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Scanning Electron Microscopic Study of the Tongue Papillae Formation

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Objectives: To clarify the morphology of the tongue papillae formation and the metabolic characteristics along the papillae distribution in rats. **Methods:** The experiment was designed as a cross-sectional study and included; a scanning electron and light microscopic examination, connective tissue core analysis, and an enzyme activity test on rat tongue samples of the embryonic (n = 60) and postnatal (n = 12) periods. **Results:** The primordium of fungiform papillae (FuP) was already formed on embryonic day 15 (E15), and filiform papillae (FiP) was formed on E17. In the postnatal period, the middle and posterior parts of the tongue quickly developed toward the anterior part of the oral cavity. Three types of FiP were found: round, conical and branched. According to the enzyme activity test, all energy, lipid, protein and sugar metabolic enzymes showed significant differences along the tongue parts. **Conclusion:** No positional difference was found in the morphology of rat tongue papillae during the embryonic period. During the postnatal period, the morphology and enzyme activities of the tongue mucosa displayed differences expressing tongue development and its functional characteristics among the described tongue parts—listed above.

Keywords: Filifrom Papillae, Fungifrom Papillae, Connective Tissue core, Enzyme Activation

Introduction

The tongue is a muscular organ whose surface is covered by a tightly adherent mucosa. The mucosa of the tongue is specific because four different types of tongue papillae: filiform, fungiform, circumvallate, and foliate are present on the dorsal mucosa of the tongue body [1]. The morphology of each type of tongue papillae is correlated with feeding behavior, functions of tongue muscles, activity of saliva glands, and other environmental factors [2]. In mammals and reptiles, the tongue is a very important organ in mastication, swallowing and phonation which are indispensable for living. Accordingly, it is very important to understand the functional potential of each type of tongue papillae as an index of the tongue's entire

function. In our previous study, we clarified the morphology and distribution of filiform and fungiform papillae of the human tongue [3].

The human tongue papillae progressively receives structural degenerations and functional failures as a result of ageing. Many elderly persons suffer from different kinds of tongue disorders such as; geographic tongue, yin-deficient tongue, atrophic change of papillae, weakness of connective tissue cores of papillae, and dry mouth which is often accompanied with a disordered papillae [4-8]. These disorders are sometime lethal for various functions of the tongue and can negatively affect quality of life due to disorders in mastication, swallowing and phonation. Therefore, keeping the tongue papillae healthy is an essential part of good oral care.

Current study focused on clarifying the morphology of tongue papillae formation and the metabolic characteristics of papillae distribution in rats.

Materials and Methods

We used the design of a cross-sectional study. Pregnant Sprague Dawley rats (SD, female, 9 weeks, SPF/VAF) were purchased from Charles River (Yokohama, Japan). The experiment included; four examinations using tongue samples (embryonic n = 60 and postnatal n = 12), a scanning electron microscopic and light microscopic examination, connective tissue core analysis, and an enzyme activity/APIZYM test. All animal experiments were conducted in accordance with institutional and national guidelines.

1. Tongue samples

1) Embryonic samples (n = 60): pregnant SD rats (n = 4) were deeply anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital (Somnopentyl®, Kyoritsu Pharm, Nara, Japan) and the anesthetized embryos were removed. The embryonic tongues were wholly removed under the stereoscopic microscope (Olympus 223033, Tokyo, Japan) in the embryonic 15, 17, 19, 21 days (each n = 15) then transferred to the scanning electron and light microscopic examinations.

2) Postnatal samples (n = 12): rats of the postnatal 0, 4, 8, 12, 16 days and 8 weeks (each n = 2) were anesthetized, and their entire tongues were removed. The rat tongue is divided into the body and root with a single circumvallate papilla on its boundary. The root of tongue was excluded from the examination because of having no papilla. The body of the rat tongue has an intermolar eminence in the mid-portion of its dorsal surface. Accordingly, we divided the body of the tongue into the anterior, middle and posterior parts for use during the scanning electron and light microscopic examinations.

2. Scanning electron microscopy (SEM)

The embryonic (n = 60) and postnatal (n = 12) tongue samples were fixed in a 2.5% glutaraldehyde in phosphate-buffered saline solution (PBS) (pH 7.4) for 4 hours at 4°C then washed in PBS for 4 hours. The samples were post-fixed in 2% OsO4 solution (Merck, Darmstadt, Germany) in PBS for 1 hour at 4°C then dehydrated in a graded series of ethanol (70-100%) twice each for 15 minutes. Samples where then immersed in a mixture of ethanol and isoamyl acetate 1:1 (Sigma-Aldrich, Tokyo, Japan) and in isoamyl acetate for each 30 minutes. The samples were dried at a critical point using CO2 (Critical Point Dryer Hitachi HCP-2, Tokyo, Japan) then coated with gold of 200 Å thickness (Ion Coater IB-3, Ibaragi, Japan). Samples were examined using a SEM (S-800, Hitachi, Tokyo, Japan) at an accelerating voltage of 10-15 kV. After taking the scanning electron micrographs, the length and width of each papillae was measured using RS Image express software (Roper Scientific, Martinsried, Germany).

3. Light microscopy (LM)

The embryonic and the postnatal tongue samples were fixed in a 4% formaldehyde solution in PBS for 4 hours at 4°C, then washed in PBS for 4 hours to be dehydrated in a graded series of ethanol 70-100% for 1 hour. Samples were treated with: 1) xylene I and II [each] for 30 minutes; 2) placed in a mixture of xylene and paraffin (Merck) 1:1 for 1 hour; 3) placed into soft and hard paraffin [each] for 1 hour and finally; 4) embedded in paraffin. The embedded samples were consecutively cut into 4 µm sections using a microtome (Microm, Walldorf, Germany) and stained with Hematoxylin and Eosin. The stained sections were examined using a light microscope (Olympus BX51, Tokyo, Japan).

4. Connective tissue core (CTC) analysis of the rat tongue papillae

The fresh tongue samples of adult rats (8 week, n = 3) were immersed in 3.5 N HCl for 2 weeks at room temperature. After immersion, the epithelium was detached from the underlying

CTCs at the boundary of the epithelium-connective tissue layer. The samples were then washed with tap water and post-fixed in a 2% OsO4 solution in PBS for 1 hour. Afterward, they were washed and dehydrated using a graded series of ethanol. After dehydration, samples were dried using CO2 and coated with gold of a 200 Å thicknesses before the examination with SEM.

5. Enzyme activity/APIZYM test

The tongues of postnatal rats (n = 2) were divided into three parts: anterior, middle, and posterior, and the mucosa of each part was homogenized (Biotron, Wollerau, Switzerland) in PBS of 1.3 ml. The prepared mucosa samples were conducted by means of the enzyme activity/APIZYM test (bioM`erieux, Marcy l'Etoile, France). It contained a set of 20 microtubes in which besides the control microtube, the remaining 19 microtubes correspond to the following 19 enzymes, respectively: alkaline phosphatase, esterase, esterase lipase, lipase, leucine amino peptidase, valine amino peptidase, cystine amino peptidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl β-glucosaminidase, α-mannosidase and α-fucosidase. Sixty-five µl of the prepared mucosa samples were dispensed to each micotube. After incubation at 37°C for 4 hours, 35 µl ZYM A reagent (Tris-hydroxymethylaminomethane, hydrochloric acid 37%, sodium lauryl sulfate, H2O) and 30 µl of ZYM B reagent (Fast Blue BB, 2-methoxyethanol) were added to develop a positive color reaction. After exposure to sun-light and fluorescent light [each] for 5 minutes, enzyme activities were measured using a spectrophotometer (NanoDrop 1000, Thermo Fisher Scientific, Chelmsford, MA, USA) based on the color reaction.

6. Statistical analysis

Structural data were automatically produced using RS Image software (Media Cybernetics, Maryland 20910 USA). For quantitative data: mean, standard deviations and ANOVA with multiple comparisons were calculated using an SPSS 19 program (version 19). Statistical significance was considered as $p<0.05$.

Results

1. Development of the tongue papillae in the embryonic period

The primordia of fungiform papillae (FuP) were observed on embryonic day 15 (E15) (Fig.1-E15-SEM). Similarly to other ectodermal appendages the epithelial layer became thick and its outer surface became convex as the underlying mesenchymal cells migrated upward, suggesting the development of FuP (Fig.1-E15-HE). The primordia of filiform papillae (FiP) were also visible at E15 (Fig.1-E15-SEM).

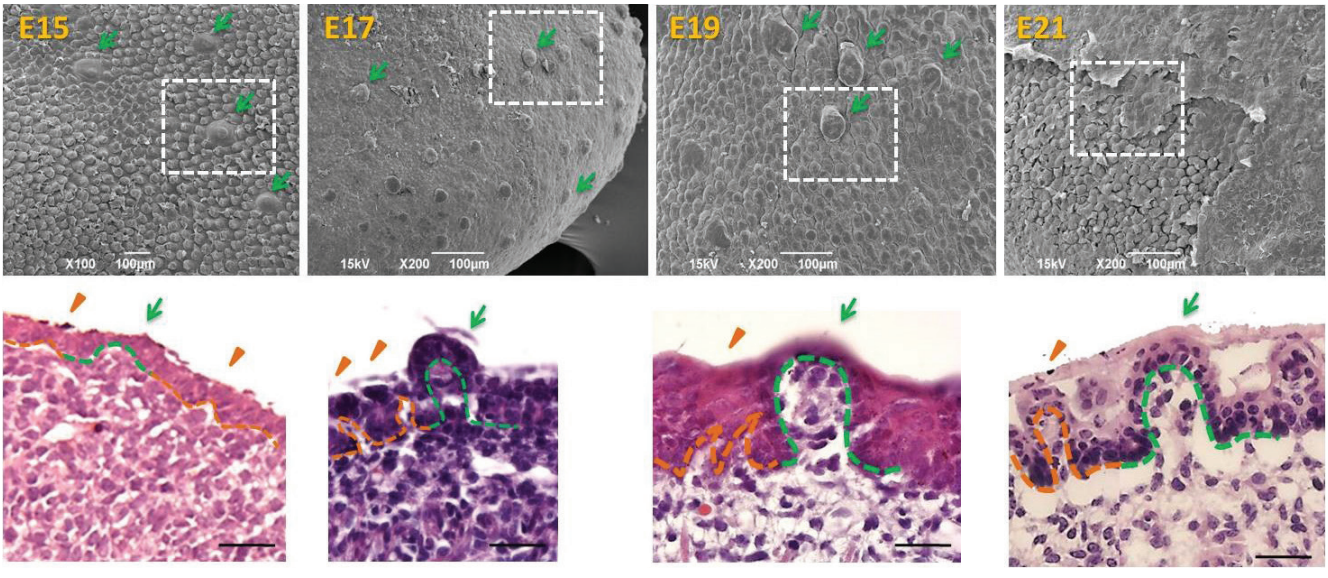

Figure 1. Development of the rat tongue papillae in the embryonic period

Upper : SEM (bar, 100 µm); Lower : HE (bar, 50 µm); Arrows : FuP; Arrowheads : FiP
The primordia of FuP were seen at E15. At E17 and E19, CTCs of FuP and FiP developed to be inserted into the epithelial thickening. At E21, resulting from detachment of periderm, the underlying FuP and FiP appeared in the surface of tongue.

At E17, the mesenchymal cells moved further upward to create the well-developed connective tissue core (CTC) of FuP. The thin CTCs of FiP also moved into the epithelial layer (Fig.1-E17-HE). The tongue was covered by a corneous squamous epithelial layer called the periderm as a result of the high turnover activity of superficial epithelial cells,. The FuP formed a dome-like structure and the primordia of FiP were now hidden by the periderm (Fig.1-E17-SEM and HE).

At E19, the CTCs of FuP and FiP increased in height and deeply inserted into the epithelial thickening (Fig.1-E19-HE).

At E21, due to the detachment of the periderm, the underlying FuP and FiP appeared on the surface of the tongue (Fig.1-E21-SEM). Before birth, both FuP and FiP become well developed (Fig.1-E21-HE), but no positional differences were found in their morphology.

2. Development of the tongue papillae in the postnatal period

The three parts of the rat tongue [samples] showed different rates of development during the postnatal period (Fig.2A). The middle and posterior parts grew rapidly forward in the oral cavity within 12 days of birth (Figs.2A and 2B). Accordingly, the length ratio compared to anterior/posterior parts significantly decreased until the postnatal 12 day (P12) (Fig.2C). Afterward, the ratio [nearly] remained the same (Fig.2C) because the middle and posterior parts were growing at the same rate as the anterior part.

The FuP did not display any significant differences except the size among the tongue parts (data not shown). However, the FiP did show a positional difference among the three parts during postnatal development.

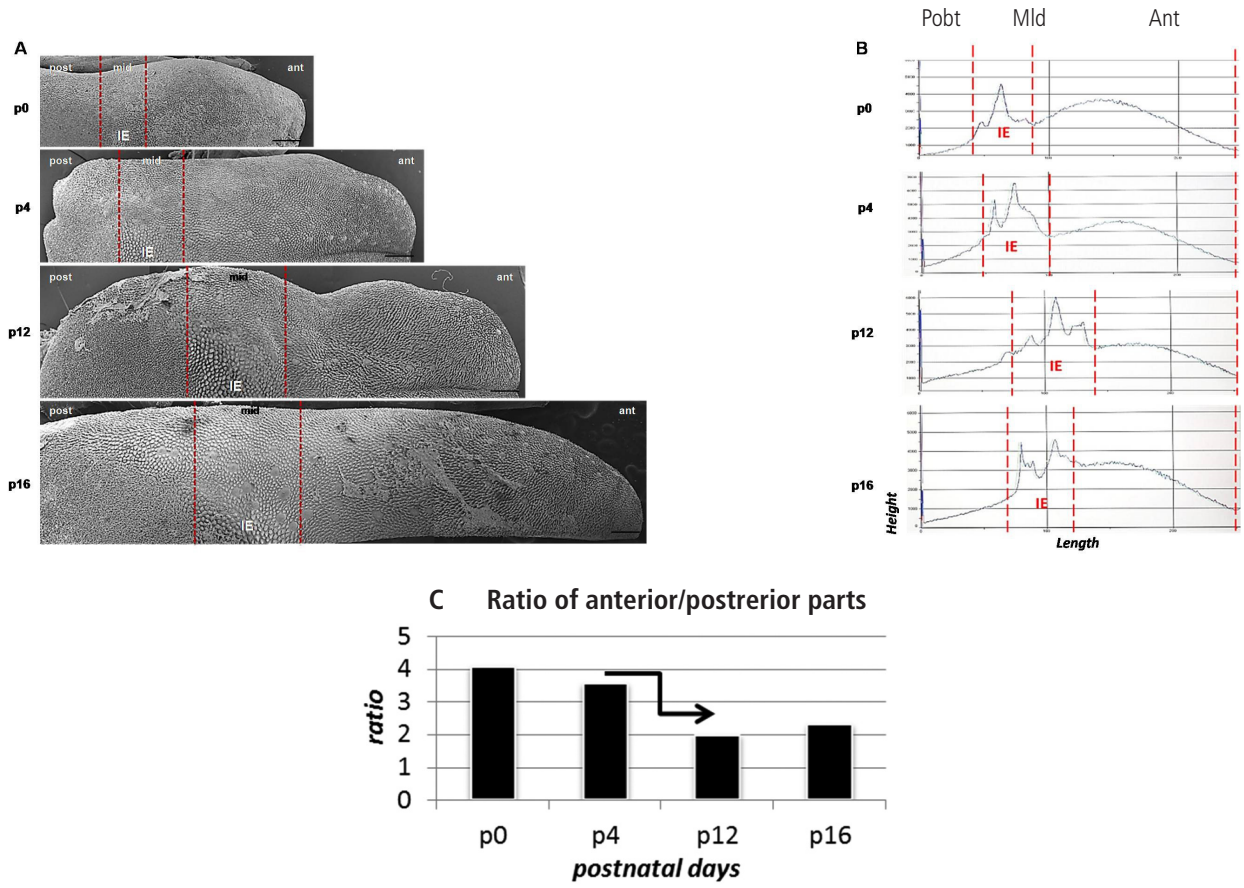

Figure 2. Development of the rat tongue in the postnatal period
A : SEM (bar, 500 μm). Dorsal view of the rat tongue;
B : The ratio of the three tongue parts. The height from the connective tissue layer junction to the tongue surface is shown in the longitudinal axis. The height displayed the peak in the middle part (the intermolar eminence, IE).
C : The ratio of anterior to posterior parts. The ratio significantly decreased up to the postnatal day 12 (P12). After then, it was kept in the approximately constant level.

The FiP of the anterior part increased in length as days passed in contrast to the indefinite change of its width (Figs.3Aa and 3Ab). The longer it became, the more it inclined backward (Fig.3Aa). Within 56 days after birth, the number of FiP per unit square gradually decreased (Fig.3Ac), conceivably resulting from the indefinite change of FiP's width in spite of increasing size of tongue.

The FiP of the middle part largely increased both in width and length within 16 days of birth (Figs.3Ba and 3Bb). Consequently, the density of FiP largely decreased (Fig.3Bc). On and after P16, the FiP inclined forward in contrast to the FiP of the anterior part which inclined backward forming a wide smooth surface on the top (Fig.3Ba, arrows).

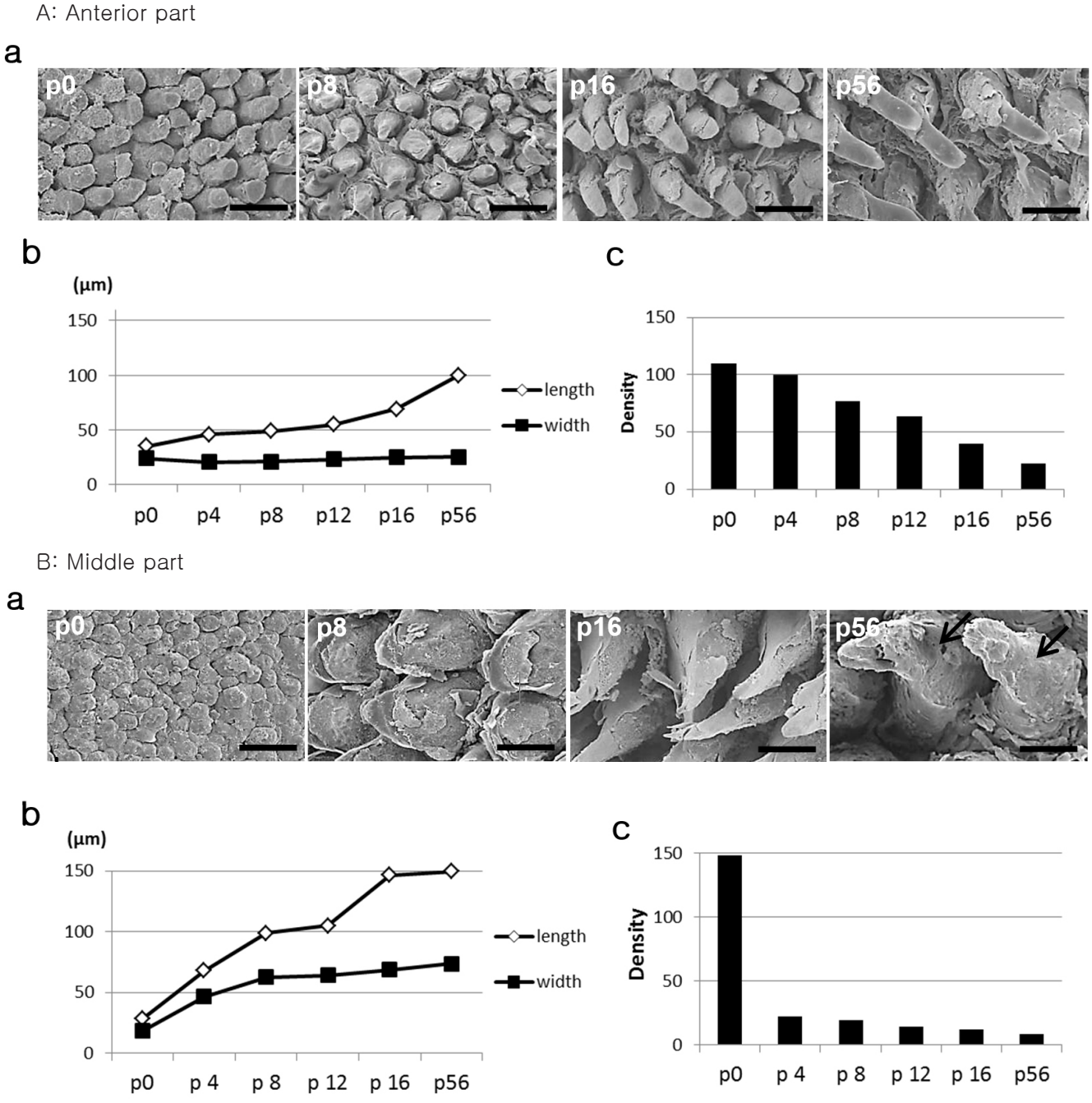

Figure 3. Postnatal development of filiform papillae (FiP) of the rat tongue

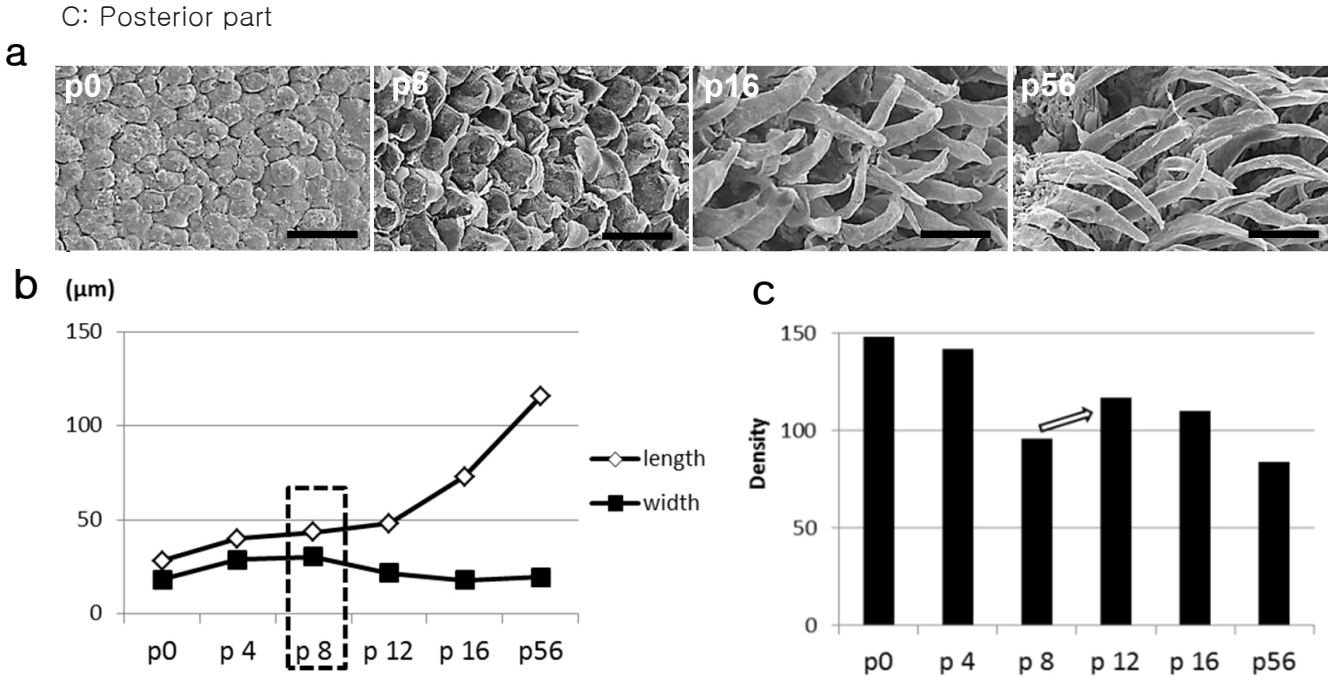

Figure 3. Postnatal development of filiform papillae (FiP) of the rat tongue
a : SEM of FiP (bar, 50 μm); b : Change of length and width of FiP ; c : Change of density of FiP The positional differences among the three parts of tongue were seen in the postnatal development.

Finally, the positional differences of the shape of FiP became clear among the three parts of the tongue in the adult rat of P56 (Figs.3Aa, 3Ba and 3Ca). Figures 4Aa, 4Ba and 4Ca display the general appearances of FiP of the respective parts of the adult rat (8weeks). We named FiPs of the anterior and middle parts as the "round tip FiP" and the "conical FiP", respectively, on the basis of their shape. With regard to the FiP shape of the posterior part, we referred to the findings of connective tissue cores (CTCs, Fig.4Cb) because the shape of CTC was probably reflected in the shape of papilla.

In the FiP of the posterior part, CTC [was observed] to have an eminence-like shape having several long slender protrusions on the top. Accordingly, we named the FiP of the posterior part as the "branched FiP". The length, width and the density of the long slender protrusion of the "branched FiP" was measured (Fig.3C).

3.Connective tissue cores (CTCs) of the rat tongue papillae

In the "round tip FiP" of the anterior part (Fig.4A), CTC had a column-like shape with a hollow on its top (Fig.4Ab). In response to the backward incline of the papilla, the underlying connective tissue layer of the front slope side of the FiP was well developed and extended upward from the basal connective tissue column (Fig.4A, arrows).

The connective tissue layer of the middle part of the tongue was the thickest (Fig.4Bc), and CTC of the "conical FiP" was larger than of the other types of FiP. It had a slender pyramid shape (Fig.4Bb). The underlying connective tissue layer of the back slope side of the FiP was well developed and consistent with the forward incline of the papilla (Fig.4B, arrows). On its front side, CTC was shabby in structure (Fig.4Bb).

In the branched FiP of the posterior part, CTC had an eminence-like shape with several long slender protrusions on the top (Fig. 4Cb).

4. Metabolic analysis of the rat tongue

All enzymes showed meaningful activity in the rat tongue specimens according to the APIZYM test. Moreover, the activities of each enzyme was different among the three tongue parts as follows: 1) Leucine amino peptidase and phospho-amidase phosphatase showed the highest activity in the anterior part; 2) Alkaline phosphatase, esterase, cystine amino peptidase, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrase, and all the sugar

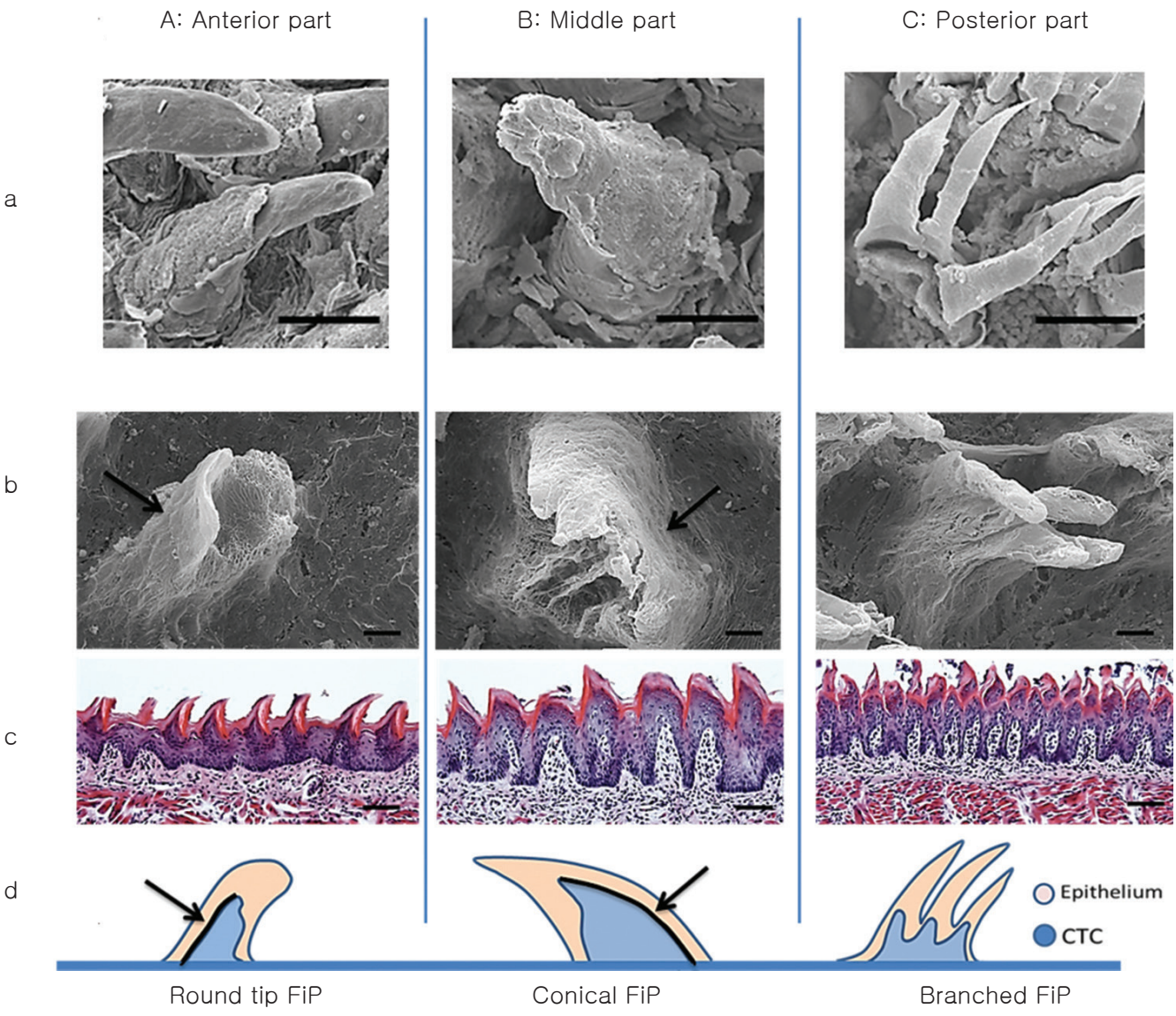

Figure 4. Connective tissue cores (CTCs) of FiP
a : SEM of FiPs (bar, 50 μm); b : SEM of CTC of the FiP in each tongue part (bar, 10 μm);
c : HE staining of the dorsal mucosa in each tongue part (bar, 100 μm); d : Schematic drawing of the FiP shape in each tongue part.
Three types of FiP were found as follows: the round tip FiP in the anterior part, the conical FiP in the middle part, and the branched FiP in the posterior part. The shapes of FiP were conceivably resulted from the shapes of underlying CTCs.

metabolic enzymes showed the highest activity in the middle part and 3) Esterase lipase, lipase, and valine amino peptidase showed the highest activity in the posterior part (Fig.5A).

The 19 enzymes within the range of measurement in the enzyme activity/APIZYM test are classified into four metabolic groups (Figure 5B).

1) The energy group: alkaline phosphatase, phosphoamidase phosphatase and naphthol-AS-BI-phosphohydrase. 2) The lipid group: esterase, esterase lipase and lipase. 3) The protein group: leucine amino peptidase, valine amino peptidase, cystine amino peptidase, trypsin and chymotrypsin. 4) The sugar group: α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl β-glucosaminidase, α-mannosidase and α-fucosidase

All four metabolic groups displayed different activities among the three parts of the tongue. In the anterior part, all groups except the lipid group showed the lowest activity. In contrast, all the energy, lipid, and sugar metabolic groups were

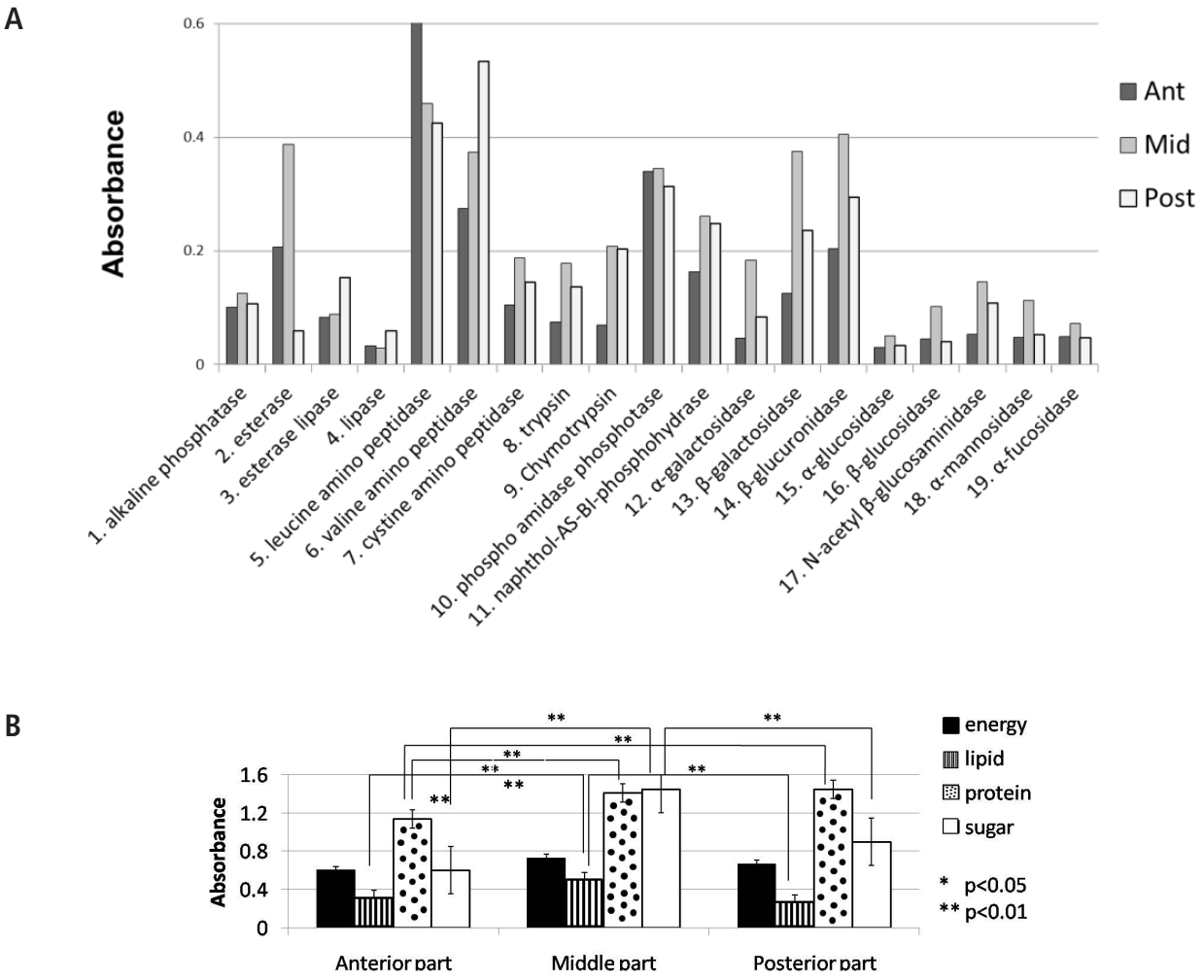

Figure 5. Enzyme activities of the rat tongue mucosa
A : Enzyme activities of mucosa in the three parts of the rat tongue; B : Metabolic conditions of mucosa in each of the three tongue parts.
In the rat tongue, all of the 19 enzymes showed the meaningful values of absorbance (A). Moreover, the values (activities) of each enzymatic group were different among the three parts of tongue (B).

in the highest activity in the middle part. However, the highest activity of the protein group was found in the posterior part (Fig.5B).

Discussion

1. Development of the tongue papillae in the embryonic period

It has been generally recognized in past detailed studies of tongue development and lingual papillae that the primordia of the circumvallate papillae appear first and followed by appearances of the respective primordia of FuP, foliate papillae and FiP [9-12]. It has been described that the homogeneous epithelium first covers the three lingual swellings at E13 [13-15]. At E14, the grooves between the separated swellings disappear to make a smooth surface to form the spatulate tongue. The formation of FuP also starts at E14 followed by the formation of FiP [15]. Therefore, we used rat embryos after E15 in the present study to make clear the formation processes of FuP and FiP.

It has been reported that the first morphological sign of the tongue papillae appears as thickenings of the epithelium and condensation of the underlying mesenchymal cells [16]. At E14, the FuP placodes were formed and arranged in two linear rows on the anterior tongue giving the tongue a distinctive topography

at E15 [13, 15]. During FuP development, the epithelial cells migrate into the primordium and thicken in layer. Before E17, the process-like mesenchyme structures vertically extend into a cornified squamous epithelium having a stereotyped pattern of arrangement [1]. Many other researchers have reported these processes of the FuP formation [13, 15, 17-19].

It was reported that the non-taste, heavily keratinized FiP is not visible until about E20 [14, 20]. There was also a report stating FiP seem to rapidly develop during a few days between E19-E21 [16]. Another opinion reported FiP starts to develop at around E15.5 [1]. This current study clarified that the first formation of FiP was visible by E15, but was hidden for a short time by the periderm. The periderm is a superficial thin sheet of cells covering the epithelium in the early stage [18, 19, 21]. Accordingly, it was suggested that development of FiP was still in progress but located under the periderm. The present study also noted the hidden FiP appeared on the surface of the tongue at E21, resulting from the detachment of the periderm. Several previous studies [9, 10, 16] also reported the FiP becomes visible between E20 to P1 after disappearance of the periderm. The confused opinions of the FiP development conceivably resulted from the above suggestion. However, very close findings were observed in our study as each hour is a very critical period for embryonic development especially in rodents who have a short life cycle. We hope our results contribute to the incontrovertible understanding about the tongue papillae formation of the experimental animals/rats and their beneficial use as research models.

2. Development of the tongue papillae in the postnatal period

Generally in rats, a single circumvallate papilla is formed in the center of the terminal groove and acts as a landmark distinguishing the body from the root of the tongue. The intermolar eminence is visible at the mid-portion of the rat tongue body and enables us to subdivide it into three parts: anterior, middle (intermolar eminence), and posterior [22].

The middle and posterior parts of the rat tongue grew rapidly within 12 days after birth. However, soon after the growth rate gradually decreased to reach the same as in the anterior part. This period is regarded as the stage on which the intermolar eminence reaches maturity and during which the feeding behavior of the rat changes from sucking milk to eating a solid food.

The postnatal FiP displayed the positional difference in morphology as follows: the round tip FiP in the anterior part, the conical FiP in the middle part, and the branched FiP in the posterior part. The round tip FiP of the anterior part tilted backward as it became longer (Figs.3Aa and 4Ad). The conical FiP of the middle part inclined forward and had a wide smooth roof (Figs.3Ba and 4Bd). Such a shape might be suitable for tight contact of the tongue to the hard palate during swallowing (Fig. 6). The branched FiP of the posterior part having several slender hair-like protrusions might be suitable for smooth passage of solid food during swallowing because of forming the cushion-like structure with keratinized surface (Fig.6).

In mice, the FiP is classified into two subtypes depending on the shape of its tip. Each subtype is known to have a different localization: the FiP with pointed tip are distributed in the intermolar eminence and those with rounded tip are distributed in other regions containing the anterior tongue [23]. In rats, the simple conical papillae, giant conical papillae, and branched papillae are classified and known to have different localization [24, 25]. The simple conical FiP is located in the anterior tongue and its body tilted backward [22]. Although its shape was described as conical, it is conceivably of the same type as the round tip FiP of the anterior part in the present study. The giant conical papillae was observed in the limited crescent region of the intermolar eminence [22]. The branched papillae was observed in the posterior region between the giant conical papillae region anteriorly and the circumvallate papilla posteriorly with tip protrusions further branched into several twigs [22, 24]. The above findings about localizations of the giant conical and branched papillae coincide well with findings of the conical and branched FiP described in the present study.

There are considerable species differences in the dorsal surface configuration of the tongue, especially in size and shape of FiP in mammals [8]. Many kinds of artiodactyl have a large type of FiP with lenticular or conical shape, which is situated on the lingual prominence corresponding to the intermolar eminence [26]. The large FiP of the Cape hyraxes was found on the lingual prominence and progressively reduced in size as tracing more posteriorly [27]. In cats and pigs, FiP of the posterior tongue is of the simple conical type, while FiP of the anterior tongue had hair-like protrusions in its top [28, 29]. The similar observations were obtained also in the rat tongue [24]. The dorsal surface of the

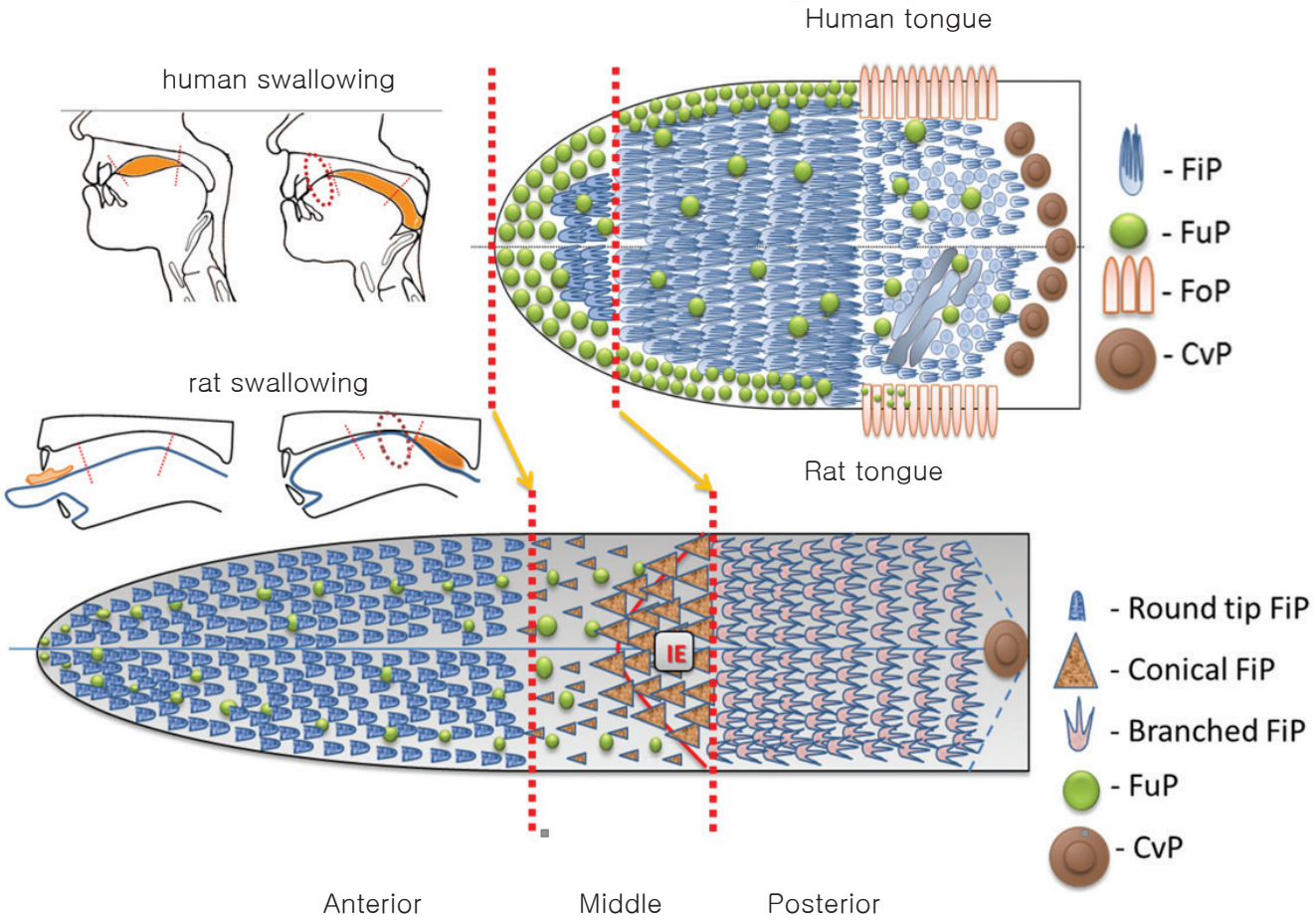

Figure 6. Comparison of the human and rat tongues
The morphology of the conical FiP of the middle part of the rat tongue was similar with those of the FuP located in the tip of human tongue. The branched FiP of the posterior part of the rat tongue was similar in morphology with the FiP located throughout the central dorsum of human tongue.

baboon tongue is covered with numerous FiPs that have several hair-like protrusions in its top similar to the human tongue but lacks of a distinct tilt of protrusions as found in rodents [30]. The morphological variations of the tongue and lingual papillae might have resulted from the different environments in which the mammals live, such as aquatic, or terrestrial and food eating habits [31-34]. Mechanical stress might be weakened by the surface configuration of the tongue dorsum as described in the human tongue [8]. The conical FiP tilt backward, as observed in rodents, may be useful for prompt transport of food before swallowing. In humans, the well developed protrusions of FiP provide the tongue with a mildly rough surface, which may be useful for the careful handling of food before swallowing [8]. In the suckling mouse, the FiP may be an important structure for weakening the mechanical stress during drinking milk [35, 36].

Human FuP and FiP were examined in our previous study (Fig.6) [3]. The FuP mainly gathered in the free margin of the dorsum of tongue. The FuP in the tip of tongue was long and had a wide and smooth surface in its top to enable the tip to tightly touch the hard palate. The FiPs with several well-developed hair-like protrusions in its top were mainly distributed in the central area of the dorsum of tongue. The well-developed protrusions may act as a cushion structure to protect the tongue surface from the mechanical stresses when receiving food masses during mastication as discussed above. It was reported that the well-developed protrusions of human FiP made the tongue surface mildly rough, creating a cushion-like structure. Comparing the human tongue with the rat tongue (Fig.6), it was also shown that the conical FiP of the middle part of the rat tongue was similar in shape to the FuP located at the tip of the human tongue [8].

The branched FiP of the rat posterior tongue was similar in shape to the FiP located throughout the central dorsum of the human tongue.

As discussed above, although various researchers use different names, the tongue papillae displays a similar external structure that may have the same functional importance as during feeding and swallowing. In other words, the morphologically similar papillae is likely to have an identical function for swallowing but have a distributional difference depending on the feeding characteristics of the individual animal. Also, the morphological variations of tongue papillae might be intended to harmonize in each phase of swallowing. Actually, if the papillae intact structure has an essential role for swallowing then in the clinical field it should be very important to maintain a quality of life by caring for and improving oral care.

3. Connective tissue cores (CTCs) of the rat tongue papillae

It is well known that the proliferation capacity and the structural formation in various mucosae are genetically determined by the connective tissue [37]. It was recently reported that a reciprocal interaction between the tongue's epithelium and underlying mesenchyme play a fundamentally important role in determining the individual position of FuP and its various stages of dependent development [19].

The CTCs of FiP of mice and rat tongues were reported to be conical in shape with a round concavity on the top [38]. The CTCs of FiP of hamsters [a insectivore mole] and the Cape hyrax are conical-shaped with a semicircular concavity on the anterior surface [27, 39, 40]. Though the descriptions are slightly different from each other, their shapes are similar to the CTC shape of the round tip FiP observed in the present study. However, it was also observed that the underlying connective tissue layer of the front slope side of the FiP developed to extend upward from the base of CTC in relation to the backward incline of papilla. Morphological modification of the underlying CTC in relation to the incline of papilla has not been reported in previous literature.

It was reported that the large conical FiP situated on the intermolar eminence in Japanese serow and Cape hyrax include the CTC with numerous small spines or small rod-shaped processes in its surfaces [41, 42]. It was also reported, that the CTC of a large conical FiP consists of three to four bundles of thick main core accompanied by numerous small rod-like cores [43]. Also, from observations of herbivorous animals, the CTC of the large conical FiP is suggested to have several secondary protrusions [41]. In contrast, with carnivorous animals, the CTC of the large conical FiP has only one well-developed main process [44, 45]. In the present study, the CTC of the rat conical FiP was the extreme type having a big core without any protrusions, in which the connective tissue layer developed well on the back slope side but poorly developed on the opposite front side in relation to the forward incline of papilla.

In contrast to the large FiP, CTC of the branched type of FiP showed nearly the same shape among different animals. In the treeshrew, crab-eating monkey and humans, the FiP has a columnar basal CTC with some rod shaped protrusions on the top, whose number and size are different among the three species [46]. According to our previous study, CTC of the human FiP consisted of a columnar base and numerous short rod-shaped secondary protrusions mainly extending from its upper surface [3]. The CTC of Mandrill FiP was fundamentally similar to that of human FiP; however, its base was narrower [43, 46]. The above CTC shape of branched FiP was similar to that of the rat branched FiP observed in the present study. Mainly, the CTCs of the morphologically similar papillae among the different kinds of animals showed similar architecture proving papillae variations must be tightly linked with the functional characteristics of the individual tongue.

4. Metabolic analysis of the rat tongue

According to the enzyme activity analysis [APIZYM test], enzyme activities were different among the three parts of the rat tongue [47]. These differences may have resulted from the developmental and functional characteristics of each part of tongue. In the present study, we considered that enzyme activities of protein metabolism may represent the proliferation and differentiation activities of epithelial and mesenchymal cells in the tongue mucosa. In the anterior part of rat tongue, it was suggested that the interpapillar epithelial cells were in high activity of proliferation because there were few changes in the width of FiP (Figs.3Aa and 3Ab) in spite of the definite decrease of its density (Fig.3Ac). Therefore, the high proliferation activity of interpapillar epithelial cells conceivably caused the relatively high activity of protein metabolic enzymes. On the other hand, the critical differentiation period of the round tip FiP of the anterior part was almost completed, which is the probable reason why the

anterior part showed the lowest activity of protein metabolism among the three parts. In contrast, the differentiation of the conical FiP of the middle part and the branched FiP of the posterior part were in progress and in pre-progress, respectively, and also the both parts were in a rapid rate of growth on the postnatal 6 day (Figs.2A and 2B), which is the probable reason why activities of protein metabolism in the both parts showed a higher activity than in the anterior part.

It is known that the Odland body corresponding to the keratinosome of fused keratohyalin granules (KHGs) compels the cell organelles to reduce the additional synthesis of extracellular matrix and induce the desquamation [37]. Moreover, the Odland body contains various lipids as its principal components [48]. It is consequently suggested that the keratinization of epithelial cells enhance lipid synthesis and reduces activation of lipid catabolic enzymes. Therefore, we considered the activity of lipid metabolism may be in inverse proportion to the keratinization activity of epithelial cells which is a common criterion demonstrating successful FiP formation. The low activity of lipid metabolism in the anterior part may result from accelerating keratinization of the round tip FiP (Fig.4Ab). The epithelial cells of conical FiP had a low activity of keratinization (Fig.4Bb). Accordingly, the activity of the lipid catabolic enzymes was the highest in the middle part. We speculate that the main mechanism of the branching process of FiP might be the keratinization. The keratinizing process strongly occurs in the posterior part during the differentiation of the branched FiP (Fig.4Cb). Consistently, the lipid catabolic enzymes had the lowest activity among the three parts. In this study, however we tried to determine enzymatic activities of the tongue mucosa and its significance, the further studies are necessary for a clear understanding.

Conflict of Interest

The authors state no conflict of interest.

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