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Assessment of intraoral mucosal pain induced by the application of capsaicin

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ABSTRACT

Objective: To develop an objective method for assessing nociceptive behaviour in an animal model of capsaicin-induced intraoral pain. Changes in nociceptive responses were also examined after injury to the inferior alveolar nerve (IAN).

Design: Nociceptive responses evoked by the intraoral application of various doses of capsaicin were analyzed in lightly anaesthetized rats. The number of c-Fos protein-like immunoreactive (Fos-LI) neurons in the medullary dorsal horn (MDH) induced by the intraoral application of capsaicin was measured. Behavioural and c-Fos responses were also examined 14 days after injury to the IAN.

Results: Larger doses of intraoral capsaicin (1, 10 and 100 μg) induced vigorous licking behaviour and c-Fos response in the MDH in a reproducible manner. The magnitudes of both behavioural activity and the c-Fos response from the 10 and 100 μg doses of capsaicin were significantly greater than that by the 1 μg dose. Injury to the IAN exaggerated the behavioural and c-Fos responses evoked by intraoral capsaicin.

Conclusions: The intraoral application of capsaicin is a valid and reliable method for studying intraoral pain and hyperalgesia following nerve injury.

1. Introduction

Assessing pain levels arising from an orofacial region in animal studies is essential for elucidating the mechanisms for the pathophysiology of orofacial pain syndromes including trigeminal neuralgia, burning mouth syndrome, and temporomandibular disorders. A widely used behavioural model of facial pain employs the formalin test.[1–4] A subcutaneous

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Abbreviations: DAB, diaminobenzidine; Fos-LI, c-Fos protein-like immunoreactive; IAN, inferior alveolar nerve; MDH, medullary dorsal horn; PAP, peroxidase anti-peroxidase; PB, phosphate buffer; PBS, phosphate-buffered saline; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1.

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injection of a formalin solution into the face of a rat has been shown to induce the early and late phases of intense grooming activity towards the injected area. A previous study also demonstrated that capsaicin treatment in the orofacial region produced similar face grooming behaviour.[5] In spite of the clinical prevalence of pain originating from intraoral tissues, experimental models to assess nocifensive behaviour following intraoral noxious stimulation have not yet been established. Therefore, an appropriate experimental model must be established to develop effective treatments for atypical orofacial pain. The experimental manipulation of intraoral tissues is particularly difficult in awake-behaving animals. A lightly anesthetized rat model was shown to permit the assessment of spinal nocifensive behaviours similar to those of unanesthetized rats.[6–9]

The use of capsaicin as a noxious stimulus offers several advantages, and is now a widely used tool to study pain mechanisms in both humans and animals. Capsaicin is known to be a specific excitant of C- and Aδ-fibres that convey nociceptive signals.[10,11] Previous studies demonstrated that capsaicin bound to the transient receptor potential (TRP) vanilloid 1 (TRPV1) channel and induced cation influx in peripheral nerve fibre terminals.[12,13] Human models of capsaicin-induced pain and hyperalgesia are now available, allowing for correlations between animal and human studies.[14–18]

Previous studies reported that thermal or mechanical noxious stimulation applied to peripheral tissue induced an intense c-Fos protein-like immunoreactivity in the spinal dorsal horn with somatotopic represented manner.[19,20] It has been shown that increasing the intensity of thermal or mechanical stimulation of the facial skin correlated to an increase in the magnitude of c-Fos protein-like immunoreactivity in the medullary dorsal horn (MDH).[21–23] The induction of c-Fos protein-like immunoreactivity may be associated with the activity of primary afferent nociceptors elicited by noxious stimulation.

The aim of this study was to develop a rat model of capsaicin-induced pain in the intraoral region in order to study trigeminal pain mechanisms. A behavioural test for the nociceptive response evoked by applying capsaicin to the dorsal surface of the tongue was conducted in lightly anesthetized rats. The induction of c-Fos protein-like immunoreactivity in the MDH was also evaluated. We further examined changes in these responses following injury to the inferior alveolar nerve (IAN) as a neuropathic pain model.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200–250 g were used in this study. Rats were housed at 20 °C with a daily light period of 12 h, and were fed food and water ad libitum. All surgical and experimental procedures described herein were reviewed and approved by the Animal Care and Use Committee, Okayama University (Protocol No. OKU-2012354), Government Animal Protection and Management Law (No. 105), Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6), and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996. Every attempt was made to minimize the number of animals used and reduce their suffering at all stages of the study.

2.2. Behavioural testing

We examined the effect of various doses of capsaicin (0.001, 0.01, 0.1, 1, 10, and 100 μg) on behavioural responses ($n = 6$ for each dose). Rats were lightly anesthetized by an i.p. injection of pentobarbital sodium (20 mg/kg). These rats had corneal and flexion reflexes, but not voluntary movement. A video camera was used to record the nociceptive response to the intraoral capsaicin treatment. Capsaicin (Wako Co., Japan) was dissolved at 1.5% in a vehicle consisting of 10% ethanol, 10% Tween 80, and 80% saline, and was then diluted with distilled water to yield the final concentrations. A 10 μl volume of diluted capsaicin was applied to the dorsal surface of the tongue using a micropipette. Care was taken to prevent the micropipette tip from contacting tissue other than the lingual surface. Following the intraoral application of capsaicin, animals were monitored for nociceptive behaviour over a 10 min period. The rats exhibited vigorous licking behaviour, i.e., quick movement of the tongue licking intra- and perioral tissues after the application of capsaicin, which continued with or without brief (<5 s) intervals. Since this behaviour started shortly after the capsaicin application and lasted for several minutes, evaluation the nociceptive response was based on measurement of two parameters; i.e., (1) latency of the onset of licking behaviour after the application of capsaicin and (2) the duration of licking (the time period between the onset and termination of licking). Licking behaviour rarely reappeared after an interval longer than 5 s. The termination of licking was determined when the rats showed no more licking behaviour for over 5 s. The behavioural test was performed by an investigator who was blinded to the group assignment of the rat.

2.3. Immunohistochemistry

We also examined the effect of intraoral capsaicin (0, 1, 10, and 100 μg) on the induction of c-Fos protein-like immunoreactivity in the MDH ($n = 5$ for each dose). Larger doses of capsaicin (10 and 100 μg) were lethal in lightly anesthetized rats and the most rats could not survive for an hour. Therefore, different groups of animals were examined for c-Fos protein-like immunoreactivity after the intraoral application of capsaicin with deep anaesthesia. Rats were anesthetized by an i.p. injection of pentobarbital sodium (40–50 mg/kg) and a 10 μl volume of diluted capsaicin or a vehicle was applied to the dorsal surface of the tongue using a micropipette. Two hours after the application, rats were re-anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brainstem with the upper cervical spinal cord attached was dissected out, postfixed in the same fixative for 24 h, and then immersed in 20% sucrose in 0.02 M phosphate-buffered saline (PBS, pH 7.4) for 48 h.

Fifty micrometre-thick transverse frozen sections from the level of the obex to the first cervical level were serially