Abstract

Under intact and spinalized conditions, we compared the responses of deep spinal dorsal horn (DH) nociceptive-specific (NS) and wide-dynamic range (WDR) neurons to subcutaneous bee venom (BV, 0.2 mg/50 μl)-induced persistent nociception. In contrast to the monophasic, long-lasting (34–81 min) WDR neuron responses in both intact and spinalized conditions, BV in NS neurons elicited short-term (<10 min) firing in intact, and long-term (>1 h) biphasic firing in spinalized rats. The BV-induced long-term biphasic NS neuron activities in spinalized condition consisted of a first, early phase (4–13 min) of firing occurred immediately after the BV injection, and a second phase of tonic firing that lasted for 28–74 min. The two phases were separated by a period that lasted 4–11 min during which there was very little neuronal activity. The data suggest that in the presence of peripheral nociception, a transitory (about 5–13 min) spinal segmental inhibitory control and a long-lasting descending inhibitory control govern deep spinal NS neuron but not WDR neuron activity. Previous reports assessing spinally organized motor activities showed a spinal WDR neuron well-controlled monophasic long-lasting withdrawal reflex in response to BV injection in both intact and spinalized conditions [44–46]. In contrast, the current data suggest that unlike spinal WDR neurons, deep spinal DH NS neurons do not modulate spinal motor output during the persistent nociception. Using the neurokinin-1 (NK-1) receptor antagonist, L-703,606 we further found that only early (within 15 min) treatment with L-703,606 produced a significant inhibition of the enhanced mechanically evoked NS neuron responses in BV-induced nociception, suggesting a dynamic function of NK-1 receptor involvement for deep spinal NS neuron mediated central sensitisation. We conclude that deep spinal DH NS neurons are strictly governed by tonic inhibitory descending controls. As this descending inhibitory control either is absent or decays, deep spinal NS neurons may play a crucial role in the development of central sensitisation in pathological nociception, for instance in spinal cord injury-induced pathological pain.

Keywords: Bee venom; Descending control; Nociceptive-specific neurons; Wide-dynamic range neurons; Neurokinin; Nociception

1. Introduction

Electrophysiological studies of dorsal horn (DH) spinal cord nociceptive signaling have uncovered the important role of wide-dynamic range (WDR) neurons. Spinal WDR neurons receive and upon them converge the peripheral, both noxious and innocuous afferent inputs that are conducted by myelinated A-δ, unmyelinated C, as well as myelinated A-β fibers. However, the role of ‘sterling’, nociceptive-specific (NS) neurons in different physiological and pathological conditions is a matter of considerable debate [1,7,11,23,30,40]. Of particular importance, the spinal NS neuron is only responsive to noxious input from myelinated A-δ and unmyelinated C afferents and constitutes a primary candidate for processing nociceptive information from the periphery [4,28]. Thus, in spite of the difficulties encountered with long-lasting recordings of spinal NS neuron activity it is important to explore the precise role of spinal NS neuron in nociception, particularly in conditions of central sensitization.

It is difficult to study the dynamic descending control modulation of the plastic modifications of spinal nociceptive neurons...
as peripheral constant noxious mechanical and/or heat stimuli may cause definite damage to the cutaneous receptive field (cRF) of the recorded spinal neuron. The central modulatory mechanisms by which either local intrinsic spinal circuits or supraspinal regions modulate the excitability of spinal DH neurons thus remain to be determined. It is important to determine the differences in functional patterns between spinal segmental and supraspinal inhibitions of DH WDR neuron and NS neuron activities. Employing behavioural and electrophysiological approaches, it has recently been found that subcutaneous (s.c.) injection of bee venom (BV) into the rat hind paw may lastingly engage peripheral nociceptors, resulting in long-lasting activation of spinal DH WDR neurons and spinally organized withdrawal reflexes that uniquely demonstrate a monophasic response that is indicative of an ongoing, stable noxious input from the periphery [24,42–46]. Thus, BV was used here as an exploring tool to generate ongoing noxious afferent input so as to reveal the dynamic roles of local spinal segmental and supraspinal descending mechanisms in nociception. Deep spinal DH nociceptive neurons (i.e., WDR neurons) are likely to be importantly involved in temporal summation (wind-up), central sensitization, and the nociceptive withdrawal reflex during physiological and pathological nociception [16,44]. Accordingly, in the present study we extracellularly recorded and compared deep spinal WDR neuron and NS neuron activities in response to BV in intact and spinal conditions. We demonstrate that spinal deep NS, but not WDR, neurons are in a dynamic functional state which is strictly inhibited by supraspinal regions. In view of the different firing patterns which BV induces in them in intact and spinalized conditions, deep spinal NS neurons do not seem to modulate motor output (i.e., the withdrawal reflex). Our results involving the NK-1 receptor antagonist, L-703,606 further suggest that within 15 min the pro-nociceptive role of deep spinal DH NS neurons may change from a relatively static condition to a dynamic intractable state.

2. Materials and methods

2.1. Animal preparation

Male age-matched Wistar rats weighing 260–320 g were obtained from the Animal Facilities of Aalborg Hospital, and housed pairwise under a 12:12 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 intraperitoneal administration. During the surgical procedures, a tracheal cannula and a left jugular vein catheter were inserted in order to ensure adequate breathing and fluid circulation. The right carotid artery was monitored by means of a homoeothermic circulating-water blanket (3–5 ml). To ensure the stability of DH neuron recordings, movement of the animal's head was reduced by tightly suspending the rat in the rigid stereotaxic frame. Oxygen (3–5 mmHg) by adjusting ventilation rate (60–80 breaths/min) and tidal volume (3–5 ml) was provided to maintain the stability of DH neuron recordings. Ventilation was monitored continuously; the end-tidal CO2 was monitored by a CO2 monitor (Normocap, Datex, Finland) and controlled within the normal range (25–35 mmHg) by adjusting ventilation rate (60–80 breaths/min) and tidal volume (3–5 ml). To ensure the stability of DH neuron recordings, movement of the spinal cord was minimized by tightly suspending the rat in the rigid stereotaxic frame by means of spinal clamps on vertebral segments T12 and L3. The dura mater and arachnoid membrane of the spinal cord were carefully removed under a dissection microscope (Leica MS 5, Germany). After that, a paraffin pool was made using ambient skin flaps around the exposed incision area of the lumbar spinal cord and filled with warm (37°C) paraffin oil to prevent drying. Rats were used only once and sacrificed at the end of the experiment by overexposure to CO2.

2.2. Electrophysiological recordings

Extracellular recordings were made in intact and spinalized conditions. In experiments involving spinalization, recordings began no earlier than 2 h after spinal surgical spinalization to allow recovery from acute spinal shock [35]. Electrophysiological responses of single spinal DH neurons with the cutaneous 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 2 μm/s by a manual hydraulic micro drive while the ipsilateral hind paw was touched by innocuous stimuli such as a gentle touch by a cotton club in order to active a neuron that had no background activity. One pair of stimulation electrodes consisted of two stainless-steel needles with a diameter of 0.15 mm were subcutaneously inserted into the plantar of the hind paw. Sometimes, an electrical stimulus with low intensity (intensity: 0.5 mA; pulse duration: 0.5 ms; frequency: 0.3–0.5 Hz) applied to the hind paw was employed to find a high-threshold spinal NS neuron. All such neurons were found throughout the deep DH field of the spinal cord at depths of 550–1100 μm. The depth of the neuron was taken from the mean micro drive readings, both on descent and during withdrawal to the spinal cord surface [32]. Each neuron was characterised by its responses to 10 s natural stimuli (brush, pressure, and pinch) before the experiment [6,18]. In addition, we used six individual von Frey monofilaments (Stoelting Co., IL, USA) with bending forces at 0.1, 1, 10, 20, 40, 75 g for the graded quantitative stimulation. The von Frey filaments were applied for 6 s to the most sensitive area of cRF. These stimulus intensities were tested on the dorsum of the operator’s hand for 6 s to mimic the situation in the rats; 75 g von Frey filament generated a strongly painful sensation. In order to reasonably compare the current data with the data obtained in the studies of spinal withdrawal reflex [43–45], we avoided the use of any muscle relaxants during the recordings of DH neurons. Neurons responding to joint movement and muscle contraction were excluded in the current study. In addition, and following earlier recommendations [42], the recorded neurons showing habituation or strong spontaneous background firing (>5 spikes/s) were not used for the remainder of the study. The cRF area was marked and measured using a marking pen and transparent plastic millimeter paper.

Spinal DH neuronal signals were amplified by a microelectrode amplifier (MEZ-8301, Nikon 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., UK).

2.3. Experimental design

2.3.1. Experiment 1: responses of spinal WDR and NS neurons in the BV-induced persistent nociception in intact and spinal rats

Characteristics of spinal WDR neuron and NS neurons were first identified during the intact and spinal conditions, respectively. After that, 50 μl of BV (0.2 mg hyaluronidase whole venom of apis mellifera (Sigma) dissolved in 0.9% saline) were subcutaneously injected into the most sensitive area of the WDR
and NS neuron RFs as established in the intact and spinal conditions. The effects of BV on the activity of different neurons were continuously recorded for 1 h. A volume of 50 µl 0.9% saline was also injected s.c. into the most sensitive area of cRF of neurons in order to establish the control group for the BV injection.

2.3.2. Experiment 2: roles of spinal NK-1 receptor in responses of spinal WDR and NS neurons to noxious mechanical stimulation in different conditions

For the investigation of substance P-related central mechanisms underlying the responses of spinal WDR neurons and NS neurons under the different conditions, the effect of the non-peptide NK-1 receptor antagonist cis-2-(diphenylmethyl)-N-[(2-iodophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine oxalate salt (L-703,606; 20–80 nmol dissolved by 50 µl 0.9% saline; Sigma–Aldrich Chemie Gmbh, Germany) was investigated by intrathecal (i.t.) administration under the different conditions of intact, spinal and the BV-induced inflammation. A cumulative dosing paradigm was used for L-703,606 (20, 40, 80 nmol) in order to investigate the possible dose-dependent effect during the intact and spinal conditions. Between the tests with different doses, the remaining drug solution on the spinal cord was carefully withdrawn using filter paper, and 1 h interval was arranged in order to maximally reduce the remaining drug effect. Under the BV-induced inflammation, only the effects of L-703,606 with the maximum dose were investigated; 0.9% saline was used as control in other animals. Each rat only received one single drug or vehicle (0.9% saline) treatment in the 1-day experiment.

For i.t. application, a 50-µl aliquot of the tested drug was administered directly onto the dorsal surface of the spinal cord after careful removal of the covering cerebrospinal fluid and paraffin oil by using filter paper. This spinal administration procedure was performed manually with a 50-µl Hamilton syringe (Hamilton Co., Reno, NV) over a period of 1–2 min.

2.4. Data analysis

For pharmacological investigations in intact and spinal rats, a neuronal response to a 6 s von Frey filament (75 g) stimulation was considered stable if less than ±10% variation was observed from the mean of the evoked 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 treatments and all of the drug effects including vehicle treatment collected per 10 min bins during the following 30 min, were expressed as a percentage of the pre-drug baseline responses (% of baseline).

During the BV-induced inflammation in the intact condition, first, the spinal NS neuron activity in responses to a 6 s von Frey filament (75 g) stimuli was tested three times in 30 min before the s.c. injection of BV, and the mean of these three responses was considered as 100%. During a period of 10 min following the BV injection, the responses of recorded NS neurons to the same stimuli were tested for 90 min. All of the data that where so obtained were calculated and expressed as a percentage of the 100% control.

Statistical analysis was performed using one-way/two-way ANOVA with post hoc Tukey’s tests, P<0.05 was considered statistically significant. Data are shown as mean ± S.E.M.

3. Results

We recorded 40 DH WDR and 67 NS neurons in the deep DH area of the spinal cord in 70 intact and 37 spinalized rats. The depth of the recorded WDR and NS neurons averaged 876 ± 29(550–1095) and 841 ± 22(603–1100) µm, respectively; the neurons were mainly (77%) located in laminae V.

3.1. Characteristics of deep spinal DH WDR (n = 40) and NS neurons (n = 67) in intact and spinalized rats

All of the recorded WDR neurons were excited by peripheral innocuous (brush and pressure), noxious (pinch) mechanical, and electrical stimulation (intensity: 10 mA; pulse duration: 1 ms). WDR neurons showed a weak response to innocuous pressure but responded markedly to noxious pinch applied to their cRFs. The mean cRFs of the WDR neurons were 55.7 ± 4.3 mm² (n = 22) and 60.8 ± 5.9 mm² (n = 18) in intact and spinalized rats, respectively. The identified cRFs were mainly located on the plantar surface of the hind paw, but partly also on the toes (Fig. 1).

We found an apparently high-sensitivity area to innocuous and noxious stimuli within the cRF area, which was surrounded by a low-sensitivity area that responded only to noxious stimuli. In accordance with different conduction latencies, two different components termed the early (5.8 ± 0.4 ms; n = 40) and the late (130.6 ± 7.6 ms; n = 40) responses, could be elicited by 1 Hz electrical stimulation (intensity: 10 mA; pulse duration: 1 ms).

Wind-up and after-discharge of the WDR neurons were induced by a 15 s, 1 Hz repeated electrical stimulation. Most recorded

![Fig. 1. Raw recordings of characteristics of spinal DH nociceptive neurons; WDR (panel a) and NS neuron (panel b) responses to 10 s mechanical stimulations (i.e., Br: brush; Pr: pressure; Pi: pinch) applied to the cutaneous receptive fields (cRFs). In contrast to the WDR neuron, the recorded NS neuron exhibited a smaller cRF with a clear borderline. Responses of the WDR and NS neurons to suprathreshold electrical stimulation are shown in insets (1) and (2), respectively (st: stimulus artefact; intensity: 10 mA; pulse duration: 1 ms). Peri-stimulus time histograms (PSTHs) representing the number of neuronal firings are also shown.](image-url)
WDR neurons showed weak spontaneous activity (<2 spikes/s). During the presence and absence of descending modulating system, there were no significant differences between intact and spinalized preparations in terms of cRFs, spontaneous activity, and mechanical threshold. Typical raw recordings of spinal WDR neuron to graded mechanical stimuli and electrical stimuli are shown in Fig. 1a.

In contrast to WDR neurons, NS neurons only responded to noxious mechanical stimulation. All of the recorded NS neurons were only excited by von Frey filament stimuli with bending force 40 and 75 g which in humans can elicit moderate and strong painful sensations, respectively. In neither intact nor spinalized animals were any spontaneous responses found in these NS neurons. Compared to WDR neurons, NS neurons exhibited a smaller cRF with a clear borderline in the intact condition (8.7 ± 0.5 mm²; n = 48). However, a significantly larger cRF of the NS neurons was found after the spinalization (19.1 ± 2.6 mm², P < 0.001, n = 19). Upon spinalization, NS neurons still showed only responses to noxious von Frey filament stimulation (bending forces: 40 and 75 g). Similar to WDR neurons, NS neurons also exhibited two different response components to supra-threshold electrical stimulation: i.e., early (8.3 ± 0.4 ms) and late (153.2 ± 3.8 ms). In 50 out of 67 NS neurons from either intact or spinalized animals, wind-up was elicited by 15 s repeated (1 Hz) electrical stimulation. Recordings from a typical NS neuron are shown in Fig. 1b.

3.2. Responses of DH WDR (n = 22) and NS neurons (n = 24) to s.c. BV injection

To investigate whether deep spinal DH WDR neurons and NS neurons are differently affected by descending modulating systems, recordings were made after s.c. BV injection (0.2 mg/50 μl) into the high-sensitivity area of the neurons’ cRF located on the hind paw. In Figs. 2 and 3, peri-stimulus time histograms show differential effects of s.c. administration of BV on the spontaneous responses of the deep DH WDR neuron and NS neuron in intact and spinalized conditions, respectively.

In WDR neurons, BV injection typically produced a rapid-onset, long-lasting (34–65 min) and monophasic increased firing compared with the sparse background activities prior to the injection of BV in intact rats. In spinal rats, deep WDR neurons also exhibited a long-lasting (47–81 min) monophasic increased firing in response to the BV injection although the BV-induced neuronal firings in spinalized rats were stronger than that of in intact rats (P < 0.05; two-way ANOVA; Fig. 4a).

In intact rats and compared to WDR neurons, NS neurons exhibited a very short (<10 min; average 5.6 ± 1.3 min) monophasic increased firing in response to BV. Most remarkably, upon spinalization, BV with NS neurons caused a long-lasting and biphasic increased firing. The BV-elicited NS neuronal response in spinal rats consisted of a first, early phase of firing (18.9 ± 5.7 spikes/s), which occurred immediately after the BV injection and lasted 4–13 min (9.5 ± 1.6 min); this was consistently followed by a second phase of a prolonged, tonic firing (13.8 ± 1.5 spikes/s) that lasted for 28–74 min (50.6 ± 8.6 min). The two phases were separated by a period
that lasted 4–11 min (6.6 ± 0.8 min) during which there was very little activity. Significant differences were found in neuronal firing of NS neurons 10 min after the BV injection in intact and spinalized conditions (P < 0.001; two-way ANOVA) (Fig. 4b).

In eight rats (four rats per group), we investigated the effects of s.c. injection of the BV vehicle (0.9% saline) on activities of WDR and NS neurons in the intact condition. Saline induced only sparse neuronal responses in both WDR and NS neurons (not shown).

3.3. Effects of the NK-1 receptor antagonist, L-703,606 on the mechanically evoked responses of WDR (n = 28) and NS neurons (n = 30) in intact and spinalized rats

As the NK-1 receptor and its native ligand, substance P (SP) are involved in the spinal processing of nociceptive signals [31], we examined the effects of intrathecal (i.t.) application of the NK-1 receptor antagonist, L-703,606 (20–80 nmol) on the neuronal response to a 6 s von Frey filament (75 g) stimulation in intact and spinalized rats. L-703,606 inhibited the WDR neuron response in a dose-dependent manner in both intact and spinalized rats (Fig. 5a). L-703,606 likewise depressed the noxious von Frey filament-evoked NS neuron activity in intact rats (Fig. 5b). In marked contrast, L-703,606 exerted no effect whatsoever on NS neuron responses to noxious stimulation in spinalized animals (P > 0.05, two-way ANOVA; Fig. 5b).

3.4. Effects of the NK-1 receptor antagonist, L-703,606 on the mechanically evoked NS neuron (n = 25) response in the presence of BV-induced nociception in intact rats

The differential effect of the NK-1 receptor antagonist on NS neuron firing in intact and spinalized preparations led us to explore whether the function of those ‘silent’ NS neurons is in either a static or dynamic state during persistent nociception.
after the BV injection.* Frey filament stimulation only when administered 15 min, but not 30 and 60 min, after the s.c. injection of BV, the NS neuron response to von Frey filament stimulation was significantly inhibited by L-703,606 when administered 15 min after the s.c. BV injection. The NS neuron response to von Frey filament stimulation was not significantly inhibited by L-703,606 when administered 30 or 60 min after the s.c. BV injection. The effect of L-703,606 on the NS neuron response to von Frey filament stimulation persisted, and the effect was significantly greater than the control treatment (data represent the mean ± S.E.M.).

When administered 15 min after the s.c. injection of BV, the NS neuron response to von Frey filament stimulation was significantly inhibited by L-703,606. The NS neuron response to von Frey filament stimulation was not significantly inhibited by L-703,606 when administered 30 or 60 min after the s.c. BV injection. The effect of L-703,606 on the NS neuron response to von Frey filament stimulation persisted, and the effect was significantly greater than the control treatment (data represent the mean ± S.E.M.).

When administered 15 min after the s.c. injection of BV, the NS neuron response to von Frey filament stimulation was significantly inhibited by L-703,606. The NS neuron response to von Frey filament stimulation was not significantly inhibited by L-703,606 when administered 30 or 60 min after the s.c. BV injection. The effect of L-703,606 on the NS neuron response to von Frey filament stimulation persisted, and the effect was significantly greater than the control treatment (data represent the mean ± S.E.M.).

4. Discussion

For the first time, the present in vivo electrophysiological study conducted in intact and spinal rats demonstrates that, unlike that of spinal DH WDR neurons, the activity of deep DH NS neurons is strictly controlled by descending modulatory systems. The results provide evidence of a transitory (about 10 min) involvement of spinal segmental inhibitory control on deep DH spinal NS neuron activity during persistent nociception. Upon spinalization and in the presence of BV-induced nociception, NS neuron activity is enhanced and modulated in part by spinal NK-1 receptors. The data strongly suggest an important role for spinal NS neurons in pathological nociception where central sensitization develops.

The different response characteristics of DH NS and WDR neurons as found in the present study are compatible with previous findings showing NS neurons to have a high threshold and a small, restricted cRF while WDR neurons demonstrate a lower threshold, more graded responses, and a larger, concentric cRF [1,7,23,30]. Unlike WDR neurons, no spontaneous activity was found in deep NS neurons in the present study in neither intact nor spinal animals; it would be of interest for further work to determine whether some intrinsic spinal or supraspinal controls may account for this absence of spontaneous activity of deep NS neurons compared with those superficial NS neurons with low frequency spontaneous firing rate [7–9,23]. It has also been reported that both descending excitatory and inhibitory systems may simultaneously control the WDR neuronal activities at the spinal cord level [13,33]. Thus, we may not be surprised by the fact in the current study that no significant difference was found in WDR neurons with respect to cRFs, spontaneous activity, and mechanical threshold in intact and spinalized conditions.

Unlike the recognized, crucial role of spinal WDR neurons in nociception, NS neuron-related nociception and analgesia, particularly in sensitised conditions (e.g., allodynia, hyperalgesia, and central sensitization) has been much debated [31,39]. The detailed mechanisms underlying spinal NS neuron-mediated nociception are as yet unresolved [7]. Furthermore, the precise role of descending control from supraspinal regions, on spinal NS neuron-involved nociceptive signalling in pathological, persistent pain states, remains to be established due to the limited availability of animal models [2,17,20]. The BV model provides an opportunity to undertake studies of the role of descending control under conditions of long-term and, in particular, monophasic peripheral nociceptive input in behavioural and electrophysiological studies [24,42,45]. The firing pattern of deep WDR neuron to the BV injection does not differ between the intact and spinal conditions. This may lead one to expect a similar longer monophasic BV-induced NS neuronal firing in spinalization while only the short-term NS neuronal firings were found in intact condition. The follow-up data, however, reveal a surprising difference in the deep DH NS neuron response to BV between intact and spinalized preparations; while BV in the intact condition elicits an initially marked but short-lived firing, in spinalized preparations, BV causes a biphasic and long-lasting (about 1 h) neuronal response. Interestingly, the temporal characteristics of this biphasic response are not unlike those found with electrophysiological [9,42,45] and behavioural responses [12] to a formalin challenge. The difference of spinal NS neuron firing between the intact and spinalized preparations indicate the presence of a robust long-term descending inhibitory control on deep NS neuronal activity in the intact condition compared with that of on deep WDR neuron. The both spinal segmental and supraspinal inhibitory controls on DH WDR neuron activity and spinally organized withdrawal reflex are thought to be active tonically [10,14,29], and the immediate firing rate response to BV in WDR and NS neurons is virtually identical (Fig. 4). One might thus expect that the intrinsic spinal segmental inhibition may also exert a long-term inhibitory control on the excitability of NS neurons. However, the decaying response of NS neurons lasted no longer than 10–13 min in spinalized rats (Figs. 3 and 4) and within the 10 min period following the BV injection, both the time course and intensity of NS neuron
firing were similar in the intact and spinalized conditions. The ‘formalin-like’, biphasic NS neuron response to the BV injection in spinal preparations suggest not only that descending controls pathways strictly immure the excitability over time of DH NS neurons, but also that the biphasic temporal shape of the NS neuron response is under spinal segmental control.

The present findings also suggest that electrophysiological responses of DH WDR neurons correlate with spinal withdrawal reflexes in response to BV injection; in intact and, also, spinalized preparations, BV induces monophasic, long-lasting (~1 h) spinal withdrawal reflex activity as assessed by single motor unit (SMU) recording from corresponding muscle [43, 45, 46]. Also, using a simultaneous recording technique followed by cross-correlation analysis, a previous study indicated that the DH WDR neuron well controls the spinal withdrawal reflex recorded from the corresponding gastrocnemius muscle [44]. In contrast, the present findings are consistent with the notion that the DH NS neuron is not involved in the control of spinally organized withdrawal reflexes [30, 44, 46].

Spinal NK-1 receptors are thought to be involved in plastic changes of pain systems that occur short-term (wind-up/temporal summation) and long-term (long-term potentiation and central sensitisation) [3, 5, 15, 21, 22, 25–27, 36–38]. The present study provides new insight in the role of NK-1 receptors in spinal NS neuron-associated nociception. In contrast to its inhibitory effects on the mechanically evoked DH WDR neuron responses, the NK-1 receptor antagonist, L-703,606 at different doses exerted no detectable inhibitory effects on the DH NS neuron activities in spinal rats whereas it did so in the intact condition. It has been suggested that the actions of spinal NK-1 receptors on NS neurons are strictly supervised by supraspinal regions via descending inhibitory control systems [21, 22]. A further question addressed here is whether any intrinsic factors would be involved in a dynamic manner in spinal NK-1 receptor-modulation of DH NS neurons in the intact condition. Thus, as it is widely accepted that descending control systems are importantly involved in central sensitisation, the present study further examined the function of NS neurons in the presence of ongoing descending inhibitory control. In the presence of BV-induced persistent inflammation in intact condition, only early (within 15 min) treatment with the NK-1 receptor antagonist produced a significant inhibition of the enhanced mechanically evoked NS neuron responses, clearly suggesting a specific time-dependent involvement of spinal NK-1 receptors in deep spinal DH NS neuron-mediated central sensitisation [34]. This may be compatible with previous studies indicating that spinal NS neurons are not functional under the static state, but rather in the dynamic state under the condition of dynamic control of descending increased facilitation and declined inhibition [1, 41].

In conclusion, the present experiment employing BV in intact and spinal rats, for the first time, show that spinal NS neurons responded to BV in a manner significantly different from WDR neurons; shorter (1.5–8 min) monophasic firings in the intact condition and biphasic, long-lasting (>60 min) responses in spinalized preparations. This indicates that a powerful descending inhibitory mechanism strictly controls the activation of spinal NS neurons. The crucial role of spinal NS neurons can not be ruled out from the abnormal nociception during the absence of the descending control, such as damage of ascending and descending pathway. It is suggested that NK-1 receptor-modulated deep spinal NS neuron activity may play an important role in persistent, chronic pain arising from central sensitization, particularly perhaps after spinal cord injury.

Conflict of interest

None.

Acknowledgments

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