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Water soluble fraction (<10 kDa) from bee venom reduces visceral pain behavior through spinal α_2-adrenergic activity in mice

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Abstract

We have previously shown that subcutaneous bee venom (BV) injection reduces visceral pain behavior in mice, but it is not clear which constituent of BV is responsible for its antinociceptive effect. In the present study, we now demonstrate that a water-soluble subfraction of BV (BVA) reproduces the antinociceptive effect of BV in acetic acid-induced visceral pain model. We further evaluated three different BVA subfractions that were separated by molecular weight, and found that only the BVAF3 subfraction (a molecular weight of <10 kDa) produced a significant antinociceptive effect on abdominal stretches and suppressed visceral pain-induced spinal cord Fos expression. Injection of melittin (MEL), a major constituent of BVAF3, also produced a visceral antinociception. However, melittin's antinociception was completely blocked by boiling for 10 min at 100 °C, while boiling either whole BV or BVAF3 did not prevent their antinociception. The antinociceptive effect of BVAF3 was completely blocked by intrathecal pretreatment with the α_2-adrenoceptor antagonist, yohimbine (YOH), while intrathecal pretreatment with the opioid antagonist, naloxone (NAL) or the serotonin antagonist, methysergide, had no effect. These data demonstrate that BVAF3 is responsible for the visceral antinociception of whole BV and further suggest that this effect is mediated in part by spinal α_2-adrenergic activity.

Keywords: Bee venom; Fraction; Visceral pain; Antinociception; Adrenoceptor

1. Introduction

We have demonstrated previously that subcutaneous bee venom (BV) injection produces a robust antinociceptive effect in several different rodent models of both somatic and visceral pain (Kwon et al., 2001a,b,c). These preliminary data imply that BV is useful for the management of both somatic and visceral pain, but it is not clear which constituent is responsible for its antinociceptive effect. We have recently reported that a water-soluble fraction of BV (BVA) produces a potent antinociceptive effect in an adjuvant-induced rheumatoid arthritis rodent model (Kwon et al., 2002). This finding has been further studied in the present investigation, by first determining if BVA is also antinociceptive in the abdominal stretch assay in mice and then further subdividing this fraction into subfractions based on molecular weight and subsequently testing which subfraction is antinociceptive in this assay. In addition, we evaluate whether melittin (MEL), a major constituent of whole BV, reproduces a visceral antinociceptive effect. The potential antinociceptive effect of each subfraction was determined by quantitative analysis of abdominal stretches and of spinal cord Fos expression

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induced by intraperitoneal acetic acid injection (Kwon et al., 2001a).

We have previously found that the visceral antinociception produced by BV is mediated by the selective activation of spinal α_2 adrenoceptors, while spinal opioid receptors do not appear to be involved (Kwon et al., 2001a). In order to determine whether the visceral antinociception produced by the BVA fraction providing the greatest level of analgesia is mediated by the selective activation of spinal cord opioidergic, serotonergic and/or adrenergic receptors, we gave intrathecal injections of antagonists to these receptors and evaluated the effect on BVA-induced antinociception.

2. Materials and methods

2.1. Experimental animals

Experiments were performed on male ICR mice, weighing 25–30 g. The Laboratory Animal Center of Seoul National University (SNU) provided all experimental animals for the study and the experimental protocols for animal usage were reviewed and approved by the SNU Animal Care and Use Committee. The principles of animal care were based on NIH guidelines (NIH publication no. 86-23, revised 1985). All assays were performed blindly and all antinociceptive tests were performed between 12:00 and 17:00 h in order to minimize circadian variability in nociceptive sensitivity.

2.2. Preparation of BV fraction

Whole BV (*Apis mellifera*) was purchased from Sigma (St. Louis, MO, USA). The BV was dissolved in water and then partitioned with hexane (1:1 vol/vol). The hexane fraction was evaporated to dryness and the resulting water layer partitioned with ethylacetate to provide an ethylacetate-soluble fraction and a water-soluble fraction. Each fraction was completely dried and stored at refrigerator temperature. Whole BV contains 90% water-soluble (BVA), 5% ethylacetate-soluble (BVE) and 5% hexane-soluble substances (BVH). Each fraction was dissolved in appropriate vehicle [BVA: 0.9% saline, BVE: ethanol and saline (1:10 vol/vol), BVH: sesame oil] prior to injection. Subsequently, the BVA fraction was further separated into three additional subfractions according to molecular weight: (1) a high molecular weight fraction (BVAF1: >20 kDa) that comprised 10% of whole BV; (2) an intermediate molecular weight fraction (BVAF2: 10–20 kDa) that comprised 20% of whole BV, and a low molecular weight fraction (BVAF3: <10 kDa) that comprised 60% of whole BV. Each subfraction of BVA was dissolved in 0.9% saline prior to injection.

Determination of dose was based on a previous study, in which we demonstrated that a subcutaneous injection of a 20-μl volume of whole BV at a concentration of 1 mg/ml produced a significant antinociceptive effect (Kwon et al., 2001c). Based on these results, the dose of each fraction in the present study was determined by considering the partial ratio of each fraction in whole BV (1 mg/ml). Thus, BVA was used at concentration of 0.9 mg/ml and BVE and BVH were each injected at doses of 0.05 mg/ml. BVAF1, BVAF2 and BVAF3 were used at concentrations of 0.1, 0.2 and 0.6 mg/ml, respectively. Each mouse was injected with a volume of 20 μl of either whole BV or one of the above BV fractions.

2.3. Determination of an antinociceptive fraction in the abdominal stretch assay

Mice were placed individually in table-top plexiglass observation cylinders (60 cm high, 40 cm diameter) and were allowed to adapt to the environment for 30 min prior to the start of the experiment. Thirty minutes prior to acetic acid injection, a specific BV fraction or its appropriate vehicle was injected subcutaneously into the Zhongwan acupuncture point (CV12) as previously described (Kwon et al., 2001a). Acetic acid (0.6 ml of a solution at a concentration of 0.9% v/v) was subsequently injected intraperitoneally to produce abdominal stretches. Following acetic acid injection each animal was recorded with a video camera over the next 30 min period to allow subsequent quantification of abdominal stretches. Generally, writhing behaviors peak during the first 10 min and become less during the final 20 min of observation, but we believe that the full 30-min observation period is relevant with respect to observing full changes in overall abdominal pain behaviors after acetic acid injection. The videotape was then viewed in slow motion by two experienced investigators, who were blinded to the experimental conditions, and the number of abdominal stretches per animal was counted separately by each observer.

2.4. Analysis of spinal Fos expression

Two hours after acetic acid injection, five to eight mice were selected for Fos immunohistochemical analysis, which was performed as previously described (Kwon et al., 2001a). These animals were chosen because their individual behavioral scores approximated the mean of the treatment group. The spinal cord and the brain stem were removed immediately after perfusion, post-fixed in the 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 6.9) for 4–5 h and then placed in 30% sucrose in PBS (pH 7.4) overnight at 4°C. Serial transverse sections (40 μm) of the brainstem and spinal cord were cut using a cryostat (Microm). The tissue sections were processed for Fos immunohistochemistry using the avidin–biotin–peroxidase procedure. Fos-like immunoreactive (FLI) neurons were visualized using a 3-3 diamino-benzidine (Sigma) reaction. For quantitative analysis the mean number of FLI neurons per spinal cord region (laminae) per section per group was determined by averaging the number of FLI cells from five thoracic spinal cord sections (containing the greatest number

of FLI neurons from the T^6–T^{13} cord segments) from each animal in the group. Spinal cord sections were first scanned manually by light microscopy as previously described (Kwon et al., 2001c) and the five sections with the greatest number of labeled cells at the T^{6-13} spinal level were selected from each animal/slide and used for subsequent quantitative analysis. Individual sections were digitized with 4096 gray levels using a cooled CCD camera (Micromax Kodak 1317; Princeton Instruments, Tucson, AZ) connected to a computer-assisted image analysis system (Metamorph; Universal Imaging, West Chester, PA). In order to maintain a constant threshold for each image and to compensate for subtle variability of the immunostaining between sections and slides, we only counted neurons that were at least 30% darker than the average gray level of each image after background subtraction and shading correction were performed. After adjusting the threshold image, individual neurons were considered to be specifically Fos-labeled when a total pixel area between 6 and 20 pixels was detected. To assess the effect of BV fraction on spinal cord Fos expression, the following four gray matter regions were selected for analysis based on cytoarchitectonic criteria: (1) superficial dorsal horn (laminae I and II), (2) nucleus proprius (laminae III and IV), (3) neck (laminae V and VI) and (4) the ventral horn (laminae VII–IX). All Fos quantitative analysis was performed as described previously (Kwon et al., 2001a).

2.5. Comparative study with melittin and evaluation of heat stability

Next, we compared the antinociceptive effect of subcutaneous injection of the different BV fractions with that of melittin. Melittin comprises 50% of the dry weight of BV (Lariviere and Melzack, 1996) and is a major component of one of the BVA fractions (BVAF3). Synthetic melittin (purity: >97%), obtained from Sigma, was used for these experiments because unlike purified melittin it is not contaminated with phospholipase A_2 (PLA). We compared the antinociception produced by melittin injection (20 μl of a 0.5 mg/ml concentration) with that produced by whole BV or its subfractions. From clinical studies, it is known that heat-resistant components of BV contribute to its therapeutic effect (Kim, 1992). To further explore this issue with respect to BV subfractions, we evaluated whether boiling BV or its components (i.e. BV fractions and melittin) for 10 min at 100 °C water affected their antinociceptive properties.

2.6. Involvement of central descending inhibitory fibers in BV-induced antinociception

The last set of experiments was designed to elucidate the involvement of the major descending antinociceptive pathways (including opioidergic, α_2-adrenergic and serotonergic neurotransmitter systems) in the antinociception produced by the BVAF3 subfraction. To evaluate the role of specific neurotransmitter receptors associated with descending and intrinsic spinal cord antinociceptive components in BV's antinociceptive effect, intrathecal injections of naloxone (NAL, dissolved in saline, Sigma), yohimbine (YOH, dissolved in distilled water, DW, Sigma) or methysergide (MET, dissolved in DW, Sigma) were performed prior to injection of BVAF3 or acetic acid. Intrathecal injections were made in conscious mice by a modification of the Hylden and Wilcox (1980) technique. A lumbar puncture was made through the intact skin into the intervertebral space located between the fifth and sixth lumbar vertebrae using a 30-gauge needle connected to a 25 μl Hamilton syringe via polyethylene tubing. Dural puncture was indicated by a flick of the tail and 5 μl of the drug was subsequently injected into the subarachnoid space. Mice were pretreated with NAL (20 μg/mouse), YOH (25 μg/mouse) or MET (20 μg/mouse) 5 min before injection of the BVAF3 subfraction. Control groups were intrathecally injected with an equivalent volume of vehicle 5 min prior to BVAF3 injection.

2.7. Statistical analysis

The number of abdominal stretches in each treatment group was expressed as the mean±S.E.M. The level of statistical significance was determined by analysis of variance (ANOVA) followed by Newman–Keul's test for multiple comparisons. Statistical significance was defined as $P<0.05$.

3. Results

3.1. Determination of the most potent BV fraction based on antinociceptive effects in visceral pain

There were no significant differences in the number of abdominal stretches elicited by injection of different vehicles (ethanol+saline or sesami oil) compared to the saline control group (data not shown). The antinociceptive effect produced by whole BV (20 μl of 1 mg/ml) was reproduced by the BVA fraction (20 μl of 0.9 mg/ml), but not by the BVE or BVH fractions, which did not show any antinociceptive effect in the abdominal stretch assay (Table 1). Among the BVA subfractions, BVAF1 (20 μl of 0.1 mg/ml) and BVAF2 (20 μl of 0.2 mg/ml) did not produce a significant reduction in the number of abdominal stretches elicited by acetic acid injection (Table 1). Only BVAF3 (20 μl of 0.6 mg/ml) produced a significant antinociceptive effect similar to that observed following injection of whole BV or BVA (Table 1). MEL pretreatment also produced a level of antinociception equivalent to that produced by BVAF3 (Table 1). Subsequently, we tested the heat stability of BV, BVAF3 and MEL. The antinociceptive effect of both BV and BVAF3 was still present following exposure to boiling for 10 min at 100 °C, while the

Table 1
The effect of BV and its subfractions on acetic acid-induced abdominal stretches

Treatment	Dose (mg/ml)	No. of abdominal stretches	*n*
Sal	–	24.3±0.7	7
BV	1	8.9±1.0*	7
BVA	0.9	7.4±1.5*	7
BVH	0.05	28.2±1.4	7
BVE	0.05	21.8±1.9	7
BVAF1	0.1	20.7±1.1	7
BVAF2	0.2	21.3±1.3	7
BVAF3	0.6	9.4±1.2*	7
MEL	0.5	9.1±1.5*	9
BV-boiling	1	7.8±1.1*	9
BVAF3-boiling	0.6	6.4±1.3*	8
MEL-boiling	0.5	24.1±3.3	7

Each BV fraction (20 μl volume) was subcutaneously injected. Data was expressed as the mean±S.E.M. Boiling = boiling for 10 min at 100 °C. *p<0.01 as compared with Sal group (ANOVA and Newman–Keul's test). n = the number of animals per group. Abbreviation: Sal: saline; BVA: water soluble fraction of BV; BVH: hexane soluble fraction of BV; BVE: ethylacetate soluble fraction of BV; BVAF1: >20 kDa subfraction of BVA, BVAF2: 10–20 kDa subfraction of BVA; BVAF3: <10 kDa subfraction of BVA; MEL: melittin.

antinociceptive effect of MEL was completely blocked by boiling for 10 min (Table 1).

3.2. Analysis of spinal Fos expression to confirm BVAF3-induced antinociception

Intraperitoneal injection of acetic acid causes a significant increase in the number of FLI neurons in the spinal cord dorsal horn (Figs. 1A and 2). Injection of BVAF3 (20 μl of 0.6 mg/ml concentration) prior to acetic acid administration significantly reduced the number of FLI neurons that were expressed in the T^6–T^{13} segments of the spinal cord (Figs. 1B and 2).

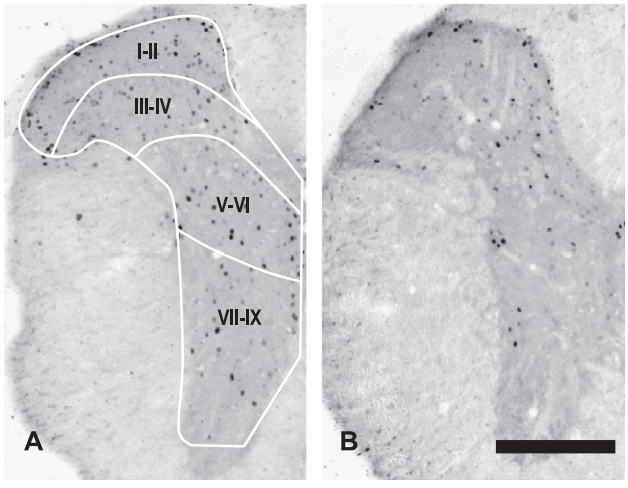

Fig. 1. Photomicrographs illustrating representative examples of Fos expression in the spinal cord. (A) Fos expression in an animal receiving saline injection and acetic acid injection (SAL+AA). (B) Fos expression in an animal receiving BVAF3 injection and acetic acid injection (BVAF3+AA). The spinal gray matter was divided into the following four regions: (1) laminae I–II, (2) laminae III–IV, (3) laminae V–VI, (4) laminae VII–IX. Scale bars = 200 μm.

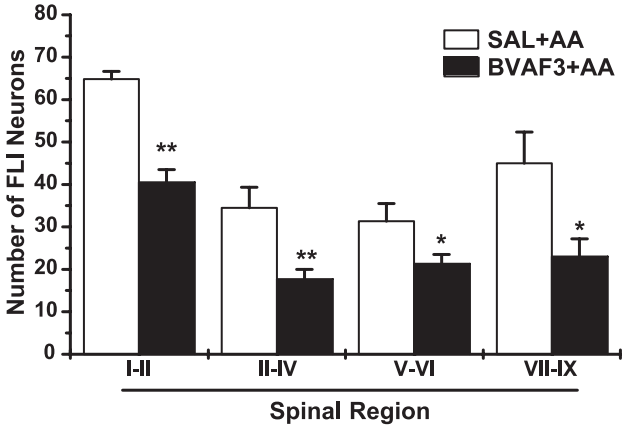

Fig. 2. Graph illustrating the number of Fos like immunoreactive (FLI) neurons in the thoracic spinal cord. Subcutaneous BVAF3 injection (BVAF3, n=8) significantly reduced the number of FLI neurons as compared with that of the saline group (SAL, n=7). *p<0.05 and **p<0.01 as compared with SAL group (ANOVA and Newman–Keul's test).

3.3. Involvement of descending spinal cord fibers in BVAF3-induced antinociception

Finally, we evaluated whether the descending opioidergic system was involved in the BVAF3-induced antinociceptive effect on acetic acid-elicited abdominal stretches. This was tested by blocking spinal opioid receptors with an intrathecal injection of NAL prior to acetic acid injection. Intrathecal injection of naloxone did not affect the BVAF3-induced antinociceptive effect (Table 2). We then evaluated the possible involvement of spinal α_2-adrenergic and serotonergic receptors on the BVAF3-induced antinocicep-

Table 2
The effect of intrathecal pretreatment with the opioid receptor antagonist naloxone (NX), the α_2-adrenoceptor antagonist (YOH) and the serotonin receptor antagonist (MET) on BVAF3-induced antinociception

Mechanism	Treatment	No. of abdominal stretch	*n*
Control	**Sal+Sal+AA**	20.1±1.1	5
	Sal+BVAF3+AA	7.6±0.6*	5
Opioid receptor	NX+Sal+AA	17.5±0.5	5
	NX+BVAF3+AA	5.6±1.2*	5
Control	**DW+Sal+AA**	17.9±1.3	7
	DW+BVAF3+AA	7.0±0.9*	7
α_2-Adrenoceptor	YOH+Sal+AA	18.7±1.4	7
	YOH+BVAF3+AA	19.0±1.0	7
Serotonin receptor	MET+Sal+AA	16.7±1.0	7
	MET+BVAF3+AA	10.6±1.4*	7

Each antagonist or its appropriate vehicle (NX: Sal, YOH and MET: DW) was injected intrathecally prior to subcutaneous injection of BVAF3 (0.6 mg/kg). Data was expressed as the mean±S.E.M. *p<0.01 as compared with each control group (bold font, ANOVA and Newman–Keul's test). n = the number of animals per group. Abbreviation: Sal: saline, DW: distilled water, AA: acetic acid.

tive effect by intrathecal injection of YOH or MET prior to acetic acid injection. Intrathecal injection of YOH significantly blocked the BVAF3-induced antinociceptive effect, while intrathecal administered of MET failed to suppress the antinociceptive effect of BVAF3 (Table 2).

4. Discussion

We have previously demonstrated that subcutaneous BV injection into the Zhongwan acupoint, produces a potent antinociceptive effect in the acetic acid-induced abdominal stretch assay (Kwon et al., 2001a). The goal of the present study was to determine whether an organically extracted or aqueous subfraction of BV contained the active constituents responsible for this antinociceptive effect. The initial experiments in this study showed that the water-soluble fraction of BV (BVA) produced the most potent antinociceptive effect on visceral pain behavior. BVA contains a variety of constituents that include the low molecular weight polypeptides apamin (2 kDa), melittin (3 kDa), mast cell degranulating (MCD) peptide (3 kDa), minimine (6 kDa) and adolapin (11 kDa) and a number of higher molecular weight glycoproteins including the enzymes PLA_2 (19 kDa), lysophospholipase (22 kDa) and hyaluronidase (38 kDa) (Kim, 1992). To determine which subcomponents of BVA might be involved in its antinociceptive effects, BVA was then further separated by molecular weight into three subfractions (BVAF1: >20 kDa, BVAF2: 10–20 kDa and BVAF3: <10 kDa) and each fraction was tested for its antinociceptive properties. The results of this phase of our studies showed that only the BVAF3 subfraction was capable of suppressing the abdominal stretch reflex. The antinociceptive effect of BVAF3 was further confirmed by its reduction of spinal Fos expression induced by intraperitoneal acetic acid injection. It was notable that BVAF3's effect equaled that of whole BV (Table 1) suggesting that the other two subfractions do not contribute to whole BV's antinociception. Furthermore, when we tested a 10 fold lower dose of BVAF3 (0.06 mg/ml; a dose that is less than that tested for the other two subfractions), it still produced a significant antinociceptive effect (unpublished data). Therefore, it reasonable that BVAF3 represents the antinociceptive subfraction of whole BV in this experimental model. Although dose response studies of other BVA subfractions certainly have merit to see if higher doses of these subfractions are capable of producing any antinociception, we do not believe this will contribute significantly to the major conclusions of the present study. Taken together, these data demonstrate that the BVAF3 subfraction contains the most potent antinociceptive constituents of whole BV.

BVAF3 contains several small peptides including melittin, apamin, MCD peptide and minimine. Like whole BV injection, which produces a brief period of both vocalizations and licking behavior, BVAF3 also evokes pain behavior upon initial injection. Melittin is the major active component of BV and is a main constituent of both whole BV and the BVAF3 fraction. Melittin was initially shown to produce local pain and inflammatory responses upon injection in mice (Hartman et al., 1991) and a recent study (Koyama et al., 2002) demonstrated that intradermal injection of melittin into the forearm in humans produces temporary pain and a subsequent sustained increase in the skin temperature due to an axon reflex. It is feasible that while subcutaneous melittin injection produces an initial pain sensation, it may subsequently produce an antinociceptive effect through stimulation of axon reflexes (Carlsson, 2002). Since melittin is a major component of BVAF3, we tested it in our behavioral assay to determine if could replicate the antinociceptive effect observed following BV or BVAF3 injection. Subcutaneous injection of melittin reduced the number of abdominal stretches induced by acetic acid injection to a level similar to that of BV and BVAF3. This initially led us to believe that melittin was the active antinociceptive factor in BV and BVAF3 and thus was responsible for their antinociceptive actions. However, after boiling melittin for 10 min at 100 °C, melittin failed to produce either the initial pain behaviors or the subsequent long lasting antinociceptive effect indicating it is extremely heat sensitive. On the other hand, boiling BV and BVAF3 for 10 min did not alter their capacity to elicit an initial pain behavior or their subsequent antinociceptive effects. Thus, while melittin may contribute to the antinociceptive effect observed in our studies, these data indicate that there is also a heat stable component present in BV and BVAF3 that is also involved in their antinociceptive effects. Apamin is another constituent of BVAF3 that could be involved in producing antinociception. While we did not test apamin in the present study, it is known to block conductance of calcium activated potassium channels (Dale et al., 2002). Since these channels are present in dorsal root ganglion (DRG) cells and blocking them with apamin increases ectopic spontaneous discharges in injured DRGs, it is feasible that apamin could alter peripheral nerve firing induced by visceral sensation. Supporting this assumption, it has been reported that the antinociceptive effect induced by diclofenac is blocked by apamin in the formalin test (Ortiz et al., 2003). MCD peptide is also present in the BVAF3 subfraction. This peptide causes mast cell degranulation and histamine release, so MCD peptide clearly is a potential agent in allergy and inflammation (Buku, 1999) and may participate in the initial short-lasting pain behaviors observed following BV or BVAF3 injection. Taken together, these data beg the hypothesis that BVAF3-induced antinociception may be produced by the interaction of several constituents of BVAF3 rather than by one specific antinociceptive component. More importantly, the interaction of these constituents may be responsible for eliciting central mechanisms of stimulation-induced analgesia.

A wide variety of noxious stimuli are known to elicit a powerful inhibition of pain sensation evoked at a remote region of the body. For example, painful heterotopic

stimulation by capsaicin may inhibit both experimental and clinical pain, an effect known as diffuse noxious inhibitory control (DNIC) of spinal activity (Witting et al., 1998). With respect to BV, we have previously shown that BV-induced noxious stimulation produces a dose-dependent suppressive effect on the abdominal stretch reflex induced by acetic acid injection (Kwon et al., 2001a). More interestingly, although the highest dose of BV (2.5 mg/kg) tested produced an antinociceptive effect in the abdominal stretch assay irrespective of site of injection (acupuncture point vs. non-acupuncture point), a lower dose of BV (0.25 mg/kg) only produced antinociception when injected into the acupuncture point (Kwon et al., 2001a). We also observed that BVAF3 produced a site-specific antinociceptive effect (data not shown). Therefore, we assumed acupuncture point stimulation of BV and its potent subfraction was more powerful regimen to induce visceral antinociception.

On the other hand, BV stimulation selectively activates the descending adrenergic system and that activation of this system is associated with the antinociceptive effect observed in the abdominal visceral pain model (Kwon et al., 2001a). The present results shown that this mechanism is also involved in BVAF3-induced visceral antinociception. Recently, we have demonstrated that bee venom injection into an acupuncture point increases Fos expression in central catecholaminergic neurons including cells in the locus coeruleus (LC) (Kwon et al., 2004). Since the LC is a major source of descending noradrenergic fibers to the spinal cord, we hypothesize that descending adrenergic activity arising from the LC is involved in decreasing nociceptive input from primary afferents at the level of the spinal cord dorsal horn probably via both pre- and post-synaptic mechanisms. It is perhaps not surprising that the LC is involved in visceral acupuncture since an earlier study utilizing the 2-deoxyglucose technique to study brain regions that were activated during acupuncture that produced visceral antinociception also identified the LC as a key component of the CNS structures that were involved in acupuncture analgesia (Shu et al., 1994). Moreover, a recent whole cell patch clamp study indicates that norepinephrine inhibits Aδ fiber- and C fiber-mediated sensory transmission to substantia gelatinosa neurons through the activation of the α_2-adrenoceptor (possibly the α^{2A} type, Kawasaki et al., 2003) further strengthening our hypothesis that acupuncture may activate descending noradrenergic pathways which inhibit incoming primary afferent input via α_2-adrenoceptors.

Clinical pharmacological therapy to reduce visceral hypersensitivity employs several agonists including those that affect opioidergic, serotoninergic and noradrenergic systems (Blackshaw and Gebhart, 2002). In particular, systemic administration of the α_2-adrenergic agonist, clonidine is currently used for reducing visceral sensitivity, but the vasomotor side effects induced by this drug limit its utility (Blackshaw and Gebhart, 2002). For this reason, intrathecal rather than systemic clonidine is used clinically for analgesia. Our results suggest that BVAF3 injected into an acupoint activates intraspinal noradrenergic mechanisms that may play an important role in the therapeutic regimen for visceral pain in humans. On the other hand, it is notable that the BVAF3 subfraction does not contain the major BV allergens including phospholipase A_2 and hyaluronidase (Kemeny et al., 1983). In general, the smaller peptides present in the BVAF3 subfraction are nonallergenic. The only exception to this is melittin and only a small percentage of patients actually have specific IgE antibodies against this component (Kettner et al., 2001). Since BVAF3 produces the same level of antinociception as whole BV, but lacks the allergenic components, it is postulated that BVAF3 may be a clinically safer alternative to whole BV treatment.

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References

Blackshaw LA, Gebhart GF. The pharmacology of gastrointestinal nociceptive pathways. Curr Opin Pharmacol 2002;2:642–9.

Buku A. Mast cell degranulating (MCD) peptide: a prototypic peptide in allergy and inflammation. Peptides 1999;20:415–20.

Carlsson C. Acupuncture mechanisms for clinically relevant long-term effects-reconsideration and a hypothesis. Acupunct Med 2002;20: 82–99.

Dale TJ, Cryan JE, Chen MX, Trezise DJ. Partial apamin sensitivity of human small conductance Ca(2+)-activated K(+) channels stably expressed in Chinese hamster ovary cells. Naunyn Schmiedebergs Arch 2002;366:470–7.

Hartman DA, Tomchek LA, Lugay JR, Lewin AC, Chau TT, Carlson RP. Comparison of antiinflammatory and antiallergic drugs in the melittin- and D49 PLA2-induced mouse paw edema models. Agents actions 1991;34:84–8.

Hylden JL, Wilcox G. Intrathecal morphine in mice: a new technique. Eur J Pharmacol 1980;67:313–6.

Kawasaki Y, Kumamoto E, Furue H, Yoshimura M. Alpha 2 adrenoceptor-mediated presynaptic inhibition of primary afferent glutamatergic transmission in rat substantia gelatinosa neurons. Anesthesiology 2003;98:682–9.

Kemeny DM, Harries MG, Youlten LJ, Mackenzie-Mills M, Lessof MH. Antibodies to purified bee venom proteins and peptides: I Development of a highly specific RAST for bee venom antigens and its application to bee sting allergy. J Allergy Clin Immunol 1983;71:505–14.

Kettner A, Hughes GJ, Frutiger S, Astori M, Roggero M, Spertini F, Corradin G. Api m 6: a new bee venom allergen. J Allergy Clin Immunol 2001;107:914–20.

Kim CM. Bee venom therapy and bee acupuncture therapy. South Korean Ed.; 1992.

Koyama N, Hirata K, Hori K, Dan K, Yokota T. Biphasic vasomotor reflex responses of the hand skin following intradermal injection of melittin into the forearm skin. Eur J Pain 2002;6:447–53.

Kwon YB, Kang MS, Han HJ, Beitz AJ, Lee JH. Visceral antinociception produced by bee venom stimulation of the Zhongwan acupuncture point in mice: role of alpha(2) adrenoceptors. Neurosci Lett 2001a;308: 133–7.

Kwon YB, Kang MS, Kim HW, Ham TW, Yim YK, Jeong SH, et al. Antinociceptive effects of bee venom acupuncture (apipuncture) in rodent animal models: a comparative study of acupoint versus non-acupoint stimulation. Acupunct Electrother Res 2001b;26:59–68.

Kwon YB, Lee JD, Lee HJ, Han HJ, Mar WC, Kang SK, et al. Bee venom injection into an acupuncture point reduces arthritis-associated edema and nociceptive responses. Pain 2001c;90:271–80.

Kwon YB, Lee JD, Lee HJ, Han HJ, Mar WC, Kang SK, et al. The water-soluble fraction of bee venom produces antinociceptive and anti-inflammatory effects on rheumatoid arthritis in rats. Life Sci 2002; 71:191–204.

Kwon YB, Han HJ, Beitz AJ, Lee JH. Bee venom acupoint stimulation increases Fos expression in catecholaminergic neurons in the rat brain. Mol Cells 2004;17:329–33.

Lariviere WR, Melzack R. The bee venom test: a new tonic-pain test. Pain 1996;66:271–7.

Ortiz MI, Granados-Soto V, Castaneda-Hernandez G. The NO-cGMP-K+ channel pathway participates in the antinociceptive effect of diclofenac, but not of indomethacin. Pharmacol Biochem Behav 2003;76:187–95.

Shu J, Li KY, Huang DK. The central effect of electro-acupuncture analgesia on visceral pain of rats: a study using the [3H] 2-deoxyglucose method. Acupunct Electrother Res 1994;19:107–17.

Witting N, Svensson P, Arendt-Nielsen L, Jensen TS. Differential effect of painful heterotopic stimulation on capsaicin-induced pain and allodynia. Brain Res 1998;801:206–10.