Differentiation of the Lingual and Palatal Gustatory Epithelium of the Rat as Revealed by Immunohistochemistry of α -Gustducin*

Ashraf EL-SHARABY, Katsura UEDA and Satoshi WAKISAKA

Department of Oral Anatomy and Developmental Biology, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan

Received July 3, 2001

Summary. We used α -gustducin, a taste-cell-specific G protein to investigate the onset of taste transduction and its relation to the development of the palatal and lingual taste buds. Frozen cryostat and paraffin sections were prepared from the palatal and lingual gustatory epithelium of the rat from birth till postnatal day 21 (PN 21d). At PN 1-7d, α -gustducin-immunoreactive solitary ovoid or bipolar cells were scattered among the oral epithelium either horizontally along the oral surface or vertically oriented between the basal lamina and oral surface. In the circumvallate and foliate papillae, these cells became wrapped in α -gustducin-immunonegative cells surrounded by an extracellular space forming a bud-like structure. Simultaneously, different stages of typical taste buds were recognized, but α -gustducin was only expressed in some neonatally developed pored buds. At PN 1d, α -gustducin was expressed in pored taste buds with a relatively higher frequency recorded in the soft palate as compared with the nasoincisor, circumvallate, and foliate papillae. The immunoreactive cells were spindle shaped with elongated processes extending from the base to the pore of the taste buds. During the second week, the solitary cells could no longer be recognized while the total counts of immunoreactive cells within the taste buds gradually increased. We argue that taste transduction is essentially required from the time of birth and can be fulfilled by both of the solitary chemosensory cells, which are immunoreactive for α -gustducin and scattered in the oral epithelium, and the taste cells within the mature taste buds. Moreover, the onset of taste transduction accomplished by the palatal taste buds developed earlier than that achieved by taste buds in the circumvallate and foliate papillae.

Taste perception is a critical sense for basic food appraisal and confers the organism with valuable discriminatory power. In mammals, it is an oral chemical sense actuated by the receptor cells congregated within different subpopulations of taste buds, which become functionally mature a few days after birth (FARBMAN, 1965 a,b; MISTRETTA, 1972; MISTRETTA and BRADLEY, 1977; MILLER et al., 1978; HOSELEY and OAKLEY, 1987; FARBMAN and MBIENE, 1991; WITT and REUTTER, 1996; HARADA et al., 2000). Electrophysiological studies on the peripheral taste fibers have revealed that taste receptor cells might selectively respond to the basic five taste stimuli (i.e. sweet, bitter, sour, salty and umami) and exhibit characteristic gustatory responses. In rats, the greater superficial petrosal nerve innervating the palatal taste buds is highly sensitive to sweet-tasting stimuli, the chorda tympani which innervates the taste buds of fungiform papillae is more sensitive to salts and acids, while the glossopharyngeal nerve fibers innervating the taste buds in the foliate papillae and circumvallate papilla respond more to bitter and acid-tasting stimuli (FRANK et al., 1983; NEJAD, 1986; AKABAS et al., 1988; FRANK, 1991; BERNHARDT et al., 1996; HARADA et al., 1997). In the last decade, these studies were strongly promoted by the discovery of α -gustducin, the α subunit of trimeric G-protein complex, which was cloned from the rat tongue tissue (McLAUGHLIN et al., 1992). Alpha-gustducin is believed to be a reliable marker for chemosensitive cells and to be primarily expressed by mature light cells, i.e. type II cells (BOUGHTER et al., 1997; YANG et al., 2000). Moreover, it is substantially expressed by

*This study was partly supported by a Grant-in-Aid of JSPS Postdoctoral Fellowship for Foreign Researcher to A.E.-S.

solitary cells in the epithelia of pancreatic ducts and the gut, and there is considerable evidence that these cells are also chemosensitive (HOFER and DRENCKHAHN, 1996; HOFER et al., 1996). Eventually, α -gustducin has come to be considered a principal mediator in sweet- and bitter-taste transduction that is selectively expressed by their cells scattered within the palatal, circumvallate, and foliate taste buds (WONG et al., 1996; BOUGHTER et al., 1997). SBARBATI et al. (1999) clarified the existence of solitary α -gustducin-immunoreactive cells in the circumvallate papilla of the neonatal rats and suggested a possible role in taste perception rather than their relevance to the early differentiation of the chemoreceptive system. This presumption brought to light the fact that mammals may have a peculiar epithelium-nerve organization consisting of a single chemosensory cell contacted by nerves, a characteristic unique to aquatic vertebrates (WHITEAR, 1992). Therefore, more investigations are required to clarify the ontogeny of the gustatory epithelium, especially for that in the newly born animals. The present study aimed to determine the existence of these solitary cells in the oral cavity and clarify their relation to the developing taste buds especially recruited in the transduction of both sweet- and bitter-tasting substances.

MATERIALS AND METHODS

All experiments used for this investigation were reviewed and approved by the Osaka University Graduate School of Dentistry Intramural Use and Care Committee. Sprague-Dawley rats of both sexes and at various postnatal ages from the first postnatal day (PN 1d) till the time of weaning (i.e. 3rd week; PN 21d) were purchased from Nihon Dobutsu, Osaka, Japan. The day of birth was designated as PN 0d, and 3 animals were used for each age group. The animals were deeply anesthetized with chloral hydrate (600 mg/kg body weight, i.p.) and perfused transcardially with 0.02 M phosphate-buffered saline (pH 7.2; PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The heads were removed and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer for 3 day. The tongues were separated at their junctions with the palates, and both the foliate and circumvallate papillae were dissected out. The palates were removed from the transverse terminal ridge caudally to the nasopharyngeal orifice, while the central part rostral to the first antemolar ruga containing nasoincisor papilla was excised. All specimens were transferred in PBS containing 20% sucrose, and tissue blocks were prepared. The naso-

incisor ducts and circumvallate papillae were oriented for sectioning in the transverse plane, while the soft palate and foliate papillae were oriented for cutting parasagittally. Complete serial frozen sections cut at a 10–20 μ m thickness were mounted on poly-L-lysine glass slides. In a parallel protocol, similarly prepared specimens were dissected from additional rats (i.e. two for each age group) and post-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 3 days. The aged specimens, including those at PN 14d, were decalcified with 7.5% ethylene diamine tetraacetic acid (EDTA) for 1–4 weeks at 4°C with continuous shaking and a weekly change. All specimens were dehydrated by ethanol, cleared in xylene, embedded in paraffin, serially cut at an 8–10 μ m thickness and mounted on poly-L-lysine glass slides. The avidin-Biotin-Complex (ABC) technique was applied for light microscopic immunohistochemistry. The paraffin sections were deparaffinized in xylene and rehydrated through a descending series of ethanol and thereafter treated in 0.1 M sodium citrate buffer (pH 6.0) for 10 min in a microwave for antigen retrieval (CATTOR-ETTI et al., 1992). All prepared sections (i.e. frozen and paraffin) were treated with absolute methanol containing 0.3% H_2O_2 for 30 min to block endogenous peroxidase activity. They were then incubated overnight at room temperature with rabbit polyclonal α -gustducin antiserum (1 : 1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA). After a PBS rinse, the sections were incubated with biotinylated swine anti-rabbit IgG (1 : 500; Dako, Copenhagen, Denmark), followed by a PBS rinse and subsequently with the ABC complex (Vector, Burlingame, CA) for 90 min each at room temperature. Peroxidase activity was detected by incubating the slides with 0.05 M Tris-HCl buffer (pH 7.5) containing 0.04% 3,3'-diaminobenzidine trihydrochloride and 0.003% H_2O_2 . Immunoreactions were enhanced with 0.08–0.1% nickel ammonium sulfate. The immunostained sections were counterstained with methyl green, dehydrated with a graded series of ethanol, cleared in xylene, coverslipped with Permount (Fisher Scientific, New Jersey, NJ), and examined with a light microscope.

For numerical analysis, all profiles containing α -gustducin-immunoreactive cells were thoroughly traced in all examined sections, serially reconstructed on schematic drawings of the palatal and lingual structures, and their numbers counted and graphically represented. In each region, twenty taste buds were randomly selected per animal to count α -gustducin-immunoreactive cells (i.e. every immunoreactive cell profile containing a nucleus was counted once). The total counts of immunoreactive cells/taste bud were recorded at different ages in all regions

Fig. 1. α -gustducin in the soft palate at PN 1d (a), nasoincisor papilla at PN 2d (b), foliate papillae at PN 3d (c) and circumvallate papilla at PN 3d (d). Arrowheads in a–c indicate α -gustducin-immunoreactive solitary cells. Inset in d. Higher magnification of α -gustducin-immunoreactive cells in the bud-like structure as indicated by arrowheads. a, b: $\times 450$, c: $\times 230$, d: $\times 200$, inset: $\times 500$

examined and graphically represented.

The preabsorbed primary antibody with an excess amount of antigen ($10 \mu\text{g/ml}$ of diluted primary antibody; Santa Cruz Biotechnology Inc.) was used to examine the specificity of the primary antibody. For immunohistochemical controls, incubation with the biotinylated secondary antibody or ABC complex was omitted. Moreover, sections were incubated with normal rabbit serum instead of the primary antibody. These control experiments resulted in no specific immunoreactions.

RESULTS

α -gustducin-immunoreactive solitary cells in the palatal and lingual epithelium

In PN 1–3d old rats, the immunoreactivity for α -gustducin was demonstrated in all investigated specimens of the nasoincisor papilla, soft palate, foliate and circumvallate papilla, which were clearly discernible. Solitary ovoid or bipolar α -gustducin-immunoreactive cells were scattered among the keratocytes of the oral epithelium. These cells, which often had a short or long process, were either horizontally extended along the oral surface or vertically oriented between the basal lamina and oral surface. In the soft palate, however, these cells were seldom recognized

Fig. 2. α -gustducin in the soft palate at PN 3d (a), nasoincisor papilla at PN 7d (b), foliate papillae at PN 7d (c) and circumvallate papilla at PN 7d (d). An arrowhead in c indicates α -gustducin-immunoreactive solitary cell. a, b: $\times 260$, c: $\times 200$, d: $\times 410$

(Fig. 1a), while they were frequently recorded in the oral surface of the nasoincisor papilla but never in the ductal epithelium (Fig. 1b). In foliate and circumvallate papillae, several solitary α -gustducin-immunoreactive cells having the same characteristics were demonstrated deep in the trench walls situated close to the basal lamina. In the meantime, two to three α -gustducin-immunoreactive cells were congregated together, became wrapped in immuno-negative cells, and were surrounded by an extracellular space forming a bud-like structure. These structures, which were often rounded, deeply situated and had no pore, could be recognized only in the foliate and circumvallate papillae (Fig. 1d). In PN 5–7d old rats, the same morphological architectures were corroborated and revealed with increasing frequency, especially in the foliate and circumvallate papillae (Fig. 2c and d, respectively). By PN 14d, these solitary cells as well as the bud-like structures with α -gustducin immunoreactivity could not longer be recognized.

α -gustducin-immunoreactivity in the palatal, foliate and circumvallate taste buds

In all specimens investigated, different stages of the developing taste buds could be recognized. The immunoreactivity for α -gustducin could not be identified in the non-pored taste buds recorded in all regions examined. In the palate, i.e. soft palate and nasoincisor papilla, the immunoreactive cells were only demonstrated in some of the pored taste buds, while others revealed no reactivity (Fig. 2a and b, respectively). On the other hand, in the lingual papillae such as the circumvallate and foliate papillae, a remarkable immunoreactivity was traced in all pored taste buds, while the non-pored taste buds completely lacked this (Fig. 1c and 2c, Fig. 1d and 2d, respectively). The immunoreactive cells had a similar morphology in all examined taste bud populations; they were spindle shaped with elongated processes extending from the base to the pore of taste buds. Intense

Fig. 3. α -gustducin in the soft palate (a), nasoincisor papilla (b), foliate papilla (c) and circumvallate papilla (d) at PN 21d. All taste buds contain spindle-shaped α -gustducin-immunoreactive cells. a, b: $\times 480$, c, d: $\times 160$

immunoreaction was demonstrated throughout the cytoplasm while the nuclei were negatively stained. At PN 21d, no great differences with regard to age were recorded in the morphologies of the taste buds among the all populations examined (Fig. 3).

The counts of taste buds containing immunoreactivity for α -gustducin as well as the counts of immunoreactive cell per taste bud were calculated at different ages and graphically represented (Fig. 4a and b, respectively). At PN 1d, the soft palate contained a substantially higher number of taste buds containing immunoreactive cells (96.7 ± 7.6 ; mean $\pm$ S.D.) compared with the nasoincisor papilla (1.3 ± 0.6), circumvallate papilla (4.7 ± 1.5) or foliate papil-

lae (5.0 ± 1.0). At the same time, the immunoreactive cells within the taste buds in the soft palate (1.6 ± 0.6) were higher compared to other populations (1.0 ± 0.0 in the nasoincisor papilla, 1.1 ± 0.3 in circumvallate papilla and 1.2 ± 0.4 in foliate papillae). During the first week, the counts of taste buds rapidly increased in all populations examined, with an evidently higher frequency in the soft palate followed by the circumvallate papilla, foliate papillae and nasoincisor papilla, respectively. At PN 7d, the number of taste buds containing α -gustducin-immunoreactive cells were 172.7 ± 7.5 in the soft palate, 83.3 ± 10.0 in the circumvallate papilla, 45.0 ± 5.0 in the foliate papillae and 28.0 ± 3.0 in the nasoincisor papilla. Concur-

Fig. 4 a. Temporal changes in the number of taste buds containing α -gustducin-immunoreactive cells in the soft palate (SP; open circles), nasoincisor papilla (NIP; closed circles), foliate papilla (FP; open squares) and circumvallate papilla (CVP; closed squares). **b.** Temporal changes in the number of α -gustducin-immunoreactive cells in a single taste bud in each gustatory epithelium.

Fig. 5. A schematic drawing of the differentiation of taste buds and its relation to the expression of α -gustducin in the palatal epithelium (a) and foliate and circumvallate papillae (b). Black cells indicate α -gustducin-immunoreactive cells.

rently, the counts of immunoreactive cells per taste bud were 3.7 ± 1.5 in soft palate, 2.8 ± 0.7 in the circumvallate papilla, 2.9 ± 0.9 in the foliate papillae, and 1.8 ± 0.7 in the nasoincisor papilla. By the second week, robust increases in the counts of taste buds were demonstrated in the circumvallate papilla (182.3 ± 7.5), foliate papillae (71.7 ± 7.6) and nasoincisor papilla (52.7 ± 3.5), while a slight decrease was recorded in the soft palate (167.3 ± 9.3). At the same time, the counts of immunoreactive cells per taste bud were 5.5 ± 1.6 in the soft palate, 5.2 ± 1.1 in the circumvallate papilla, 5.1 ± 1.4 in the foliate papillae, and 6.1 ± 1.7 in the nasoincisor papilla. At the end of our investigation, the same pattern of increase was traced with the highest frequencies recorded in the circumvallate papilla, soft palate, foliate papillae and nasoincisor papilla, in that order.

Figure 5 summarizes the expression of α -gustducin in the solitary and taste cells and its relation to the development of the taste buds in the palatal (Fig. 5a) and lingual epithelium (Fig. 5b).

DISCUSSION

α -gustducin immunoreactivity and the onset of sweet- and bitter-taste transduction

Aquatic vertebrates have, in addition to the olfactory and gustatory system, a peculiar epithelium-nerve unit known as solitary chemosensory cells, which serves in feeding and the exploration of chemicals (WHITEAR, 1992). Morphological observations have indicated that the taste perception in mammals is accomplished in the oral cavity by the taste buds, which become functionally mature few days after birth. Moreover, there is a general consensus that the development of a pore in a taste bud is the most authoritative feature required for their maturation (MILLER et al., 1978; HOSELEY and OAKLEY, 1987; FARBMAN and MBIENE, 1991; WITT and REUTTER, 1996; HARADA et al., 2000). In these studies, conclusions were evidently elicited after the application of only conventional staining techniques. Recently, α -gustducin has proved not only an indisputable immunoreactivity with mature light cells but also a rather specific implication in both sweet- and bitter-taste transduction (WONG et al., 1996; BOUGHTER et al., 1997; YANG et al., 2000). In the present report, we traced the existence of α -gustducin-immunoreactive cells among the gustatory epithelium of the newly born rats. These cells were either solitary or within the pored taste buds. The solitary immunoreactive cells could be frequently recorded in the circumvallate papilla, foliate papillae and nasoincisor papilla, but were rare in the soft palate. Subsequently, these cells transiently developed into bud-like structures in the foliate and circumvallate papillae. It is conceivable that these cells are chemosensory and therefore the whole gustatory epithelium including the tongue can potentially participate in taste perception from birth. This is in agreement with SBARBATI et al. (1999) who found cells having the same characteristics in the circumvallate papilla and suggested that they were chemosensitive.

Physiological studies have shown that the palatal gustatory epithelium of the rat is highly sensitive to sweeteners (NEJAD, 1986; TRAVERS and NORNGREN, 1991; HARADA et al., 1997), while the circumvallate and foliate epithelium are more responsive to bitter tasting substances (AKABAS et al., 1988; FRANK, 1991). In the neonatal rats, we noticed that the counts of taste buds containing α -gustducin-immunoreactive cells as well as the number of immunoreactive cells per taste bud were evidently higher in the soft palate and nasoincisor papilla when compared with those in the foliate and circumvallate papillae. By the second

week, the total counts of taste buds containing immunoreactive cells within the foliate and circumvallate papillae exceeded those within the palatal epithelium. Therefore, we can presume that the taste buds in the soft palate patronized by solitary α -gustducin-immunoreactive cells in the nasoincisor papilla can achieve a substantially earlier responsiveness to sweet substances in neonatal rats. Likely, this correlates with finding by HALL and BRYAN (1981) that rats as young as PN 1-3d can differentially respond to oral infusions of sucrose. On the other hand, the sensitivity to bitter tasting substances achieved by solitary immunoreactive cells and taste buds of the foliate and circumvallate papillae could be efficiently triggered toward the end of the second week, and subsequently increased with age.

Differentiation of the mammalian chemoreceptive system

It is commonly believed among morphologists that taste buds should pass two basic morphological stages (i.e. immature or non-pored and mature or pored) to achieve competence for taste transduction. MISTRETTA and BRADLEY (1977) were the first to assert that the existence of elongated, longitudinally oriented cells should be an additional characteristic feature of the mature taste buds. Recently, our previous advert (EL-SHARABY et al., 2001) supported by the present results revealed that the development of pores in the palatal taste buds is insufficient and the subsequent differentiation of mature receptor cells would be rather essential for taste transduction. On the contrary, in the foliate and circumvallate papillae, a substantial number of α -gustducin-immunoreactive cells either solitary or contained in bud-like structures (i.e. without pores) preceded the appearance of pores in the taste buds. Despite these discrepancies, we can reassert the notion that these cells have a significant relevance to taste transduction in suckling rats. Nonetheless, their potential as a precursor for the prospective taste buds is plausible and can not be refused.

According to the sequences of α -gustducin immunoreactivity as illustrated in Figure 5, the differentiation of taste buds seems to differ with respect to the populations examined – the lingual and palatal taste buds. In the palate, a non-pored taste bud developed from a primordial, which was situated at the base of oral epithelium. Subsequently, the bud acquired an access to the oral cavity via a taste pore; however, no immunoreactivity for α -gustducin could be traced within the whole bud. Finally, a typically mature taste bud presumably develops when the

mature receptor cells (i.e. spindle-shaped α -gustducin-immunoreactive cells) can be recognized. These mature taste buds were frequently identified at PN 1d and PN 2d in the gustatory epithelium of the soft palate and nasoincisor papilla, respectively. Simultaneously, solitary α -gustducin-immunoreactive cells were recognized, albeit rarely in the soft palate or only restricted to the oral epithelium of the nasoincisor papilla. Presumably, these cells may not be quietly involved in the differentiation of taste buds but can perform a collaborative gustatory function, especially in the nasoincisor papilla (Fig. 5a). This is supported by our previous findings in the prenatal rats where we could not trace any α -gustducin-immunoreactive cells in either the soft palate or nasoincisor papilla, although substantial development of the taste buds with pores was recorded (EL-SHARABY et al., 2001).

On the other hand, the pattern of α -gustducin-immunoreactivity in the foliate and circumvallate papillae revealed another mechanism for taste bud differentiation (Fig. 5b). At PN 1–7d, a substantial number of solitary α -gustducin-immunoreactive cells appeared in the gustatory epithelium, followed by bud-like structures, which apparently had no pores but contained immunoreactive cells. Despite their extraordinary morphological simulation with the non-pored taste buds, they were conceivably different i.e. these bud-like structures were deeply situated and transiently recorded. With age, a typical mature taste bud comprising a pore and mature receptor cells could be subsequently traced and gradually increased in both the foliate and circumvallate papillae. Meanwhile, neither the solitary α -gustducin-immunoreactive cells nor the bud-like structures could be recorded any longer. In agreement with SBARBATI et al. (1999), these extraordinary α -gustducin-immunoreactive cells should have a crucial role in taste transduction, which seems a requisite in the foliate and circumvallate papillae during the first few days after birth. Moreover, they might be also involved in the early differentiation of taste buds in these regions. This corresponds with the hypothesis that taste buds may be compound sensory organs containing several cell types, one of which may be a solitary chemosensory cell (SBARBATI et al., 1990; OSCULATI and SBARBATI, 1995; FINGER, 1997). However, at this level of our investigation, we cannot discriminate such conflicts in the ontogeny of taste buds within these populations, and further investigations will be required to determine this.

REFERENCES

- AKABAS, M. H., J. DODD and Q. AWQATI: A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. *Science* 242: 1047–1050 (1988).
- BERNHARDT, S. J., M. NAIM, U. ZEHAVI and B. J. LINDEMANN: Changes in IP₃ and cytosolic calcium²⁺ in response to sugars and non-sugars sweeteners of sweet taste in the rat. *J. Physiol.* 490: 325–336 (1996).
- BOUGHTER, J. D., D. W. PUMPLIN, C. YU, R. C. CHRISTY and D. V. SMITH: Differential expression of α -gustducin in taste bud populations of the rat and hamster. *J. Neurosci.* 17: 2852–2858 (1997).
- CATTORETTI, G., M. H. BECKER, G. KEY, M. DUCHROW, C. SCHLUTER, J. GALLE and J. GERDES: Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J. Pathol.* 168: 357–363 (1992).
- EL-SHARABY, A., K. UEDA, K. KURISU and S. WAKISAKA: Development and maturation of taste buds in the palatal epithelium of the rat: A histological and immunohistochemical study. *Anat. Rec.* 263: 260–268 (2001).
- FARBMAN, A. I.: Electron microscope study of the developing taste bud in rat fungiform papilla. *Devel. Biol.* 11: 110–135 (1965a).
- : Fine structure of the taste bud. *J. Ultrastr. Res.* 12: 328–350 (1965b).
- FARBMAN, A. I. and J. P. MBIENE: Early development and innervation of taste bud-bearing papillae on the rat tongue. *J. Comp. Neurol.* 304: 172–186 (1991).
- FINGER, T. E.: Evolution of taste and solitary chemoreceptor cell systems. *Brain Behav. Evol.* 50: 234–243 (1997).
- FRANK, M. E.: Taste-responsive neurons of the glossopharyngeal nerve of the rat. *J. Neurophysiol.* 65: 1452–1463 (1991).
- FRANK, M. E., R. J. CONTRERAS and T. P. HETTINGER: Nerve fiber sensitivities to ionic taste stimuli in chorda tympani of the rat. *J. Neurophysiol.* 50: 941–960 (1983).
- HALL, W. G. and T. E. BRYAN: The ontogeny of feeding in rats. IV. Taste development as measured by intake and behavioral responses to oral infusions of sucrose and quinine. *J. Comp. Physiol. Psychol.* 95: 240–251 (1981).
- HARADA, S., T. YAMAMOTO, K. YAMAGUCHI and Y. KASAHARA: Different characteristics of gustatory responses between the greater superficial petrosal and chorda tympani nerves in the rat. *Chem. Senses* 22: 133–140 (1997).
- HARADA, S., K. YAMAGUCHI, N. KANEMARU and Y. KASAHARA: Maturation of taste buds on the soft palate of the postnatal rat. *Physiol. Behav.* 68: 333–339 (2000).
- HOFER, D. and D. DRENCKHAHN: Cytoskeletal markers allowing discrimination between brush cells and other epithelial cells of the gut including enteroendocrine cells. *Histochem. Cell Biol.* 105: 405–412 (1996).

- HOFER, D., B. PUSCHEL and D. DRENCKHAN:** Taste receptor-like cells in the rat gut identified by expression of α -gustducin. *Proc. Nat. Acad. Sci. USA* 93: 6631-6634 (1996).
- HOSELEY, M. A. and B. OAKLEY:** Development of the vallate papilla and taste buds in rats. *Anat. Rec.* 218: 216-222 (1987).
- MCLAUGHLIN, S. K., P. L. MCKINNON and R. F. MARGOLSKEE:** Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature* 357: 563-569 (1992).
- MILLER, I. J., M. M. GOMEZ and E. H. LUBARSKY:** Distribution of the facial nerve to taste receptors in the rat. *Chem. Senses Flav.* 3: 397-411 (1978).
- MISTRETTA, C. M.:** Topographical and histological study of the developing rat tongue, palate and taste buds. In: (ed. by) J. F. BOSMA: Third symposium on oral sensation and perception. The mouth of the infant. Charles C. Thomas, Springfield, IL. 1972 (p. 163-187).
- MISTRETTA, C. M. and R. M. BRADLEY:** Taste in utero: Theoretical considerations. In: (ed. by) J. M. WEIFFENBACH: Taste and development: the genesis of sweet preference. DHEW Publications No. (NIH) 77-1068, Bethesda, MD, 1977 (p. 51-64).
- NEJAD, M. S.:** The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. *Chem. Senses* 11: 283-293 (1986).
- OSCUлатI, F. and A. SBARBATI:** The frog taste disc: a prototype of the vertebrate gustatory organ. *Prog. Neurobiol.* 46: 351-399 (1995).
- SBARBATI, A., C. ZANCANARO, F. FRANCESCHINI, G. BALERCIA, M. MORRONI and F. OSCULATI:** Characterization of different microenvironments at the surface of the frog's taste organ. *Amer. J. Anat.* 188: 199-211 (1990).
- SBARBATI, A., C. CRESCIMANNO, P. BERNARDI and F. OSCULATI:** α -gustducin-immunoreactive solitary chemosensory cells in the developing chemoreceptorial epithelium of the rat vallate papilla. *Chem. Senses* 24: 469-472 (1999).
- SBARBATI, A., C. CRESCIMANNO, D. BENATI and F. OSCULATI:** Solitary chemosensory cells in the developing chemoreceptorial epithelium of the vallate papilla. *J. Neurocytol.* 27: 631-635 (1999).
- TRAVERS, S. P. and R. NORGREN:** Coding the sweet taste in the nucleus of the solitary tract: differential roles for anterior tongue and nasoincisor duct gustatory receptors in the rat. *J. Neurophysiol.* 65: 1372-1380 (1991).
- WHITEAR, M.:** Solitary chemoreceptor cells. In: (ed. by) T. J. HARA: Fish chemoreception. Elsevier, Amsterdam, 1992 (p. 103-125).
- WITT, M. and K. REUTTER:** Embryonic and early fetal development of human taste buds: a transmission electron microscopical study. *Anat. Rec.* 246: 507-523 (1996).
- WONG, G. T., K. S. GANNON and R. F. MARGOLSKEE:** Transduction of bitter and sweet taste by gustducin. *Nature* 381: 796-1003 (1996).
- YANG, R., S. TABATA, H. H. CROWLEY, R. F. MARGOLSKEE and J. C. KINNAMON:** Ultrastructural localization of gustducin immunoreactivity in microvilli of type II taste cells in the rat. *J. Comp. Neurol.* 425: 139-151 (2000).

Dr. Satoshi WAKISAKA
 Department of Oral Anatomy and
 Developmental Biology
 Osaka University
 Graduate School of Dentistry
 1-8, Yamadaoka, Suita, Osaka
 565-0871 Japan
 Tel: +81-6-6879-2872
 Fax: +81-6-6879-2875
 E-mail: wakisaka@dent.osaka-u.ac.jp

脇坂 聡
 565-0871 吹田市山田丘1-8
 大阪大学大学院歯学研究科
 口腔分化発育情報学講座
 口腔解剖学第一教室