Qualitative analysis of pupillary responses to taste stimulation
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In order to investigate if it is possible to objectively assess gustatory sensation, which is one of the oral senses, we qualitatively analyzed pupillary responses using an infrared electronic pupillometer after stimulation with taste-testing preparations that elicit the taste sensations of sweet, sour, salty, and bitter. The Iriscorder® C7364 (Hamamatsu Photonics, Shizuoka, Japan) was used to measure pupillary responses in 8 adult subjects. Taste discs® (Sanwa Kagaku Kenkyusho, Nagoya, Japan) were used to stimulate the taste sensations of sweetness, sourness, saltiness, and bitterness. Water was used for no taste. Pupillary responses were measured before and after each taste stimulation, and the pupillary area and maximum pupillary contraction rate were analyzed. We found no significant difference in the maximum pupillary contraction rate, which is an index of parasympathetic activity, during any of the taste stimulations. However, the pupillary area, which is an index of sympathetic activity, showed significant increases on stimulations involving saltiness and bitterness. These results suggest the possibility of objectively assessing the sense of taste using an infrared electronic pupillometer. (J Osaka Dent Univ 2011; 45: 69–74)

Key words: Pupillary light reflex; Autonomic nervous function; Gustatory stimulation

INTRODUCTION

Recently, various attempts have been made to objectively evaluate somesthesias, special senses, and psychological state using brain waves, cerebral blood flow, and cardiovascular dynamics.1-4 The sense of taste is a special sense that is currently examined employing electric taste-testing5 and the filter paper disc method.6 However, as the evaluation criteria of these testing methods are dependent on the subjects' perception, the results are affected by the subjects' preferences, as well as physical and psychological conditions. People are not capable of objectively quantifying taste sensations.

Basic research in the field of ophthalmology has shown that the autonomic responses can be evaluated by pupillary responses.7 The effects of bathing in a hot spring3 and occlusal intervention8 have been reported to produce autonomic activities. In addition, the objective evaluation of olfactory and acoustic sensations, which are also special senses, has been attempted using pupillary responses.9

We quantitatively analyzed the sense of taste to objectively quantify taste using pupillary responses. Taste was stimulated using taste-testing preparations, and the pupillary responses were measured using an infrared electronic pupillometer. We then qualitatively analyzed the pupillary responses to the basic tastes of sweet, sour, salty, and bitter.

MATERIALS AND METHODS

Subjects

Eight adults (6 males and 2 females) with an average age of 26 years and no systemic, gustatory, or visual abnormalities were selected from undergraduate and postgraduate students at Osaka Dental University. Prior to the study, the approval by the Ethical Review Board of Osaka Dental University was obtained. The objective and methods of the study
were sufficiently explained to the subjects, and their consent was obtained according to the Helsinki Declaration (Ethical Review Board, Osaka Dental University No.071131).

Measurement of pupillary responses
Pupillary responses were measured using the Iris-corder C 7364 infrared electronic pupillometer. The settings were Photographic field of the CCD camera: 30 × 22.5 mm; Illumination: Infrared LED with a peak wavelength of 890 nm; Sampling time: 1/60 seconds; Light stimulation: peak wavelength of 660 nm/maximum quantity of light 10 μw; Light simulation time: 1 second; and Measurement time: 5.25 seconds. Because pupillary responses are affected by the time of day, illumination, and physical conditions, the pupillometry was performed between 9:00 and 15:00, during which time the pupillary diameter is relatively stable.10,11 All tests were done after dark adaptation by turning off the lights in the room and having the subjects wear goggles. The subjects were confirmed to be in good physical condition, and it was repeatedly confirmed that they were not sleepy during the measurements. Pupillary light reflex measurements were limited to 15 times per experiment to avoid the effect of asthenopia.

Gustatory stimulation
Gustatory stimulation was performed using the Taste discs. The four stimulants of sweet, sour, salty, and bitter were tested, and water was used as a tasteless control. Since the objective of this study was the qualitative analysis of pupillary responses to basic tastes, preparations at the maximum concentration (level 5) were used for gustatory stimulation. Stimulation was performed employing the filter paper disc method. A taste solution was dropped on a round filter paper disc 5 mm in diameter, and the disc was placed with forceps on the simulation site at the tip of the tongue.6

Experimental procedure
To evaluate the effect of taste stimulation, the pupillary responses to the maximum taste stimulation were compared with those before stimulation. The pupillary light reflex was first measured during rest before taste stimulation. Stimulation was then carried out using a level 5 taste disc or a disc saturated with water as a control, and the pupillary light reflex was measured 5 seconds later. Although the order of taste stimulations was randomized, bitterness was tested last based on the instructions for use of the Taste discs. The mouth was rinsed sufficiently after each stimulation, and tests were conducted at intervals of at least 1 minute (Fig. 1).6

Analytical methods
On the basis of the reports that the pupillary area increases during excitation and decreases during the suppression of sympathetic activity,12,13 the pupillary area immediately after exposure to light has been used as a parameter of sympathetic activity. We investigated the pupillary area before and after taste stimulation and compared the results using the t-test. On the basis of previous reports,14-16 the maximum pupillary contraction rate was selected as a parameter of parasympathetic activity, the distribution of the maximum pupillary contraction rate and pupillary area were compared on a binary scattergram, and analysis of covariance was performed.
using the maximum pupillary contraction rate as a covariant (Fig. 2). Statistical analyses were performed using SPSS (Ver.13.0 J, SPSS Japan Inc., Tokyo, Japan).

RESULTS

Pupillary area
Figure 3 shows the pupillary areas before and after gustatory stimulation using water and the four basic tastes, and the results when the t-test was applied to the data. No significant difference was observed in the pupillary area before and after stimulation using water, sour, or sweet. However, the pupillary area increased significantly (p<0.05) after bitter and salty stimulation.

Maximum pupillary contraction rate
Figure 4 shows scattergrams before and after stimulation with water and the basic tastes. Although the variation increased after stimulation compared with before for all of the taste stimulants, no difference was observed in the distribution area. Analysis of covariance showed no significant difference among any of the taste stimulations (Table 1).

DISCUSSION

Gustatory stimulation
Gustatory sense can be classified into 5 basic categories, sweetness, saltiness, sourness, bitterness and tastiness. They are perceived as signals generated by the binding of taste-inducing substances

| Table 1 Analysis of covariance using the maximum pupillary contraction rate as a covariant |
|---------------------------------|-----------------|------------------|-----------------|-----|-----|
| Source of Variation             | Sum of Squares  | Degrees of Freedom | Mean sum of Squares | F   | P   |
| Water                           | 4.375           | 1                 | 4.375            | 0.113 | 0.740 |
| Experimental condition          | 0.793           | 1                 | 0.793            | 0.020 | 0.887 |
| Maximum pupillary contraction rate | 1049.512      | 27                | 38.871           | 11.346 | 0.005 |
| Sum                             | 1058.007        | 29                |                  |      |     |
| Salty                           | 1433.228        | 27                | 53.083           | 2.492 | 0.050 |
| Experimental condition          | 22.999          | 1                 | 22.999           | 0.433 | 0.644 |
| Maximum pupillary contraction rate | 1473.841      | 1                 | 14.738           | 0.278 | 0.603 |
| Sum                             | 1466.303        | 29                |                  |      |     |
| Sour                            | 24.555          | 1                 | 24.555           | 0.612 | 0.441 |
| Experimental condition          | 8.734           | 1                 | 8.734            | 0.218 | 0.644 |
| Maximum pupillary contraction rate | 1062.894      | 27                | 40.107           | 5    |     |
| Sum                             | 1116.071        | 29                |                  |      |     |
| Sweet                           | 2.492           | 1                 | 2.492            | 0.050 | 0.826 |
| Experimental condition          | 176.085         | 1                 | 176.08           | 3.497 | 0.072 |
| Maximum pupillary contraction rate | 1359.361       | 27                | 50.347           | 5    |     |
| Sum                             | 1564.487        | 29                |                  |      |     |
| Bitter                          | 20.076          | 1                 | 20.076           | 0.425 | 0.520 |
| Experimental condition          | 11.346          | 1                 | 11.346           | 0.240 | 0.628 |
| Maximum pupillary contraction rate | 1274.056      | 27                | 47.187           | 29   |     |
| Sum                             | 1305.635        | 29                |                  |      |     |
with the gustatory receptors located at the upper end of taste cells of the taste buds distributed over the tongue, soft palate, epiglottis, and pharynx. The signals are transmitted to the central nervous system via the gustatory nerve. There are a variety of taste-inducing substances for each basic taste, and a taste-inducing material must be present at a certain concentration (threshold) for taste to be perceived. The test solutions absorbed in the taste discs used in this study were purified sugar for sweetness, sodium chloride for saltiness, tartaric acid for sourness, and quinine hydrochloride for bitterness. Their level 5 concentrations were 80%, 20%, 8% and 4%, respectively. Humans will generally respond to a level 2 or 3 stimulation for each taste using the taste discs. Since we used the level 5 taste stimulation, which is 16 times in sweetness and saltiness, 20 times in sourness, and 200 times in bitterness compared with level 2 stimulation, the intensity of stimulation is considered to have been sufficient.

**Autonomic and pupillary responses**
Evaluation of autonomic activities is generally done using cardiovascular dynamics and pupillary activity. Evaluation based on the cardiovascular dynamics requires measurements during rest and cannot be done during normal activities. In addition, as the cardiovascular dynamics are likely to be affected by such factors as respiratory rhythm, body position and swallowing reflex, their use as an index is limited. We therefore directed our attention to the pupillary test as a method for the assessment of autonomic activity. It allows simple, objective, and quantitative analyses of the measurements. In addition, while the sympathetic and parasympathetic activities can be measured simultaneously, they can be evaluated separately. Since the infrared electronic pupillometer used in this study has been applied to the pathological investigation of neuropathies, pre- and postoperative evaluations, and, more recently, the pathological evaluation in patients with chemical hypersensitivity, it is possible to evaluate detailed effects on autonomic activities.

Ophthalmologic experiments that have controlled the sympathetic and parasympathetic activities, and analyzed pupillary responses have shown that the pupillary diameter is adjusted by sympathetic activities, being dilated by excitation and contracted by relaxation of the sympathetic nervous system. We found no significant difference in the pupillary area before and after sweet stimulation. However, bitter and salty stimulation produced significant dilation, suggesting that they changed somatesthesia, causing activation of the sympathetic nervous system.

Parasympathetic activities alone without any involvement of sympathetic activities have been reported to cause miosis and mydriasis in the pupillary light reflex. Some reports on parameters of parasympathetic activities have suggested that the maximum pupillary contraction rate during the pupillary light reflex is a useful parameter of parasympathetic activity. In studies evaluating parasympathetic activities using scattergrams, as in our study, the maximum pupillary contraction rate was reduced during the suppression of parasympathetic activities. Because our study showed no significant difference in the distribution area on any taste stimulation, we concluded that taste stimulation has no effect on parasympathetic activity.

**Pupillary responses to basic tastes**
Gustatory sensation is the most important factor in human preferences in taste. However, these preferences are also affected by odor, texture, color, and sounds of mastication. Among the senses of taste, sweetness, saltiness, and tastiness are generally perceived as pleasant, while sourness and bitterness are unpleasant. However, perception also depends on the concentration of the taste-causing material, and changes with increases in the concentration for the various tastes. Sweetness is perceived as more pleasant as the concentration of the taste-causing material increases for low concentrations, but as less pleasant as it increases further, and becomes increasingly unpleasant at high concentrations. Sourness and bitterness are perceived as more unpleasant with increases in the concentration from a low level. As sympathetic ac-
tivities are reportedly excited when intense pressure is applied to the skin,\textsuperscript{33} they are considered to be enhanced by external stimulations such as pain, cold and emotions (joy, agony, anger, sorrow, fear, and surprise).\textsuperscript{3,8,9} Also, as parasympathetic activities reportedly become dominant on applying warm sheets over the eyes, they are enhanced by pleasant stimulations and in a relaxed state.\textsuperscript{34}

Stimulation in this study was conducted using level 5 taste discs, which were considered sufficient for all subjects. However, no effect on sympathetic activity was noted on stimulation with sour or sweet, presumably because human preferences to tastes are modified after birth by learning through dietary habits and experience,\textsuperscript{31} causing individual differences. Since saltiness significantly enhanced sympathetic activity, it is considered unpleasant at high concentrations in all subjects, and its preference appears to differ between high and low concentrations, as has been suggested in the literature. Bitterness is basically perceived as an unpleasant taste; it enhanced sympathetic activity in this study. Therefore, there seem to be no individual differences with regard to the preference for bitterness. Sympathetic responses to it are thought to change stepwisely with increases in concentration.

Since saltiness and bitterness elicited sympathetic activity in some of our subjects, we concluded that its objective and quantitative assessment can be accomplished using an infrared electronic pupillometer. However, as there was no uniform tendency among the subjects, and as the responses varied from day to day and even within the same subjects, we concluded that pupillometry may be affected by other factors. Further detailed evaluation is considered necessary.

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