Comparison of mechanical pain thresholds among various orofacial areas in human

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[Received 31 May 2004, Accepted 7 July 2004]

Abstract

We compared mechanical pain thresholds in various orofacial areas. Twelve subjects (6 males, 6 females) aged 28 to 64 years participated in this study. They were seated in a supine position on a dental chair during measurement. The pain thresholds were measured at 38 sites in oral region, 7 sites in facial region, and 4 sites in the hand using an electronic von Frey instrument. The subjects were instructed to lift their left hands when they started to feel pain. The pain thresholds as the subjective sensation intensity were calculated on the basis of the Weber-Fechner law. The threshold values in 4 areas were as follows; red margin of the lip < oral mucosa < facial skin < skin of the hand (Bonferroni, $p < 0.05$) and those in 9 oral areas were; frenum $\approx$ floor of mouth < red margin of the lip $\approx$ free gingiva $\approx$ tongue < lip mucosa < hard palate $\approx$ attached gingiva < Kiesow zone (Bonferroni, $p < 0.05$). It is suggested that the mechanical pain thresholds in orofacial areas might be influenced by the density of free nerve endings, the degree of keratinization, and tissue thickness.

Keywords: oral mucosa, facial skin, von Frey test, mechanical pain, threshold, human

INTRODUCTION

It is often observed that various diseases can cause pain in the orofacial region^{16,17,22,23}. In order to analyze the degree of pathological pain, the information of normal physiological pain threshold in human is needed. However, there were few quantitative data concerning physiological pain thresholds in the oral mucosa or in the facial skin^{4,8,11,12,21,24,25,27,32}. Therefore, the present study was undertaken to compare mechanically-induced pain thresholds in various orofacial areas using a modified von Frey test.

METHODS

Twelve subjects (6 males, 6 females; 26 to 64 years old) took part in this study. They were systemically healthy, and had no organic or functional disease in all areas tested in the present study. In addition, they did not wear full or partial denture. Each subject was informed about the aim and the methods of the present study, and signed a written consent before taking part in the study, and the study protocols conformed to the ethical guidelines of the Declaration of Helsinki.

Table 1 The numbers of test sites, laterality, 4 areas, and 9 oral areas

No.	test sites	Laterality	4 areas	9 oral areas
1	red margin of the upper lip	R	red margin of the lip	red margin of the lip
2	red margin of the upper lip	C	red margin of the lip	red margin of the lip
3	red margin of the upper lip	L	red margin of the lip	red margin of the lip
4	red margin of the lower lip	R	red margin of the lip	red margin of the lip
5	red margin of the lower lip	C	red margin of the lip	red margin of the lip
6	red margin of the lower lip	L	red margin of the lip	red margin of the lip
7	upper labial mucosa	R	oral mucosa	labial mucosa
8	upper labial mucosa	C	oral mucosa	labial mucosa
9	upper labial mucosa	L	oral mucosa	labial mucosa
10	lower labial mucosa	R	oral mucosa	labial mucosa
11	lower labial mucosa	C	oral mucosa	labial mucosa
12	lower labial mucosa	L	oral mucosa	labial mucosa
13	upper anterior labial free gingiva	R	oral mucosa	free gingiva
14	superior labial frenum	C	oral mucosa	frenum
15	upper anterior labial free gingiva	L	oral mucosa	free gingiva
16	lower anterior labial free gingiva	R	oral mucosa	free gingiva
17	lower anterior labial free gingiva	L	oral mucosa	free gingiva
18	upper anterior labial attached gingiva	R	oral mucosa	attached gingiva
19	upper anterior labial attached gingiva	L	oral mucosa	attached gingiva
20	lower anterior labial attached gingiva	R	oral mucosa	attached gingiva
21	lower anterior labial attached gingiva	L	oral mucosa	attached gingiva
22	Kiesow zone	R	oral mucosa	Kiesow zone
23	Kiesow zone	L	oral mucosa	Kiesow zone
24	inferior lingual surface	R	oral mucosa	tongue
25	inferior lingual surface	L	oral mucosa	tongue
26	lingual frenum	C	oral mucosa	frenum
27	floor of mouth	R	oral mucosa	floor of mouth
28	floor of mouth	L	oral mucosa	floor of mouth
29	incisive papilla	C	oral mucosa	hard palate
30	hard palate	R	oral mucosa	hard palate
31	hard palate	C	oral mucosa	hard palate
32	hard palate	L	oral mucosa	hard palate
33	lateral edge of tongue	R	oral mucosa	tongue
34	dorsum of tongue	R	oral mucosa	tongue
35	dorsum of tongue	C	oral mucosa	tongue
36	dorsum of tongue	L	oral mucosa	tongue
37	lateral edge of tongue	L	oral mucosa	tongue
38	apex of tongue	C	oral mucosa	tongue
39	forehead	R	facial skin	-
40	forehead	L	facial skin	-
41	cheek	R	facial skin	-
42	cheek	L	facial skin	-
43	mentum	C	facial skin	-
44	earlobe	R	facial skin	-
45	earlobe	L	facial skin	-
46	palm of the hand	R	skin of the hand	-
47	palm of the hand	L	skin of the hand	-
48	back of the hand	R	skin of the hand	-
49	back of the hand	L	skin of the hand	-

Laterality: right (R), center (C), left (L)

They were seated in a supine position on a dental chair with their heads and necks on the headrest. The mechanical pain thresholds were measured at 38 sites (No. 1 to 38) in oral region

and 7 sites (No. 39 to 45) in the facial region, as shown in **Table 1** and **Fig. 1**. The threshold value was also measured at 4 sites (No.46 to 49) in the hand for comparison. A modified

Fig. 1 Test sites in the present study.

The numbers in the figure correspond to those in Table 1. The values of stimulus intensity (gf) were decided when the subjects started to feel pain in 38 oral sites, 7 facial sites, and 4 sites in the hand. On measurement, a modified electronic von Frey instrument (Model-2290; IITC Life Science, California, USA) was used. Every site was tested 5 times on 5 different days.

electronic von Frey instrument (EFI) (Model-2290; IITC Life Science, California, USA) was used. The EFI consisted of a disposable tip, a hand-held probe, a connector cable, and a display unit. The tip made of polypropylene was mounted on the probe as the pipette tip (tip diameter: 0.5 mm) for mechanical stimulation.

For pain threshold measurements, the tip of the EFI was placed gently over each test site and applied manually in a vertical direction with gradually increasing intensity. The subjects were instructed to lift their left hands when the sensation evoked by stimuli applied to the test site had just changed from tactile to painful. When the subjects felt pain, the investigator immediately stopped the mechanical stimuli and recorded the force shown on the digital display (accuracy: 0.1 gf). An interval of 30 to 40 s was allowed between each test site, when the EFI was zeroed for precise data

collection. We made 5 trials on each test site on 5 different days.

During measurement session, we took care that the subjects never saw the digital display of the EFI. The tip was applied vertically to the test site except for hard palate and Kiesow zone (a part of buccal mucosa facing the second molar). The threshold of the red margin of the lip was measured with the subjects' mouths loosely closed. When the threshold of lip mucosa, superior labial frenum, or gingiva was measured, the investigator opened the subjects' lips lightly with a dental mirror, and the subjects were instructed to bite lightly. In case of the measurements of the thresholds in the dorsum, apex, and lateral edge of tongue, the subjects were instructed to thrust out the tongue. On the measurements of thresholds in hands, the subjects were instructed to put their hands on the upper legs and to lift the opposite hands

Fig. 2 Stimulus intensity on the threshold at 49 test sites (mean $\pm$ SD).

Ordinate, stimulus intensity; Abscissa, 49 test sites

Fig. 3 Mechanical pain thresholds (subjective sensation intensity) at 49 test sites (mean $\pm$ SD).

Ordinate, mechanical pain thresholds; Abscissa, 49 test sites

as to signal pain.

As to the data analysis, we determined the subjective sensation intensity as the mechanical pain thresholds instead of the stimulus intensity applied to the test sites. We calculated the values of sensation intensity as the logarithm values of measured stimulus intensity based on the Weber-Fechner law ^{5,20}.

The 49 test sites were grouped into 4 areas; red margin of the lip, oral mucosa, facial skin,

and skin of the hand (**Table 1**). The thresholds of 4 areas were analyzed by multiple comparisons of Bonferroni method. The 5% level of significance was used for comparison. Thirty eight test sites in oral region were grouped into 9 areas; red margin of the lip, lip mucosa, free gingiva, frenum, attached gingiva, tongue, floor of mouth, Kiesow zone, and hard palate. The thresholds of 9 areas were compared in the same way.

Fig. 4 Comparison of mechanical pain thresholds in 4 areas (mean $\pm$ SD).

The statistical differences were recognized among all of 4 areas by Bonferroni method. The comparison of pain thresholds was as follows; red margin of the lip < oral mucosa < facial skin < skin of the hand ($p < 0.05$).

Fig. 5 Comparison of mechanical pain thresholds in 9 oral areas (mean $\pm$ SD).

There were some statistical differences among some areas by Bonferroni method. The comparison of pain thresholds was as follows; frenum $\approx$ floor of mouth < red margin of the lip $\approx$ free gingiva $\approx$ tongue < lip mucosa < hard palate $\approx$ attached gingiva < Kiesow zone ($p < 0.05$). No significant differences were recognized between frenum and floor of mouth, among red margin of the lip, free gingiva, and tongue, and between hard palate and attached gingiva.

RESULTS

The means and SDs of the stimulus intensity at 49 test sites are shown in **Fig. 2**. The values of sensation intensity according to the Weber-Fechner law were also calculated (**Fig. 3**).

The values of sensation intensity as the mechanical pain thresholds on 4 areas of red margin of the lip, oral mucosa, facial skin, and skin of the hand were shown in **Fig. 4**. Significant differences were detected among all 4 areas. The relations among threshold values were as

follows; red margin of the lip < oral mucosa < facial skin < skin of the hand (Bonferroni, $p < 0.05$).

The mechanical pain thresholds in 9 oral areas, red margin of the lip, lip mucosa, free gingiva, frenum, attached gingiva, tongue, floor of mouth, Kiesow zone, and hard palate, are shown in **Fig. 5**. It was clearly observed among the threshold values; frenum $\approx$ floor of mouth < red margin of the lip $\approx$ free gingiva $\approx$ tongue < lip mucosa < hard palate $\approx$ attached gingiva < Kiesow zone (Bonferroni, $p < 0.05$). No signifi-

cant differences were observed between frenum and floor of mouth, among red margin of the lip, free gingiva and tongue, and between hard palate and attached gingiva.

DISCUSSION

Methods of experimental pain stimulation, recording and analysis

To evoke a painful sensation in the skin, 4 different methods are usually used ^{9,11,20,34}. Electrical stimulation has a relatively easy control of intensity, duration, and frequency ^{7,20}. However, the electrical stimulation is not a natural stimulation in sensation and perception. It often bypasses receptor transduction and depolarizes the afferent fibers directly ²⁶. Even on the skin, there is a problem that the current may spread to adjacent structures. The current spread might be greater in the oral region because of the existence of saliva. As for thermal stimuli, radiant heat has been used in order to avoid touch and pressure disturbance ²⁹. The major disadvantage of photic stimulation is wavelength-dependent reflections from the skin. In recent years, argon or carbon dioxide laser stimulation has been employed ^{26,31}. However, it is not easy to measure thermal pain thresholds at various oral sites. Pain producing substances like bradykinin, capsaicin cream, and mustard oil are used for the chemical stimulation ^{14,26}. However, in the oral region, this kind of stimulation may be critically disturbed by saliva. Therefore, these materials might not be adequate to measure pain thresholds in specific oral sites because of dilution by the saliva, spread on other sites, and absorption into the mucosa.

Mechanical stimulation was first developed as a quantitative stimulation to estimate somatosensation including pain ³⁵. Von Frey apparatuses made from horse hair are now substituted for nylon filaments ²⁶. In the present study, we used mechanical stimulation, because von Frey

method was comparatively easy, not disturbed by saliva, and causes no injury to oral mucosa or skin.

In the previous studies on mechanical pain thresholds, pressure was used as a mechanical stimulation ^{4,24,25,27,28}. However, the contact area of the pressure tip was too large (2 to 4.8 mm in diameter) to be applied to specific sites in the oral region.

On the other hand, there are few studies related to pain threshold measurement in the orofacial region using a fine tip like that of von Frey filaments ³². In this study, we used polypropylene pipette tips, which are not affected by the oral temperature or saliva. Because a pressure sensor is attached to the EFI, we could easily read the pressure intensity as a continuous value.

In this study, mechanical pain thresholds were compared in terms of sensation intensity based on the Weber-Fechner law: the subjective sensation intensity is a logarithmic function of the stimulus intensity ^{5,20}. Our present study is the first study of mechanical pain thresholds in the orofacial region to perform statistical analysis based on the Weber-Fechner law.

Variation of pain thresholds

Individual differences between subjects were reported in other studies ^{2,3,4,25,27}. In the present study, the individual differences were supposed to be due to age, gender, and anatomical differences such as density of free nerve endings, the degree of keratinization, and tissue thickness. In addition, emotional aspect like anxiety may be concerned with individual differences of the pain thresholds.

To compare the thresholds, we separated red margin of the lip from other oral areas because the red margin of the lip is a muco-cutaneous junction, while other oral areas are mucosal. Histologically, the covering epithelium of red margin of the lip is orthokeratinized but thin. In addition, the distribution of free nerve endings

in the anterior part of the mouth is richer than that in the posterior part ^{6,33}). When mechanical force is applied to red margin of the lip, free nerve endings may be easily stimulated because thin epithelium has little effect of alleviation of force. Therefore, the mechanical pain threshold of red margin of the lip was low as shown in the present study (**Fig. 4**). Functionally, the low mechanical pain threshold of this area is reasonable to disturb the intake of the dangerous substances.

There are many sites exposed to more intense mechanical stimulation during the mastication of food in oral mucosa compared with in red margin of the lip. Attached gingiva, hard palate, and dorsum of the tongue belong to these areas. The epithelium in these areas is thick and keratinized ³³). This may be the reason why the mechanical pain threshold of oral mucosa was higher than that of red margin of the lip (**Fig. 4**).

Skin has an important function of protecting the body against invasion from outside of the body. The surface of the skin consists of the firm structure of epidermis, and horny cell layer of epidermis alleviates mechanical force. Dermis and subcutaneous tissue also alleviate mechanical force as cushions. When the mechanical force is given for a long period, epidermis increases thickness ¹⁵). As the skin tends to be subject to stronger force than the mucosa, the mechanical pain threshold of skin must be physiologically higher than that of mucosa to adapt the force.

The skin of the hand tends to be caught more intense force for a longer time than facial skin. Thus horny cell layer of the palm (0.090 mm) and back (0.065 mm) of the hand is thicker than that of the face (forehead: 0.024 mm, cheek: 0.015 mm) ¹³). Especially, horny cell layer in the skin of the palm of the hand is extremely thick like the sole, and is occupying more than half of epidermis. Epidermis in the

skin of the hand is also thicker than that in the facial skin. Thus, it is suggested that the mechanical pain threshold of skin of the hand was higher than that of facial skin (**Fig. 4**).

The main tissue components of oral mucosa are a stratified squamous epithelium, called the oral epithelium, and an underlying connective tissue layer, called the lamina propria or corium ³³). The sensory nerves in oral mucosa lose their myelin sheaths in the reticular layer of the lamina propria, and terminate in a subepithelial plexus. The density of free nerve endings and development of nerve plexus in oral mucosa may affect the mechanical pain thresholds ⁶). The degree of keratinization of covering epithelium and thickness of lamina propria may be concerned with the alleviation of mechanical stimulation to nociceptors.

The structure of oral mucosa reflects a variety of functional adaptations. The oral mucosa is generally divided into three main types; masticatory mucosa, lining mucosa, and specialized mucosa ^{18,33}). Masticatory mucosa is exposed to compressive forces during the mastication of food. Accordingly, the epithelium of masticatory mucosa is thicker than that of other oral mucosa and is orthokeratinized or para-keratinized. Masticatory mucosa is firmly attached to cementum or periosteum via collagen fibers ¹⁹). On the other hand, the covering epithelium of lining mucosa is movable. The epithelium of lining mucosa is thin except for labial and buccal mucosa. The epithelium of lining mucosa is nonkeratinized except for the lips. The mucosa of the dorsum of tongue is classified as a specialized mucosa, because it is covered by movable masticatory mucosa with lingual papillae.

Covering epithelium of frenum and floor of mouth is thin, nonkeratinized, stratified squamous epithelium. Especially, the epithelium of floor of mouth is the thinnest (100 μ m) in all of oral mucosa ³³). It is thought that this was the

reason for the lowest mechanical pain thresholds (see **Fig. 5**). Red margin of the lip, free gingiva, and tongue showed higher thresholds than frenum and floor of mouth. The epithelium of red margin of the lip is thin but keratinized. Covering epithelium of free gingiva is non-keratinized and thin but not so thin as floor of mouth. Although the red margin of the lip is keratinized and free gingiva is nonkeratinized, the mechanical pain thresholds were not significantly different (**Fig. 5**). The reason for this result was supposed to be due to richer distribution of free nerve endings in the anterior part of the mouth. As to mucosa of the tongue, inferior surface of tongue is covered with lining mucosa like floor of mouth. Dorsum of tongue is covered with specialized mucosa, which is functionally masticatory mucosa. Apex of tongue and lateral edge of tongue is supposed to be the junction between specialized and lining mucosa. As the threshold of tongue was the average of these different types of tissue, it was not significantly different from that of red margin of the lip or free gingiva. The threshold of labial mucosa was a little higher, because the covering epithelium of labial mucosa is nonkeratinized but much thicker than that of red margin of the lip or free gingiva. The mechanical thresholds of hard palate and attached gingiva were high, because these two areas are covered with masticatory mucosa, where the epithelium is thick (250 μm) and orthokeratinized or parakeratinized, and the lamina propria is also thick with a dense network of bundles of collagen fibers. The reason why the threshold of Kieszow zone was highest may be that the epithelium is the thickest in oral mucosa and the lamina propria is also thick, and that distribution of free nerve endings in the posterior part of the mouth is sparse.

In conclusion, the mechanical pain thresholds in the orofacial areas are not uniform. In this study, we did not investigate the differences in

gender, age, and laterality. Further investigation is needed to clarify these differences ^{1,10,30}. Anatomical analysis that will support the results of this physiological study is also expected.

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