgG (1:600; Vector Labs, Burlingame, CA, USA) for two hours at room temperature. After washing, the sections were incubated in peroxidase-conjugated avidin-biotin complex (1:100; ABC, Vector Labs) for two hours at room temperature. After washing in 0.05 M Tris Buffer (TB), the sections were incubated in 0.035% 3,3'-diaminobenzidine-tetra HCl (DAB, Sigma), 0.2% nickel ammonium sulfate, and 0.05% peroxide in 0.05 M TB (pH 7.4). The sections were washed in PBS, serially mounted on gelatin-coated slides, dehydrated in alcohols and cover slipped. The number of pERK-LI cells in the Vc was counted from every 8th section. Three sections with the largest and following number of pERK-LI cells were chosen from the Vc and upper cervical spinal cord. Then the number of pERK-LI cells was counted and the total number of pERK-LI cells from these three sections was calculated and the mean number of pERK-LI cells (/ three sections / rat) was obtained from each animal.

For double immunofluorescence, 3 rats with subcutaneous capsaicin into the whisker pad region were perfused through the aorta with 500 ml 0.9% saline followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH7.4). Thirty-micron-thick sections were cut and were processed for pERK and NeuN double labeling immunohistochemistry. Free-floating tissue sections were rinsed in PBS, 10% normal goat serum in PBS for 1 hour, and then incubated in rabbit anti Phospho-p44/42 MAP Kinase Antibody (1:300) and rats anti-NeuN Antibody (1:1000, Chemicon, Temecula, CA) over night at 4°C and secondary antibodies (FITC- and rhodamine-, 1:100; Jackson ImmunoResearch, West Grove, PA) conjugated for 1 hour at room temperature in a dark room. Then the sections were washed in PBS three times for 5 min. Sections were mounted on slides and cover slipped in PermaFluor.

Statistical analysis

Results are presented as means $\pm$ SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Fisher's PLSD post hoc test or Dunnett's test.

Student's t test or Welch's t test was conducted when two groups were compared. Differences were considered significant at $p < 0.05$.

Results

Blood pressure and eye blink reflex

The mean blood pressure (BP) during PRO infusion ($n=5$) was as follows; $97.94 \pm 3.77 / 73.07 \pm 1.76$ mmHg (systolic / prediastolic $\pm$ SE) in the light level and $80.17 \pm 2.67 / 61.09 \pm 0.76$ mmHg in the deep level during PRO infusion. On the other hand, BP during PEN infusion ($n=5$) was $101.05 \pm 1.42 / 79.76 \pm 0.68$ mmHg in light level and $80.89 \pm 0.56 / 60.92 \pm 0.79$ mmHg in deep level. We observed that the number of eye blink reflexes elicited by eyelash stimulation was different between LIGHT and DEEP hypnotic levels ($p < 0.01$) as follows: LIGHT level (PRO: 26.0 ± 2.7 , PEN: 14.5 ± 1.7) and DEEP level (PRO: 11.6 ± 1.8 , PEN: 6.4 ± 1.7). These indicate that the hypnotic level during PEN or PRO infusion is confirmed by the BP and the number of eye blink reflexes elicited by eyelash brush.

pERK- and NeuN-like immunoreactive neurons

Fluorescent photomicrographs of pERK-LI cells and their double labeling with anti-NeuN antibody, a neuronal marker, were illustrated in Fig. 3. The pERK-like immunoreactivity was observed in Vc neurons (Fig. 3B). All pERK-LI cells were also labeled with NeuN (Fig. 3C). The time-course change in the expression of pERK-LI cells in the middle Vc is illustrated in Fig. 3D. The pERK-LI cells were detected at 2 min, peaked at 5 min and there were no significant differences in the number of pERK-LI cells between control (saline) and that at 60 min after capsaicin injection into the whisker pad. Photomicrographs of pERK-LI cells in the middle Vc following saline (PRO: A and B, PEN: E and F) or capsaicin (PRO: C and D, PEN: G and H) injection into the whisker pad region are illustrated in Fig. 4. A small number of pERK-LI cells was observed in the middle Vc after saline injection into the whisker pad region in rats with LIGHT (PRO: 1.72 ± 0.04 mg/kg/min, PEN: 1.03 ± 0.04 mg/kg/min) or DEEP (PRO: 2.52 ± 0.21 mg/kg/min, PEN: 2.25 ± 0.15 mg/kg/min) level of hypnosis after PRO (Fig. 4A and B) or PEN injection (Fig. 4E and F). After capsaicin injection into the whisker pad region, a large number of pERK-LI cells were expressed in the superficial laminae of the middle Vc in the rats with PRO or PEN injection. The number of pERK-LI cells expressed following capsaicin injection was increased after an increase in the depth of the hypnosis in the rats with PRO injection (Fig. 4C and D). On the other hand, the number of pERK-LI cells was decreased following an increase in the depth of hypnosis following PEN infusion (Fig. 4G and H).

Rostro-caudal distribution of pERK-LI cells after capsaicin injection in PRO-injected rats

Figure 5 illustrates camera-Lucida drawings of pERK-LI cells from Vi/Vc to Vc/C2 of the rats with PRO administration and following saline or capsaicin injection into the whisker pad region. The pERK-LI cells were detected in the Vi/Vc zone, middle Vc, Vc/C2 zone, nucleus tractus solitarius (NTS), reticular formation and inferior olive in the rats with PRO injection. We focused on the pERK-LI cells in the Vi/Vc zone, middle Vc and Vc/C2 zone of the spinal trigeminal complex. A small number of pERK-LI cells was observed in the superficial laminae of the rostral Vc, middle Vc and Vc/C2 zone after subcutaneous saline injection into the whisker pad region. Most of pERK-LI cells were located in the dorsoventral middle portion of these regions. After capsaicin injection, the density and distribution areas were increased compared with that of saline injection in rats with PRO infusion (see Fig. 5 capsaicin). We also observed a small number of pERK-LI cells on the side contralateral to capsaicin injection. The rostro-caudal distributions of the mean number of pERK-LI cells were illustrated in Fig. 6. In the rats with saline injection to the whisker pad, pERK-LI cells appeared from slightly rostral to the obex to 2800 μ m caudal from the obex and peaked at the obex level (Fig. 6A). On the other hand, a large number of pERK-LI cells was observed in the Vi/Vc zone and middle Vc after

capsaicin injection into the whisker pad (Fig. 6C). The largest number of pERK-LI cells was obtained at 1200 μ m caudal to the obex in capsaicin treated rats (Fig. 6C). The number of pERK-LI cells was significantly larger in the ipsilateral Vi/Vc zone at the DEEP level as compared with that of the LIGHT level in the rats with saline injection into the whisker pad and that was significantly larger in Vi/Vc zone and middle Vc in rats with capsaicin injection (Fig. 6E). We also observed significantly larger number of pERK-LI cells in the DEEP level than LIGHT level on the contralateral side to PRO injection in capsaicin injected rats but not observed any differences in saline injected rats (Fig. 6B, D and F).

Rostro-caudal distribution of pERK-LI cells after capsaicin injection in the PEN-injected rats

Fig. 7 illustrates the camera Lucida drawings of pERK-LI cells in the rats with PEN administration following subcutaneous saline or capsaicin injection into the whisker pad region. A small number of pERK-LI cells was observed in the NTS, Vi/Vc zone, middle Vc and Vc/C2 zone following saline injection into the whisker pad region in the rats with PEN infusion (Fig. 7). After capsaicin injection, a larger number of pERK-LI cells were observed in these nuclei compared with that of saline-injected rats. The pERK-LI cells were decreased in all of these nuclei in the PEN injected rats compared with those of PRO injected rats at DEEP hypnotic level as illustrated in Figs. 5 and 7. A small number of pERK-LI cells was also observed on the contralateral side to capsaicin injection in the PEN treated rats (Fig. 7). Rostro-caudal arrangement of mean number of pERK-LI cells was illustrated in Fig. 8. A small number of pERK-LI cells were observed on the ipsilateral side to saline injection but almost no pERK-LI cells was expressed on the contralateral side (Fig. 8). After capsaicin injection into the whisker pad region, a large number of pERK-LI cells was observed on the side ipsilateral to capsaicin injection but not on the contralateral side as illustrated in Fig. 8 E and F. We also observed that the number of pERK-LI cells was significantly larger in middle Vc at deep level compared with that of light level in capsaicin injected rats (Fig. 8C).

Effect of infusion speed, infusion through the femoral vein and lidocaine infusion on ERK phosphorylation

We also studied the effect of infusion speed and infusion through the femoral vein on pERK phosphorylation in Vc, upper cervical spinal cord and L5 dorsal horn neurons (Figs. 9 and 10). A large number of pERK-LI cells was observed following bolus injection of PRO, but not in slow injection (Fig. 9). We also observed that pERK-LI cells in Vc following bolus injection of PRO through the femoral vein and those after lidocaine + bolus injection of PRO through the femoral vein2 was similar in their distribution pattern as illustrated in Fig. 9C and D. A number of pERK-LI cells was also observed in L5 spinal dorsal horn following bolus injection of PRO through the femoral vein (Fig. 9E and F).

Rostro-caudal arrangement of the mean number of pERK-LI cells expressed after bolus injection of PRO (16 mg/kg, 22mg/kg or 28 mg/kg i.v.) and the effect of i.v. lidocaine (10 mg/kg) infusion through the jugular vein on ERK phosphorylation were illustrated in Fig. 10 A. We observed a large number of pERK-LI cells with two peaks at about obex level and 1600 mm caudal to the obex. The number of pERK-LI cells was increased in the Vi/Vc and middle Vc following increases in the amount of PRO administration as illustrated in the inset bar graphs of Fig. 10A. The number of pERK-LI cells in the Vi/Vc and middle Vc was significantly decreased after lidocaine infusion prior to PRO administration (Fig. 10B and C). The number of pERK-LI cells in middle Vc and Vi/Vc in the rats with bolus infusion of PRO was significantly larger compared with that of slowly infused rats. We could not observe the significant difference in the number of pERK-LI cells in middle Vc and Vi/Vc following slow PRO infusion via the jugular vein and lidocaine + PRO infusion through the jugular vein. On the other and, many pERK-LI cells were expressed in middle Vc and Vi/Vc in the rats with bolus PRO infusion through the femoral vein (Fig. 10B and C).

Discussion

PRO is frequently used as an intravenous hypnotic agent in clinic, because the depth of hypnotic level induced by PRO is easy to control and it has no severe side effects during infusion.^{7, 25,33} It has been shown that PRO has a depressive action on neuronal activity in the whole brain areas, reflected by a decrease in EEG activity.²⁶ We observed a similar EEG depression in rats that is observed in humans during PRO infusion. The effect of PRO on neuronal activity is thought to be induced through GABAA receptor mechanisms.^{12,41,50} There is a large number of GABAergic interneurons in the brain. The activity of those neurons is increased after PRO administration.⁵⁰ It is, therefore, possible that the ascending pain pathways are modulated by PRO as well as other brain areas as there are GABAergic interneurons in the medulla and spinal cord that are involved in modulation of nociception.^{27,39,45} However, some previous studies described that PRO lacked antinociceptive action during infusion and sometimes it caused deep pain in the i.v. injected regions.^{32,34} Therefore, it is still controversial whether PRO can produce antinociception.

We observed a very restricted distribution of pERK-LI cells in superficial laminae of the Vc and upper cervical spinal cord following capsaicin stimulation of the whisker pad. Recently, it has been reported that ERK is phosphorylated in dorsal horn neurons within 10 min after noxious stimulation of the peripheral structures.²¹ This is a very early event in the intracellular transduction cascades compared with those of other intracellular molecules such as Fos protein.² Phosphorylation of ERK is known as a result of Ca²⁺ influx into neurons which is involved in generation of action potentials.^{20,21,24} These results strongly suggest that ERK phosphorylation is directly correlated with neuronal activation.

The distribution pattern of pERK-LI cells was obviously different from that of Fos-positive neurons.²⁶ Fos-positive neurons were observed in both superficial and deep laminae, and also those were distributed rostro-caudally wider areas of these nuclei following peripheral noxious stimulation compared with pERK-LI cells.^{3,5,13,16,35} It has been reported that Fos protein is expressed in the Vi/Vc zone, middle Vc and Vc/C2 zone 0.5-1.0 hr after noxious stimulation of the face, whereas pERK is expressed within 10 min.³⁵ The time-course difference in the expression between Fos protein and pERK may be related to their sequential participation in signal transduction after orofacial noxious stimulation. Furthermore, there are multiple intracellular transduction pathways involved in producing Fos protein.²² The ERK phosphorylation cascade is thought to be one of the pathways for Fos production. It is likely that the different intracellular transduction cascades are involved in Fos production and ERK phosphorylation, which may explain the distribution differences between Fos and pERK positive neurons.

We also observed that the number of capsaicin-induced pERK-LI cells was significantly larger in DEEP hypnotic level compared with LIGHT level in rats with PRO infusion, whereas that was significantly smaller in LIGHT hypnotic level than DEEP level in the rats with PEN infusion. These data suggest that PRO, but not PEN, could enhance nociceptive neuronal activity in the CNS. Furthermore, we observed that the bolus PRO injection itself induced many pERK-LI cells in Vi/Vc and middle Vc without capsaicin treatment and they were increased following an increase in the dose of PRO. Previous clinical studies described that PRO did not have an antinociceptive effect, but could induce deep pain in the i.v. injection regions.^{32,34} Together with previous clinical observations our data suggest that PRO activates and enhances activity in trigeminal nociceptive pathways.

We observed that the ERK phosphorylation in Vc neurons was significantly depressed following i.v. injection of lidocaine through the jugular vein. The lidocaine is known as a strong sodium channel blocker.^{6,23,49} The venous nociceptors and CNS neurons are thought to be

the possible targets for lidocaine. It is therefore possible that the lidocaine affects the venous nociceptors and Vc neuronal excitability, resulting in a decrease in the number of pERK-LI cells in Vc. To clarify whether PRO activates nociceptors innervating the venous structures during infusion, we tested the effect of intravenous lidocaine infusion given prior to PRO administration. We observed a significant reduction of PRO-induced pERK-LI cells in Vi/Vc zone and middle Vc following preceding lidocaine infusion. The present results support the observation that intravenous administration of PRO causes deep pain in human subjects, suggesting that the activation of nociceptors innervating in the venous structures are involved in ERK phosphorylation of Vi/Vc zone and middle Vc neurons. Furthermore, the number of pERK-LI cell in middle Vc and Vi/Vc was significantly smaller following slow infusion of PRO compared with bolus infused rats. This also suggests that the mechanical nociceptors in the vein may be involved in ERK phosphorylation of middle Vc and Vi/Vc neurons following bolus infusion of PRO.

The present findings reveal that propofol is capable of enhancing nociceptive neuronal activity in the trigeminal region, suggesting that propofol may produce deep pain through nociceptors innervating the venous structures during infusion. It is very important to use antinociceptive drugs in clinic before propofol infusion, in order to eliminate the enhancement of neuronal activity in the ascending pain pathways.

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Fig. 1. Rectified neck EMG activities at awake level (A) and LIGHT level (B) during PRO infusion. Rectified EEG at awake level (C), LIGHT level (D) and DEEP level (E) during propofol infusion. The threshold amplitude for i-EEG analysis was above 2SD of the mean EEG amplitude indicated by the dotted line. F: Mean relative time period of isoelectric EEG appearance in PEN and PRO injected rats. EEG and EMG activities were recorded 30 min after continuous infusion of PRO or PEN, **: $p < 0.01$.

Fig. 2.

Schematic illustration of the present experimental protocol. The time-course of experimental procedure in rats with capsaicin or saline injection to the whisker pad skin (A) and that in rats with bolus infusion of PRO without capsaicin injection (B). Jugular: jugular vein, Femoral: femoral vein, Lido: lidocaine, Cap: capsaicin.

Fig. 3.

Capsaicin-induced pERK in trigeminal spinal subnucleus caudalis neurons. A-C: Photomicrographs illustrating examples of pERK-LI cells and their double labeling with the anti-NeuN antibody in the middle Vc. The NeuN immunoreactive cells are labeled with red (A) and pERK-LI cells are labeled with green (B). The double labeled cells were observed as yellow (C). Arrows indicate neurons double labeled with pERK-LI and NeuN. D: Time-course change in pERK-LI cells in the spinal trigeminal complex and upper cervical spinal cord following subcutaneous capsaicin injection into the whisker pad region. Capsaicin (10 mM, 50 μ l) was injected to the whisker pad subcutaneously 30 min after PRO infusion through the jugular vein. Rats were perfused at 2, 5, 10, 30 and 60 min after capsaicin injection. **: $p < 0.01$.

Fig. 4. Photomicrographs of pERK-LI cells following saline (A, B, E and F) or capsaicin (C, D, G and H) injection into the whisker pad region in rats with LIGHT (A, C, E and G) or DEEP level (B, F, D and H) of hypnosis. A-D: rats with propofol infusion, E-H: those with pentobarbital infusion.

Fig. 5.

Camera Lucida drawings of pERK-LI cells in the medulla and upper cervical cord of rats with propofol administration following saline or capsaicin injection into the whisker pad region. Capsaicin was injected to the whisker pad skin subcutaneously 30 min after the continuous infusion of PEN or PRO. Rats were perfused 5 min after capsaicin injection. Brains were obtained from rats in a LIGHT or DEEP level of hypnosis. The numbers beside drawings indicate distance from the obex (positive numbers: rostral to the obex, negative numbers: caudal to the obex) in this and Fig. 6.

Fig. 6.

Rostro-caudal distribution of mean number of pERK-LI cells in rats with PRO infusion following saline or capsaicin injection into the whisker pad region. Capsaicin was injected to the whisker pad subcutaneously 30 min after the continuous infusion of PEN or PRO. Rats were perfused 5 min after capsaicin injection and the rat brains were removed for the immunohistochemical analysis. A: ipsilateral to saline injection, B: contralateral side to saline injection, C: ipsilateral side to capsaicin injection, D: contralateral side to capsaicin injection. E and F: total number of pERK-LI cells in rats with PRO infusion in ipsilateral and contralateral sides to capsaicin injection respectively. *: $p < 0.05$, **: $p < 0.01$.

Fig. 7.

Camera Lucida drawings of pERK-LI cells in the medulla and upper cervical cord of rats with pentobarbital administration following saline or capsaicin injection into the whisker pad region. Capsaicin was injected to the whisker pad subcutaneously 30 min after the continuous infusion of PEN or PRO. Rats were perfused 5 min after capsaicin injections. Brains were obtained from rats in a LIGHT or DEEP level of hypnosis.

Fig. 8.

Rostro-caudal distribution of pERK-LI cells in rats with pentobarbital infusion following saline or capsaicin injection into the whisker pad region. Capsaicin was injected to the whisker pad subcutaneously 30 min after the continuous infusion of PEN or PRO. Rats were perfused 5 min after capsaicin injection. A: ipsilateral side to saline injection, B: contralateral side to saline injection, C: ipsilateral side to capsaicin injection, D: contralateral side to capsaicin injection. E and F: total number of pERK-LI cells in rats with PEN infusion in ipsilateral and contralateral sides to capsaicin injection respectively. *: $p < 0.05$.

Fig. 9.

Photomicrographs of pERK-LI cells in middle Vc following bolus infusion of PRO through the jugular vein (A), slow injection of PRO through the jugular vein (B), bolus infusion of PRO through the femoral vein (C) and bolus infusion of PRO after lidocaine infusion through the femoral vein (D). E and F: photomicrographs of pERK-LI cells in L5 spinal dorsal horn following bolus infusion of PRO through the femoral vein ipsilateral side and contralateral sides to PRO infusion respectively. Jugular: jugular vein, Femoral: femoral vein, Lido: lidocaine, ipsi: ipsilateral side to PRO infusion, contra: contralateral side to PRO infusion.

Fig. 10.

Rostro-caudal distribution of mean number of pERK-LI cells expressed in Vc and upper cervical spinal cord in the ipsilateral side to PRO infusion. A: mean number of pERK-LI cells following bolus infusion of different doses of PRO through the jugular vein, B and C: mean number of pERK-LI cells following bolus infusion of different dose of PRO through the jugular vein, i.v. lidocaine infusion (2% lidocaine 10mg/kg) + PRO infusion through the jugular vein, bolus or slow infusion of PRO through the jugular or femoral vein. Bolus injection of PRO was done 10 min after continuous infusion of lidocaine. The number of pERK-LI cells is increased following an increase in the dose of PRO. *: $p < 0.05$, **: $p < 0.01$. Jugular: jugular vein, Femoral: femoral vein, Lido: lidocaine.