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Comparative lectin histochemistry on taste buds in foliate, circumvallate and fungiform papillae of the rabbit tongue

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Summary. Taste buds (TB) in the foliate, circumvallate and fungiform papillae of the rabbit tongue were examined with lectin histochemistry by means of light (LM) and electron (EM) microscopy. Biotin- and gold-labeled lectins were used for the detection of carbohydrate residues in TB cells and subcutaneous salivary glands. At the LM level, the lectins of soybean (SBA) and peanut (PNA) react with material of the foliate and circumvallate taste pores only after pretreatment of the section with neuraminidase. This indicates that the terminal trisaccharide sequences are as follows: Sialic acid–Gal–GalNAc in *O*-glycosylated glycoproteins or Sialic acid–Gal–GlcNAc in *N*-glycosylated glycoproteins. In fungiform taste buds the lectins of *Dolichos biflorus* (DBA) and *Helix pomatia* (HPA), also specific to GalNAc residues, are reactive without preincubation with neuraminidase. Wheat germ agglutinin (WGA), specific to GlcNAc, reacts with TBs of all papillae; and the lectin from *Ulex europaeus* (UEA I), specific to fucose, binds to individual TB cells. The presence of sialic acid may protect mucus or other glycoproteins in TB cells and inside the taste pore from premature enzymatic degradation. In a post-embedding EM procedure on LR-White-embedded tissue sections, only gold-labeled HPA was found to bind especially on membrane surfaces of the microvilli which protrude into the taste pore; however HPA did not bind to the electron-dense mucus inside the taste pore. The mucus situated in the trough and at the top of the adjacent epithelial cells also is strongly HPA-positive, but is of different origin and composition than that found in the taste pore. These results demonstrate distinct carbohydrate histochemical differences between fungiform and circumvallate/foliate taste buds. The different configuration of galactosyl residues and the occurrence of mannose in circumvallate and foliate TBs leads to the suggestion that the lectin reactivities of TBs are not only due to the presence of mucins, but also to *N*-linked glycoproteins, possibly with a hormone-like, paraneuronal function. A possible relationship to v. Ebner glands in these papillae is discussed.

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Introduction

Lingual taste buds (TBs) of the rabbit are contained in three different kinds of papillae with distinct locations on the tongue. These include the foliate, circumvallate and fungiform papillae. TBs consist of neuroepithelial cells with secretory and/or taste receptor functions. The mechanisms of taste receptor transduction are complex. Mucous substances, which are secreted by taste cells themselves and by subcutaneous v. Ebner glands or by salivary glands of the oral cavity, have been implicated in the taste stimulation process. Bannister (1974) interpreted the mucus as an ion reservoir, which could be important during the chemoreception stimulation. The significance of salivary secretion for taste perception has been pointed out by Gurkan and Bradley (1988) and Ohsawa et al. (1988) who compared Bowman's nasal glands in olfaction (Getchell and Getchell 1984) to the v. Ebner glands in the tongue. Schmale et al. (1990) found a glycoprotein in the mucus of v. Ebner glands, which was chemically related to the "odor binding protein" (OBP) that may play a key role in olfactory reception (Pevsner et al. 1988). The relationship between the secretory process and TBs in fungiform papillae is of particular interest, because these papillae are not supplied with subcutaneous salivary glands corresponding to v. Ebner glands in circumvallate and foliate papillae. Furthermore, the kinds of taste stimuli that generate taste responses vary considerably within the different lingual regions (Miller and Bartoshuk 1991). These facts lead us to hypothesize that different morphological and physiological conditions might be reflected in the differences in the carbohydrate composition of the taste buds.

The distribution and occurrence of glycoconjugates has been investigated by Scalzi (1967), Brouwer and Wiersma (1978) and by Reutter and Klessen (1979) in

the rabbit foliate TBs with conventional carbohydrate histochemistry. More detailed studies with carbohydrate-specific (glyco)proteins, the lectins, have been performed recently at both the light microscopical (Witt and Reutter 1988) and electron microscopical levels (Ohmura et al. 1989). Lectins are proteins or glycoproteins that interact specifically with mono- or oligosaccharide residues (see review by Sharon and Lis 1972). In the last decade, techniques have been developed to visualize lectin-binding sites histochemically at both the light (e.g. Hsu and Raine 1982) and electron microscopical level (Roth 1983).

The aim of the present work was to determine possible differences in the carbohydrate composition of TBs within different papillae by lectin histochemical methods. Especially the carbohydrate composition of the mucus inside the taste pore can be elucidated with lectin conjugates. Another feature of the investigation was to obtain information about carbohydrate moieties at the ultrastructural level.

Table 1. Lectins used for the histochemical demonstration of carbohydrate residues in taste bud cells of the rabbit

Lectin	Specificity[a]
Helix pomatia agglutinin, (HPA)	GalNac-α1,3 GalNac > αGalNAc
Dolichos biflorus agglutinin (DBA)	GalNac-α1,3 GalNac > αGalNAc
Arachis hypogaea agglutinin (PNA)	Gal-β1,3 GalNAc > Gal
Glycine max agglutinin (SBA)	α and βGalNAc > α and βGal
Ricinus communis I agglutinin (RCA I)	Gal > GalNAc
Ulex europaeus I agglutinin (UEA I)	α-L-Fuc
Triticum vulgare agglutinin (WGA)	$(GlcNAc\ \beta1,4)_3$ > GlcNAc

[a] GalNAc, *N*-acetyl-D-galactosamine; Gal, galactose; GlcNAc, *N*-acetyl-D-glucosamine; Fuc, fucose (for references see Goldstein and Poretz 1986)

Materials and methods

Tissue preparation

Eight rabbits (*Oryctolagus cuniculus*) were sacrificed by an overdose of pentobarbital. The tongues were excised and fixed either with 2% freshly prepared paraformaldehyde or with Bouin's solution. After about 1 h of fixation, fungiform, circumvallate and foliate papillae were excised, postfixed in the same solutions for ca. 4–8 h and processed for light microscopy. The tissue specimens were dehydrated in ethanol and methylbenzoate, embedded in Paraplast, and sectioned at thicknesses of 5–8 µm. Sections were mounted on slides that were pretreated with gelatin-chromalum or poly-L-lysine chemicals (Sigma, St Louis, Mo., USA).

Electron microscopy

After initial fixation of about 30 min, small pieces (ca. 1 mm^3) of foliate taste buds were excised and fixed in a solution of 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.4) for 2 h at 4° C. The specimens were dehydrated in a graded series of ethanol and immersed into pure LR White (medium grade, London Resin Co., Woking, UK; Newman and Hobot 1987). After three changes of resin, the tissues were placed into gelatin capsules and polymerized at 60° C for 24 h. Ultrathin sections were mounted on Formvar-coated Nickel grids.

Lectin histochemistry

For light microscopy, paraffin sections were hydrated and treated according to the procedure of Hsu and Raine (1982). Briefly, sections were exposed to a solution of 0.3% H_2O_2 (Sigma) in demineralized water for 15 min in order to eliminate endogenous peroxidases. Then, a 20–40 µg/ml solution in phosphate-buffered saline (PBS) of biotinylated lectins (Vector, Burlingame, Calif.; HPA: Sigma) was dropped on the sections for 45 min. Subsequently, the sections were incubated for 30–45 min in an avidin-biotin/peroxidase complex (ABC; Vectastain Elite, Vector, Burlingame, Calif.). The sections were developed in 3–3′-diaminobenzidine (DAB)-H_2O_2 medium (Sigma) under microscopical control to visualize the activity of peroxidase (Graham and Karnovsky 1966). Controls included omission of lectin or ABC complex, respectively. Preincubation of the lectins with their respective inhibitory sugars (Table 1) led to a diminution of the reaction.

Neuraminidase-test

In order to demonstrate sialic acids situated on terminal positions of oligosaccharide side-chains, some sections were treated with a 0.3 U/ml solution of neuraminidase from *Vibrio cholerae* (E.C. 3.2.1.18; Boehringer, Mannheim, FRG; or Behring, Marburg) for 4–18 h at 37° C and then incubated with the lectins. Control sections were incubated with an enzyme-free buffer solution.

Preparation of colloidal gold particles (15 nm)

The colloidal gold was prepared using the citrate method according to the procedure of Frens (1973). Lectins were conjugated to colloidal gold as described by Roth (1983). For electron microscopy, the ultrathin sections were floated on a drop of PBS containing 0.1% bovine serum albumin (BSA) for 5–10 min and then incubated with the gold-labeled lectin (diluted 1:30–1:100 in PBS) for 45 min. Subsequently, the sections were jet-washed with demineralized water and contrasted with uranyl acetate (4 min) and lead citrate (1 min). The sections were viewed with a Philips (Eindhoven, The Netherlands) EM 300 electron microscope.

Results

Taste buds consist of neuroepithelial cells which are gathered to a bulb-like formation at the top of a corium papilla. In the tongue of the rabbit, as in other mammals, TBs occur on different types of papillae. On the anterior part of the tongue, they are concentrated on fungiform papillae, most prevalently on the ventral side of the tip. Foliate papillae are found on the dorsal lateral margin of the tongue, and the circumvallate papillae are located bilaterally beneath the midline of the dorsal lingual surface. Five to eight TBs are situated on the top of each fungiform papilla, whereas TBs in circumvallate and fo-

Table 2. Lectin binding[a] in taste bud cells and salivary glands in foliate, circumvallate and fungiform papillae of the rabbit

Lectin	TB cells	Porus	v. Ebner glands	Pap. Epith.
Foliate papillae:				
PNA	−	−	+++	++str. corn.
N/PNA	+	+	++ducts	+
DBA	(+)	−	++	+
N/DBA	++	++	++	+
RCA I	+	+	(+)	+
N/RCA I	++	+	+	+
HPA	+(+)	++	+	+
N/HPA	n.d.			
SBA	−	−	+	−
N/SBA	+	++	+	−
UEA I	+	+	++ducts	+
WGA	++	++	++	+
Circumvallate papillae:				
PNA	−	−	+++	++str. corn.
N/PNA	++	++	++	−
DBA	+	++	+	+
N/DBA	+	++	+	+
RCA I	+	+	+	(+)
N/RCA I	++	++	+	+str. corn.
HPA	++	++	+	(+)
N/HPA	n.d.			
SBA	−	−	−	−
N/SBA	(+)	++	(+)	−
UEA I	+	+	(+)	(+)
WGA	+	+	(+)	(+)

	TB cells	Pap. Epith.
Fungiform papillae:		
PNA	−	+
N/PNA	−	+
DBA	++	−
N/DBA	++	−
RCA I	−	−
N/RCA I	−	−
HPA	+	+
N/HPA	n.d.	
SBA	−	+
N/SBA	(+)−	+
UEA I	+	+
WGA	++	+

TB, Taste bud; Pap. Epith., papillary epithelium; N, neuraminidase; str. corn, stratum corneum

[a] n.d., Not determined; −, no reaction; +++, maximal reaction

liate papillae are lined up in great numbers on the side wall facing a narrow trough between the papillae. The submucosa of the latter papillae contain tubulo-alveolar glands producing a serous secretion (v. Ebner 1873). The corium of fungiform papillae is devoid of these glands. The morphology of the circumvallate and foliate TBs is well known (Fujimoto and Murray 1970; Murray 1986), whereas information concerning ultrastructural and histochemical details of fungiform TBs in rabbit is scarce (Murray 1973). Comparative morphological studies about TBs of different papillae in the golden hamster have been performed by Miller and Chaudhry (1976).

Fixation and lectin histochemistry

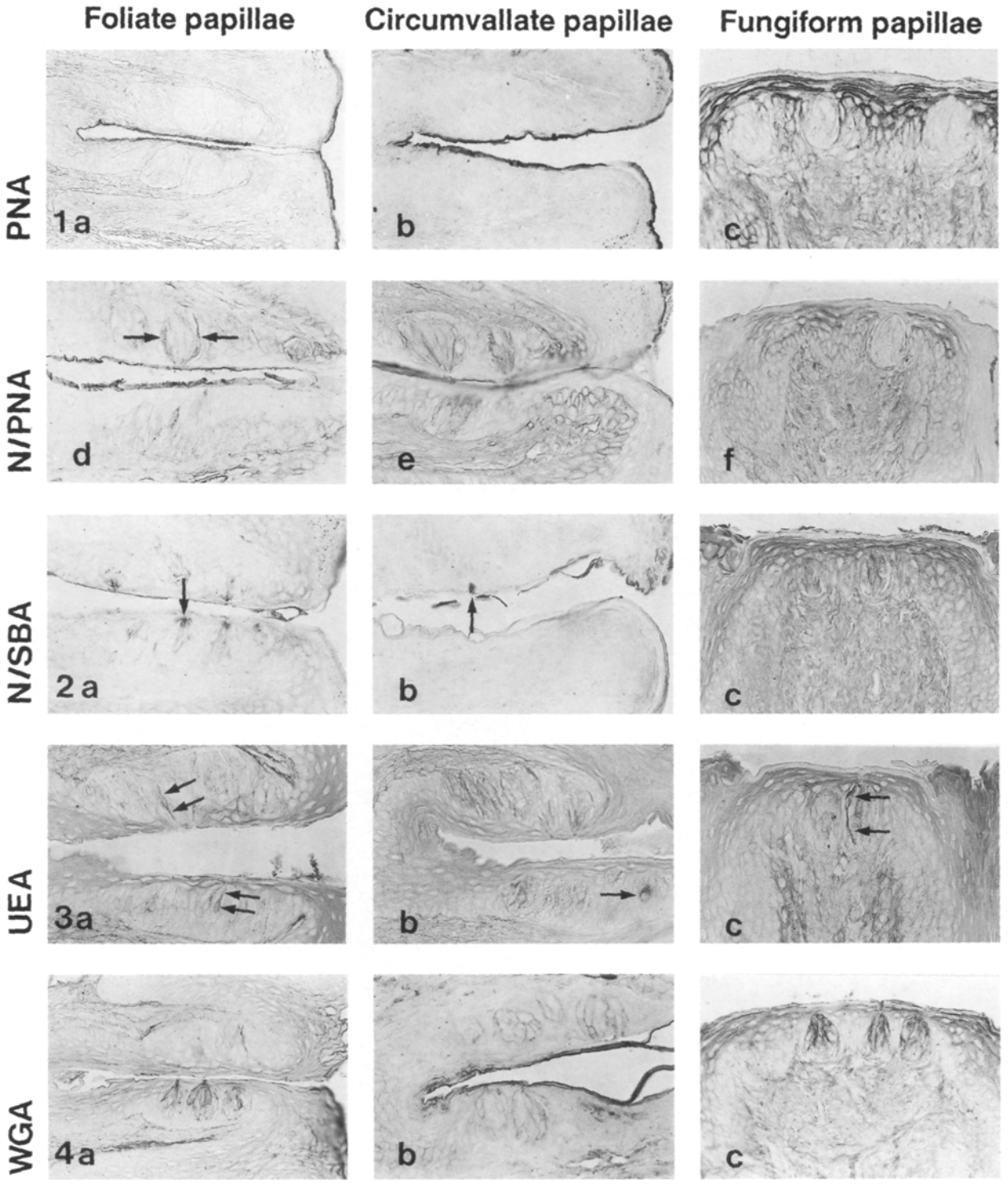
Lectin histochemistry is influenced by the fixation procedure. The best fixation results were obtained with Bouin's solution, while a minimal "background staining" was achieved with paraformaldehyde. The results of the lectin binding studies are shown in Table 2.

Light microscopical histochemistry

Peanut agglutinin (PNA) did not react with the TB cells of all papillae examined unless the sections were preincubated with neuraminidase (Figs. 1a, b). Enzyme-treated fungiform TBs, however, did not exhibit PNA-binding sites (Fig. 1c). Many salivary gland cells were strongly PNA positive also without pretreatment with neuraminidase (Fig. 8b). There was no affinity of soybean agglutinin (SBA) to TB cells. Only after treatment of the section with neuraminidase, SBA reacted more intensely with the taste pore material than with intracellular material of the foliate and circumvallate TBs (Figs. 2a, b). The TBs of fungiform papillae were diffusely marked (Fig. 2c). The reactivity of v. Ebner glands with SBA was less intense than with the other galactosyl-specific lectins.

Ulex europaeus agglutinin (UEA I) exhibited a strong affinity to a few, singular cells in TBs of all papillae (Fig. 3). Wheat germ agglutinin (WGA) showed a high affinity to cells of all TBs examined (Fig. 4) and to v. Ebner glands (Fig. 8c).

Ricinus communis agglutinin (RCA I) also exhibited specific binding reactions to material of the TB cells. In circumvallate and foliate TBs (Fig. 5a), a distinct reaction of individual TB cells was observed. After preincubation of the section with neuraminidase, the binding reaction was strongly enhanced (cf. Fig. 5b and a). TBs in fungiform papillae, however, remained unreactive in all cases both before and after enzymatic removal of sialic acids (Fig. 5c). The binding reaction of *Dolichos biflorus* agglutinin (DBA) varied within the different types of papillae. There was a weak reaction of DBA in circumvallate (Fig. 6a) and foliate (not shown) TB cells, which was considerably enhanced after removal of sialic acids. In contrast to PNA this lectin binds to fungiform TB cells also in the absence of neuraminidase (Fig. 6b). The v. Ebner glands are positive, too (Fig. 8a). *Helix pomatia* agglutinin (HPA) bound to TBs in all papillae. Fungiform papillae reacted less intensely with this lectin than TBs of circumvallate and foliate papillae (Fig. 7). The lingual salivary glands were moderately reactive with HPA.



Figs. 1–8. Lectin histochemistry of foliate, circumvallate and fungiform papillae of the rabbit

Fig. 1. a Peanut agglutinin (PNA), foliate papilla: PNA does not bind to taste bud (TB) cells, but only to the stratum granulosum or stratum corneum of the papillary epithelium; ×160. **b** PNA, circumvallate papilla: the same binding pattern as in **a**; ×160. **c** PNA, fungiform papilla: the lectin binds exclusively to corneal epithelial cells, but not to TB structures; ×200. **d** Neuraminidase/PNA, foliate papilla: some TB cells (*arrows*) and cells of the stratum basale are marked; ×200. **e** Neuraminidase/PNA, circumvallate papilla: the same situation as in **d**; ×160. **f** Neuraminidase/PNA, fungiform papilla: the incubation with neuraminidase does not lead to lectin binding of the TB cells. Strong reaction with the epithelium and with connective tissue; ×160

Fig. 2. a Neuraminidase/soybean agglutinin (SBA), foliate papilla: some TB cells are weakly stained with SBA, but the most intense reaction product is located in the TB taste pore (*arrow*); ×200. **b** Neuraminidase/SBA, circumvallate papilla: the reaction product is exclusively visible in the taste pore (*arrow*), but not in TB cells; ×200. **c** Neuraminidase/SBA, fungiform papilla: in contrast to the neuraminidase/PNA sequence, TB cells are diffusely labeled with SBA; ×160

Fig. 3. a Ulex europaeus agglutinin (UEA I), foliate papilla: a few singular TB cells are reactive with UEA I. They exhibit a strong supranuclear reaction (*arrows*); ×200. **b** UEA I, circumvallate papilla: the same reaction pattern as observed in foliate TBs. Note the supranuclear reaction product (*arrow*); ×200. **c** UEA I, fungiform TB: one individual cell with a long process near the presumed basal lamina is marked with this fucose-specific lectin (*arrows*); ×200

Fig. 4. a Wheat germ agglutinin (WGA), foliate papilla: strong reaction especially in supranuclear material of TB cells and taste pores; ×260. **b** WGA, circumvallate papilla: the reaction is confined to cell border structures, possibly to cell membranes; ×260. **c** WGA, fungiform papilla: strong reaction to several TB cells and to the taste pore material; ×200

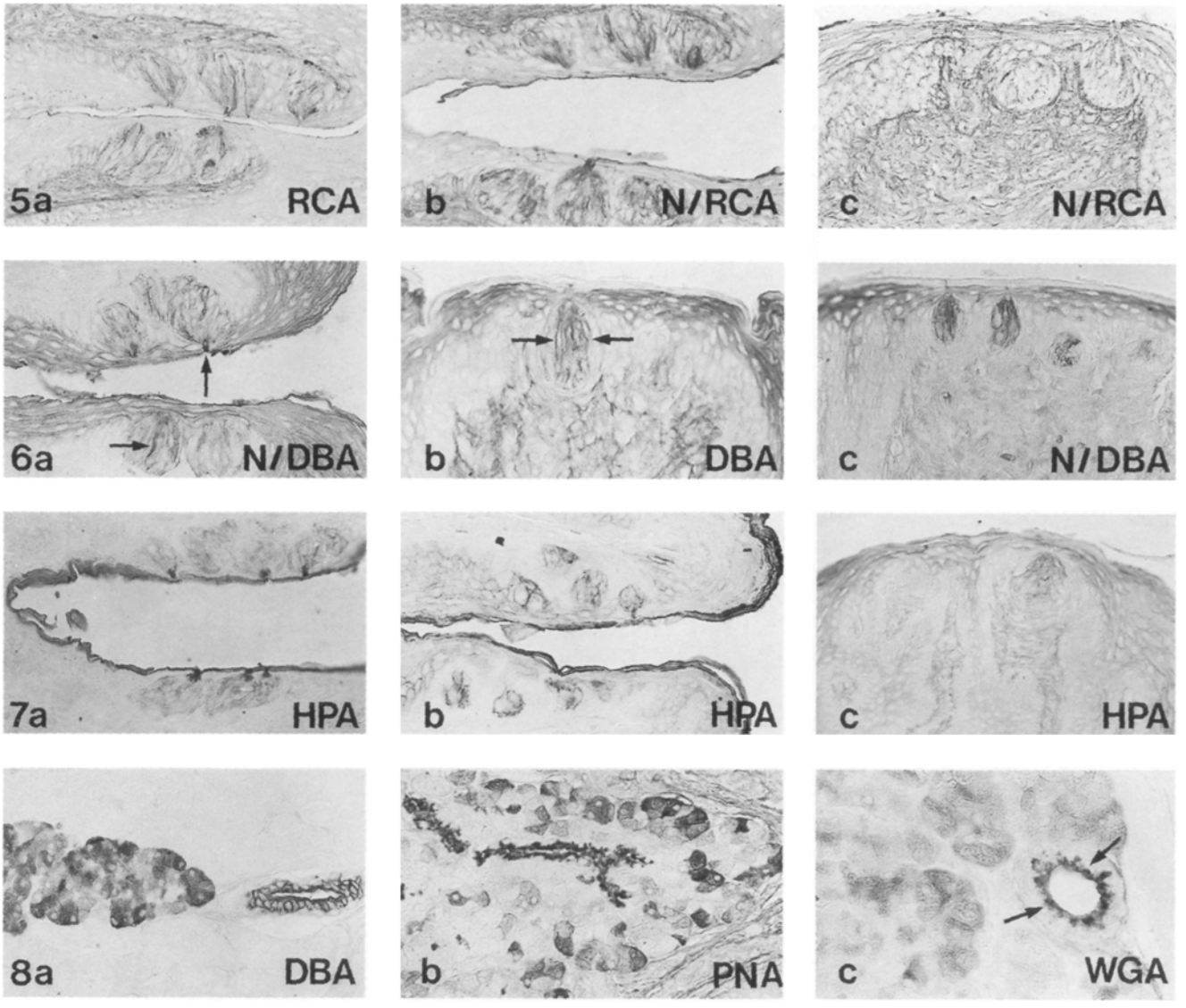


Fig. 5. a *Ricinus communis* agglutinin (RCA I), foliate papilla: a moderate lectin reactivity is confined to singular TB cells and to the subepithelial connective tissue; ×200. **b** Neuraminidase/RCA I, foliate papilla: the reaction is considerably enhanced and the taste pore material is also clearly marked; ×200. **c** Neuraminidase/RCA I, fungiform papilla: there is a moderate reaction at connective tissues, but taste buds are no more reactive than surrounding epithelial cells; ×200

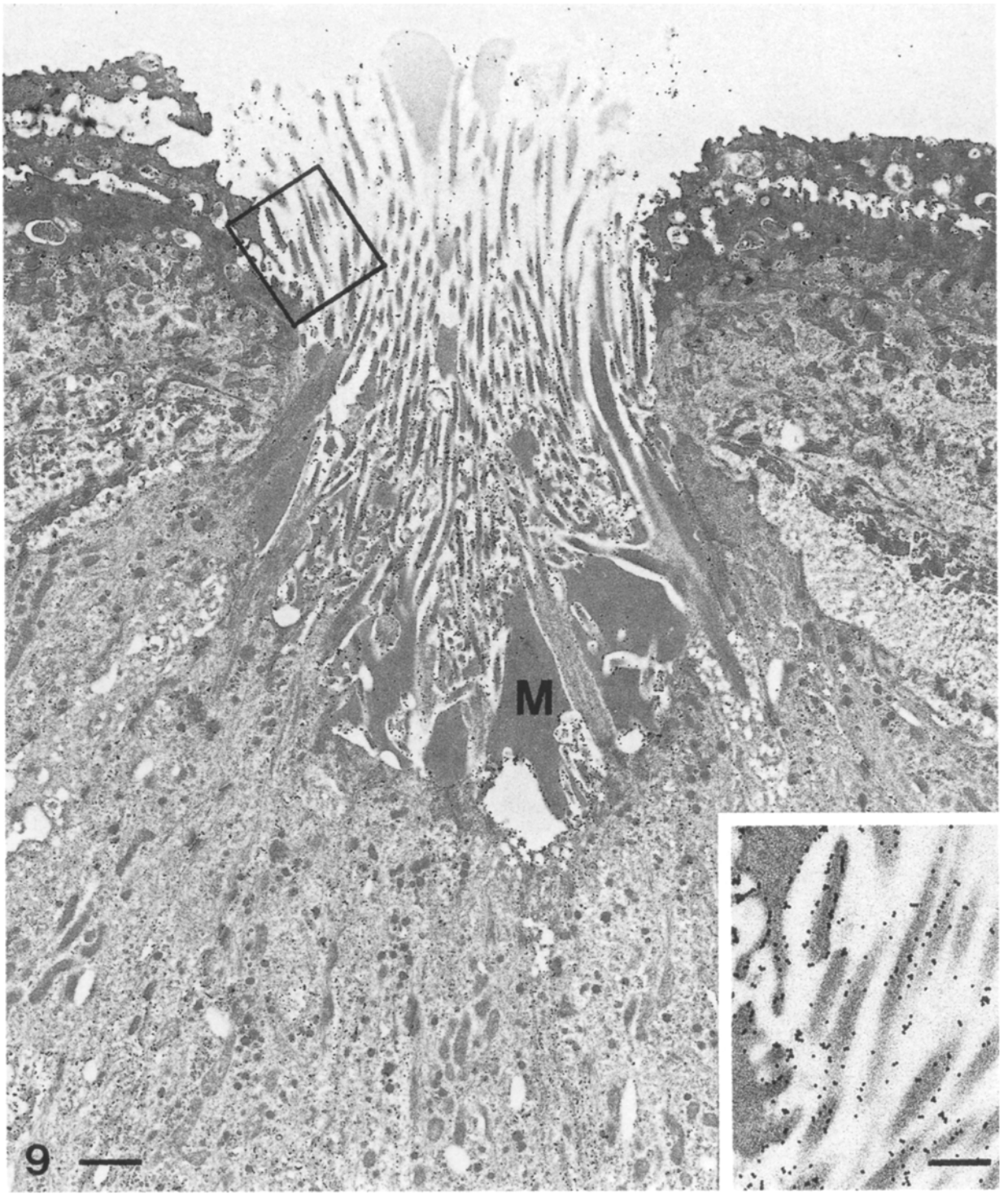
Fig. 6. a Neuraminidase/*Dolichos biflorus* agglutinin (DBA), foliate papilla: the lectin reacts with the epithelium and with TB cells as well as taste pore material (*arrows*); ×200. **b** DBA, fungiform papilla: positive reaction within the TB (*arrows*): ×200. **c** Neuraminidase/DBA, fungiform papilla; after neuraminidase, the reaction product is somewhat more pronounced than in **b**; ×200

Fig. 7. a *Helix pomatia* agglutinin (HPA), foliate papilla: the lectin labels the stratum corneum, the taste pores and the TB cells; ×160. **b** HPA, circumvallate papilla: similar reaction pattern as **a**; ×200. **c** HPA, fungiform papilla: weak reaction of the epithelium and TB cells; ×160

Fig. 8a–c. Lectin reactivity of v. Ebner glands of the foliate papillae. **a** DBA: the lectin reacts differently to various acinar cells, while the cells of the excretory duct are homogeneously stained; ×200. **b** PNA: the same reaction pattern as in **a**; ×200. **c** WGA: no essential difference is to be seen from the previous figures (**a, b**). Note the supranuclear location of glucosamine residues in the duct (*arrows*); ×200

Electron microscopy

TBs of rabbit foliate papillae consist of at least three types of elongated cells (Murray 1986). Microvillar processes of these cells protrude into the taste pore, and the pore is filled with an amorphous electron-dense mass (De Lorenzo 1958). From all the investigated lectins specific to *N*-acetyl-galactosamine, only HPA reacted with structures of the foliate papilla. Plasmalemmata of all TB cells were densely labeled (Fig. 9). Dark granules in the apical part of type I cells were, in part, reactive with lectin, as shown by the presence of gold particles; filaments and compartments of the rough endoplasmic reticulum (rER) were HPA negative. HPA also bound



Figs. 9–10. Electron microscopic detection of HPA binding sites at LR White-embedded foliate TBs.

Fig. 9. Taste pore region with numerous microvilli, which exhibit dense labeling with HPA at their membranes. The electron-dense mucous material (*M*) is devoid of HPA-binding sites. ×10300, *bar* = 1 μm; Inset: ×27400, *bar* = 0.5 μm

to components of some, but not all, dense-cored vesicles of type III cells (Fig. 10). HPA bound to interdigitated cell membranes of squamous epithelial cells (Fig. 9) and to mucous substances in the papillary furrow, which appear to be secreted by acinar cells of the v. Ebner glands and also by some specialized secretory duct cells. Inside the taste pore, HPA-colloidal gold bound only to locations near the surface of the amorphous mucus. Nerve fiber profiles were HPA positive, but type II cells and basal cells were not reactive with HPA.

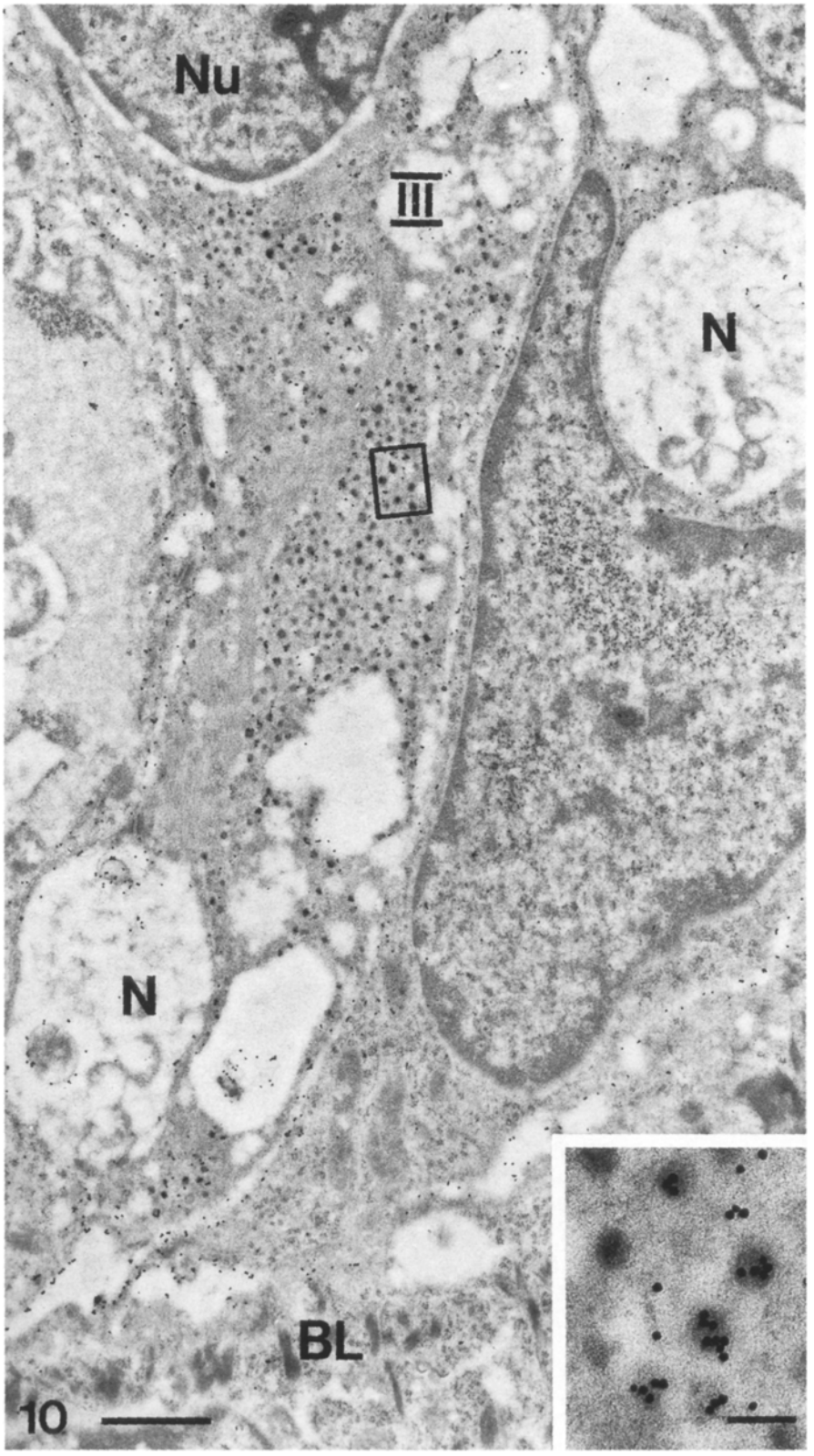


Fig. 10. Basal part of a type III cell. Numerous dense-cored vesicles contain galactosyl residues. The typical membrane around the vesicles is not contrasted well because of the omission of osmium post-fixation. *BL*, basal lamina; *Nu*, nucleus; *N*, nerve fiber. ×16700, *bar* = 1 μm; inset: ×79800, *bar* = 125 nm

Discussion

In the present study, taste buds (TBs) of foliate, circumvallate and fungiform papillae of the rabbit tongue were compared by means of lectin histochemistry. The results show that homologous structures of TBs in circumvallate and foliate papillae provide an almost characteristic reaction to various lectins. Some authors have already described morphological diversity between the fungiform TBs and the foliate and circumvallate buds. Nada and Hirata (1976) demonstrated serotonin analogues in rabbit foliate and circumvallate TBs, but not in fungiform ones. Miller and Chaudhry (1976) found morphological features in fungiform TBs of the Syrian hamster, which were different than in foliate and circumvallate TBs.

The lectin from *Arachis hypogaea* (peanut, PNA) demonstrates the presence of the penultimate disaccharide D-galactose-β(1–3)-D-galactosamine (Gal–GalNAc) or galactose (Gal) in various tissues (Lotan et al. 1975; Stoward et al. 1980; Schulte and Spicer 1985). TB cells and the taste pore substance of these papillae exhibit a strong affinity to PNA only after removal of *N*-acetylneuraminic acid (sialic acid). In contrast to the observations of Scalzi (1967) by conventional carbohydrate histochemistry, which demonstrated only neutral mucopolysaccharides, we now have indirect evidence that the taste pore material of the circumvallate and foliate papillae contains sialic acid. Also the lectin from *Glycine max* (soybean, SBA), which is most specific to *N*-acetyl-galactosamine (Pereira et al. 1974), demonstrates this terminal carbohydrate configuration and shows that the substance inside the taste pore is unique in the tongue. There seem to be no other sources of this substance than the TB cells themselves, since the secretion of v. Ebner glands is barely reactive with this lectin. Furthermore, the weak reactivity of v. Ebner glands with SBA and the high reactivity with PNA and DBA can be observed also without pretreatment of the section with neuraminidase. Of particular interest are the small differences between SBA and PNA with regard to TB affinity. At the light microscopical level, SBA can be observed almost exclusively in the taste pore region and to a lesser extent on the borders, presumably plasmalemmata, of TB cells. The dense reaction product of the taste pore, which is visualized light microscopically, could be traced to the enormously enlarged surface area of the microvilli protruding into the taste pore. PNA, however, reacts with both the pore substance and the cytoplasm of individual TB cells.

The comparative light and electron microscopical lectin studies suggest, indeed, that the reactivity of the taste pore material to lectins may be due to different characteristics. Ohmura et al. (1989) showed that the electron-dense taste pore material of hamster TBs exhibited the same affinity to WGA and UEA I as the content of dark granules of type I cells. However our experiments with the lectin from the edible snail (*H. pomatia*, HPA) demonstrated that the strongly reactive taste pore material, which was visualized light microscopically, is apparently due to membrane-bound glycoproteins of the microvillar processes of TB cells. Dark secretory granules of type I cells were only randomly labeled, and the amorphous mucous mass in the taste pore was entirely HPA-negative. The affinity of HPA to different types of complex galactosamine-containing carbohydrates, i.e., to secretory mucins as well as to structural glycoproteins has been shown by Roth (1984) and by Wasano et al. (1988).

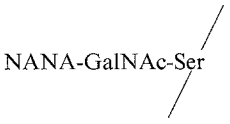
The castor bean lectin (*R. communis* agglutinin, RCA I) also presents a difference between circumvallate and foliate TBs, on the one hand, and fungiform TBs on the other. In circumvallate and foliate taste buds, neuraminidase is required to unmask galactosyl residues. Fungiform TBs do not respond to this treatment; apparently they do not contain sialic acid. Our results show that sialic acids occur in TB cells of foliate and circumvallate papillae and mask penultimate Gal- or GalNAc residues of oligosaccharide side-chains. The possibility should be considered that the occurence of sialic acids could be related to the existence of v. Ebner salivary glands, which are obligatorily associated with circumvallate and foliate papillae.

The biological significance of sialic acids is commonly ascribed (for review see Schauer 1975) to the following features: cell-to-cell interaction, prevention from premature enzymatic degradation of circulating glycoproteins by glycosidases (e.g. glycoprotein hormones; Ashwell and Morell 1974), and prevention of cell adhesion due to the negative charge of the sialic acid residues coating the cell membranes (e.g. red blood cells; Marikovsky and Danon 1969).

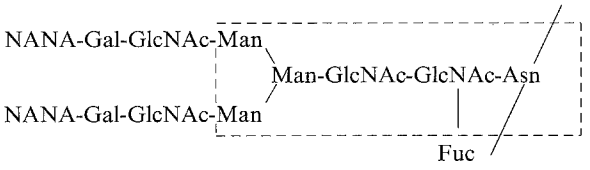
We offer two hypothetical models for the function of sialic acids in TBs. 1. Sialic acids as constituents of cell membranes prevent the long microvilli inside the narrow taste pore from adhering together. 2. Sialic acids not only mask the terminal oligosaccharide chain of *O*-glycosidically linked glycoproteins, as they occur commonly in mucins (Gottschalk 1972; Kornfeld and Kornfeld 1980; Table 3a), but they can also terminate *N*-glycosidically linked glycoproteins which are constituents of glycoprotein hormones. Histochemical studies using the sequence neuraminidase-PNA or neuraminidase-DBA have shown a characteristic reaction to gonadotrophins in cells of the anterior pituitary (Nakagawa et al. 1985; Witt and Klessen 1987). These gonadotrophins carry the terminal sequence Sialic acid–Gal–GlcNAc in their carbohydrate side-chains (Kessler et al. 1979). The constant core unit in all *N*-glycosidically linked oligosaccharide side-chains contains three mannose residues (Table 3b).

These mannose residues probably are responsible for the binding of the pea lectin (*Pisum sativum* agglutinin, PSA) to TB cells (Witt and Reutter 1988). Since mucins do not contain mannose (Allen 1983), the binding of PSA to some TB cells points to an *N*-linked glycoprotein and, possibly, to the hormone-like contents of some TB cells. This concept supports the idea of Fujita and Kobayashi (1979) that the contents of taste cells might serve a paraneuronal function by influencing the secretory activity of v. Ebner glands. The HPA binding to some dense-cored vesicles of type III cells gives a further indication of the occurrence of a glycoprotein in these vesi-

Table 3a. Oligosaccharide side-chain that is *O*-linked to serine (mucin of the bovine submandibular gland; Gottschalk and Graham 1959); NANA, *N*-acetyl-neuraminic acid



b. Oligosaccharide side-chain that is *N*-linked to asparagine (human chorionic gonadotrophin; Kessler et al. 1979); the oligosaccharide core unit is displayed in the dotted area



cles. The absence of HPA-binding sites in many vesicles shows their functional diversity. GAlNAc-containing glycoproteins which are located outside the vesicles could either be transferred into the dense-cored vesicles or have no relation to the former product. Cytochemically, this material is not identical with the amorphous substance in the taste pore, but the release of very small amounts of this substance either into the taste pore or through the basolateral plasmalemma is possible. So far, there is no histochemical evidence for this specific release.

Taste buds of fungiform papillae exhibit different lectin-binding affinities from circumvallate and foliate TBs, the former TBs are PNA- and RCA-negative, DBA-positive, and do not respond to pretreatment with neuraminidase. Fungiform TBs are also WGA- and UEA I-positive, as are TBs in the other papillae. The strong reactivity to these lectins is related to the dark granules of type I cells (Ohmura et al. 1989). Ultrastructurally, no differences were observed between the type I cells of different TBs (Miller and Chaudhry 1976). Fungiform papillae are not associated with local salivary glands. In this context, we can speculate that the secretory product of v. Ebner glands in foliate and circumvallate papillae influences not only the taste response (Gurkan and Bradley 1988; Ohsawa et al. 1988), but also may influence the glycochemical characteristics of the respective taste buds.

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