Selective Inhibitory Effects of Pregabalin on Peripheral C but Not A-Delta Fibers Mediated Nociception in Intact and Spinalized Rats

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Abstract—Effects of pregabalin (PGB, 20–80 mg/kg i.v. injection) on spinally-organized nociception were investigated in isoflurane-anesthetized intact and spinalized rats. Responses of single deep spinal dorsal horn (DH) (laminae IV–V) nociceptive-specific (NS) neurons receiving peripheral inputs from A-δ and C fibers to repetitive electrical stimulation (intensity: 3–5 mA; frequency: 1 Hz; pulse duration: 1 ms), mechanical/heat stimulation were recorded extracellularly during physiological condition and s.c. bee venom (BV) induced inflammation. PGB significantly inhibited C-fiber mediated spinal NS neurons’ late responses including phenomena of wind-up (temporal summation) and after-discharge. However, the antinociceptive effects of PGB on nociception were not observed until 30 min after its administration. In contrast, no significant inhibitory effect of PGB on A-δ fiber mediated early responses was observed during the experiments. Compared with intact rats, the inhibitory effects of PGB upon nociception vanished in the spinalized animals. This suggests that PGB-induced selective antinociceptive effect on C-fiber mediated nociception is mainly central effects involving supraspinal centers via descending inhibitory controls. Furthermore, pre-treatment, but not post-treatment, with PGB (80 mg/kg) markedly inhibited s.c. BV elicited spontaneous neuronal responses, and noxious mechanical/heat stimuli evoked hyperactivities of spinal NS neurons, indicating that PGB has efficacy of pre-emptive analgesia on pathological pain associated with central sensitization. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: pregabalin, spinal nociceptive-specific neurons, nociception, pre-emptive analgesia, bee venom.

The function of pregabalin (PGB, an anticonvulsant drug), the pharmacologically active S-enantiomer of 3-amino-methyl-5-methyl-hexanoic acid, has both been preclinically and clinically used for the control of chronic neurological disorder, i.e. epilepsy, and for the treatment of pathological pain, such as fibromyalgia (Zareba, 2008; Jan et al., 2009; for reviews see Gajraj, 2005; Lyseng-Williamson and Siddiqui, 2008). Upon nociception/pain, PGB and its develop-

mental precursor gabapentin effectively exhibit not only persistent antinociceptive effects at treating differential pathological nociception, but also some enhancing actions to reinforce the antinociceptive effects of conventional analgesics such as opioids (Tippana et al., 2007). Due to low potential abuse and a limited addiction liability, PGB may have promising hope to become the first-line medication in treatment of intractable pathological pain (Reuben et al., 2006; Million et al., 2007).

Even though PGB and gabapentin have efficacies in treating neuropathic pain, the effectiveness of PGB/gabapentin on wind-up (temporal summation) of nociception/pain is still not well documented (Dickenson and Ghanehkhari, 2007). Gabapentin has almost no effect on wind-up in vitro preparations (Patel et al., 2001; Villette et al., 2003), whereas wind-up of single motor unit activities and the “wind-up like” pain in humans can be depressed by gabapentin (Harding et al., 2005; Curros-Criado and Herrero, 2007). Similar to gabapentin, PGB is structurally related to central inhibitory neurotransmitter GABA. It has been, however, reported that neither GABAA-B receptors nor GABA metabolism can be bound and interacted by gabapentin/PGB during the treatment of epilepsy and pathological pain (Gee et al., 1996; Field et al., 2006). Accordingly, the central mechanisms associated with efficacy of PGB on pain, particularly pathological pain, need to be further explored.

With regard to the study of nociception conducted in the spinal cord, one thing should be noted here that most of electrophysiological experiments are designed to investigate the activities of spinal dorsal horn (DH) wide-dynamic range (WDR) neurons, which receive multiple sensory afferent information from both innocuous and noxious fibers (Melzack and Wall, 1988). Less study directly concentrates on the nociceptive responses of spinal “immaculate” nociceptive neurons: spinal nociceptive-specific (NS) neurons. In contrast to spinal WDR neurons, the activities of deep spinal NS neurons are strictly controlled by the descending inhibitory controls (You et al., 2008a). Deep spinal NS neurons are, however, comparable with WDR neurons exhibiting dynamic neuronal plastic sensitivities, i.e. wind-up to repeated electrical stimulation, as well as playing an important pro-nociceptive role during pathological nociception (You et al., 2008a).

Thus, the main aim of the present experiment employing extracellular recordings in isoflurane-anesthetized rats is twofold. First, we evaluated and confirmed potential effects of PGB on electrically-evoked spinal DH NS neurons’ nociceptive responses including phenomena of
wind-up and after-discharge. Second, influence of PGB on the facilitated spinal DH NS neuron responses during s.c. bee venom (BV, 0.2 mg/50 \( \mu l \)) induced pathological inflammation were further investigated.

A preliminary report of the present study was presented in abstract form (You et al., 2008b).

**EXPERIMENTAL PROCEDURES**

**Animal preparation**

Male Wistar rats (10 weeks age) weighing 260–300 g were obtained from the Animal Facilities of Aalborg Hospital, and housed pairwise under a 12-h light/dark cycle with food and water available ad libitum. The experimental procedures were approved by the institutional Animal Ethics Committee. All experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC), and efforts were made to minimize suffering and reduce the number of animals used.

The rats were initially anesthetized with pentobarbital (50 mg/kg) by i.p. administration. During the surgical procedures, a tracheal cannula and a left jugular vein catheter were inserted in order to ensure adequate breathing, fluid circulation, and drug administration. The right carotid artery was cannulated via a catheter for heart rate and mean arterial systolic pressure monitoring (80–140 mm Hg; 5011V Stoelting Co., IL, USA). Body temperature was maintained at 37.5 ± 0.5 °C by means of a homeothermic circulating-water blanket (T/Pump@, TP-420 Gaymar Industries Inc., NY, USA) beneath the rat abdomen. All vital signs were kept within physiological range. Spinal laminectomy was performed from T13 to L1 vertebrae to expose the corresponding lumbar enlargement for spinal DH neuron recording. In some experiments to exclude the effect of supraspinal descending modulation on spinal DH neuronal activity, a separate mini laminectomy was performed at T8–T9 and the spinal cord was subsequently transected with a surgical knife under a microscope.

After the above surgery (about 1 h), the animal was placed in a stereotactic frame (MP8003a, RWD Co., China), anesthetized and artificially ventilated using an anesthesia workstation (Hallowell EMC, Pittsfield, USA). The main anesthesia implemented isoflurane inhalation (Forene®, Abbott Scandinavia AB, Sweden; 1.4% isoflurane in 98.6% oxygen) as this level of anesthesia has the cutaneous receptive field (cRF) located on the ipsilateral hind limb. Ventilation was monitored continuously; the end-tidal CO\(_2\) was 1.4% isoflurane in 98.6% oxygen) as this level of anesthesia has the cutaneous receptive field (cRF) located on the ipsilateral hind limb. The main anesthesia implemented isoflurane inhalation (Forene®, Abbott Scandinavia AB, Sweden; 1.4% isoflurane in 98.6% oxygen) as this level of anesthesia has the cutaneous receptive field (cRF) located on the ipsilateral hind limb. The main anesthesia implemented isoflurane inhalation (Forene®, Abbott Scandinavia AB, Sweden; 1.4% isoflurane in 98.6% oxygen) as this level of anesthesia has the cutaneous receptive field (cRF) located on the ipsilateral hind limb.

**Electrophysiological recordings and spinal neuron identification**

Extracellular recordings were made as described elsewhere (You et al., 2003, 2008a). In experiments involving spinalization, recordings began no earlier than 2 h after surgical spinalization to allow recovery from acute spinal shock (You et al., 2003, 2008a). Electrophysiological responses of single spinal DH neurons with the cutaneous receptive field (cRF) located on the ipsilateral hind paw were recorded extracellularly using tungsten microelectrodes (impedance: 1–2 MΩ; FHC Inc., USA). The recording electrode was advanced at a speed of approximately 2 \( \mu m/s \) by a manual hydraulic micro drive while the ipsilateral hind paw was stimulated by an electrical stimulus with low intensity (intensity: 0.5 mA; pulse duration: 0.5 ms; frequency: 0.3–0.5 Hz) in order to promptly locate a high-threshold spinal NS neuron. All spinal NS neurons were found throughout the deep (laminae IV–V) DH field of the spinal cord. The depth of the neurons was taken from the mean micro drive readings, both on descent and during withdrawal to the spinal cord surface (Paxinos and Watson, 1998).

Each recorded spinal DH neuron was initially characterized by its responses to 10 s natural stimuli (brush, pressure, and pinch) performed by wood brush, blunt metal probe, and fine forceps, respectively (You et al., 2008a). For further qualitative identification, we used six individual von Frey monofilaments (Stoelting Co., IL, USA) with bending forces at 0.1, 1, 10, 20, 40, 75 g to generate the graded quantitative stimulation. The von Frey filaments were applied for 6 s to the most sensitive cRF area of the recorded spinal neurons as this 6 s mechanical stimulation was tested previously to be adequate in generating stable neuronal responses and preventing ischemia due to the reduced tissue blood flow. In addition, these stimulus intensities were tested on plantar area of hind paw in conscious rats as well as on the dorsum of the operator’s hand for 6 s to mimic the actual situation in the rats: 40 and 75 g von Frey filaments generated obvious withdrawal flinching behavior in conscious rats, and moderate to strongly painful sensation in humans, respectively. In the current study, the recorded spinal DH NS neurons responded only to 10 s pinch stimuli and the stimulation of 40–75 g von Frey filament, but not brush and pressure stimuli.

Following earlier recommendations (You and Chen, 1999; You et al., 2008a), the recorded spinal neurons showing habituation or strong spontaneous background firing (>5 spikes/s) were not recruited for the remainder of the study. In order to mimic the normal physiological state, any muscle relaxants were not used (You et al., 2003, 2008a). Spinal DH neurons in response to joint movement and muscle contraction were strictly excluded in the current study to ensure the spinal neurons receiving afferent inputs only from cutaneous area. The cRF area was detected with a fine forceps, marked and measured using a marking pen and transparent plastic millimeter paper.

Spinal DH neuronal signals were amplified by a microelectrode amplifier (MEZ-8301, Nihon Kohden, Japan). The neuronal signals were simultaneously sampled by CED-1401 (CED Co., UK) at 10 kHz, and analyzed off-line using Spike 2 software (CED Co.).

**Drug administration and intervention**

PGB (dissolved by 0.9% saline; Pfizer Co., UK) was administrated by i.v. injection performed slowly over a period of 40–50 s via the left jugular vein catheter during the intact/spinalized conditions and the BV-induced persistent nociception. Three doses of PGB (20, 40, 80 mg/kg) were selected in order to investigate the possible dose-dependent effect. Under the BV-induced inflammation, only the effects of PGB with the maximum dose were investigated; 0.9% saline was served as control in other animals.

To establish the condition of persistent nociception and inflammation, 50 \( \mu l \) volume of BV [0.2 mg lyophilized whole venom of apis mellifera (Sigma) dissolved in 0.9% saline] were s.c. injected into the most sensitive cRF area of the recorded spinal NS neuron in the intact condition (You and Chen, 1999; You et al., 2008a). The effects of PGB on BV-induced spontaneous neuronal activities, mechanical/heat evoked hypersensitivities of spinal neuron responses were investigated for 1-h in BV-induced inflammation.
Electrical, mechanical and heat stimulation

As elsewhere (You et al., 2003, 2008a), a train electrical stimulation containing 16 square wave pulses (pulse duration: 1 ms, frequency: 1 Hz) were generated from a constant current electrical stimulator via a pair of stainless steel electrodes (diameter: 0.3 mm; length: 1 cm), which were inserted into the most sensitive cRF of the recorded spinal NS neuron. The intensity threshold (T) to evoke the late neuronal responses (latency >75 ms) by a single electrical stimulus was initially measured and considered as the current of stimulus intensity (mA) evoking the late neuronal responses to 50% of the test stimuli. After that, the intensity of electrical stimulation was selected as 1.5×T (about 3–5 mA, pulse duration: 1 ms) (Fig. 1).

As mentioned above, 75 g von Frey filament bended for 6 s was used to generate noxious mechanical stimulation. The responses of spinal NS neurons to a 10 s non-touched heat stimuli were measured with a computer controlled intensity adjustable radiant heat stimulator (Taimeng Instruments Co., Cheng Du, China). In preliminary experiments, the intensity of this 10-s heat stimulation was adjusted adequately to evoke obvious withdrawal flinching behavior in conscious rats. Besides, painful, but tolerable, sensation could be generated from the operator’s stimulated hand using this heat stimulation. The interval between mechanical and heat stimulations was 10 min.

Data analysis

The electrically-evoked spinal NS neuron responses were clearly classified as two components according to its different conduction latencies (Fig. 1). Using off-line Spike 2 template analysis, the minimum latency of these two components was about 5 and 75 ms, respectively. The number of the electrically-evoked spikes of DH NS neuron was counted and divided into early (latency around 5–20 ms) and late (latency >75 ms) responses (Melzack and Wall, 1988). The electrically-evoked after-discharges of NS neuron were evaluated by counting the remaining spikes for 10 s starting from 1 s after the last stimulus artefact of the train stimulation (Fig. 1).

A neuronal response to repeated electrical stimulation was considered stable if less than ±10% variation was observed from the mean of the evoked neuronal responses during the last three consecutive tests within 30 min; these responses obtained were then considered as baseline (i.e. 100%). The control group without any pharmacological treatments and all of the drug effects including vehicle treatment collected per 10 min during 1-h observation were expressed as a percentage of the pre-drug baseline responses (% of baseline).

For the observation of PGB effects on spinal NS neuron activities during the BV-induced inflammation, 30 min prior to the BV injection the spinal NS neuron activities responding to a 6 s mechanical stimulation and a 10 s heat stimulation were initially tested, and the responses were considered as 100%. After that, PGB was i.v. administered either 30 min prior to or 30 min post the BV injection. Sixty minutes post the BV injection, the recorded spinal NS neuron responses to mechanical and heat stimuli were tested again. All of the data obtained during this period were calculated and expressed as a percentage of the 100% control.

Statistical analysis was performed using paired t-test, one-way/two-way analysis of variance (ANOVA) with post hoc Tukey’s tests. P<0.05 was considered statistically significant. Data are shown as mean±SEM.

RESULTS

In total, 97 NS neurons in the deep DH area of the spinal cord from 79 intact and 18 spinalized rats were recorded. The depth of the recorded spinal NS neurons located in the DH of the spinal cord averaged 792±24 μm, which were mainly (79%) located in laminae IV–V (Paxinos and Watson, 1998). The recorded spinal NS neurons showed restricted small cRF around 9.1±0.7 mm² (n=79 in 79 rats) in the intact condition and significantly larger cRF about 16.8±3.2 mm² in the spinalized condition (n=18 in 18 rats). For mechanical stimulation, all the recorded spinal DH NS neurons responded only to noxious pinch and 40–75 g von Frey stimuli, but not to innocuous brush and pressure stimuli in both the intact and spinalized conditions. Using electrical stimulation (intensity: 3–5 mA, pulse duration: 1 ms), the recorded spinal NS neurons exhibited early and late responses according to its different conduction latencies (early response: 9.2±0.9 ms; late response: 134.5±4.8 ms). In the current experiment, the distance from the stimulation site located on the plantar area of hind paw to the recording site from dorsal horn of the spinal cord was about 15 cm. According to the suggested criteria (Cervero et al., 1976), these recorded spinal NS neurons were considered to receive and conduct peripheral nociceptive inputs from both A-δ and C afferents.

Effects of PGB on electrically-evoked spinal DH NS neuronal activates in intact and spinalized rats

Effects of PGB on three different components termed early response, late response, and after-discharge of the recorded spinal NS neurons to the repeated electrical stimulation were analyzed. During 90 min observation time after the PGB treatment, early responses of the spinal NS neurons did not vary significantly compared with the untreated control and vehicle (0.9% saline) treatment (Fig. 2A). In contrast, both late responses and after-discharges of the spinal NS neurons were depressed significantly after the i.v. administration of PGB with different doses (P<0.05, paired t-test) (Fig. 2B, C). However, these depressive effects of PGB on both late responses and after-discharges were not observed until 30 min after the drug administration (P>0.05). For instance, 40 min after 20 mg/kg PGB injection the number of spikes of late responses and after-discharges of the spinal NS neuron was significantly reduced to 85±6% and 87±8% of the pretreatment control level, respectively (P<0.05). In Fig. 2B, C, although 40–80 mg/kg PGB seemed to produce more pronounced inhibition relative to 20 mg/kg PGB treatment, however, the dose-dependent antinociceptive effects of PGB on the spinal NS neuronal late responses and after-discharges were not observed (P>0.05, one-way ANOVA). This suggests that the antinociceptive effects of 20 mg/kg dose of PGB on nociception might be close to the maximum extent of inhibition during the normal physiological state.

The effects of PGB on wind-up were specially evaluated. Raw recordings of spinal NS neuronal wind-up and after-discharges affected by PGB treatment are shown in Fig. 3. For 20 mg/kg PGB treatment, seven out of nine spinal NS neurons exhibited wind-up in intact condition (Fig. 4). Likewise, no inhibitory effect of PGB on the spinal NS neuronal wind-up was found until 30 min post the drug administration. In Fig. 4, wind-up of spinal NS neuronal responses to the

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repeated electrical stimulation vanished 60 and 90 min after the PGB treatment ($P<0.001$). Apparent depressive effects of PGB with doses of 40–80 mg/kg on wind-up of the spinal NS neuron responses were also found.

During the absence of descending modulating system, i.e. the spinalized condition, we further investigated the effects of 80 mg/kg PGB on the electrically-evoked early response, late response, and after-discharge of the spinal

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**Fig. 1.** Raw recordings of spinal DH NS neuron response elicited by a repetitive electrical stimulation (intensity: $1.5 \times T$ (T: threshold for the neuronal late response); pulse duration: 1 ms; frequency: 1 Hz). The electrically-evoked spinal NS neuron activities shown here are selected from the responses elicited by the 1st, 4th, 7th, 10th, 13th, 16th electrical stimulus. Early responses, late responses, and after discharges were accounted according to its different conduction latencies, respectively (E.S.: electrical stimulation; E.R.: early response; L.R.: late response; A.D.: after-discharge).
NS neurons. The 80 mg/kg dose of PGB exerted no inhibitory effect on the early and late responses, and the after-discharges in the spinalized animals (\( P < 0.05 \), two-way ANOVA; Fig. 5). Within the 90 min of the observation time following the PGB injection, the repetitive electrical stimulation obviously elicited wind-up of the spinal NS neurons over time (data not shown).

Effects of pre- and post-treatment with PGB on BV-induced spontaneous nociception and mechanical/heat hyperactivities

In accordance with previous finding (You et al., 2008a), upon NS neurons in intact condition BV s.c. produced a rapid-onset, short-lasting (less than 10 min) increased neuronal firing relative to the sparse background activities prior to the injection of BV. To investigate whether PGB differently affect the BV-induced spontaneous nociception and follow-up mechanical and heat hypersensitivities, 80 mg/kg PGB was injected i.v. either 30 min before or 30 min after the BV injection.

Pre-treatment with PGB significantly depressed the BV induced spontaneous neuronal responses. In six out of nine NS neurons, less than 2–3 min spinal NS neuronal firings elicited by the BV injection were observed (\( P < 0.05 \)). Likewise, pre-treatment with PGB markedly inhibited the BV-enhanced NS neuron response to noxious mechanical and heat stimuli compared with the untreated control and saline treatment (\( P < 0.05 \) and \( P < 0.001 \)). By contrast, 30 min after the BV injection 80 mg/kg PGB did not significantly alter the spinal neurons’ hyperactivities to mechanical and heat stimulations (\( P > 0.05 \)) (Fig. 6).

DISCUSSION

Using extracellular recording approach, the current electrophysiological study demonstrates that PGB significantly depressed peripheral C, but not A-\( \delta \) fibers mediated spinal NS neuronal late responses and after-discharges, including phenomenon of wind-up (temporal summation). The PGB-induced inhibition of nociception is supraspinal dependent. Pre-treatment, but not post-treatment, with PGB markedly inhibited s.c. BV elicited spontaneous nociception, and mechanical/heat evoked hypersensitivities of spinal NS neurons, suggesting a pre-emptive analgesic effect of PGB on pathological nociception associated with central sensitization.

Extracellular spinal nociceptive neuron recordings in animals

During the past four decades, pain research explored by spinal extracellular recordings in animals has intensively been developed. One thing brings our attention that electrophysiological experiments related to the investigation of spinally-organized nociception have mostly been designed to assess the activities of spinal DH WDR neurons, which receive the multiple sensory information from both innocuous and noxious afferents (Cervero et al., 1976). On the one hand, the electrically-evoked spinal neuronal early responses no doubt contain both noxious information mediated by peripheral A-\( \delta \) fibers and innocuous message mediated by A-\( \alpha/\beta \) afferents. Thus, to some extent the spinal WDR neuronal early response in animals is not a
good index for the study of acute nociception. On the other hand, pharmacological or non-pharmacological treatments on spinal WDR neurons' activities are probably influenced by innocuous inputs mediated by A-α/β afferents via gate control mechanisms (Melzack and Wall, 1988). Accordingly, insofar the experimental data concerning central mechanisms of nociception assessed by the recordings of spinal DH WDR neurons activities would not account for rational interpretation on some aspects.

We recently found that deep spinal DH NS neurons with restricted small cRFs differ significantly from WDR neurons in their responses to peripheral tonic noxious stimuli, i.e. s.c. BV insult, in both the intact and spinalized conditions (You et al., 2008a). Additionally, deep spinal NS neurons exhibit dynamic neuronal plastic sensitivities, i.e. wind-up to the repeated electrical stimulation compared with other spinal NS neurons located in the superficial spinal DH area, suggesting that deep spinal DH NS neurons play an important pro-nociceptive role during pathological condition (our unpublished experimental observations). Together with previous report (You et al., 2008a), the present findings have further provided the essential consideration that recordings of the spinal cord NS neuron, relative to WDR neuron, might be a better model to assess nociception and its transmission involving the spinal and supraspinal mechanisms.

Role of PGB on nociception/pain associated with central sensitization

Recent studies have demonstrated that PGB, which has a better pharmacokinetic profile to that of its developmental
precursor gabapentin, shows significant analgesic effects in various experimental pain models, i.e. neuropathic pain, postoperative pain, etc. (Chen et al., 2001; Tiippana et al., 2007; Vranken et al., 2008). Pathological nociception associated with central sensitization, i.e. mechanical and cold allodynia in neuropathic pain (Field et al., 1999; Wallin et al., 2002; Gustafsson and Sandin, 2009), and noxious mechanically and thermally evoked hyperalgesia (Takeuchi et al., 2007; Yokoyama et al., 2007), has also been proven to be apparently prevented by PGB treatment. Interestingly, the threshold of pain/nociception and pain behaviour, i.e. noxious heat elicited tail-flick reflex and paw flinching behavior, were failed to be influenced by PGB and gabapentin during physiological state as well as short-lasting nociception (Field et al., 1997; Hunter et al., 1997; Lynch et al., 2006; Curros-Criado and Herrero, 2007; Takeuchi et al., 2007). Likewise, intrathecal and systemic administration of PGB apparently resulted in a significant depression of the noxious flinching responses observed only in the second (late) phase, but not the first (early) phase, of formalin test (Field et al., 1997; Gustafsson and Sandin, 2009). These suggested that PGB might play differential antinociceptive roles in acute and tonic pain models. Recently, our behavioral experiment performed in conscious rats initially revealed that descending inhibitory controls were not tonic in physiological nociception, but rather be initiated by peripheral noxious information carried by C-fibers, not A-δ fibers, during pathological state, i.e. muscle pain (our unpublished observations). From this, we are not surprised by the finding that PGB only showed the antinociceptive effects on peripheral C, but not A-δ, fibers mediated spinal NS neuronal nociception in the current study.

In the present study, the effects of the blood-spinal cord barrier and blood–brain barrier for small molecules, PGB after the i.v. administration could result in PGB reaching the spinal DH area as well as the supraspinal structures, and thus affecting the activities of DH NS neuron. Due to influence of absorption, distribution, metabolism, and excretion (ADME) on pharmaceutical compound, the slow onset performance of PGB on the spinal DH NS neuronal responses are considerable to be accepted. However, these antinociceptive effects of PGB vanished

**Fig. 4.** Effects of 20 mg/kg of PGB on electrically-evoked wind-up phenomenon of the spinal DH NS neuronal responses. Sixty minutes and 90 min after the PGB treatment, no wind-up of spinal DH NS neuron activities was observed. *P<0.05 and **P<0.001 as compared with the untreated control (n=8 in untreated control group; n=7 in PGB treatment group).

**Fig. 5.** Effects of PGB treatment (80 mg/kg) on the electrically-evoked spinal NS neuron responses during the intact and spinalized conditions. *P<0.05 as compared with the untreated control in intact rats. *P<0.05 and **P<0.001 as compared with PGB treatment in intact rats (n=9 in each group).
that PGB might not effectively govern intractable pain in patients with special central lesion disease such as the spinal cord injury due to its supraspinal-dependent manner. However, PGB may prompt us one promising hope to pre-emptively prevent and treat operative pain such as postoperative pain (Hill et al., 2001; Ho et al., 2006; Jokela et al., 2008b). Further testing in different animal models for detailed documentation would be necessary and useful to substantiate this notion.

CONCLUSION

In summary, during normal physiological state and BV-induced inflammation we have demonstrated that PGB selectively suppressed C-fiber, but not A-δ fiber, mediated spinal NS neuronal late responses including central neuronal plastic changes, i.e. wind-up and after-discharges. However, the depressive effects of PGB on nociception vanished after the spinalization, indicating that supraspinal organization is involved in the PGB mediated antinociceptive effects. It is further suggested that PGB has efficacy of pre-emptive analgesia and important clinical implications to the management of persistent pain and follow-up central manifestations such as hyperalgesia.

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Fig. 6. Effects of pre-treatment and post-treatment with 80 mg/kg of PGB on mechanical and heat evoked spinal NS neuronal responses during the BV-induced inflammation. * P<0.05 and ** P<0.001 as compared with the untreated control group. * P<0.05 and ** P<0.001 as compared with saline treatment.

Potential antinociceptive mechanisms of PGB and its importance in clinical usage

Despite gabapentin and PGB are documented structurally related to GABA, it has been, however, reported that neither GABAA/β receptors nor GABA metabolism can be bound and interacted by gabapentin and PGB during the treatment of epilepsy and pathological pain (Bauer et al., 2009). Also unlike other anticonvulsants, PGB and gabapentin seem not to alter the activating level of sodium channels (Rogawski and Bazil, 2008). Hence, the molecular mechanisms of PGB and gabapentin on nociception are not well revealed although involvement of specific subtypes of glutamate receptors, i.e. protein kinase C-dependent NMDA receptors, and voltage-dependent calcium channel such as αδ subtype have been reported (Fehrenbacher et al., 2003; Cunningham et al., 2004; Field et al., 2006; Bauer et al., 2009; Gee et al., 1996). So far, studies concerning effects of PGB and gabapentin on wind-up (central temporal summation) are few, and the conclusion is controversially debated. Gabapentin has almost no effect on wind-up in vitro preparations (Patel et al., 2001; Villetti et al., 2003). Our data exhibited the pronounced antinociceptive effects of PGB on wind-up, which are in line with others showing that wind-up of withdrawal reflex and “wind-up like pain” in humans can significantly be depressed by gabapentin (Curros-Criado and Herrero, 2007; Harding et al., 2005).

Although the slowly occurred antinociceptive action of PGB was found in our study, synergic effects of PGB apparently reinforcing the antinociceptive action of other conventional analgesics has been reported (Jokela et al., 2008a). The present study also provides new insight in the role of PGB on pathological inflammation. We speculate during the absence of descending controls. Therefore, the current data suggest that the spinal cord is probably not the main targeting site of PGB. These findings are consistent with previous reports (Takeuchi et al., 2007; Yokoyama et al., 2007), clearly indicating a supraspinal-associated efficacy of PGB upon nociception.


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