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# Substantial role of locus coeruleus-noradrenergic activation and capsaicin-insensitive primary afferent fibers in bee venom's anti-inflammatory effect

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**Abstract**

Several lines of evidence indicate significant interactions between the immune and nervous systems. Our recent study reveals that 'bee venom (BV) induced anti-inflammatory effect' (BVAI) was produced by sympathetic preganglionic neuronal activation and subsequent adrenomedullary catecholamine release in a zymosan-induced inflammation model. However, the specific peripheral input and the supraspinal neuronal systems that are involved in this BVAI remain to be defined. Here we show that subcutaneous BV injection into left hind limb significantly reduces zymosan-induced leukocyte migration and that this effect is completely inhibited by denervation of the left sciatic nerve. This BVAI was not affected by the destruction of capsaicin-sensitive primary afferent fibers using either neonatal capsaicin or resiniferatoxin (RTX) pretreatment. BV injection into the left hind limb significantly increased Fos expression in the contralateral locus coeruleus (LC) in non-inflamed mice. In zymosan-inflamed mice, BV injection produced a further increase in LC Fos expression as compared with non-inflamed mice. This BV-induced Fos increase in the LC was not affected by RTX pretreatment. Pharmacological blockage of central noradrenergic activity by either central chemical sympathectomy (i.c.v. 6-hydroxydopamine) or alpha2 adrenoceptor antagonism (i.c.v. idazoxan) completely blocked BVAI. Taken together, these results suggest that BVAI is mediated by peripheral activation of capsaicin-insensitive primary afferent fibers and subsequent central noradrenergic activation including the LC.


*Keywords:* Bee venom; Inflammation; Locus coeruleus; Capsaicin-sensitive primary afferent fibers; Fos

## 1. Introduction

We have previously demonstrated that subcutaneous injection of bee venom (BV) produces a significant anti-inflammatory effect in animal models of both acute and chronic rheumatoid arthritis (Kwon et al., 2001; Lee et al., 2001). We have also observed that subcutaneous injection of BV produces an anti-inflammatory effect in a zymosan-induced inflammation model (Kwon et al., 2003). This murine air-pouch model is considered to be a model of synovial-like tissue inflammatory diseases similar to that observed in rheumatoid arthritis (Cabrera et al., 2001, 2004).

Growing evidence supports the hypothesis that alterations of the stress response and interactions between the neuroendocrine and immune systems contribute to the pathogenesis of rheumatoid arthritis (Wahle et al., 2002). In particular, the hypothalamus–pituitary–adrenal (HPA) axis and the autonomic

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nervous system (ANS) are of special interest (Black, 2002). Recently, we observed that adrenomedullary catecholamines are involved in producing a 'BV-induced anti-inflammatory effect' (BVAI) in the zymosan-induced inflammation model (Kwon et al., 2003). This BVAI is mediated through activation of peripheral β-adrenoceptors, whereas the HPA axis is not involved. In a subsequent study we further demonstrated that BV stimulation selectively activates sympathetic preganglionic neurons that project to the adrenal medulla (Yoon et al., 2005). This finding parallels what has been demonstrated clinically in that norepinephrine has been shown to significantly inhibit the release of proinflammatory mediators (interleukin 8, tumor necrosis factor, etc.) in the synovial tissue of rheumatoid arthritis patients (Straub et al., 2002). These results imply that peripheral catecholamine release plays an important role in producing anti-inflammatory effects in rheumatoid arthritis patients. Based on this data it is likely that BVAI on rheumatoid arthritis is also associated with the specific activation of the ANS. While the ANS is clearly involved in BVAI, it is not clear which specific peripheral inputs or supraspinal mechanisms participate in BVAI and this is a focus of the present study.

It is known that BV contains a variety of different constituents including melittin, histamine and phospholipase $A_2$ that produce an acute tonic nociception following subcutaneous injection (Lariviere and Melzack, 1996). Recently, it has been reported that capsaicin-sensitive primary afferent fibers play a partial role in this BV-induced pain sensation (Chen and Chen, 2001). However, since it is unknown whether primary afferents also play a role in BVAI, the first goal of present study was to evaluate whether primary afferent fibers and particularly capsaicin-sensitive primary afferents are involved in BV's ability to reduce peripheral inflammation. To investigate this we tested BVAI in mice that had received either a unilateral sciatic nerve denervation or depletion of capsaicin-sensitive primary afferent fibers using either neonatal capsaicin pretreatment or excitotoxic TRPV1 agonist, resiniferatoxin (RTX) pretreatment.

It has been documented that central norepinephrine depletion by intracerebroventricular (i.c.v.) injection of the selective, catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) augments paw edema in Brewer's yeast-induced peripheral inflammation (Hore et al., 1997). Conversely, i.c.v. injection of norepinephrine suppresses formaldehyde-induced paw edema as does i.c.v. injection of clonidine suggesting that central noradrenergic pathways acting via $\alpha_2$-adrenoceptors are involved in this anti-inflammatory effect (Dumka et al., 1996). In this regard, it is thought that specific activation of the locus coeruleus (LC)-central noradrenergic system plays an important role in neuroimmune adaptive responses against inflammation (Elenkov et al., 2000). In light of this it is perhaps not surprising that we previously observed that subcutaneous BV injection selectively increases the neuronal activity (as measured by Fos expression) of central catecholaminergic neurons including the LC (Kwon et al., 2004). As a logical extension of this study, the present investigation was designed to evaluate whether BVAI is mediated by activation of the LC-central noradrenergic system. To accomplish this we first evaluated whether BV injection increased neuronal activity in the LC of normal mice compared to mice with zymosan-induced inflammation. Next we pharmacologically confirmed the involvement of the central noradrenergic system in BVAI by depleting central norepinephrine with 6-OHDA in one set of experiments and by central blockage of $\alpha_2$-adrenoceptors with idazoxan in another set of experiments. Finally, we verified whether the activation of capsaicin-sensitive primary afferent fibers are involved in this BV-induced LC neuronal activation by pretreating animals with the RTX.

## 2. Materials and methods

### 2.1. Animals

Experiments were performed on male ICR mice (from the Laboratory Animal Center of Seoul National University, South Korea) weighing 24–30 g. All of the experimental protocols for animal usage were reviewed and approved by the Animal Care and Use Committee at Seoul National University and conformed to NIH guidelines (NIH publication No. 86-23, revised 1985).

### 2.2. Mouse air-pouch model and BV treatment

The air pouch was prepared in mice as previously described (Kwon et al., 2003; Yoon et al., 2005). About 5 ml of air was subcutaneously injected into the back of the experimental animal on day 0 and the pouch was reinforced with an additional 2.5 ml of air on days 2 and 5 to maintain the pouch cavity. Six days after the initial air injection, animals were injected with 0.5 ml of 1% zymosan (Sigma, St. Louis, MO, USA) to induce local inflammation in the air pouch. Four hours after zymosan administration, the animals were anesthetized with 3% isoflurane in a mixed $N_2O/O_2$ gas and the pouch exudates were collected with 2 ml of saline. The collected exudates were immediately diluted with Turk's solution at a ratio of 1:20. The total number of leukocytes in the exudate fluid was counted using a Neubauer hemacytometer counting chamber. Data are expressed as $10^6$ leukocytes per millilitre of exudate harvested from each mouse.

BV (Sigma Chemical, St. Louis, MO, USA, Cat #: V3125) was diluted in physiological saline. We selected a dose of BV that showed maximal anti-inflammatory effects based on previously reported data from our laboratories (Kwon et al., 2001; Kim et al., 2005). For all experiments BV (1:1000 in 20 μl saline) or vehicle (20 μl saline) was administrated subcutaneously into the left hind knee 5 min prior to zymosan injection as previous reported (Kwon et al., 2003).

### 2.3. Involvement of the sciatic nerve or capsaicin-sensitive primary afferent fibers in BVAI

In order to test whether peripheral nerves are involved in the BVAI effect, the left sciatic nerve was surgically transected just proximal to the point of trifurcation of the tibial, common