Spinal processing of bee venom-induced pain and hyperalgesia

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Abstract: Subcutaneous injection of bee venom causes long-term neural activation and hypersensitization in the dorsal horn of the spinal cord, which contributes to the development and maintenance of various pain-related behaviors. The unique behavioral ‘phenotypes’ of nociception and hypersensitivity identified in the rodent bee venom test are believed to reflect a complex pathological state of inflammatory pain and might be appropriate to the study of phenotype-based mechanisms of pain and hyperalgesia. In this review, the spinal processing of the bee venom-induced different ‘phenotypes’ of pain and hyperalgesia will be described. The accumulative electrophysiological, pharmacological, and behavioral data strongly suggest that different ‘phenotypes’ of pain and hyperalgesia are mediated by different spinal signaling pathways. Unraveling the phenotype-based mechanisms of pain might be useful in development of novel therapeutic drugs against complex clinic pathological pain.

Key words: spinal cord dorsal horn; signaling pathway; persistent spontaneous pain; hyperalgesia; allodynia; bee venom model

1 Introduction

Peripheral tissue or nerve injury can induce persistent spontaneous pain and hyperalgesia (e.g., hypersensitivity or enhanced pain to normally noxious stimuli) or allodynia (e.g., pain evoked by normally non-painful stimuli)\(^{(1,2)}\). In the past thirty years, it has been well established that activity-dependent central or spinal neural plasticity or sensitization contributes to the generation of the long-term state of persistent pain and hypersensitivity\(^{(3-7)}\). For the neural processing of peripheral nociceptive information, dorsal horn of the spinal cord has been believed to be a gate monitoring and integrating different sources of information from primary afferent input, segmental interaction and supraspinally-derived descending modulation, as well as a relay station sending the integrated nociceptive information, on one hand, to the higher brain structures producing multidimensional experience of pain stimulation including sensory-discriminative, affective-motivational and cognitive-evaluative functions of the brain, while, on the other hand, to the spinally-organized nociceptive reflex motor output and effectors producing nocifensive escape or withdrawal of the injured limbs\(^{(8-11)}\). Unlike the physiological pain state, it has been gradually known that a variety of signaling pathways in the spinal cord dorsal horn are involved in the induction and maintenance of pathological pain state in terms of activation, modulation and modification of the nervous system following peripheral tissue or nerve injury\(^{(5-7)}\). Therefore, understanding about the spinal processing of nociceptive information will be of particular importance for the novel strategies of pain treatment because spinal block can distinctly eliminate pain in the clinical practice\(^{(12)}\).

However, despite the knowledge about the involvement of a variety of spinal signaling pathways in different processing (activation, modulation and modification) of the nervous system plasticity, so far little is known about the specific roles of spinal signaling pathways in the induction and maintenance of different ‘phenotypes’ of pain observed in behavioral animal models. In fact, similar to the clinical symptoms and signs of different types of pain, genetic studies successfully identify at least five phenotypic clusters of nociception and hypersensitivity by using 12 strains (14 sub-strains) of inbred mice and 22 conventional pain measurements and animal models with various etiology (tissue injury, inflammation or nerve injury), stimulus modalities (thermal, mechanical or chemical), onset and time course (acute, persistent or chronic), and injury site (topical, intradermal, subcutaneous, visceral or deep tissue)\(^{(13-17)}\).
These include: (1) type 1, baseline thermal nociception; (2) type 2, spontaneous responses to noxious chemical stimuli; (3) type 3, thermal hypersensitivity; (4) type 4, mechanical hypersensitivity; and (5) type 5, afferent input-dependent hypersensitivity\[13-17\]. Among the five phenotypic clusters, types 2-5 are relevant to pathological pain state, while type 1 is related to physiological pain state. The above genetic results highly support the idea that different phenotypic clusters of pain (nociception and hypersensitivity) might be mediated by different signaling pathways in the nervous system. However, to test this presumption, the following criteria for the animal models of pain should be requested: (1) to minimize the inter-individual and inter-model difference, only one species or strain of animal should be used; (2) the animal model can display utmost all of the above ‘phenotypes’ of pain (nociception and hypersensitivity) following a single insult treatment; (3) the animal model of pain must be acute in onset and tonic and protracted in time course so that the effects of a single drug on different types of pain can be evaluated on the same experimental day; (4) the behavioral ‘phenotypes’ of pain have distinct neuronal basis mediating different processing of pain information; (5) there is no distinct species difference in the animal model of pain.

In the past ten years, a novel experimental animal model of pain, the bee venom (BV) test, has been well established by using behavioral, electrophysiological and neurochemical assays, which permits measurement of all of the five types of nociception and hypersensitivity identified in the above genetic studies in an individual rodent\[13,18-23\]. Besides type 1 (baseline thermal nociception), the rodent shows four types of nociception and hypersensitivity relevant to pathological pain state following subcutaneous (s.c.) injection of BV solution into one hind paw, including: (1) immediate persistent spontaneous nociception (type 2); (2) primary thermal hypersensitivity (type 3) and (3) primary mechanical hypersensitivity (type 4) identified at the injection site; (4) secondary heat hypersensitivity at the site remote to the injured region on the injected paw (similar to type 5); (5) mirror-image heat hypersensitivity (type 5) at the non-injected paw\[13,18-20,23\]. The BV-induced immediate persistent spontaneous nociception is tonic in response and lasts for more than 1 h, while the following thermal or mechanical hypersensitivity is phasic and last for 3-4 d with the plateau hyperalgesic effect at 2-8 h after injection\[18,19,20,23\]. The BV-induced tonic nociception and hypersensitivity has been demonstrated to be mediated by capsaicin-sensitive primary afferent input and spinal neuronal plasticity\[18-20,23,25-29\].

Based upon the unique behavioral ‘phenotypes’ of nociception and hypersensitivity, the BV test is appropriate to the study of the roles of different signaling pathways in induction and maintenance of different ‘phenotypes’ of pain. In this review, only the roles of spinal signaling pathways in the development and maintenance of the BV-induced nociception and hypersensitivity are described and discussed.

2 Behavioral ‘phenotypes’ of nociception and hypersensitivity induced by s.c. BV injection

Honeybee sting-induced inflammatory pain and allergy is a clinical phenomenon well experienced by human beings. As a consequence, the local injured area is featured by typical inflammatory responses, including dolor (pain), rubor (redness), tumor (edema or swelling) and calor (heat) which is also accompanied by allergy shown as anaphylaxis with wheal and itch (desire to scratch). Intradermal injection of melittin, a major toxic polypeptide (occupying more than 50% of the whole dry venom, Apis mellifera) with a 26 amino acids sequence, into a human subject volar forearm experimentally results in generation of both primary and secondary thermal and mechanical hyperalgesia as well as persistent spontaneous pain\[30-33\]. In accord with the human subject study, our series of previous studies show that s.c. injection of both BV and melittin in the plantar surface of one hind paw of rats also produces persistent spontaneous pain-related behaviors such as paw flinching, licking, lifting and guarding, followed by a prolonged primary thermal and mechanical hypersensitivity identified at the injection site as well as a secondary or a mirror-image thermal hypersensitivity identified at the area remote to the injection site of the same side or contralateral to the injected paw\[18-20,23,25,26,28,34,35\]. Moreover, by comparing the effects and costs of six different venoms (honeybee venom and filtered honeybee venom, yellow jacket venom, paper wasp venom, bumble bee venom, yellow hornet and white faced hornet), it was found that the two types of honeybee venom are the most effective agents to induce persistent nociception and the costs are the least expensive (150-230 times lower in price, honeybee venom: other 5 venoms are 0.72:109-166 US$/mg, according to Sigma, MO, USA)\[23\]. This effect and cost comparison makes the BV model more practical in widespread use for experimental study of pathological pain mechanisms. Through investigation of the pharmacological effects of different polypeptides structurally identified from whole venom of
honeybee by using gel chromatography, reverse-phase high pressure liquid chromatography (RP-HPLC), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and amino acid sequence analysis, melittin is known to be the major chemical substance producing long-term biotoxic effect consisting of tonic nociception and hypersensitivity as described above\(^{26,28,36}\). The BV- or melittin-induced persistent nociception and hypersensitivity are also accompanied by a long-term inflammatory response such as increase in skin temperature, paw edema and local plasma extravasation\(^{21,22,37}\). Dose-response study shows that the BV- or melittin-induced number of paw flinches and the time spent in paw lifting and licking are dose-dependent, however, the extent of the heat and mechanical hypersensitivity has no dose-dependent characteristic\(^{21,28}\). Based on this dose-response result, it is found that 0.2 mg/50 \(\mu\)L of BV and half of the dose for melittin could be an optimal dose to induce the most intense tonic nociception in rats. Inter-species studies show no species difference between rodents and cats that can be seen in the formalin test\(^{20,27,38,39}\).

In summary, rats receiving single injection of s.c. BV can show multiple behavioral ‘phenotypes’ of pain relevant to clinical pathological pain state including persistent spontaneous nociception, primary heat hypersensitivity, primary mechanical hypersensitivity, and secondary or mirror-image heat hypersensitivity as well.

### 3 Spinal neuronal responses to s.c. BV injection

Wide-dynamic-range (WDR) neurons in the deep layers (IV-VI) of the spinal cord dorsal horn have been believed to be a ‘central encoder’ of spinal nociceptive withdrawal reflex circuitry\(^{40-43}\). To test whether the activity of the spinal cord dorsal horn is involved in development of BV- or melittin-induced persistent spontaneous nociception and hypersensitivity, electrophysiological recordings were made in both sensory (input) and motor (output) nerve cells along the spinal nociceptive withdrawal reflex circuitry. First, a novel setup of experimental design was obtained by simultaneously extracellular single unit recordings of a paired lumbar spinal dorsal horn WDR unit and single motor unit of the related muscle in the urethane-chloralose or spinal and halothane-anesthetized rats\(^{41}\).

Following a single injection of BV (0.2 mg/50 \(\mu\)L) into the common cutaneous receptive field of a dorsal horn WDR unit and a single motor unit, along the spinal nociceptive withdrawal reflex circuitry, a long-lasting parallel increase in persistent spontaneous spike discharges was observed in both the dorsal horn WDR unit and the single motor unit simultaneously recorded\(^{18,19}\). Moreover, following 1 h of the tonic increase in ongoing spike discharges following either BV or melittin injection, both the dorsal horn WDR neuron and single motor unit recorded became hypersensitive to radiant heat, mechanical von Frey or pressure and pinch stimuli as well as electrical stimulation\(^{18,19,27,28,44}\). Furthermore, the BV-induced long-term tonic firing of WRD neurons could be completely blocked by both peripheral sciatic nerve blockade and local administration of NMDA and non-NMDA receptor antagonists as well as propofol, suggesting a peripheral primary afferent input-dependence of the spinal dorsal horn WDR neuron activities\(^{27,44-47}\). Taken together, it is suggested that WDR neurons should be a major population of spinal dorsal horn cells persistently activated by both BV and melittin insults at the periphery\(^{18,19,45-47}\).

It is clearly noted that the response pattern and time course of the BV- or melittin-induced tonic spike discharges and thermally or mechanically hypersensitive state in the spinal dorsal horn WDR neuron and the relevant single motor unit are similar and in parallel with that of the BV- or melittin-induced persistent spontaneous behaviors of pain and hypersensitivity\(^{18-20,26-29,46}\). These electrophysiological results highly support that spinal neuronal changes contribute to the development of the BV-induced persistent nociception and hypersensitivity. Moreover, the behavioral ‘phenotypes’ of nociception and hypersensitivity induced in the BV test are separately mediated by different spinal neuronal changes in response to different stimulus modalities at the periphery such as chemically-induced persistent firing, thermally- or mechanically-evoked increase in responsiveness. This is the cellular basis of the nervous system underlying different ‘phenotypes’ of pain under pathological state.

To further investigate the spatial and temporal activities of the spinal cord dorsal horn, c-Fos protein immunostaining was conducted in urethane-anesthetized rats 0.5, 2, 4, 8, 24, 48, 72 and 96 h after s.c. BV injection\(^{49}\). The results show that c-Fos-like immunoreactive neurons are localized in both superficial and deep layers of the spinal dorsal horn following s.c. BV injection, however, there is no c-Fos expression in the ventral horn. Moreover, the time course of c-Fos expression in the deep layers of dorsal horn, in particular, is much longer and well consistent with that of the heat and mechanical hypersensitivity\(^{18,19,46}\), suggesting a pattern of network response of the dorsal horn neurons to peripheral pain stimulation produced by s.c.
injection of BV.

As a common feature of pain models, the BV-induced persistent nociception and hypersensitivity in both behavioral and electrophysiological assays are sensitive to morphine in a naloxone-reversible manner; however, non-steroidal anti-inflammatory drugs (NSAIDs) can only block the BV-induced persistent nociception without significant effect on the BV-induced hypersensitivity\(^\text{[5-7]}\), suggesting different neural basis should be involved between the chemically-induced nociception and physically-induced hypersensitivity following s.c. BV injection.

4 Processing of different ‘phenotypes’ of BV-induced pain by different spinal signaling pathways

As introduced above, it has been gradually known that a variety of signaling pathways in the spinal cord dorsal horn are involved in the induction and maintenance of pathological pain state in terms of activation, modulation and modification of the nervous system following peripheral tissue or nerve injury\(^\text{[11,12,49]}\). Among them, excitatory amino acids (EAAs) glutamate and neuropeptides such as substance P (SP) are thought to be the most likely candidates for nociceptive transmission because they are morphologically localized and synthesized in non-myelinated (C-) primary afferents or small myelinated (Aδ-) primary afferents\(^\text{[12]}\). It has also been believed that co-activation of glutamate NMDA and NK1 (SP) receptors in the spinal cord plays a key role in development and maintenance of central sensitization as well as hyperalgesia or allodynia\(^\text{[4,50]}\). Additionally, voltage-dependent calcium channel (VDCC) subtypes such as N-, L- and P/Q-subtypes are localized in the spinal cord as well as in the small DRG cells and are also believed to play a role in spinal processing of nociceptive transmission\(^\text{[51-55]}\). Activations of the spinal cellular membrane receptors and VDCC may lead to increase in intracellular calcium concentration and intracellular cascade activations which in turn enhance actions of the membrane receptors and ion channels\(^\text{[5-7,50]}\). To look insight into the underlying mechanisms of different types of pain, the roles of these membrane receptors/channels and intracellular cascade in development and maintenance of the BV-induced different ‘phenotypes’ of pathologically-relevant pain are well studied and established in our laboratory.

To see whether the spinal neurochemical signal transduction system is shared by the BV-induced different ‘phenotypes’ of pathologically-relevant pain, we tested the effects of i.t. pre- or post-treatment of glutamate NMDA and non-NMDA receptor antagonists (MK-801 and CNQX, respectively), metabotropic glutamate receptor (mGluR) subunits [AIDA for mGluR1, MPEP (6-methyl-2-(phenylethynyl)pyridine) for mGluR5, (S)-MAP4 hydrochloride ((S)-2-amino-2-methyl-4-phosphonobutyric acid hydrochloride) for mGluR4/6/7], NK1/2 and NK3 receptor antagonists (spantide and SR142801, respectively), blockers of VDCC L-, N- and P/Q-subtypes (verapamil, ω-conotoxin-GVIA and ω-agatoxin-IVA, respectively), selective and non-selective inhibitors of COX2 and COX1/2 (etodolac and indomethacin, respectively), inhibitor of NOS (L-NAME), potent or selective inhibitors of PKC (chelerythrine chloride), PKA (H-89) and cAMP (SQ22536), non-selective PKA/PKG inhibitor (HA1004), selective mitogen-activated protein kinases (MAPKs) inhibitors (U0126 for ERK1/2, SB239063 for p38 MAPK and SP600125 for c-Jun N-terminal kinase) on the induction and maintenance of the BV-induced persistent nociception, primary thermal and mechanical hyperalgesia (allodynia), and mirror-image thermal hyperalgesia. The major results are summarized as follows.

Glutamate NMDA and non-NMDA receptors: activation of both NMDA and non-NMDA receptors in the spinal cord is involved in the induction and maintenance of the BV-induced persistent nociception and secondary or mirror-image heat hypersensitivity; however, neither NMDA nor non-NMDA receptor is involved in the development or maintenance of the primary heat or mechanical hypersensitivity\(^\text{[18,19,23,25,34]}\).

mGluRs: activation of mGluR1/5 is involved in induction of both primary heat and mechanical hypersensitivity, as well as maintenance of mechanical hypersensitivity. While activation of both mGluR2/3 and mGluR4/6/7 is involved in maintenance of primary heat hypersensitivity\(^\text{[39]}\) (and Yan et al., unpublished data). The roles of mGluRs in generation
of the BV-induced persistent nociception have not been evaluated due to the limit of drug amounts.

NK1/2 and NK3 receptors: activation of NK1/2 receptors in the spinal cord is involved in induction of persistent nociception, primary as well as secondary heat hypersensitivity; however, it is not involved in the inducing process of primary mechanical hypersensitivity. Unexpectedly, NK1/2 receptors only play an action in the maintaining process of persistent nociception, while they are not likely to be involved in the long-term expression of either primary or secondary (and mirror-image) heat hypersensitivity and primary mechanical hypersensitivity. NK3 receptor is not involved in any types of the BV-induced pathological pain. The actions of highly selective antagonists to NK1 or NK2 receptors should be further studied in the BV model.

VDCC: on the basis of its subtype distribution in the spinal cord and DRG, only the actions of L-, N- and P/Q-subtypes were studied by i.t. pre- or post-treatment, respectively. N- and P/Q-subtypes seem to be co-activated by s.c. BV and are involved in both induction and maintenance of the persistent nociception, primary and secondary (and mirror-image) heat hyperalgesia; however, they seem not to play a role in either inducing or maintaining process of the primary mechanical hypersensitivity. In contrast, L-subtype of VDCC is activated in the maintaining process of the BV-induced primary and secondary (and mirror-image) heat hyperalgesia, while it is not activated in any other processes.

Nitric oxide (NO): release of NO in the spinal cord can be induced by s.c. BV; however, NO seems to be involved in both inducing and maintaining processes of primary mechanical hypersensitivity as well as persistent nociception. Moreover, NO seems to play an important role in maintenance, but not induction, of BV-induced primary and secondary (and mirror-image) heat hypersensitivity.

Cyclooxygenase (COX)-1/2: by intrathecal application of selective COX2 inhibitor, etodolac, or a non-selective COX1/2 inhibitor, indomethacin, it is found that activation of spinal COX2 is involved in either inducing or maintaining process of the BV-induced persistent nociception; however, it seems not to play any role in either process of the BV-induced heat or mechanical hypersensitivity. COX1 is found to be probably involved in either process of the persistent nociception or the maintaining process of primary heat and mechanical hypersensitivity induced by s.c. BV.

Intracellular cascades: intrathecal pre- or post-application of potent or selective inhibitors of PKC, PKA, cAMP, or a non-selective PKA/PKG inhibitor shows different results of drug actions. Generally, co-activation of both PKC and cAMP-dependent PKA pathways is involved in either inducing or maintaining process of the BV-induced persistent nociception as well as mirror-image heat hyperalgesia. However, for the BV-induced primary hyperalgesia, PKC is selectively activated in both inducing and maintaining processes of the heat hyperalgesia, while cAMP-dependent PKA pathway is selectively activated in either inducing or maintaining processing of the mechanical hyperalgesia.

MAPKs: spinal ERK1/2 and p38 MAPK are activated by s.c. BV and involved in both induction and maintenance of persistent nociception and heat hypersensitivity, but without any role in those processing of mechanical hypersensitivity.

In summary: (1) the BV-induced persistent nociception, a consequence of peripherally-dependent spinal neuronal hyperexcitable state, and the BV-induced mirror-image heat hypersensitivity, a consequence of central sensitization, are mainly induced and maintained by co-activation of spinal NMDA/non-NMDA-PKC/cAMP-PKA/MAPKs (ERK1/2 and p38 MAPK) pathways; (2) the BV-induced primary heat hypersensitivity is predominantly induced and maintained by VDCC (N- and P/Q-types)-PKC pathway, while in contrast the BV-induced primary mechanical hypersensitivity is predominantly induced and maintained by persistent release of NO and activation of mGluR1/5-cAMP-dependent PKA pathway; (3) spinal NK1/2 receptor is mainly involved in the inducing processing of the BV-induced persistent nociception, primary and secondary heat hyperalgesia, but is not involved in the development of primary mechanical hyperalgesia. These results implicate that the BV-induced multiple ‘phenotypes’ of pathologically-relevant pain and hypersensitivity are mediated by different spinal signaling pathways, suggesting that the drugs, regardless of west or east medicine, which are with ability to block multiple pharmacological targets in the spinal cord, are required to realize better control of multiple types of clinical pathological pain.

It is fascinating to note that experimental sting of the rat’s skin by a honeybee toxin can cause a series of wonderful pathophysiological processes of pain and inflammation which can mimic multiple manifestations of clinical pathological pain. It is worthy of noting that the BV-induced different ‘phenotypes’ of pain exert different transmembrane-intracellular signal transduction systems in the
spinal cord, and these biological features provide a possibility to dissociate different neurochemical signal transduction pathways that mediate different ‘phenotypes’ of pain. Getting a good understanding of the peripheral as well as spinal processing of pathological pain information would no doubt help to get understanding of the whole pathogenesis and controlling strategies of clinical pathological pain.

The purpose of this review is to set up a hypothesis that different ‘phenotypes’ of pain under pathological state might be mediated by different spinal signaling pathways. This hypothesis is highly supported at least by lines of exhausting experimental evidence showing distinct pharmacological actions of various drugs antagonizing or inhibiting membrane receptors, ion channels and intracellular cascades associated with processing of noceceptive information in the dorsal horn of the spinal cord in rats. The principle of the hypothesis is that the ‘phenotype’ of the pain-related behaviors might be ascribed to the organization of the somatosensory system which shows ability to distinguish functional responses to different stimulus modalities in terms of thermal, mechanical and chemical features[62].

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