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Research Report

The anti-inflammatory effect of peripheral bee venom stimulation is mediated by central muscarinic type 2 receptors and activation of sympathetic preganglionic neurons

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Abstract

The anti-inflammatory effect (AI) induced by peripheral injection of diluted bee venom (dBV) involves activation of spinal cord circuits and is mediated by catecholamine release from adrenal medulla, but the precise neuronal mechanisms involved are not fully understood. In a recent study, we demonstrated that an increase in spinal acetylcholine is involved in mediating the anti-inflammatory effect of dBV and that this mediation also involves adrenomedullary activation. The present study utilized the mouse air pouch inflammation model to evaluate the involvement of spinal acetylcholine receptors and sympathetic preganglionic neurons (SPNs) in dBV's anti-inflammatory effect (dBVAI). Intrathecal (IT) pretreatment with atropine (muscarinic cholinergic antagonist) but not hexamethonium (nicotinic cholinergic antagonist) significantly suppressed dBVAI on zymosan-evoked leukocyte migration. Subsequent experiments showed that IT pretreatment with methoctramine (a muscarinic receptor type 2; M_2 antagonist), but not pirenzepine (an M_1 antagonist) or 4-DAMP (an M_3 antagonist), suppressed the dBVAI. In addition, dBV stimulation specifically increased Fos expression in SPNs of the T7–T11, but not the T1–T6 or T12–L2 spinal cord segments, in animals with zymosan-induced inflammation. Moreover, IT methoctramine pretreatment suppressed this dBV-induced Fos expression specifically in SPNs of T7–T11 level. Peripheral sympathetic denervation using 6-hydroxydopamine (6-OHDA) treatment (which spares sympathetic adrenal medullary innervation) did not alter dBVAI. Collectively these results indicate that dBV stimulation leads to spinal cord acetylcholine release that in turn acts on spinal M_2 receptors, which via a hypothesized disinhibition mechanism activates SPNs that project to the adrenal medulla. This activation ultimately leads to the release of adrenal catecholamines that contribute to dBVAI.

Theme: Endocrine and Autonomic regulation
Topic: Neuroendocrine regulation: other

Keywords: Bee venom; Inflammation; Sympathetic preganglionic neuron; Muscarinic M_2 receptor; Adrenomedullary activity

Abbreviations: dBV, diluted bee venom; dBVAI, diluted bee venom-induced anti-inflammatory effect; IT, intrathecal; IP, intraperitoneal; SPNs, sympathetic preganglionic neurons; M_1 (M_2, M_3) receptor, muscarinic type 1 (type 2, type3) receptor; 6-OHDA, 6-hydroxydopamine; ChAT, choline acetyltransferase; T1–L2, thoracic 1–lumbar 2 segment; IML, intermediolateral cell column; FLI, Fos-like immunoreactivity

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1. Introduction

There are several reports in the literature indicating that intraplantar injection of bee venom evokes a local inflammatory response with accompanying nociception in naive animals [2,4,23,42,44]. Despite the well-known nociceptive and local inflammatory responses produced by a bee sting or by the injection of bee venom, apitherapy has been practiced since ancient times and bee venom is currently used to treat arthritis and other inflammatory diseases. In this regard previous reports from our laboratory have demonstrated that peripheral injection of diluted bee venom (dBV) significantly reduces or prevents the development of an inflammatory response in human arthritic disease as well as in several animal models of inflammatory disease [20–22,24].

Moreover, we recently reported that 'dBV-induced anti-inflammatory effect' (dBVAI) was mediated by catecholamine release from adrenal medulla [22]. Because the adrenal medulla is controlled by sympathetic preganglionic neurons (SPNs) and these SPNs receive synaptic inputs from descending fibers as well as spinal interneurons [13,25], we hypothesize that dBV stimulation alters descending bulbospinal neuronal activity and this ultimately regulates the activity of SPN [16]. However, the specific spinal neuronal circuits and neurochemistry underlying dBV-induced adrenomedullary activation remain to be determined.

There are a number of reports indicating that intrathecal administration of cholinergic agonists (such as neostigmine or carbachol) activate spinal SPNs [3,27,31]. Moreover, a recent study from our laboratories showed that increased spinal acetylcholine (ACh) suppressed zymosan-induced leukocyte migration via activation of the sympatho-adrenomedullary system [41]. Based on these lines of evidence, we hypothesize that dBV stimulation increases spinal Ach, which subsequently activates the sympatho-adrenomedullary system to produce dBV's anti-inflammatory effect.

A zymosan-induced local inflammatory response in the air pouch is considered a model of a synovial-like tissue inflammatory response [1,15]. In the present study, we used this model to investigate spinal cholinergic involvement and the role of sympathetic preganglionic neurons in dBVAI. This was accomplished by first examining the effect of intrathecal pretreatment with selective muscarinic or nicotinic cholinergic receptor antagonists on the number of leukocytes migrating into the air pouch following zymosan injection. Secondly, we determined whether dBV stimulation specifically increased the activity of SPNs by performing double-labeling immunohistochemistry for choline acetyltransferase (ChAT, marker of SPNs) and Fos (cellular activity marker) based on the approach used by Marson and Gravitt [28]. Finally, we examined whether dBVAI was mediated by catecholamine released from peripheral sympathetic nerve endings (except for the adrenal medulla) by performing a chemical sympathectomy using 6-hydroxydopamine treatment [8].

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

The air pouch was prepared in mice as previously described [17,22]. Five milliliters 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 into the air pouch. Zymosan was prepared by incubating it in a dry oven at 39 °C for 50 min just prior to injection. 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.

2.3. Treatment with diluted bee venom

Bee venom (BV; obtained from Sigma, St. Louis, MO) was diluted to a dose of 0.6 mg/kg in 20 μl of saline and subcutaneously administered into the left lateral hindlimb 5 min before zymosan injection. This dose of dBV was selected for these experiments because we have previously shown that it produces the maximal anti-inflammatory effect [20,22,24]. For control groups, saline was injected instead of dBV.

2.4. Intrathecal injection of drugs

For intrathecal (IT) injection, drugs were dissolved in 5 μl of vehicle and an IT injection was performed according to the procedure of Hylden and Wilcox [12] using a 30-μl Hamilton syringe with a 30-gauge needle. The flick of the tail was considered indicative of a successful IT injection. The control group received an IT injection of vehicle.

To determine whether inhibition of spinal muscarinic receptors or nicotinic receptors affects the dBVAI, we intrathecally injected atropine (a muscarinic receptor antagonist, 20 ng; Sigma) [35,41] or hexamethonium (a nicotinic receptor antagonist, 100 ng; Sigma) [32,41] 10 min before