Differential actions of intrathecal nociceptin on persistent spontaneous nociception, hyperalgesia and inflammation produced by subcutaneous bee venom injection in conscious rats

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Abstract: Nociceptin is an endogenous ligand for the opioid receptor-like 1 (ORL1) receptor. The present study was designed to investigate spinal actions of nociceptin on the spontaneous nociception, hyperalgesia and inflammation induced by subcutaneous bee venom injection. Subcutaneous injection of bee venom into one hindpaw of conscious rat produced a persistent spontaneous nociception followed by a long-lasting primary heat and mechanical hyperalgesia as well as local inflammation. Compared with the pre-saline group, pretreatment with intrathecal injection of three doses (3, 10 and 30 nmol) of nociceptin produced significant suppression on the spontaneous paw flinching reflex. The inhibitory rates were 37±7, 43±6 and 57±11%, respectively, which were enhanced with an increase in the concentration of nociceptin. The inhibitory action of nociceptin was completely antagonized by a new selective ORL1 receptor antagonist CompB (30 nmol). Pre-treatment with 10 nmol nociceptin prior to bee venom produced no significant effect on the occurrence of primary heat and mechanical hyperalgesia, nor did post-treatment with the same dose again 3 h after bee venom injection. Additionally, pre-treatment with nociceptin had no effect on the bee venom-induced increase in paw thickness and volume and the plasma protein extravasation. These results indicate that intrathecal nociceptin has no effect on primary heat and mechanical hyperalgesia as well as inflammation, but has dose-related anti-nociceptive effect on the bee venom-induced persistent spontaneous nociception via activation of spinal ORL1 receptor.

Key words: nociceptin; bee venom test; persistent spontaneous pain; hyperalgesia; inflammation; anti-nociception; anti-inflammation
Nociceptin (NOC), also known as orphanin FQ (OFQ), is an endogenous heptadecapeptide ligand for the orphan opioid receptor-like 1 (ORL1) receptor. The ORL1 receptor, which belongs to the superfamily of G-protein coupled receptor, has approximately 65% homology to the µ-, δ-, and κ-opioid receptors without specific affinity to any endogenous opioid substances such as enkephalins, β-endorphin and dynorphin[1-2]. Central administration of NOC showed various pharmacological effects on analgesia, food intake, cardiovascular and renal responses, etc.[3]. The ORL1 receptors are widely distributed throughout the central and peripheral nervous system, and have broad overlap with opioid receptors, especially in the areas involved in pain processing[4].

However, functional studies about the effects of NOC on pain or nociception have generated conflicting results and the diversity might be dependent on various factors including different animal models of pain, species, administration routes, dose ranges and nociceptive assays[1, 2, 5-8]. Therefore, to minimize individual difference and inter-model differences, it is necessary to re-evaluate the spinal effects of NOC in an animal model showing most types of pain in an individual subject. Recently, we developed a novel animal model of pain by subcutaneous (s.c.) injection of bee venom (BV) into one hindpaw of rat and the animal displays: (1) long-lasting persistent spontaneous nociceptive (PSN) response such as paw flinching reflex for 1 h; (2) occurrence of primary heat and mechanical hyperalgesia; (3) mirror-image heat hyperalgesia; and (4) distinct local inflammation (paw swelling, edema and plasma extravasation). The behavioral expressions of hypersensitivity and inflammation last for 3~4 d and beyond the process of PSN responses, implicating existence of a difference in underlying mechanisms between spontaneous pain and hyperalgesia and inflammation. The multiple behavioral expressions of nociception and hyperalgesia observed in the BV model are likely to be in correspondence with the genetic phenotypes of pain described recently. By studying genetic correlations of the sensitivity of 11 inbred strains of mice on 22 common assays of nociception and hypersensitivity, Lariviére et al.[9] identified at least five fundamental genetic phenotypes of nociception and hypersensitivity (hyperalgesia): (1) baseline thermal nociception; (2) spontaneous responses to noxious chemical stimuli; (3) thermal hypersensitivity; (4) mechanical hypersensitivity; and (5) afferent input-dependent hypersensitivity. Thus, in this study we designed to use the novel BV model to evaluate the spinal actions of NOC and to see whether it has the same or different effects on spontaneous nociception, hyperalgesia and inflammation? Meanwhile, to ask whether the actions of NOC is mediated via the ORL1 receptor, the antagonism of CompB, (1-[(3R, 4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one), a novel selective non-peptide ORL1 receptor antagonist, was also studied[10, 11].

1 MATERIALS AND METHODS

1.1 Animal. Fifty Sprague-Dawley albino male rats (weighing from 180~220 g) were used [provided by Laboratory Animal Center of the Fourth Military Medical University (FMMU)]. The use of animals was reviewed and approved by the FMMU Animal Care and Use Committee and the IASP’s guidelines for pain research in conscious animals were followed. All rats received intrathecal (i.t.) chronic catheterization, a polyethylene tubing was placed from T1-4 level into the subarachnoid space of lumbosacral enlargement[12]. Experiment was performed at least 5 d after the operation. Each animal was housed in plastic box alone at 22~26°C with food and water available ad libitum in the colony room.

1.2 Experimental design. To investigate whether NOC is effective in suppression of the BV-induced spontaneous pain and hyperalgesia, i.t. administration of three doses of 3, 10 and 30 nmol NOC (Peptide Institute, Osaka, Japan) or vehicle (saline 0.9%) were performed in 20 rats 10 min prior to BV injection (n=5 per group). Behavioral surveys were carried out at three time point: prior to BV injection (the baseline), 0~60 min (the time course of flinches), 2 or 4 h after BV injection (the peak duration of hyperalgesia). To further examine whether the actions of NOC is mediated by ORL1 receptor, effects of a mixture of 10 nmol NOC and 30 nmol CompB (dissolved in 30% DMSO, kindly provided by Banyu Pharmaceutical, Tokyo, Japan) were also studied by pre-treatment 10 min prior to BV injection. To study the action of NOC on the BV-induced plasma extravasation, another index of inflammatory response, rats were injected with a solution of Evans blue (50 mg/kg) via tail vein 20 min prior to BV. The thickness and volume of bilateral hindpaws were also measured 30 min prior to BV and 4 h after BV, respectively. At the end of the experiment (about 4 h after BV), the animals were killed by overdose of pentobarbital (200 mg/kg) and the two hindpaws were transected for measurement of plasma protein extravasation. I.t. administration prior to BV injection could be used to evaluate whether the drug has preventive effect, while administration of 10 nmol NOC 3 h after BV could be used to evaluate whether the drug has a therapeutic
effect (for methodological details see reference\(^{(12)}\)).

1.3 Quantitative measurement of spontaneous nociception. A 30 cm × 30 cm × 30 cm transparent plastic test box with a transparent glass floor was placed on a supporting frame of 30 cm high above the experimental table to allow the experimenters to observe the paws of the animals without obstruction. The rat was placed in the test box for at least 30 min before administration of BV. The spontaneous behavioral response of rats was determined by counting the number of paw flinches during each 5 min interval of 1 h time course following injection of BV\(^{(13)}\).

1.4 Quantitative measurement of hyperalgesia. Thermal and mechanical hyperalgesia were determined by measuring the paw withdrawal thermal latency (PWTL) and the paw withdrawal mechanical threshold (PWMT). As described previously, the rat was placed on the surface of a 2 mm thick glass plate covered with a plastic chamber (20 cm × 20 cm × 25 cm), the latency of paw withdrawal reflex to laser stimuli was measured with a RTY-3 radiant thermal stimulator (Xi’an Fenglan Instrumental Factory, P.R. China). Five stimuli were repeated to the same site and the mean PWTL was obtained from the latter 3 stimuli. The inter-stimulus interval for each thermal test was more than 10 min at the same region. For examination of PWMT, mechanical stimuli were applied by using 10 von Frey filaments (forces ranging from 21 to 490 mN). The rat was placed on a metal mesh floor covered with the same plastic chamber and von Frey filaments were applied in an upgrade intensity order from underneath the metal mesh floor to the testing sites. A single von Frey filament was applied 10 times (once every several seconds) to each testing site. A bending force being able to evoke 50% of the paw withdrawal occurrence was expressed as the PWMT\(^{(13)}\).

1.5 Evaluation of inflammation. The dorsoventral thickness of the paw was measured with precision calipers (MC 2616, Harbin Measure Factory, P.R. China). Care was taken to assure that the caliper was placed at the same site for each measurement. Paw volume was measured by water displacement of the hindpaw immersed in a 50 ml cylinder to the rostral edge of the heel, using a 1 ml syringe to collect and measure the water. Plasma protein extravasation was measured as the amount of dye concentration in the paw. The hindpaw was cut from the ankle joint and the skin was dissected after the rat was killed, then the skin and the remainder were placed in 4 ml of formamide, incubated for 96 h at room temperature. Samples in the formamide were then analyzed for dye concentration by spectrophotometric measurement of absorbance at 620 nm.

1.6 Statistical analysis. All results are expressed by mean ± SEM. The data between the experimental and control groups were compared by using ANOVA followed by post hoc analysis (Scheffé test). \(P<0.05\) is considered to be statistically significant.

2 RESULTS

2.1 Effect of intrathecal NOC on BV-induced persistent spontaneous nociception

Similar to our previous reports\(^{(13)}\), s.c. injection of BV into the plantar surface of one hindpaw of rats immediately produced 1 h period of persistent nociceptive response displaying as spontaneous flinching reflex in a monophasic manner (Fig. 2, open circle). The total mean number of the paw flinches of the NOC-treated (3, 10 or 30 nmol) and the vehicle-treated groups were 302.4 ± 34.14, 273.4 ± 29.26, 207.80 ± 54.81 and 480.00 ± 21.57 times during 1 h after BV injection, respectively. Compared with the control group, three doses of NOC produced significant inhibitory effects upon the BV-induced nociceptive responses (\(P<0.001\), Fig. 1). The inhibitory rate was enhanced with an increase in the concentration of NOC (37 ± 7, 43 ± 6 and 57 ± 11%, respectively) although ANOVA showed no significant difference among three doses used.

2.2 Antagonistic effect of selective ORL1 receptor antagonist CompB
As shown in Fig. 2, the mean numbers of the BV-induced paw flinches per 5 min in 1 h time course was significantly suppressed by 10 nmol NOC compared with the control group. I.t. co-administration of CompB (30 nmol) and NOC (10 nmol) could significantly reverse the inhibitory effect of NOC. The antagonistic effect of Comp B was shown at the first 5 min time block and reached the highest time point at 15 min after BV and 25 min after NOC (Fig. 2).

2.3 Effect of intrathecal NOC on BV-induced primary thermal and mechanical hyperalgesia

Similar to our previous reports[13], s.c. injection of BV produced distinct primary thermal and mechanical hyperalgesia in the injection site. NOC (10 nmol) can significantly suppressed the paw flinches (see result 2.1), but neither i.t. administration 10 nmol NOC prior to BV nor i.t. administration same dose 3 h after BV injection produced significantly inhibitory effect on the occurrence and maintenance of the primary thermal and mechanical hyperalgesia (Fig. 3).

2.4 Effect of intrathecal NOC on BV-induced inflammation

In the vehicle group, the thickness and volume of the paw receiving injection of BV solution were significantly increased compared with the values prior to BV chemical injury (P<0.05). I.t. administration with three doses of NOC (3, 10 or 30 nmol) prior to BV did not influence the BV-produced increase in paw thickness and volume (Fig. 4A and B), nor was the increased plasma extravasation affected by the same three doses of NOC compared with the control (Fig. 4C).

3 DISCUSSION

3.1 Anti-nociceptive effect of intrathecal NOC on persistent spontaneous nociception

Persistent and prolonged spontaneous paw flinches can be consistently observed in the BV test or the formalin test although the time course response patterns are different between the two chemical pain models[13, 14]. This behavioral expression has been proved to be mediated by in-
crease in spontaneous firing of spinal dorsal horn wide-
dynamic-range (WDR) neurons which locate along the
spinally organized nociceptive flexion reflex circuitry and
can be quantified objectively as a spontaneous pain-related
response[15-18]. In the current study, we found that i.t. NOC
produced suppressive effect upon the BV-induced paw
flinches, suggesting that NOC can act directly or indirectly
on the spinal cord dorsal horn WDR neurons and pro-
duced anti-nociceptive actions on the persistent spontane-
ous nociception. Moreover, the NOC-produced anti-
nociception or analgesia can be completely reversed by a
novel selective ORL1 receptor antagonist, CompB[7, 11, 19],
suggesting that the NOC-produced analgesia is mediated
by spinal ORL1 receptors. Our present results can be sup-
ported by previous studies on the formalin-produced noci-
ceptive response[20, 21] and electrically-evoked flexor reflexes
[22]. Taken together with these previous and the present
results, we conclude that i.t. treatment of NOC can block
the occurrence of persistent spontaneous pain produced
by the peripheral tissue chemical injury regardless of
the model difference between the BV test and the for-
malin test[13, 15].

3.2 Actions of intrathecal NOC on BV-produced pri-
mary heat and mechanical hyperalgesia

Unlike the anti-nociceptive actions of i.t. NOC on the
spinally-organized paw flinches, the actions of NOC on the
baseline PWTL and PWMT (normal heat or mechanical
sensitivity) or post-injury PWTL and PWMT (hypersensitivity to heat or mechanical stimuli) are various. For an example, i.t. NOC has been demonstrated to be effective in elevation of heat threshold in the tail flick test
[22, 20], however, it has not been effective in 52°C hot-plate
test[2]. The baseline PWMT to von Frey filament stimuli
has also shown not to be affected by i.t. NOC[8, 24].

Under abnormal state such as peripheral tissue or nerve
injury, i.t. NOC was shown to have dose-related effect in
suppression of the carrageenan- or peripheral nerve injury-induced heat hyperalgesia[8, 21, 23]. In contrast, our
present results showed that neither i.t. NOC prior to BV
nor post-treatment with 10 nmol NOC can produce any
anti-nociceptive effect on the BV-induced primary heat
hyperalgesia. The reason for the discrepancy is unknown,
but is likely to be caused by the difference in animal mod-
els used. It is likely that the anti-nociceptive actions of i.t.
NOC on heat hyperalgesia are not consistent and need to
be further compared by using more existing animal models.

In previous studies, the anti-nociceptive actions of i.t.
NOC on mechanical hyperalgesia have been reported in
peripheral nerve or spinal injury models[23, 25], while, to our
best knowledge, the effects of i.t. NOC on inflammation-
induced mechanical hyperalgesia have been poorly studied. We found that i.t. NOC produced no suppression on the BV-induced primary mechanical hyperalgesia. Since there is a report showed that lack of anti-nociceptive effects of peripheral NOC on the carrageenan-induced mechanical hyperalgesia\cite{28}, we propose that neither peripheral nor spinal NOC play anti-nociceptive actions on mechanical hyperalgesia induced by inflammation although it might be effective in nerve injury-induced mechanical hyperalgesia.

Taken together, the anti-nociceptive actions of i.t. NOC on pain hypersensitivity are not consistent and probably depend upon etiology of injury, the animal models of pain, stimulus-modalities and probably animal species.

3.3 Actions of intrathecal NOC on BV-produced inflammation

The present results suggest that i.t. treatment with NOC is not able to relieve inflammatory response although it is effective in suppression of persistent spontaneous nociception. However, i.t. NOC has been demonstrated to be effective in suppression of carrageenan- and capsaicin-induced paw swelling\cite{19,19}. This discrepancy is likely to be caused by difference in etiology of injury in animal models. Moreover, dry BV solution contains a great mixture of components such toxins, peptides and small organic molecules and it probably produces more complicated state of inflammatory responses than carrageenan and capsaicin do. More recently, Calixto et al.\cite{28} showed that s.c. BV injection caused release of many peripheral pro-inflammatory and inflammatory mediators including histamine, 5-HT, bradykinin and tachykinins as well as activations of H$_1$, B$_1$, B$_2$, neurokinin (NK)-2 and NK-3 receptors and COX-2 enzyme. Our preliminary data also showed that s.c. BV injection could produce neurogenic inflammation which is mediated by both dorsal root reflex and axonal reflex\cite{27}.

3.4 Spinal mechanisms of NOC-produced antinociception on BV-induced PSN responses

The BV-induced spontaneous paw flinches have been proved to be initiated and maintained by ongoing primary afferent impulses originating from the injury site and sensitization of spinal dorsal horn WDR neurons\cite{13,15-18}. Our previous studies suggest that the BV-induced PSN responses are mediated by co-activation of spinal NMDA/non-NMDA/NK1/2/VDCCs – PKC/PKA signal transduction pathways\cite{28}, the BV-induced primary mechanical hyperalgesia is only sensitive to PKA inhibitor, while the primary heat hyperalgesia is sensitive to the NK1/2 antagonist and VDCC blockers and PKC inhibitor, implicating existence of differential spinal mechanisms between the PSN responses and primary heat and mechanical hyperalgesia produced by s.c. BV\cite{28}. NOC might exert anti-nociceptive effect on the BV-induced PSN response pre-synaptically and post-synaptically mediated by ORL1 receptors\cite{28,30}.

Acknowledgments: The authors are grateful to H.S. Chen, J.H. Zheng and H.L. Li for their cooperation during the whole process of the experiments, and to Dr. Toshihiko Saeki from Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Japan for kindly providing CompB.

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