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ORIGINAL ARTICLE

Response of human oral mucosa and skin to histamine provocation: laser Doppler perfusion imaging discloses differences in the nociceptive nervous system

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Abstract

Objective. To investigate the existence of histamine-excitable nerve fibers in the oral mucosa and to compare the response to histamine provocation in healthy volunteers with that in a small group of patients with chronic oral pain. ***Material and methods.*** Thirteen healthy volunteers and six patients suffering from chronic oral pain took part in the study. Blood perfusion was monitored in the hard palate, the tongue, and the skin of the cheek using laser Doppler perfusion imaging (Perimed; Sweden). Baseline scannings were performed, followed by 15 scannings after iontophoresis of histamine (1%). A free description of the sensations was then obtained from the participants after finishing the measurements. ***Results.*** Compared to pre-histamine scanning, histamine application resulted in a considerable increase in blood perfusion in all regions ($p < 0.001$) that was significantly higher in skin than in oral mucosa ($p < 0.001$). There were no significant differences between the healthy volunteers and the patients regarding baseline blood flow, increased blood perfusion, or flare size after histamine provocation. The sensory impression was reported to be more persistent and intense in the skin than in the oral mucosa. No effect on mucosa could be detected by visual inspection. ***Conclusions.*** Intra-oral flare could be induced by activating histamine-excitable nerve fibers. Both duration and intensity of the flare were considerably less pronounced than in the control skin site. Histamine application was not clearly associated with itch.

Key Words: *Axon reflex, histamine-excitable nerve fibers, laser-Doppler perfusion imaging, nociceptors, oral pain*

Introduction

Itch is regarded as an important defense mechanism of the nociceptive system. In skin, histamine is the primary itch-inducing substance [1]. In response to application of histamine, a visible wheal and flare develop. The flare is a remote increase of blood flow within the innervation territory of a specific subgroup of histamine excitable C fibers (itch fibers), and is elicited by release of vasodilatory neuropeptides (e.g. CGRP) in the entire terminal tree of the axon (axon reflex) [2]. The wheal is attributed to direct effects of histamine on the vessel and does not involve the nerve fibers [3]. Hence, the histamine-induced flare is an indicator for itch fiber activation [4].

Several pathological conditions involving both skin and oral mucosa typically elicit different extra- and intra-oral sensory responses. For example, fungal infections and lichen planus give rise to itch on the skin but burning or smarting sensations in the mouth. It is unclear why the sensory response differs in skin and oral mucosa. Little is known about the presence of histamine-excitable nerve fibers in the oral mucosa. Histamine-excitable nerve fibers may be more sparsely distributed in the oral mucosa, or may be coded to respond to sensations other than itch.

Detailed study of the mechanisms underlying the sensory response of the oral mucosa to histamine is clinically relevant, as it may contribute to our knowledge of such poorly understood clinical conditions as burning mouth syndrome (BMS).

The aims of the present study were to investigate the existence of histamine-excitable nerve fibers in the oral mucosa and to compare the response to

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histamine of healthy volunteers and a small group of patients with chronic oral pain.

Material and methods

Subjects

The participants comprised 13 healthy volunteers and 6 patients with persistent symptoms consistent with BMS. The healthy group comprised 11 females and 2 males, with a mean age of 45 years (range 25–59 years). None of the volunteers had a history of cardiovascular or neurological disease or was receiving medication that might influence cardiovascular functions. Two of the volunteers were taking the drugs Ipren® and Celebra® (Ibuprofen and Celecoxib, respectively). The six patients were recruited from the Division of Hospital Dentistry (Institute of Odontology, Karolinska Institutet) and had symptoms consistent with BMS (mean age 66 years range 50–76; all females). The patients are described in Table I.

Informed consent was obtained from all subjects and the study was approved by the local ethics committee at Karolinska Institutet.

Measurement procedure

The experiment was performed in a quiet, dark room, with the subject positioned in a dental chair in a comfortable, half-reclined position. Medical history taking was followed by intra-oral inspection, and thereafter the scanning procedure started.

Scanning was conducted of three regions. There were two intra-oral test sites (tongue and hard palate) and the skin of the cheek (scanned as a control). During intra-oral scanning, the subjects were instructed to breathe through the nose, as breathing through the mouth influences superficial blood perfusion.

The scanning procedure followed the same protocol for all subjects and all areas: the baseline blood flow was measured three times and scanning started every 60 s. Saline was then applied by iontophoresis, followed by three scans. Thereafter, histamine was applied by iontophoresis and the stimulated region was scanned 15 times at a rate of one scan per minute. In all, 21 scans were conducted in each region. To keep unintentional movements at a low level, the subjects were allowed to withdraw the tongue and rest for 12 s between each measurement. This procedure did not affect the timing of the measurements.

Iontophoresis of saline and histamine

Saline and histamine were applied using iontophoresis (Stimulus Isolator A360; World Precision Instruments, Sarasota, Fl., USA). The probes had a slightly concave metal tip, 5 mm diameter, and served as an anode for current delivery. To avoid contamination, separate probes were used for saline and histamine.

A small cotton pad was soaked in saline (9 mg/ml, prod. no. 021205; Fresenius Kabi, Halden, Norway) or 1% histamine dihydrochloride (Lot. 39H0678, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and placed in the probe cavity. The probe was gently applied to the site. A large (3×2 cm) silver-plated cathode placed on the forehead was deoxidized (37% phosphoric acid) before application of electrode gels (Blågel, Cefar, Lund, Sweden). A constant current of 0.25 mA was delivered for 20 s.

Table I. Detailed description of the patients with chronic oral pain

Patient	Gender	Age (years)	Symptoms	PPI	Pain duration (years)	Medication
PG 1	F	73	Burning pain from the tongue. Periodic.	1.5	>5	Nifedipin (Adalat®), Atenolol (Atenolol®), Simvastatin (Simvastatin®), Bendroflumetiazid (Salures K®)
PG 2	F	72	Burning pain from the tongue. Continuous.	7.3	10	Alendronat (Fosamax®)
PG 3	F	76	Burning pain from the tongue and throat. Continuous.	5.5	3	Levotyroxin (Levaxin®)
PG 4	F	72	Burning pain from the tongue and throat. Continuous.	4.6	11	Metoprolol (Seloken Zoc®), Amlodipin (Norvasc®), Spironolakton (Spironolakton®), Klobetasol (Dermovat®), Lidokain (Xylokain® Kutanspray), Ketoprofen (Orudis retard®)
PG 5	F	53	Burning pain from the tongue, periodically from the oral cavity as a whole. Continuous but intensity varies.	5.9	>2	Sibutramin (Reductil®), Klorzoxazon (Paraflex® comp), Paracetamol (Alvedon®)
PG 6	F	50	Burning pain from the tongue. Periodic	1.4	2	None

PPI = present pain index, where 0 is no pain and 10 unbearable pain.

Laser Doppler perfusion imaging

Superficial blood perfusion was quantified by a laser Doppler perfusion imaging system (Periscan PIM II, serial no 1046; Perimed, Järfälla, Sweden) recording laser Doppler image scans (30×30 pixels, distance 17 cm to region of stimulation). A low power, 670 nm, solid-state laser beam is used in this system. The spectrum of the back-scattered light is analyzed for Doppler components generated by moving blood cells in the superficial micro-vascular network. At each tissue site the monochromic laser light penetrates the tissue to a depth of 0.5–1.0 mm. All scanning data were stored in LDPIwin format (Perimed).

Psychophysics

After completion of the measurement procedure in each region, the subject was asked to describe the sensations experienced during and after application of saline and histamine. In order not to influence the answers, we did not use any standardized form. The subjects were asked to describe the sensations in their own words.

Data analysis

Blood perfusion is calculated by the concentration and mean velocity of moving blood cells related to the magnitude of the Doppler signal and the frequency shift, respectively. The system includes a function in which a region of interest (ROI) can be delineated on an image for subsequent analysis. The ROI in a sequence was adjusted to fit the area affected by iontophoresis. The ROI was adjusted for movements between the images. Blood perfusion was calculated within the ROI, and is a measure of the flare intensity. The area of the flare was determined by the total number of pixels (0.8 mm^2/pixel) in which the mean perfusion exceeded the baseline perfusion by 2 SD (from the six first readings).

Statistics

The software package Statistica (v. 7.1, Statsoft, Tulsa, OK, USA) was used for statistical analysis. To identify differences between the studied regions, ANOVA was used, with repeated measure design based on the first 12 scans. Contrast analysis was undertaken to compare the pre- and post-histamine scans. To identify significant differences between patient group and volunteers, ANCOVA for repeated measures was performed.

The measurement error was assessed by analyzing a duplicate series of experiments. A p-value of 0.05 or less was considered statistically significant.

Results

Perfusion differences

The baseline perfusion differed significantly among the three regions (ANOVA; $p < 0.001$; Figure 1), and was lower for the cheek skin than for the intra-oral regions. The application of saline by iontophoresis did not affect baseline blood perfusion (ANOVA; $p = 0.13$): In the cheek region, the baseline mean (SD) perfusion was 0.9 (0.3) arbitrary perfusion units ((A)PU), in the tongue 1.5 (0.4) (A)PU, and in the palate 1.6 (0.5) (A)PU.

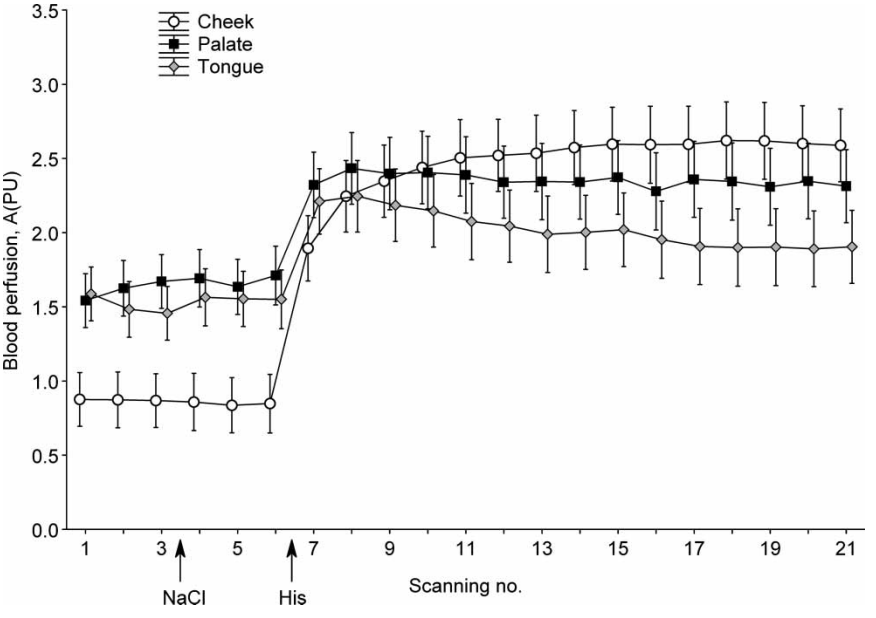

Figure 1. Blood perfusion (arbitrary perfusion units, A(PU)) in 19 subjects (13 volunteers and 6 patients) before and after iontophoresis application of saline (NaCl) and 1% histamine (His). Three regions were monitored: tongue, palate, and cheek. Dots: mean value; bars: 95% confidence interval.

Histamine application resulted in a considerable increase in blood perfusion in all three regions: the increase varied from region to region, with the greatest increase in the cheek (ANOVA; $p<0.001$; Figure 1). There were also regional differences in the flare area (ANOVA; $p<0.001$), the largest flares occurring in the cheek (Figure 2) and exceeding the scanned area in some subjects. The flare intensity peaked at scan number 18 in the cheek and number 8 in the palate and tongue, corresponding respectively to 12 and 2 min after histamine application in each region. Figure 3 shows every second scan in all three regions for one of the subjects (volunteer no. 1).

There were no significant differences between the healthy volunteers and the patients regarding baseline blood flow (ANOVA; $p=0.59$), increased blood perfusion (ANOVA; $p=0.33$), or flare size after histamine provocation (ANOVA; $p=0.33$). As a result, the data from all the subjects were treated as one group. The measurement error for mean perfusion was 0.28 (A)PU and 0.54 cm^2 for flare size.

Sensory and clinical differences

The description of sensations is summarized in Table II. Generally, the patients reported fewer symptoms than the volunteers. The most common sensations were prickling (tongue 37%; palate 26%; cheek 42%) and a sense of cold or warmth (tongue 16%; palate 32%; cheek 58%), a burning or smarting feeling (tongue 37%; palate 26%; cheek 26%). One of the patients did not report any sensation at all in the tongue, while another did not report any sensation from the palate. The remaining patients and volunteers reported at least one sensation for each area. In about half of cases these sensations were only transient (<2 min). Itch was not reported at all from the tongue and only in one case from the palate, whereas 7 subjects (37%) reported itching of the cheek (persistent in 5 subjects). In general, the cheek flare was visible to the naked eye, but this was not the case in the intra-oral regions.

Discussion

To our knowledge, this is the first work evaluating histamine-induced axon reflexes in oral mucosa. Intra-oral laser scanning of the tongue and the palate and scanning of the cheek skin were undertaken in 13 healthy controls and six patients.

Regional differences in baseline flow and flow reactivity

No vascular effect of the iontophoresis in itself was observed. The higher baseline blood perfusion in the intra-oral regions is presumably due to the fact that epithelium is relatively thin compared to skin [5].

Compared to the saline iontophoresis control, histamine iontophoresis resulted in a clear rise in regional blood flow. The intensity and size of the flare were considerably less pronounced intra-orally than in the skin of the cheek. This may have been attributable to differences in the size of innervation territory or the density of histamine excitable nerve fibers in the oral mucosa. The vascular capacity of oral tissues to increase blood perfusion upon provocation could be smaller, i.e. result in a less pronounced flare. The high baseline flow in itself could

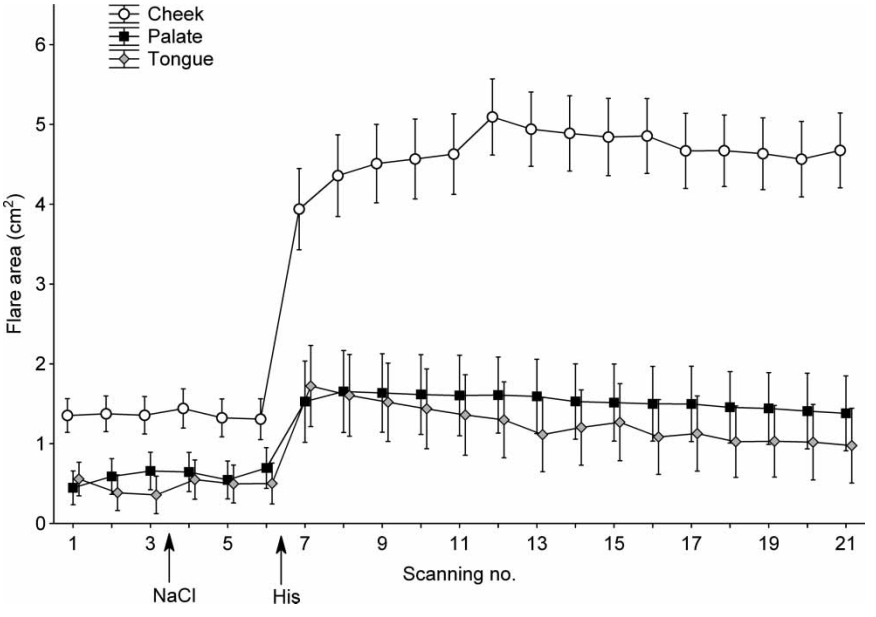

Figure 2. Flare size in 19 subjects (13 volunteers and 6 patients) before and after iontophoresis of saline (NaCl) and 1% histamine (His) determined by total number of pixels (0.8 mm^2/pixel) in which the mean perfusion exceeded the baseline perfusion by 2 SD. Three regions were monitored: Tongue and palate (intra-oral) and cheek (skin). Dots: mean value; bars: 95% confidence interval.

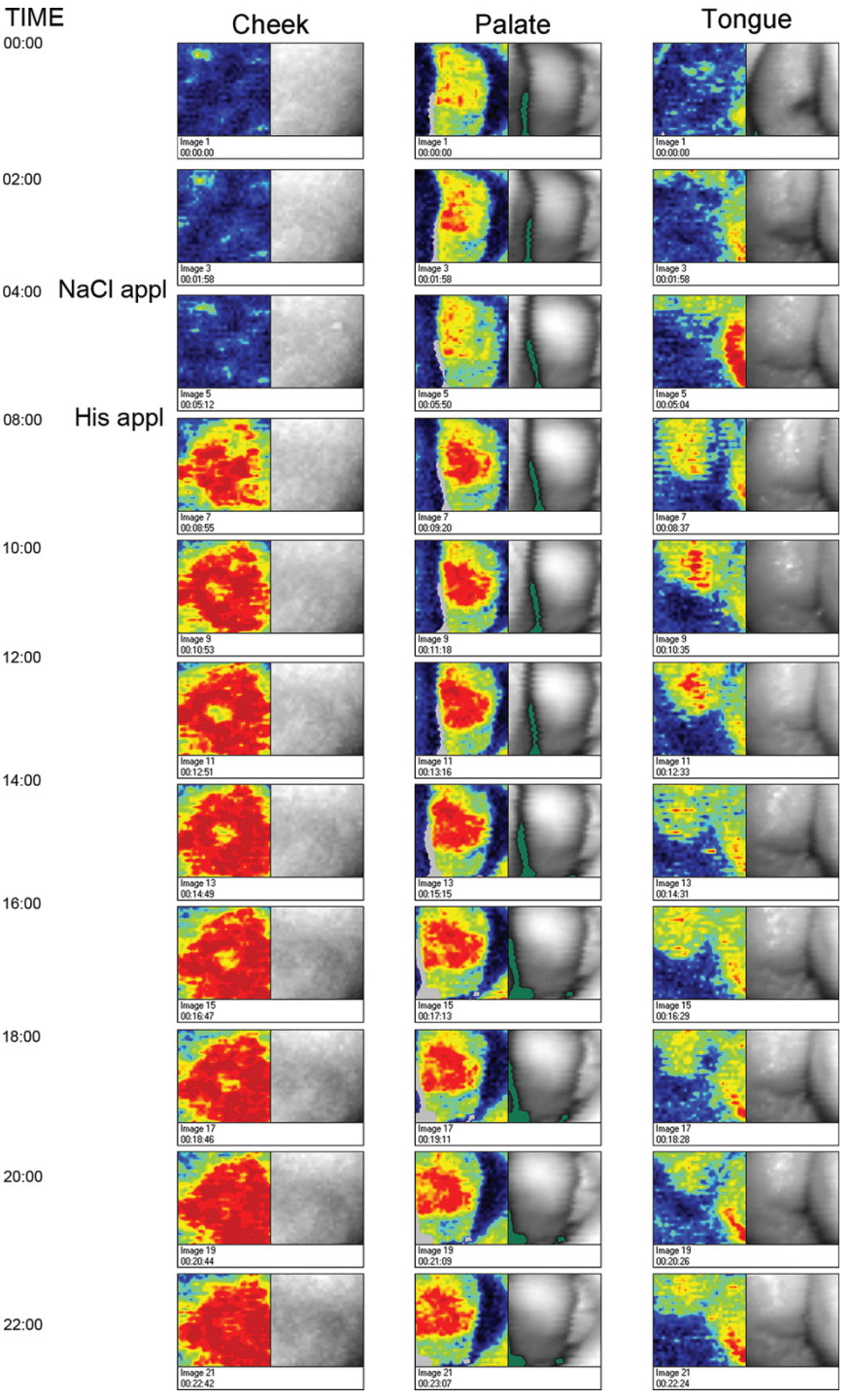

Figure 3. Scanning images (LDPI; Perimed, Sweden) of volunteer no. 1: tongue, palate, and cheek, showing every second scan in a series of 21. Using iontophoresis, saline was applied after scan no. 3 and histamine after scan no. 6.

eliminate histamine from the tissue quicker than in the skin site.

However, the possibility that the intra-oral flares may have been due to a direct histamine effect on the vessels cannot be discounted. A remote effect of histamine application on flux could also be explained by horizontal diffusion and pre-capillary vasodilation with an increase of blood flow also further downstream (i.e. remote from the point of histamine effect). Still, the diameter of the intra-oral flare was on average 1.3 cm, thus exceeding the probe diameter by 0.8 cm. It is considered unlikely that histamine would have such a far-reaching direct effect.

The results confirm the existence of histamine-excitable nerve fibers in the oral mucosa. In human skin, a specific histamine-sensitive population of C fibers has been shown to be responsible for the axon reflex flare. These fibers have large territories and produce a long-lasting flare [2,6,7].

Table II. Various sensations reported by the volunteers and patients after histamine application by iontophoresis for 20 s at 0.25 mA*

Sensation	Tongue: persist (trans)[2]	Palate: persist (trans)[2]	Cheek: persist (trans)[2]	Total: persist (trans)[2]
Prickling				
Volunteers[3]	3 (4)	3 (2)	6 (2)	12 (8)
Patients[4]	0 (0)	0 (0)	0 (0)	0 (0)
Warmth/Cold				
Volunteers[3]	1 (2)	1 (4)	7 (2)	9 (8)
Patients[4]	0 (0)	0 (1)	1 (1)	1 (2)
Burning/Smarting				
Volunteers[3]	1 (2)	2 (1)	1 (1)	4 (4)
Patients[4]	1 (3)	0 (2)	1 (2)	2 (7)
Itching				
Volunteers[3]	0 (0)	0 (1)	4 (1)	4 (2)
Patients[4]	0 (0)	0 (0)	1 (1)	1 (1)
Battery/Metal				
Volunteers[3]	1 (2)	1 (0)	0 (0)	2 (2)
Patients[4]	1 (3)	0 (2)	0 (0)	1 (5)
Pulsating/Pecking				
Volunteers[3]	0 (0)	1 (0)	1 (0)	2 (0)
Patients[4]	0 (0)	0 (1)	0 (0)	0 (1)
Other[1]				
Volunteers[3]	0 (0)	0 (0)	1 (1)	1 (1)
Patients[4]	0 (0)	0 (0)	0 (0)	0 (0)

[1]Other: paresthesia, touch. [2] Persist: persistent sensations (>2 min). Trans: transient sensations (<2 min). [3]$n=13$; [4] $n=6$.
*Some subjects reported more than one sensation in each region.

Nerve fibers of this class of mechano-insensitive C nociceptors, but with smaller receptive fields, could explain the smaller flare size in the oral cavity. Alternatively, another fiber class may exist in the oral cavity. In support of this theory is the fact that the peak of flux in the oral cavity occurred very early (2 min). This is in contrast to the persistent histamine-induced discharge of the "itch fibers" in the skin, which corresponds well with the late peak of flux recorded in the cheek (12 min).

No evidence for pathological flare reactions in the patient group

BMS is a condition of uncertain cause and pathogenesis [8]. However, in recent research the role of neurogenic dysfunction in the pathogenesis is supported. Lauria et al. [9] demonstrated a trigeminal small-fiber sensory neuropathy, characterized by a significant loss of epithelial and sub-papillary nerve fibers in BMS patients. In another study, Heckmann et al. [10] reported that vasoreactivity after dry ice stimulation was significantly elevated in BMS patients compared to controls. Altered thermal and nociceptive thresholds have been reported in BMS patients, as well as abnormalities in the trigeminofacial reflex responses by small-diameter nerve fibers [11]. The present study failed to disclose any differences in baseline blood flow or in flow or flare size after histamine iontophoresis in the BMS patients compared to the healthy controls. However, the patients did not report sensations to the same extent as the volunteers did. Because the number of patients was limited, and the subjects used their own words to describe the sensations, it was not possible to analyze whether the reported sensations differed significantly between the groups. Hypothetically, the presence/occurrence of ongoing pain in the patient group may affect the modulation of sensory input [12].

Regional and inter-individual differences in histamine-induced sensations

In contrast to an earlier report of histamine application to skin [2], itch was not the major sensation reported in any of the monitored regions, but was more commonly reported from the cheek than from the intra-oral sites. This could partly explain the inconsistencies of sensory symptoms from skin and oral mucosa reported in pruritogenic diseases.

In the present study, almost two-thirds of the subjects reported no itching of the cheek. This is in accordance with several previous studies of histamine application to the skin. Itch rating after histamine application has been shown to vary considerably between body regions, the forehead rating being lower than all other tested regions (shoulder, arm, hand, thigh, and foot) [13]. In a study of the scalp by Rukwied et al. [14], a subpopulation of 40%

of subjects did not experience itch upon histamine application. It has been speculated that this could be due to a lower innervation density and/or different central processing of itching in this group of subjects [14].

Clinical implications

The effects of the axon reflex were clearly visible in the cheek skin but not in the oral mucosa, even though blood flow changes were clearly shown by the laser Doppler measurements. This finding is clinically relevant: it confirms that blood flow changes, indicative of several pathological conditions with classical inflammatory components or suspected neurogenic inflammation, are difficult to detect by visual inspection. This finding might also imply that most erythematous changes in oral mucosa do not reflect blood flow changes but rather thickness of the epithelium (atrophy/hypertrophy).

Drawbacks of the method

In order to minimize errors, the scanning process requires that movement of both the scanner and the ROI be kept to a minimum. The tongue was particularly difficult due to unintentional movements, but by allowing withdrawal of the tongue between each reading the protocol was accomplished. Intra-oral scanning is also difficult due to the anatomical limitations.

In conclusion, the present study shows that an intra-oral flare could be induced by activating histamine-excitable nerve fibers. The duration and intensity of the flare were considerably less than at the control skin site. Histamine application was not clearly associated with itching but with prickling, burning, or smarting, which could partly explain the sensory differences between affected mucosa and skin in certain diseases.

As blood flow changes in oral mucosa are not detectable by visual inspection, the laser Doppler perfusion imager may be a suitable investigative or diagnostic tool.

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