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Chunxiao Zhang · Bruce Oakley

The distribution and origin of keratin 20-containing taste buds in rat and human

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Abstract Sections of tissues containing lingual and extra-lingual taste buds were evaluated with monoclonal antibodies against cytokeratins. In the caudal third of the rat's tongue, keratin 20 immunoreactivity was restricted to taste buds, whereas keratins 7, 8, 18, and 19 were expressed in vallate and foliate taste buds and in cells of salivary ducts that merge with these taste epithelia. Hence, antibodies against keratin 20 most clearly distinguished differentiated taste cells from all other cells. In rat epiglottis, taste buds and isolated bipolar cells were keratin-20-positive. In rat nasopalatine papilla and palate, antibodies against keratin 20 identified Merkel cells, none of which was near to the keratin-20-negative taste buds. Nor were Merkel cells present at epiglottal taste buds or the keratin-20-negative fungiform taste buds or elsewhere in rat tongue. Hence, Merkel cells make no contribution to rat fungiform, epiglottal, nasopalatine, or palatal taste buds. Human and rat keratin-20-positive tissues are reported to be endodermal derivatives with the exception of Merkel cells and luminal urothelial cells. In rats the distribution of keratin-20-positive taste buds was in full agreement with the classical view that the posterior third of the tongue is derived from endoderm (keratin-20-positive taste buds), whereas the anterior two-thirds of the tongue is derived from stomadeal ectoderm (keratin-20-negative taste buds). The equally intense keratin 20 immunoreactivity of human fungiform and vallate taste buds violates this traditional rostro-caudal segregation and suggests that endodermally derived tissues may be present in the tip of the human tongue.

Introduction

Keratins 7, 8, 18, and 19 are associated with simple epithelia [11, 17, 26, 32] and are present in intragemmal cells of taste buds in mice, rats, gerbils, rabbits, and humans [2, 9, 10, 21, 22, 24, 25, 31, 34, 35, 39, 41]. The present investigation was prompted by the observation that human taste buds are strongly keratin-20-immunoreactive [14]. Keratin 20 is the most recently discovered and the most divergent of the 20 cytokeratins [13]. Its rod domain has only a 58% sequence identity with keratin 14 [15]. With the probable exceptions of urothelial umbrella cells and Merkel cells, the keratin 20 expression pattern indicates that keratin-20-positive tissues share an endodermal origin [14, 15]. Cells of human pancreatic ducts express keratin 20 as do many cells lining the gastrointestinal tract, including enterocytes, foveolar epithelial cells, goblet cells, and some columnar cells, but not neuroendocrine or Paneth cells [3, 13, 14]. Sequence data and in situ hybridization indicate that the gastrointestinal system of laboratory rats also express keratin 20 [4, 15, 27].

There is general agreement that adult taste buds of several amphibian species have two types of basal cells: stem basal cells whose daughters replace aged progeny, and Merkel-like basal cells that are synaptically connected to adjacent receptor cells and to sensory axons (reviewed in [29] and [30]). Recent experiments in mammals indicate that both taste cells and Merkel cells originate from local epithelium rather than migrating in from remote sources like the neural crest [12, 16, 33]. In addition to sharing a local origin, both Merkel cells and type III taste receptor cells have dense core vesicles, neuron-specific enolase and keratins 8 and 19 [5, 9, 39, 40]. While this is suggestive, there is little additional evidence linking Merkel cells or Merkel-like cells with mammalian taste buds [18, 30]. Since antibodies against keratin 20 are preferred markers of cutaneous Merkel cells [16], we used them to probe for spatial associations between Merkel cells and taste buds in the rat. We demonstrate several instances where Merkel cells and taste buds must be independent because they occupy different tissue sites.

The specific contributions of stomadeal ectoderm to the developing oropharynx are not precisely known and may, in any event, vary among mammals [20]. We consider whether rostral taste buds might arise from endo-

C. Zhang · B. Oakley (✉)
Department of Biology, 3127 Natural Science Building,
University of Michigan, Ann Arbor, MI 48l09, USA

dermal cells or, as generally believed, might arise from stomodeal ectoderm that slides caudally into the oropharynx [19]. It is convenient that taste buds are distributed from the rostral to caudal recesses of the oropharynx. This distribution allowed us to use keratin 20 immunoreactivity to probe extant taste buds as reflections of the historical merger between oropharyngeal endoderm (keratin-20-positive) and stomadeal ectoderm (keratin-20-negative). The observed restriction of keratin-20-negative taste buds to the anterior two-thirds of the rat tongue is consistent with the traditional ectodermal origin of rostral taste buds. However, we find that keratin 20 expression in human taste buds does not conform to this classical pattern.

Methods

Tissue collection

The Tissue Procurement Core of the University of Michigan's Comprehensive Cancer Center Laboratory provided lingual tissue from two individuals. Fresh frozen biopsies of fungiform and vallate papillae were obtained during surgical removal of a carcinoma of the anterior tongue of a 68-year-old male. Oral radiation therapy had been administered 5 months earlier. We also sampled fungiform and vallate papillae from tongue tissue obtained at an autopsy of a 36-year-old male.

Sprague-Dawley albino rats (Harlan Sprague-Dawley, Indianapolis, Ind., USA) were kept on a 12-h light:12-h dark cycle and housed with ad libitum food and water. For tissue collection, 14 neonatal rats aged 0, 1, 2, 3, 4, 7, and 10 days were euthanized with carbon dioxide gas. Ten adult rats were deeply anesthetized with an i.p. injection of sodium pentobarbital (70 mg/kg body weight). The anesthetized adult rats were perfused intracardially with either 0.9% NaCl solution containing 0.02% sodium heparin and 0.5% procaine hydrochloride or TRIS-buffered mammalian Ringer's solution [23], followed by acid alcohol fixative composed of 70% ethanol and 10% acetic acid.

Denervation of the foliate papillae (unilateral transection of the IXth and chorda tympani nerves) and vallate papilla (bilateral transection of the IXth nerve) was carried out in three rats each. Anesthesia was induced by a mixture of xylazine (Rompun) at 5 mg/kg body weight and Ketamine-HCl at 125 mg/kg body weight. Recovery occurred within 24 h without postoperative difficulties in feeding or drinking. After 3 weeks the six rats were euthanized and perfused as described above. The methods of animal care and euthanasia were approved by the University of Michigan Committee on the Use and Care of Animals.

Normal or partially denervated rat tongues were excised and, along with human lingual tissues, were immersed in acid alcohol fixative, generally for at least 1 day. Tongue tissue was cryoprotected in a 2:1 mixture of 20% sucrose and the embedding compound OCT (Miles Inc, Elkhart, IN 46515, USA). Ten-micrometer-thick cryosections were cut at –25°C, mounted on gelatin-coated slides, and stored at –20°C.

Immunohistochemistry

The primary monoclonal antibodies (MAbs) were: MAb RCK105 to keratin 7 (1:40–80 dilution; Monosan, 5400 AM Uden, The Netherlands; [28]); MAb LE41 to keratin 8 (1:200–400; Amersham Corp., Arlington Heights, IL 60005, USA; [10]); MAb LE65 to keratin 18 (1:20–100; Amersham [10]); MAb 4.62 to keratin 19 (1:100–200; Sigma Chemical Co, St. Louis, MO 63178, USA; [7]); MAbs CK 20.5 (1:10) and CK 20.10 (1:1 supernatant) to keratin 20 (American Research Products, Belmont, MA 02178, USA; [14–16]); and MAb CK 20.8 to keratin 20 (1:25, DAKO Corp., Carpinteria, CA 93013, USA; [14]).

Immunohistochemistry was carried out using an avidin-biotin peroxidase (ABC) method (Vector Labs., Burlingame, CA 94010, USA). For the ABC method, mounted tissue sections were hydrated in four 5-min washes of 0.1 M phosphate-buffered saline (PBS), pH 7.4, containing 0.4% Triton-X 100 (TX-100, Sigma). The slides were then incubated for 30 min with 3% normal goat serum (Cappel/OTC, Durham, NC 27704, USA) in PBS/TX-100, followed by a 1-h incubation at room temperature with the primary antibody diluted in 0.1 M PBS containing 0.4% TX-100 and 3% NGS. The slides were washed with PBS four times for 5 min each, followed a 45-min application of the secondary antibody, biotin-conjugated goat anti-mouse IgG preadsorbed with rat serum proteins (1:200 dilution; B-8774, Sigma). Three additional washes in PBS preceded both the 30-min application of Vectastain avidin-biotin complex (PK-4000 kit, Vector) and the 5- to 10-min incubation with a PBS solution containing 0.5 mg/ml 3,3′-diaminobenzidine (Sigma), 0.01% hydrogen peroxide and 0.04% $NiCl_2$ to tint the reaction product blue. MAb 20.5 was effective with human but not with rat oral tissues. MAbs 20.8 and 20.10 were tested on all tissues containing taste buds. They were equivalent in effectiveness at the concentrations employed.

For immunocytochemical controls each trial routinely employed a slide lacking the primary antibody, a slide lacking both the primary and secondary antibodies, and a positive tissue control slide. To optimize comparisons of staining intensity across tissues, constant values of time and of primary and secondary antibody concentrations were used for a given primary antibody. Uniformly processed photographs are presented in the figures.

Results

Taste buds are similarly distributed on rat and human tongues. On the anterior two-thirds of the tongue more than 100 fungiform taste papillae are scattered in loose rostro-caudal rows. On the posterior one-third of the tongue taste buds are found laterally in trenches of the foliate papillae and more caudally in trenches of the single dorsally situated vallate papilla in rat, or in trenches of the multiple vallate papillae in human. In the *roof* of the rat's mouth the transverse stripe of palatal taste buds lies at the margin of the soft and hard palates positioned immediately anterior to the foliate papillae in the tongue, whereas the taste buds in the rostrally situated nasopalatine canal lie above the tip of the tongue. As summarized in Table 1, we evaluated the keratin 20 immunoreactivity of human fungiform and vallate taste buds and rat fungiform, foliate, vallate, nasopalatine, palatal, and epiglottal taste buds.

Keratin-20-like immunoreactivity was intense for human vallate taste buds and for rat vallate and foliate taste

Table 1 Keratin 20 immunoreactivity of adult taste buds. Entries are based on reactions to the similarly effective primary monoclonal antibodies CK 20.8 and/or CK 20.10 (*FF* fungiform, *Val* vallate, *Fol* foliate, *Epi* epiglottis, *Pal* palate, *NP* nasopalatine papilla, +++ uniformly positive; ++ intense staining of the apical portion of some taste buds, ± no staining in most taste buds, – no staining, *ND* not determined, *NA* not applicable)

	FF	Val	Fol	Epi	Pal	NP
Human	+++	+++	ND	ND	ND	NA
Rat	-	+++	+++	++	±	±

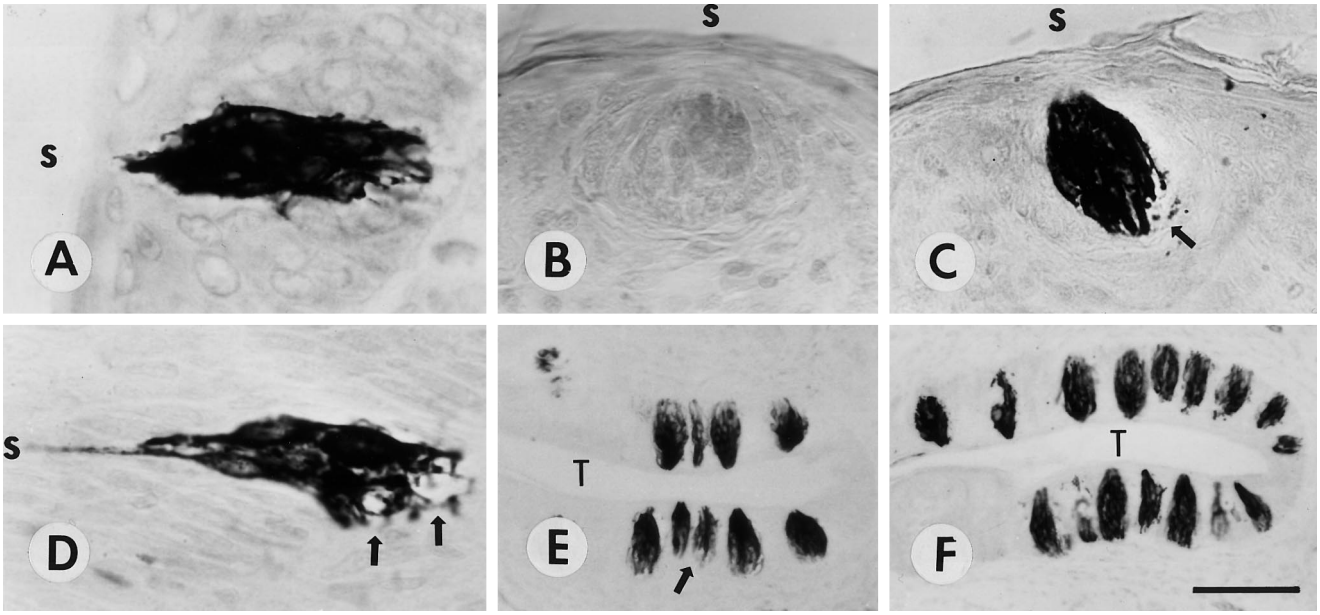

Fig. 1 Cryostat sections of fungiform taste buds were immunostained for keratin 20 in human (CK 20.8; **A**) and rat (CK 20.10; **B**), and for keratin 7 in rat (**C**). Vallate taste buds were stained for keratin 20 in human (CK 20.8; **D**, **E**) rat (CK 20.10; **D**), and for keratin 7 in rat (**F**; *arrows* **C–E** putative basal or immature suprabasal cells that are keratin-7-negative or keratin-20-negative, *s* surface, *T* vallate trench; *Scale bar* **A–D** 20 μm, **E**, **F** 90 μm)

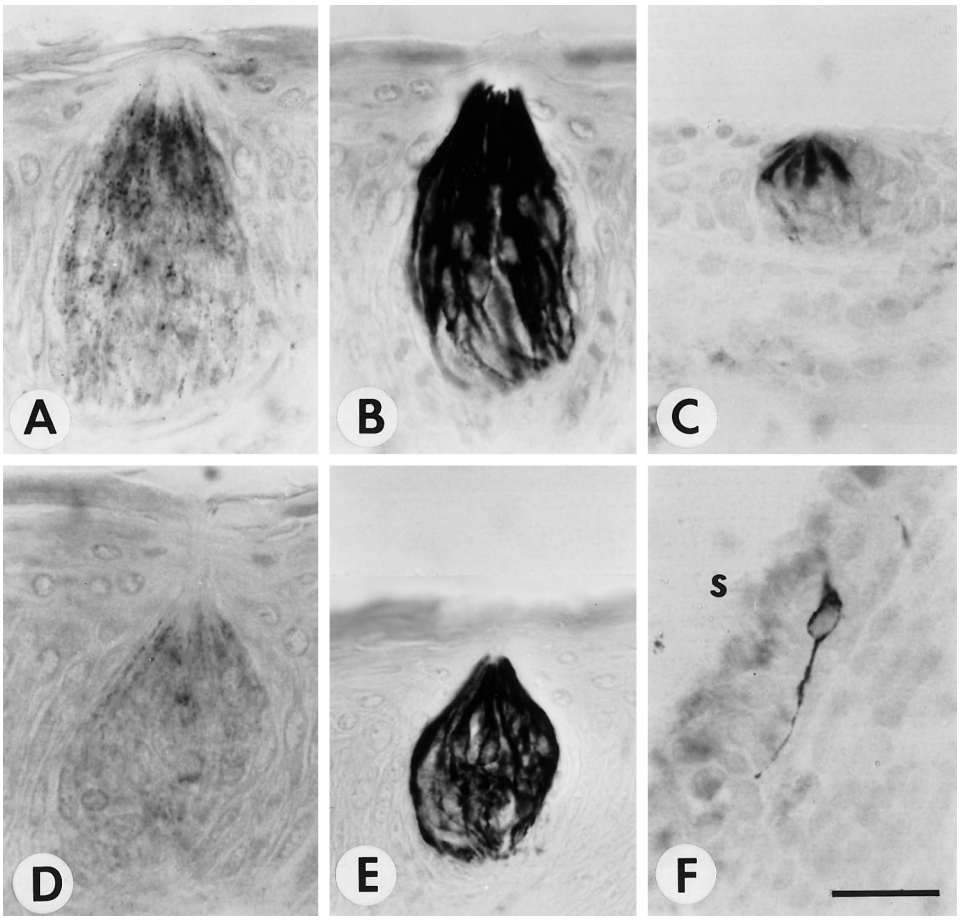

Fig. 2 Immunostaining of taste buds in the rat nasopalatine canal for keratin 20 (CK 20.10; **A**) and keratin 7 (**B**). **C** Rat epiglottal taste bud immunoreactive for keratin 20 (CK 20.8). Immunoreactivity of taste buds shown in rat soft palate for keratin 20 (CK 20.10; **D**) and keratin 7 (**E**). **F** Keratin-20-positive, isolated, neuron-like receptor cell in rat epiglottis (CK 20.8). The granular appearance of staining in **A** and **D** is characteristic of the weakest reactions of anti-keratin antibodies with such taste buds (*s* surface; *scale bar* 20 μm)

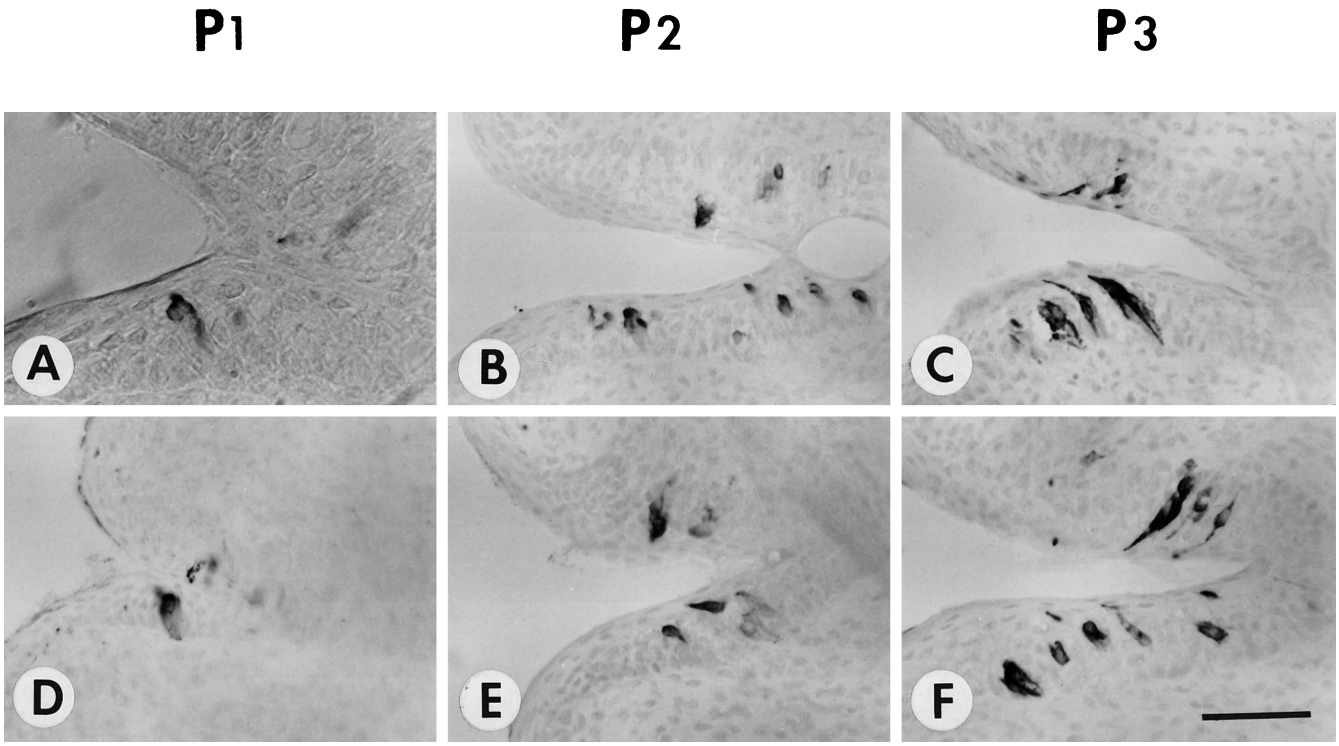

Fig. 3 Immunoreactivity of cells in the rat vallate papilla at P1, P2, P3 for keratin 20 (CK 20.8 for **A**, **B** and CK 20.10 for **C**), and keratin 7 (**D–F**). **A** A Nomarski view (*scale bar* 100 μm)

buds. As with taste cell immunoreactivity for keratins 7, 8, 18, and 19 [9, 39, 41], keratin 20 immunoreactivity appeared to be restricted to the elongated intragemmal cells, although not every intragemmal cell was intensely stained. In contrast to the strong immunoreactivity in human fungiform taste buds, rat fungiform taste buds lacked keratin 20 immunoreactivity. The most intense staining ever observed within a rat fungiform papilla is presented in Fig. 1B.

The absence of keratin 20 immunoreactivity in rat fungiform taste buds was paralleled by infrequent and minimal staining of palatal taste buds. Only a few taste buds in the nasopalatine ducts and in the soft palate were even faintly stained for keratin 20. The weak keratin 20 staining depicted in Fig. 2A and D represent the most intensely stained taste buds observed in the nasopalate and the soft palate, respectively (Fig. 2). In contrast, the most caudal of the extra-lingual taste buds – the epiglottal taste buds – frequently contained taste cells with keratin 20 immunoreactivity in their apices. Somewhat fewer than half of the epiglottal taste buds were strongly keratin-20-positive. The epiglottis also contained a few keratin-20-positive, isolated bipolar cells. Solitary, bipolar receptor cells have not been described previously in the oral cavity of mammals.

The development of keratin 20-like immunoreactivity was examined in postnatal rat tongues. All tissue sections of the vallate papilla at postnatal day 0 (P0), and most at P1, lacked keratin 20 staining. By P2, keratin-20-positive, polygonal cells were present in each vallate section. By P3, the immunopositive cells were more abundant and more frequently elongated. These results for keratin 20 were similar in developmental time and appearance to those for immunostaining with keratin 19 (not shown) or with keratin 7 (Fig. 3). Polygonal (immature) taste cells identified in adult rats were keratin-20-negative.

Keratin-20-like immunoreactivity in the tongue was restricted to taste cells. Salivary ducts, such as those beneath the base of the vallate papilla, were keratin-20-negative in both human and rat tongue. Immunostaining of adjacent sections revealed that cells of the same salivary duct were keratin-7-positive and keratin-20-negative (Figs. 4A,B,D,E, 5A,B).

Denervation selectively eliminates taste buds but not the salivary ducts [9]. Hence, after denervation of the rat vallate papilla, salivary duct cells remained intact at the base of the denervated vallate trenches. The duct cells were immunopositive for keratin 19, but not keratin 20. The denervated vallate contained neither taste buds nor keratin-20-immunopositive cells (Fig. 5).

Scattered keratin-20-positive Merkel cells were present at the junction between the epithelium and lamina propria in both nasopalate and hard palate (Fig. 4C,F). In addition to previously reported Merkel cell immunoreactivity for keratins 8, 18, 19 and 20, we observed that Merkel cells were moderately keratin-7-positive. Merkel cell clusters of the hard palate were positioned at the base of those epithelial rete whose apex supported heavily keratinized ridges. Merkel cells of the hard palate were displaced more than 1000 μm horizontally from the nearest taste buds and were at least 350 μm deeper than the base of taste buds. No Merkel cells were identified near epiglottal taste buds or near the keratin-20-negative fungiform taste buds or elsewhere in rat tongue.

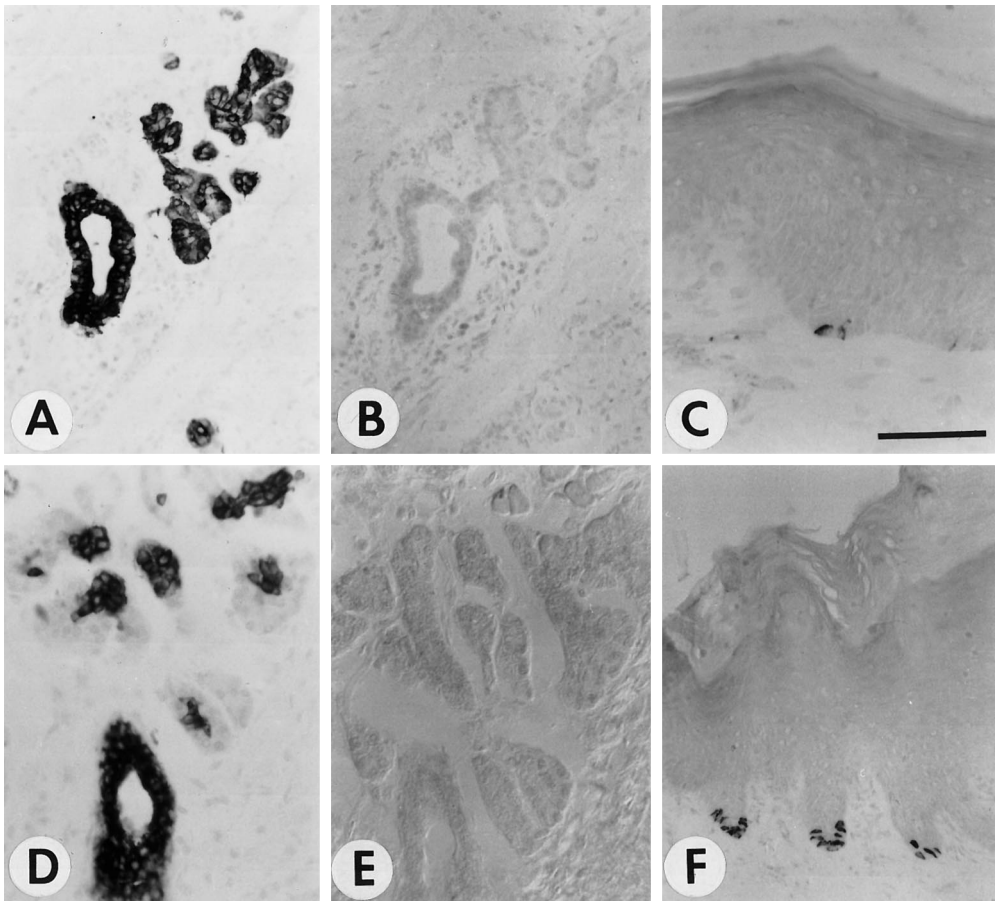

Fig. 4 **A**,**B** Salivary ducts beneath the vallate papilla were immunopositive for keratin 7 in human (**A**) and rat (**D**), but were immunonegative for keratin 20 in human (CK 20.8; **B**) and rat (CK 20.10; **E**). **A**,**B** and **D**,**E** are pairs of adjacent sections. Keratin-20-positive Merkel cells were present in the nasopalatine canal (CK 20.10; **C**), and more abundantly at the base of rete in the hard palate (CK 20.10; **F**; *scale bar* **A–E** 100 μm, **F** 50 μm)

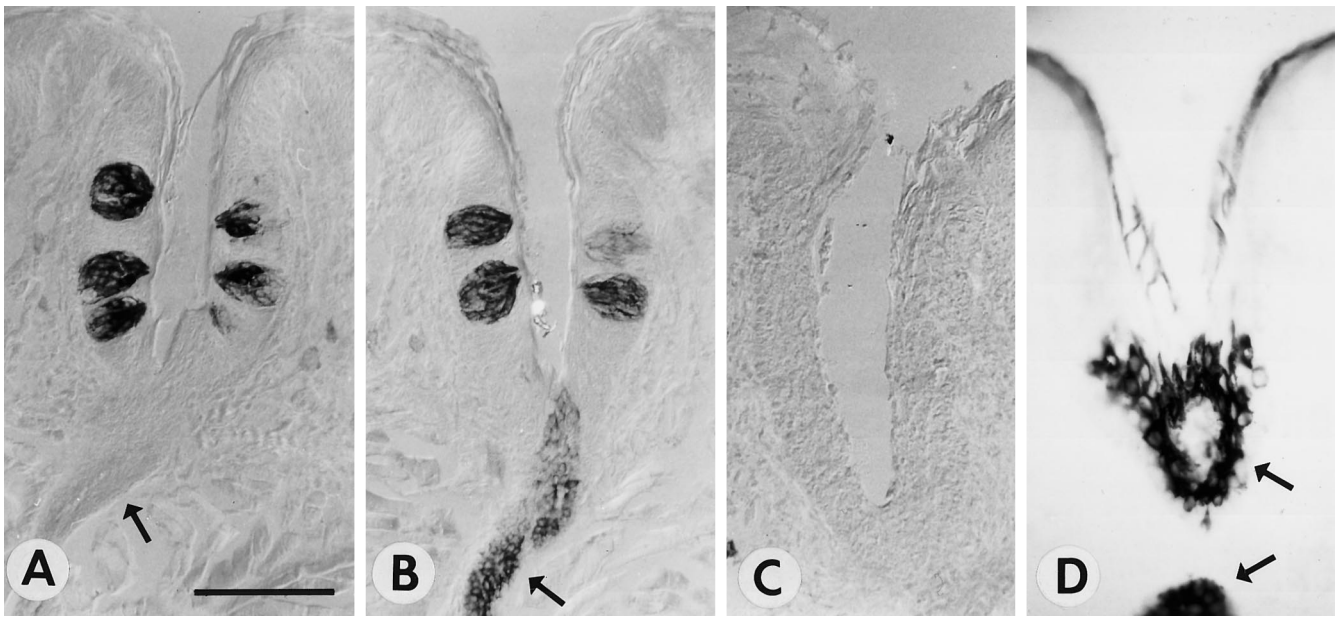

Fig. 5 **A** In a foliate trench of a normal adult rat, the taste buds were immunoreactive for keratin 20 (CK 20.8) but not for salivary ducts of von Ebner's glands (*arrow*), whereas in an adjacent section the salivary duct cells (*arrow*; **B**) as well as the taste buds were immunoreactive for keratin 7. Denervation eliminated rat vallate taste buds and (**C**) eliminated their immunoreactivity for keratin 20 (CK 20.8), yet (**D**) the keratin 19 immunoreactivity of the salivary duct cells (*arrow*s) remained (MAb 4.62; *scale bar* 100 μm)

Discussion

Neither MAb 20.8 nor MAb 20.10 stained rat lingual salivary ducts or fungiform taste buds that are readily stained by MAbs monospecific for keratin 7 or 8 or 18 or 19 [9]. Such restriction of keratin 20 staining to caudal taste buds rules out cross-reaction of MAbs 20.8 and 20.10 with any of the other lingual cytokeratins within or around taste buds.

Both intragemmal cells and salivary duct cells contain keratins 7, 8, 18, and 19 [9, 32, 39, 41]. It is therefore noteworthy that in rat vallate and foliate papillae the taste cells are keratin-20-positive whereas the cells of the salivary ducts are uniformly keratin-20-negative. Consequently, antibodies against keratin 20 represent highly specific markers for use in taste research because they are selective for intragemmal taste cells. In an application pertinent to the present examination of the neonatal rat tongue, keratin-20-positive polygonal cells must have been developing taste cells since salivary cells are keratin-20-negative [41]. By P3 many keratin-20-positive polygonal cells have become elongated – an attribute strongly associated with taste receptor cells. We detected no keratin-20-positive polygonal cells in adult rat tongue.

Cellular patterns of X chromosome inactivation in transgenic mice indicate that lingual taste cells arise locally from epithelial cells [33]. Cutaneous xenografts suggest that human Merkel cells also arise locally from epidermal cells [12, 16]. While the rat's palate contains both taste cells and keratin-20-identified Merkel cells [38], they are too far apart for Merkel cells to contribute cells to these taste buds during postnatal taste bud development or during normal taste cell turnover. Similarly, in large fungiform papillae of monkey, Merkel cells are laterally situated and therefore remote from the dorsally placed fungiform taste buds [37]. Thus, because rat and monkey taste buds are remote from Merkel cells, Merkel cells cannot be sufficient to give rise to taste buds.

It has been suggested that the progeny of Merkel-like cells give rise to the frog taste organ [36]. Merkel cells are also believed to attract mechanosensory axons [6]. So it is possible that keratin 20-positive mammalian taste buds also contain Merkel-like cells that attract taste axons or perform other functions. Both Merkel cells and mammalian type III taste cells contain dense core vesicles and neuron-specific enolase, and express keratins 7, 8, 19, and 20 (present results and [5, 9, 14, 16, 39, 40]). Nonetheless, by their lack of cytoplasmic spines, and in more subtle ways, the type III mammalian taste receptor cells have been considered to be distinct from Merkel cells [18]. To determine whether Merkel cells are necessary participants in taste buds, we used antibodies against keratins 8 or 18 or 19 or 20 that stain Merkel cells. No Merkel cells were detected in the tongue [9, 16, 39, 41]. It is possible that the intense keratin-20-positive staining of vallate and foliate taste buds obscured Merkel cells that were nerve-dependent and therefore not revealed by vallate denervation – no keratin-20-positive cells survived denervation (Fig. 5C). However, Merkel cells were also absent from normal taste buds that were keratin-20-immuno*negative*. These include fungiform papillae, nasopalate, and soft palate taste buds that were entirely keratin-20-negative and therefore lacking Merkel cells. The unstained basal half of epiglottal taste buds also lacked Merkel cells. We conclude that Merkel cells make no contribution to the structure of varied taste buds of rat. This does not invalidate a probable active role of Merkel-like cells in taste buds of amphibians [30, 36].

Recent experimental evidence that taste cells originate in local epithelium has renewed interest in the ectodermal versus the endodermal germ layer origin of taste buds [1, 33]. If all vertebrate taste cells originate from local epithelium [33], the external taste buds scattered over the trunk epithelium of many teleosts must be ectodermal [8]. In terrestrial mammals all taste buds lie internally in the oropharynx. Here the melding of tissues and the commingling of cells may produce a blurred ectodermal-endodermal boundary that differs among mammals [20]. We considered that the early developmental mobilities of germ layers may be reflected in adult keratin 20 expression patterns. With the exceptions of Merkel cells and urothelial umbrella cells, the known human tissue distribution of keratin 20 is restricted to gastrointestinal endoderm and its derivatives [3, 14, 15]. In rats the observed distribution of keratin-20-positive taste buds was in full agreement with the traditional view that taste buds in the posterior third of the tongue and more caudal oral tissues are derived from endoderm (keratin-20-positive), whereas those in the palate and anterior two-thirds of the tongue are derived from stomadeal ectoderm (keratin-20-negative). If an endodermal germ layer origin is required for keratin 20 immunoreactivity of taste cells, then all rat vallate taste buds might be expected to be keratin-20-positive whether situated in a protective trench or exposed on the papilla's dorsal surface. We tested for this expectation by examining solitary dorsal vallate taste buds. Although they resembled fungiform taste buds in their exposed location and appearance, dorsal vallate taste buds were strongly keratin-20-positive.

The keratin 20 staining of human vallate taste buds confirms an earlier report that human vallate taste buds are keratin-20-immunoreactive ([14] and W.W. Franke, personal communication). The present demonstration that human fungiform taste buds are also keratin-20-immunoreactive is consistent with an endodermal origin for the human tongue. Alternatively, the impressive migratory potential of streams of developing cells [19] makes the selective rostral migration of fungiform taste bud precursors a possibility. Recent evidence on the local origins of taste buds does not exclude such migratory streams [33]. In these two alternatives either the human tongue epithelium is endodermal and stomadeal ectoderm is constrained to more rostral tissues, or taste precursor cells glide far rostrally to enter a sea of lingual ectoderm where they deposit islands of endodermal fungiform taste buds [1]. The provocative rostral location of cytokeratin-20-positive human fungiform taste buds in-

vites more detailed characterization of germ layer migrations in the human oropharynx.

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