Maturation of taste buds on the soft palate of the postnatal rat

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Abstract

Taste bud distribution on the soft palate and within three types of tongue papillae (fungiform, foliate, and circumvallate) were examined histologically in the rat at different postnatal ages. After paraffin embedding, serial sections (10 μm) were made and stained by HE, and digitized images of each section were examined. The existence of a taste pore was used to identify mature taste buds. At birth, 53% (68 of 127 observed) of the taste buds on the soft palate, but only 14% (14 of 110 observed) within fungiform papillae, contained a taste pore. One week after birth, the number of mature taste buds increased rapidly, resulting in 90% of soft palate taste buds and 80% of fungiform taste buds containing taste pores. In contrast, no taste buds with pores were observed at birth within foliate and circumvallate papillae; however, at two weeks after birth 52% (71 of 132 observed) of the foliate and 68% (180 of 267 observed) of the circumvallate taste buds examined contained taste pores. These results suggest that taste buds within the soft palate play an important role in the detection of nutrients in the neonatal rat. © 2000 Elsevier Science Inc. All rights reserved.

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1. Introduction

The gustatory system in newborn mammals is apparently important for the selective ingestion of nutrients through the oral cavity. Behavioral experiments revealed that the human neonate discriminated certain sapid solutions [1]. Also, an examination of the behavioral responses to taste stimuli in newborn rat pups suggested that the gustatory system becomes functionally mature during the first postnatal days, which allows the animal to discriminate sucrose, sodium saccharin, citric acid, and quinine from distilled water [2]. Histological studies in mammals, including humans, demonstrated that several subpopulations of taste buds are distributed on the tongue and throughout the oral cavity [3–5]; however, the appearance and maturation of taste buds are different among these subpopulations [6–8]. The maturation of taste buds on the soft palate (SP) precedes that of taste buds in other areas in the oral cavity, suggesting that the functional importance of the palatal taste buds is for suckling behavior in neonatal animals. Quantitative analysis of the development of taste buds in the rat, especially for the SP at early postnatal stages, was previously performed [9]. In this previous report, however, the developmental changes of palatal taste buds were not compared with those of taste buds in other areas within the oral cavity. Furthermore, the “primordial” taste buds on the soft palate in newborn rats were not included in the count. Thus, in the present report, to clarify the functional importance of palatal taste buds in newborn rats, developmental changes in the number, size, shape, and maturation of taste buds on the SP were quantitatively investigated and compared with those within the fungiform (FF), circumvallate (CV), and foliate (FL), papillae in the postnatal rat.

2. Materials and methods

2.1. Animals

Pregnant female rats (Sprague–Dawley) were purchased from the Kyudo Laboratory Animal Center, and were kept in separate cages. The rats were checked twice a day at approximately 0900 and 1700 h, and the day in which a litter was found was estimated to be Day 0. Pups were fed by the mother until 4 weeks of age. Male and female pups were placed into separate cages after weaning. Data were obtained from 36 rats of both sexes. Animals were sacrificed at postnatal ages of day 0, or 1, 2, 3, 4, 8, or 9 weeks (n = 3 for each age). All animals were maintained on a 12:12 light:dark cycle and had chow pellets and water available ad lib.

2.2. Tissue processing

Rats were given an overdose of Nembutal® (Abott, 3 ml/kg i.p., containing 150 mg/kg sodium pentobarbital) and
their heads were removed. The skin was peeled away from each head and the attached were transected. Each head was placed directly in 10% neutral formaldehyde fixative to minimize shrinkage by fixation. In rats younger than 4, the SP and the tongue were removed after fixation for 3 to 7 days. In adult rats, after fixation for a few days, the tongue and soft palate were removed and continued to be fixed for more than 2 days. Several millimeter cube of tongue epithelium containing a CV, and two cubes containing the FL papillae were surgically removed per animal with the aid of a binocular microscope. Tissue blocks of SP, whole tongue, CV, and FL were put into a 45 × 73-mm nylon mesh bag (Sample Pack, Eiken Kizai Co. Ltd.) and washed overnight with tap water. The tissue blocks in the bags were automatically dehydrated and dipped in melted paraffin (Tissue Prep, melting point 58.0°C, Fisher Scientific) by an automated device (RH-12DM, Sakura Co. Ltd.). The tissue blocks were embedded in three 2 × 1 × 1-cm (B × W × H) plastic boxes with the aid of a binocular microscope, oriented in transverse. Complete serial sections were cut on a rotary microtome at 10 μm, and mounted on egg albumin-coated slides. In the case where sections were missing or the tissue damaged, the specimen was not included in the study. The sections on the glass slide were stained by Mayer’s hematoxylin procedure [10] and counter stained with 0.25% eosin-alcohol.

Each section was examined carefully by a light microscope (40–200×, BH2, Olympus), and the height, width, and the existence of a taste pore representing functional matura- tion were recorded for each taste bud. To distinguish individual taste buds, the image was digitized by a high-resolution digital camera (HC-2000, Fuji-Film) and stored on line as a pict file (1280 × 1000 pixels, 32 bits/pixel color) on a microcomputer (Power Macintosh 7300/180, Macintosh). The digitized image was processed by Photoshop® software (4.0.1J, Adobe Systems Inc.), and printed out in 600 dpi resolution (Deskjet 850C, Hewlett Packard). Because taste buds are densely distributed and overlap each other, it is impossible to discriminate each taste bud by simply observing each section of the CV and FL by microscope. Observing the sequence of photographs of each section made it possible to check and identify each taste bud (Fig. 1). (The largest extent of the height and width of each taste bud with or without pore was measured, and the size of the taste buds was calculated by the equation: area = π × height × width/4, which estimates the cross-section of a taste bud as an ellipse. Also, the ratio of height divided by width was calculated to obtain the general shape of each taste bud. A centrum width at the top of each CV papilla, and a mean length of intergroove lengths of each FL papilla were measured. Maximal groove depth in each CV, and a mean of maximal depth in each groove for each FL papilla were also measured.

![Fig. 1. Three serial sections from one groove of a foliate papillae showing six discriminable taste buds (A–F). Each arrow shows a taste pore. Data are from a nine week-old rat.](image-url)
2.3. Statistical analysis

To analyze difference between each of the developmental changes, values in the four groups were analyzed by two-way analysis of variance (group versus age). Differences in the values among the four groups were analyzed using independent measures two-tailed t-test.

3. Results

3.1. The number and maturation of taste buds

At birth, more than a hundred taste buds (with or without pores) were observed on the SP and FF (SP; 126.7 ± 23.7, FF; 110.3 ± 13.7). The number of SP and FF taste buds increased and reached a steady level (SP; 203.7 ± 8.0, FF; 184.3 ± 26.3) at 1 week of age (Fig. 2). The developmental curves for the SP and FF were similar. On the other hand, the CV and FL contained only a few taste buds at birth (CV; 1.0 ± 1.4, FL; 3.0 ± 4.2), and the number continuously increased until 8–9 weeks of age, reaching 588.7 ± 57.5 and 247.0 ± 77.2 (one side), respectively. The developmental curves for the CV and FL were similar (Fig. 2).

Taste pores were recognized easily in SP, but not FF taste buds at birth (Fig. 3A and B). Although throughout postnatal age the mean numbers of taste buds on the SP was similar to that for the FF (Fig. 2), the percentage of SP taste buds containing a taste pore (52.9 ± 6.8%) at birth was four times larger than that for FF taste buds (12.2 ± 2.6%) (Table 1; Fig. 4). During the first week after birth, the percentages of SP and FF taste buds with taste pore rapidly increased, and at 1 week of age 90% of the SP and 75% of the FF taste buds contained taste pores at 1 week of age (Table 1; Fig. 4). In contrast, at birth, no CV or FL taste buds with pores were observed (Table 1; Fig. 3C and D; Fig. 4). The increase in the number of mature taste buds in the CV and FL was slower than that for the SP and FF, but at 2 to 3 weeks of age, 70–80% of the CV and FL taste buds contained pores (Table 1).

3.2. Developmental changes of the size and shape of taste buds

The mean heights of the SP and FF taste buds at birth were 21.4 ± 0.4 and 17.3 ± 0.5 μm, respectively, and both types increased similarly until 2 weeks of age (Fig. 5; height). At 1 and 2 weeks of age there were no significant differences between the height for SP and FF taste buds. The height of SP taste buds increased from 2 to 4 weeks of age more rapidly than FF taste buds, and reached approximately the same heights as CV and FL taste buds age (Fig. 5; height). The height of FF taste buds increased until 4 weeks of age, and was significantly less than that of the CV or FL taste buds (60–80%) beyond that time (Fig. 5; height). At 2 weeks of age the height of the CV and FL taste buds were 37.0 ± 1.5 μm and 38.9 ± 1.5 μm, respectively, about two times larger than SP taste buds age (Fig. 5; height). A two-factor ANOVA across all ages indicated no significant difference in taste bud height between the SP and FF and between the CV and FL, while the height of the SP and FF taste buds were significantly smaller than CV and FL taste buds (p < 0.01).

At birth through 1 week of age, the width of the SP taste buds was significantly larger than FF taste buds (Fig. 5; width). From 2–4 weeks of age, both SP and FF taste buds increased in width similarly, attaining 47.2 ± 2.3 and 41.2 ± 4.0 μm, respectively. From 1 to 4 weeks of age, the width of SP and FF taste buds was significantly greater than that of the CV and FL taste buds (Fig. 5; width). A two-factor factorial ANOVA indicated no significant difference in width between the SP and FF and between the CV and FL, while the width of SP and FF taste buds was significantly larger than that of CV and FL taste buds (p < 0.05).

Although taste bud populations increased in overall size and reached a plateau at 4 weeks of age, there was no significant difference in the overall size (area) of the taste buds for the different populations (Fig. 5; area). However, the overall size of SP taste buds continued to increase after 4 weeks of age, and were significantly larger (p < 0.05) than other taste buds at 9 weeks of age (Fig. 5; area). The ratio of height/width during development of SP and FF taste ranged between 0.76 to 1.31 from birth to adult, indicating that the shape for these subpopulations of taste buds was nearly spherically (Fig. 5; height/width). In contrast, the ratios of height/width for CV and FL taste buds was 2.4 and 1.9, respectively, at 1 week of age, showing that the taste buds were elongated along with their long axis to the apical surface. The elongation rapidly decreased with increase in age until 4 weeks of age. Subsequent to maturation, the height/width ratio remained 1.8, indicating that CV and FL taste buds remained slender (Fig. 5; height/width).

Fig. 2. Developmental changes in the number of taste buds located within the soft palate (SP), fungiform papillae (FF), circumvallate (CV), and foliate papillae (FL). The numbers indicated for the FL per animal were twice that observed on one side. Data are from three animals for each age. Error bars depict SD.
Fig. 3. Taste buds on the soft palate (A), fungiform papillae (B), sections of the circumvallate (C), and foliate (D) papillae in the rat at Day 0. Note a taste pore shown by an arrow. No taste buds were observed in C and D. Vertical bars indicate 10 μm in A and B, 40 μm in C and D.
3.3. Development of CV and FL

The depth of the groove of the CV papilla rapidly increased until 3 weeks of age, and reached a steady level of \(434.3 \pm 33.1\ \mu m\), while that for the FL rather slowly increased until 4 weeks of age reaching \(264.0 \pm 22.9\ \mu m\) (Fig. 6). The size of the CV papillae increased continuously until 9 weeks of age, while the increase in the size of FL papillae ceased at 3 weeks of age (Fig. 6).

4. Discussion

At birth, more than a hundred FF and SP taste buds existed in the rat. The functional maturation of SP taste buds preceded that of FF taste buds, in that at birth 53% of SP taste buds compared to only 12% FF taste buds possessed a taste pore. At 1 week of age, 90% of SP taste buds contained a taste pore, indicating that maturation was almost complete. A similar maturation of FF taste buds occurred weeks later. In a scanning electron microscopical study, 72% of 12-day-old rat FF taste buds possessed a pore, whereas only one taste pore was observed in 1-day-old rats [8]. Also, in the hamster, 39% of SP taste buds possessed a taste pore at 1 day of age [6].

Although numerous taste buds were observed on the rat SP, it was previously reported that no taste buds were found on the SP of newborn rats [9]. This discrepancy between our results and those by Srivastava and Vyas [9] may depend on the difference between the methods for counting the number of taste buds. In our experiment, careful attention was paid to distinguishing a taste pore by observing the sequence of sections for one taste bud, which made it possible to find even small immature taste buds.

Taste buds in the nasoincisorduct (NID) were also analyzed. At birth, only four to seven taste buds occurred within the NID, and only one taste bud with a pore was observed. Also, it was slightly harder to measure the height and width of each taste bud than those of taste buds in other areas at later ages because of the difficulty to set the tissue block in an appropriate orientation. Therefore, the data for...
taste buds within the NID were not compared in the present experiment.

The development of rat taste buds within the CV and FL was delayed compared to SP and FF taste buds. The number of CV and FL taste buds at birth were few, but they continuously increased over the next 9 weeks. At birth, no taste pores were observed in CV and FL taste buds. Similar results were shown previously in the rat, where taste buds were not observed at birth and reached a maximum of 610 ± 87 taste buds with a pore in the CV at 90 days [7]. In rat FL taste buds, it was reported that pores were observed at 10 days of postnatal age [11]. Also, in the hamster, few CV and FL taste buds were observed at birth; however, 121 FL taste buds were observed at 71 days of age, and 150 FL taste buds were reported at 120 days [12].

A comparison of height and width of taste buds among the four subpopulations revealed that the shape of SP and FF taste buds was approximately spherical, while CV and FL taste buds were more slender and elongated. Similar results were obtained by recalculation of previously published data [7], in which the rate of height/width for CV taste buds was 1.6–1.8. The reason for the elongation of CV and FL taste buds cannot be explained due to a high density of taste buds occurring within these two papillae, because the rate of elongation was greatest at 1 week where less than a hundred taste buds existed, and elongation decreased and ceased at 4 weeks, during which the number of taste buds were a few hundreds and continuously increased. Despite the difference between the development of the CV and FL papillae, the curves for the ratio of height/width and ages were similar.

Although there are significant differences in the age at which the different types of taste buds occurred during development and in their shapes, there were no significant differences in the volume of the taste buds until 4 weeks of age. This similarity of the increasing volume among different types of taste buds may be due to the increasing size of the cells comprising the taste buds during development.

Among these four subpopulations in the rat, SP taste buds are innervated by the greater superficial petrosal nerve (GSP), a branch of the facial nerve.

Electrophysiological recordings in adult rats and hamsters revealed that stimulation of the SP with sweet substances produced robust neural responses in the GSP [13–15]. Behavioral experiments indicated that the GSP was important in the perception of sucrose taste by showing that rats with transection of the GSP alone or with a combined
transection of both the GSP and the CT exhibited a significant decrease in the mean lick ratio to sucrose [16]. The GSP in the hamster was shown by a conditioned taste aversion paradigm to also contribute greatly to the taste of a sucrose [17]. The degree of the conditioned taste aversion to 0.1 M sucrose significantly decreased when the GSP and/or the CT were sectioned bilaterally. Also, it is suggested that the spatial distribution of palatal taste buds are functionally important for suckling behavior in preweanling animals because palatal taste buds are stimulated by the milk spouting out from the nipple much more than the taste buds on the tongue [18,19]. These neurophysiological and behavioral data, along with the present results, suggest that the GSP plays an important role in mediating taste information for nutrients, and that it functions during early postnatal development in rodents.

Acknowledgments

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References