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Trigeminal nerve stimulation triggers oral mast cell activation and vascular permeability

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ABSTRACT

Background: The nervous system contributes to the pathophysiology of allergic and inflammatory diseases, including oral inflammation. Mast cells (MCs) are involved in their pathogenesis through proinflammatory mediator release.

Objective: To investigate the effect of trigeminal nerve (TN) stimulation compared with sham operation on MC activation and oral vascular permeability in the gingiva, palate, buccal mucosa, and tongue of the rat and to examine the possible role of substance P using rats treated with capsaicin as neonates to deplete substance P.

Methods: Six male Sprague-Dawley rats (250 g) were anesthetized and injected intravenously with Evans Blue (EB). Six other rats were injected neonatally with capsaicin (n = 3) or solvent (n = 3) and then injected with EB when they reached 250 g. The mandibular branch of the TN was stimulated for 1 minute (n = 3), and the remaining rats (n = 3) were subjected to sham operation. The ipsilateral and contralateral sides of the mouth were examined for EB extravasation, and tissue sections were removed for light and electron microscopy.

Results: TN stimulation resulted in EB extravasation in the ipsilateral side compared with the contralateral side or the ipsilateral side of sham-operated rats. Significant degranulation of MCs also was evident only on the ipsilateral side ($P < .0001$). There was no difference in MC degranulation between the vehicle- and capsaicin-treated rats, implying that neuropeptides other than substance P may be involved.

Conclusion: This is the first time that TN stimulation has been shown to result in MC activation and oral vascular permeability, suggesting that MC inhibitors may be used for the treatment of oral inflammatory diseases.

Introduction

Mast cells (MCs) are critical for allergic diseases, innate and acquired immunity, and inflammation through the release of numerous mediators.[1–4] These are often released selectively without degranulation[5] and have potent vasodilatory, inflammatory, and nociceptive properties.[3] MCs secrete preformed and newly generated tumor necrosis factor (TNF)[6] and adenosine triphosphate, bradykinin, platelet-activating factor, prostaglandins, serotonin, and tryptase.[7] These can excite sensory nerve fibers, thus resulting in a feedforward loop and potentiation of neurogenic inflammation.[8] MCs also stimulate T cells through TNF and cell-surface costimulatory molecules.[9,10]

Mast cells participate in the brain–skin axis[8] as targets of corticotropin-releasing hormone (CRH) and related peptides.[11] In fact, MC-dependent skin inflammation is impaired in the absence of sensory nerves.[12] The authors previously showed that trigeminal nerve (TN) activation stimulates dural MCs, thus contributing to pathogenesis of migraines.[13,14] Moreover, MCs augment adaptive immunity by recruiting dendritic cells to infected tissues.[15] MCs are considered critical in allergy and innate immunity in the skin[16] and might promote and regulate inflammation.[17]

Electrical stimulation of peripheral nerves increases vascular permeability and plasma extravasation in the oral cavity.[18,19] The role of MCs in such changes has not been investigated or has been insufficiently clarified. The aim of the present study was to

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investigate the effect of TN stimulation (TNS) on MC activation and vascular permeability in the oral cavity of rats and the possible involvement of substance P (SP) because it has been localized in the TN.[20,21]

Methods

Male Sprague-Dawley rats (N = 12; Charles River Laboratories, Cambridge, Massachusetts) were kept from birth until they were used (at 250 g) under standard living and feeding storage conditions at a 10-hour light and 14-hour dark cycle and were provided with food and water ad libitum. Some rats (n = 3) were injected with capsaicin, whereas others (n = 3) were treated with vehicle neonatally. The remaining rats (n = 6) were not treated neonatally and were kept on their cages until they were used (at 250 g). Of these rats, 3 were subjected to sham operation and the remaining 3 underwent TNS, as described below.

Evans Blue Extravasation

Rats were anesthetized with a combination of 0.5 mL of xylazine HCl and 0.5 mL of ketamine HCl (20 mg/mL each) intraperitoneally. After the animal was asleep, 0.6 mL of 1% Evans Blue (EB) in normal saline was injected into the tail vein. EB binds to large-molecular-weight proteins (eg, albumin) in the blood and serves as an indicator of vascular permeability.[22]

Neonatal Capsaicin Pretreatment

Capsaicin treatment was as follows.[23] Littermates (n = 3) were injected subcutaneously in the first 2 days of life with 50 mg/kg of capsaicin (Polysciences, Inc, Warrington, Pennsylvania) diluted in 0.05 mL of a solution containing 0.9% NaCl, 100% ethanol, and Tween 80 (8:1:1) or a solvent (n = 3, control). During capsaicin injection, animals were kept in a tent containing an aerosol of isoproterenol (0.25 mg/mL for 10 minutes). After injection, all neonates were returned to their cages. They were maintained on a diurnal lighting cycle and allowed access to food and water ad libitum. Eight weeks later, TNS was carried out as described below. The 6 rats were sacrificed over carbon dioxide and decapitated immediately after TNS. The oral cavity was exposed and photographed. EB staining appeared dark blue owing to the vascular background. Tissue specimens from the tongue, palate, buccal mucosa, and gingiva were taken and prepared for morphologic examination of MCs.

Surgical Exposure of the TN

The mandibular branch of the TN on the right side was exposed surgically as it branches off (Fig 1A). During the surgical exposure of the nerve, anesthesia was maintained by inhalation of methoxyflurane. A vertical incision was made distal to the ascending ramus of the mandible and in front of the ear on the right side. A second incision was made from the top of the first incision in an oblique direction toward the eye of the rat. The mandible was gently moved forward and to the outside to expose the TN as it exits from the brain (Fig 1B). This protocol was approved by the Tufts Medical Center animal care and use committee.

Stimulation of the TN

In some rats (n = 3), the mandibular branch of the TN was stimulated using a disposable nerve stimulator (VARI-STEM, III, Bristol-MyersSquibb Company, Jacksonville, Florida). The nerve stimulator was tested for its function by applying the tip of the stimulator on the superficial facial nerve and monitoring the muscular activity. After this, the tip was applied directly over the

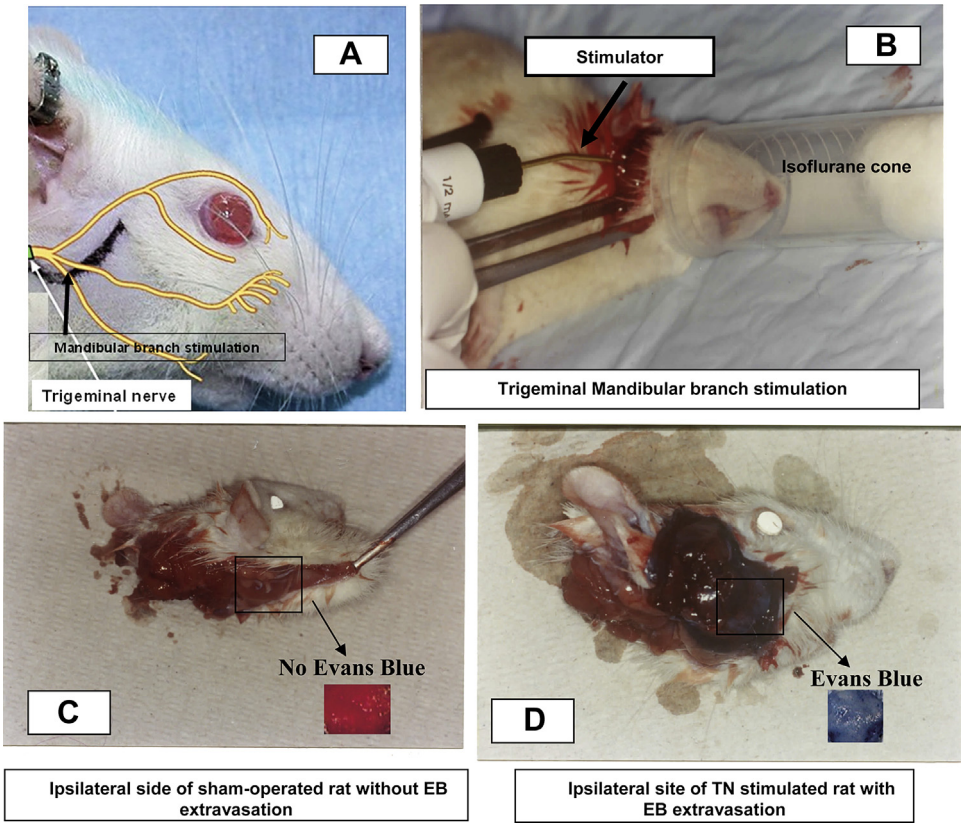

Figure 1. *(A)* Diagrammatic representation of how the mandibular branch of the trigeminal nerve was stimulated. *(B)* Photograph showing actual stimulation of an anesthetized rat. Photographs of the effect of trigeminal nerve stimulation on oral vascular permeability in *(C)* a sham-operated rat (red buccal mucosa) and *(D)* an experimental rat (dark-blue buccal mucosa indicating Evans Blue extravasation).