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Electron microscopic demonstration of lectin binding sites in the taste buds of the European catfish *Silurus glanis* (Teleostei)*

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Summary. Taste buds in the European catfish *Silurus glanis* were examined with electron microscopic lectin histochemistry. For detection of carbohydrate residues in sensory cells and adjacent epithelial cells, gold-, ferritin- and biotin-labeled lectins were used. A post-embedding procedure carried out on tissue sections embedded in LR-White was applied to differentiate between the sensory cells: The lectins from *Helix pomatia* (HPA) and *Triticum vulgare* (WGA) bound to *N*- acetyl-galactosamine and to *N*-acetylglucosamine residues occurring especially in vesicles of dark sensory cells. This indicates a secretory function of these cells. Most light sensory cells – with some exceptions, probably immature cells –, are HPA-negative. The mucus of the receptor field and at the top of the adjacent epithelial cells was strongly HPA-positive. Pre-embedding studies were performed in order to obtain information about the reaction of the mucus with lectins under supravital conditions. The mucus of the taste bud receptor field exhibited intensive binding to WGA, but not to the other lectins tested. Most lectins bound predominantly to the surface mucus of the nonsensory epithelium and to the marginal cells close to the receptor field. The strong lectin binding to mucins and the relatively weak lectin binding to cell surface membranes in pre-embedding studies suggest that the mucus possibly serves as a barrier which is passed selectively only by a small amount of lectins or lectin-carbohydrate complexes. Lectin-carbohydrate interactions may play a role in recognition phenomena on the plasmalemmata of the taste bud sensory cells. Recognition processes directed to bacteria or viruses should be considered as well.

Introduction

The carbohydrate content of fish taste buds (TBs) was described in previous studies by the use of conventional carbohydrate histochemistry and lectin histochemistry at the light microscopic level (Reutter and Klessen 1979; Witt and Reutter 1988). The sensory cells and the mucous substances covering the receptor field of a fish TB contain complex polymeric glycoconjugates. They protect the TB receptor field mechanically and probably also fulfil functions involving taste perception processes: Bannister (1974) interpreted the mucus to be an ion reservoir; the ions might be active in the chemoreception event. In the olfactory organ of vertebrates lectins have been used to demonstrate the important function of carbohydrate residues on top of the olfactory epithelium (fish: Kalinoski et al. 1987; frog: Key and Giorgi 1986; mouse: Plendl and Schmahl 1988; Lundh et al. 1989). Therefore lectins are not only a useful tool for detection of particular carbohydrate residues and for characterization of sensory cell types and mucous substances, but, moreover, they also seem to have important physiological functions with regard to taste perception, as shown recently by Kalinoski et al. (1989) in TBs of the catfish *Ictalurus punctatus*. In contrast to the olfactory organ, where membrane glycoproteins and an "odor binding protein" (OBP) have been isolated (Pevsner et al. 1988), transduction events in taste systems are not well understood yet. Recently, Schmale et al. (1990) isolated a protein from v. Ebner glands which is chemically related to the OBP and suggested an analogue function for taste perception.

Besides membranous ion channels (reviewed by Kinnamon 1988), ligand-gated channels (Roper 1989) also seem to play a role in transduction mechanisms for taste sensation.

The objectives of the present study were:

1. to obtain further information about the composition and the distribution of carbohydrate residues on the apical TB region and about the mucus on top of both sensory and adjacent nonsensory cells of *Silurus glanis* at the electron microscopic level.
2. to differentiate the morphologically well-known TB sensory cells (Grover-Johnson and Farbman 1976; Reutter 1978, 1986) by the aid of lectin histochemistry.

In order to detect carbohydrate residues in and around TBs by means of post- and pre-embedding procedures, we applied biotin-, gold- and ferritin-labeled

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lectins which bind specifically to galactose (Gal), *N*-acetyl-galactosamine (GalNAc), *N*-acetyl-glucosamine (GlcNAc), mannose (Man) and fucose (Fuc).

Material and methods

The investigations were carried out on the TBs situated in the barbel epithelium of the European catfish, *Silurus glanis*. Barbel pieces were cut off from the anaesthetized fish (MS 222, Sandoz, Basel, Switzerland) and immediately immersed into the respective solution (see below).

Preparation of colloidal gold particles (14 nm)

Colloidal gold particles were prepared according to Frens (1973): Briefly, 12 ml tetrachlorauric acid ($HAuCl_4$, 1%, Merck, Darmstadt, FRG) were added to 100 ml of double distilled water. This solution was heated to boiling, and 4 ml of trisodiumcitrate (1%, Merck) were added rapidly. Upon gentle boiling the solution became first blue, then red (approx. 10 min). The pH of colloidal gold measured at room temperature was adjusted to the required values given below. For electrode protection, aliquots of colloidal gold were stabilized with a few drops of 1% polyethylenglycole (PEG = Carbowax, Fluka, Buchs, Switzerland). Only siliconized glassware was used.

Preparation of lectin gold complexes

Lectins were labeled with gold particles according to Roth (1983): To stabilize 10 ml of the colloidal gold solution, the following amounts of lectins are necessary (for abbreviations see Table 1) (Zsigmondy's test – Horisberger 1983): HPA: 65 µg, pH of colloidal gold: 7.4; UEA I: 250 µg, pH 6.3; Con A: 250 µg, pH 8.0; PNA: 130 µg, pH 6.3, 20 ml of pH-adjusted colloidal gold solution and the required amount of lectin were centrifuged at $60000 \times g_{(max)}$ for 45 min at 4° C. The supernatant was removed and the deep red pellet was resuspended in 1 ml phosphate buffered saline (PBS, pH 7.4) containing (0.2 mg PEG and 0.02% sodium azide (Merck).

Preparations of Con A-*gold* and DBA-*gold* were purchased from Sigma (Munich, FRG) and Medac (Hamburg, FRG). In one series of pre-embedding experiments ferritin-labeled lectins (Medac) were used.

Post-embedding studies

Tissue preparation. Maxillary barbel pieces were fixed with 1.5% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.2) for 2 h at 4° C. After fixation, the specimens were washed in cacodylate buffer several times and then dehydrated by graded ethanols. Subsequently the specimens were immersed in pure LR White (medium grade, London Resin Co., Woking, UK) (Newman and Hobot 1987). After three additional changes of resin, the tissues were placed into gelatine capsules and polymerized at 60° C for 24 h.

Treatment of tissue sections with gold- resp. ferritin-labeled lectins. For post-embedding labeling the ultrathin sections were floated on a drop of PBS containing 0.1% BSA for 5–10 min. and then incubated with the lectin-gold (diluted 1:30–1:100) or lectin-ferritin (1:50) conjugates for 45 min (all reactions were performed at room temperature unless otherwise indicated). Then the grids were thoroughly washed in demineralized water. The sections were contrasted with uranyl acetate (4 min) and lead citrate (1 min). For comparison, sections from Epon-Araldite embedded barbels were also incubated with lectins in exactly the same way as described above. However, in these sections the background staining was too intense.

Table 1. Lectins used for the histochemical demonstration of carbohydrate residues in taste bud cells and adjacent epithelial cells of *Silurus glanis*

Lectin	Specificity[a]
Helix pomatia agglutinin (HPA)	GalNAc-α1,3 GalNAc > αGalNAc
Dolichos biflorus aggl. (DBA)	GalNAc-α1,3 GalNAc > αGalNAc
Arachis hypogaea aggl. (PNA)	Gal-β1,3 GalNAc > Gal
Glycine max aggl. (SBA)	α and β GalNAc > α and βGal
Ricinus communis I aggl. (RCA)	Gal > GalNAc
Canavalia ensiformis aggl. (CON A)	αMan > αGlc > GlcNAc
Pisum sativum aggl. (PSA)	αMan > αGlc, GlcNAc
Ulex europaeus aggl. I (UEA I)	α-L-Fuc
Triticum vulgare aggl. (WGA)	$(GlcNAc\ \beta1,4)_3$ > GlcNAc
Succinylated *Trit. vulg.* aggl. (S-WGA)	GlcNAc

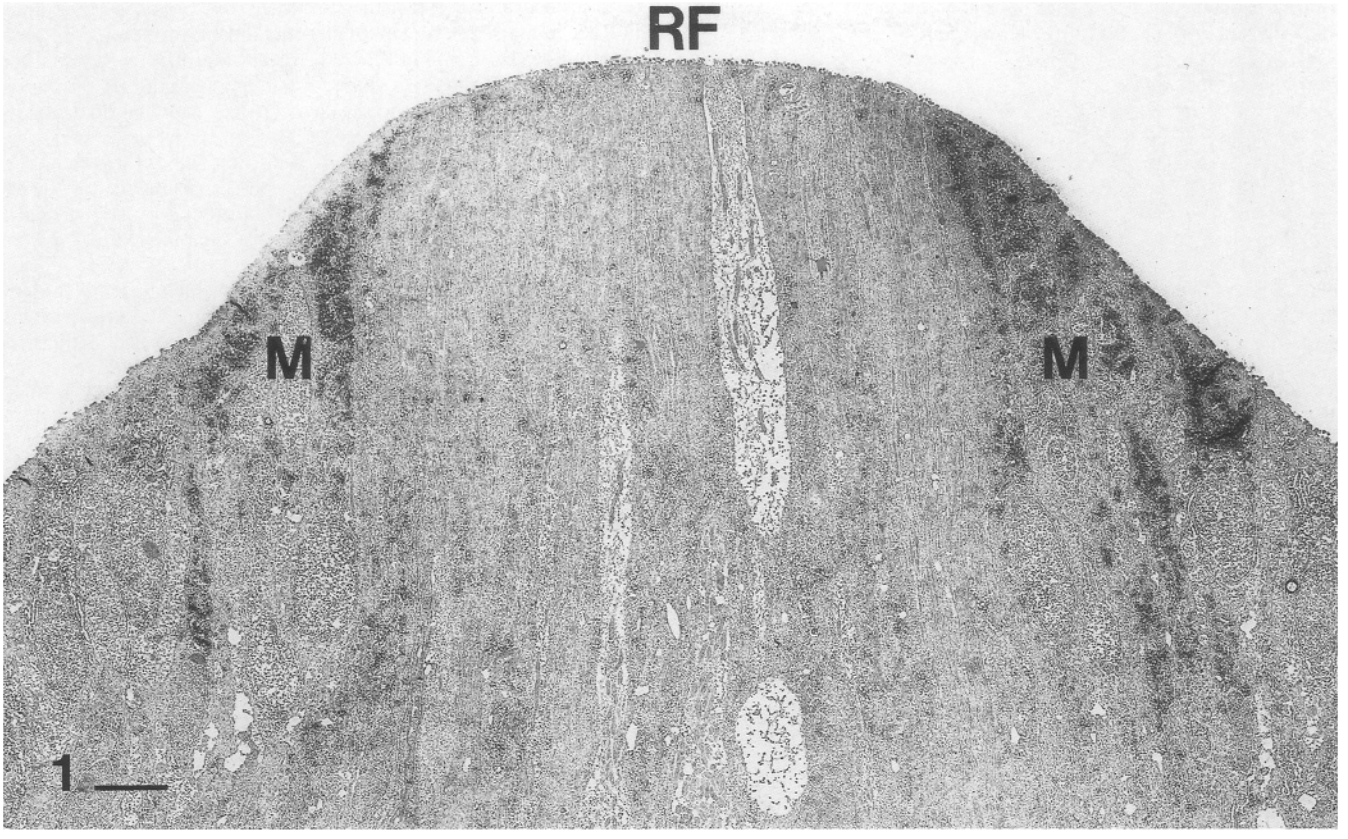
[a] For references see: Goldstein and Peretz (1986)

GalNAc = *N*-acetyl-D-galactosamine, GlcNAc = *N*-acetyl-D-glucosamine, Gal = galactose, Glc = Glucose, Man = mannose, Fuc = fucose

Cytochemistry with biotinylated lectins. For post-embedding labeling with biotinylated lectins, we modified the protocol of Jones and Stoddard (1986): Sections were floated on a drop of PBS for 5–10 min. Then the activity of endogenous peroxidases was blocked by treatment of the section with 3% H_2O_2. After being washed in demineralized water, the sections were incubated with biotinylated lectins (Vector, Burlingame, Calif., USA) for 60 min at a concentration of 10–20 µg/ml. Subsequently, the sections were thoroughly washed in PBS and incubated with a streptavidin-biotin/peroxidase-complex (ABC, diluted 1:100, Amersham-Buchler, Braunschweig, FRG) for 60 min. After a wash in PBS the reaction

Figs. 1–2. Taste buds of *Silurus* barbels which were fixed, embedded in LR White, cut and then labeled with lectins (post-embedding). **Fig. 1.** DBA: Longitudinal section through the apical part of a *Silurus* taste bud. Development and visualization of the reaction product with the ABC-method and DAB-H_2O_2. DBA reacts strongly with supranuclear material of marginal cells (*M*) and less intensely with the surface of the receptor field (*RF*). Section not contrasted. *Bar* = 3 µm, × 3400

Fig. 2. HPA-gold: TB receptor field. Lectin binding on the apical cell surfaces and the basolateral membranes of both light (*L*) and dark (*D*) sensory cells (*arrows*). Mucus-vesicles of dark sensory cells contain GalNAc residues (*arrowheads*), whereas the cytoplasm of light sensory cells normally lacks lectin binding sites. Enlarged cytoplasmic membrane systems (Golgi-vesicles?) in two light sensory cells (*right*) also react with HPA which normally does not occur. Contrast agents: Uranyl acetate (UA) and lead citrate (Pb). *Bar* = 0.5 µm, × 39800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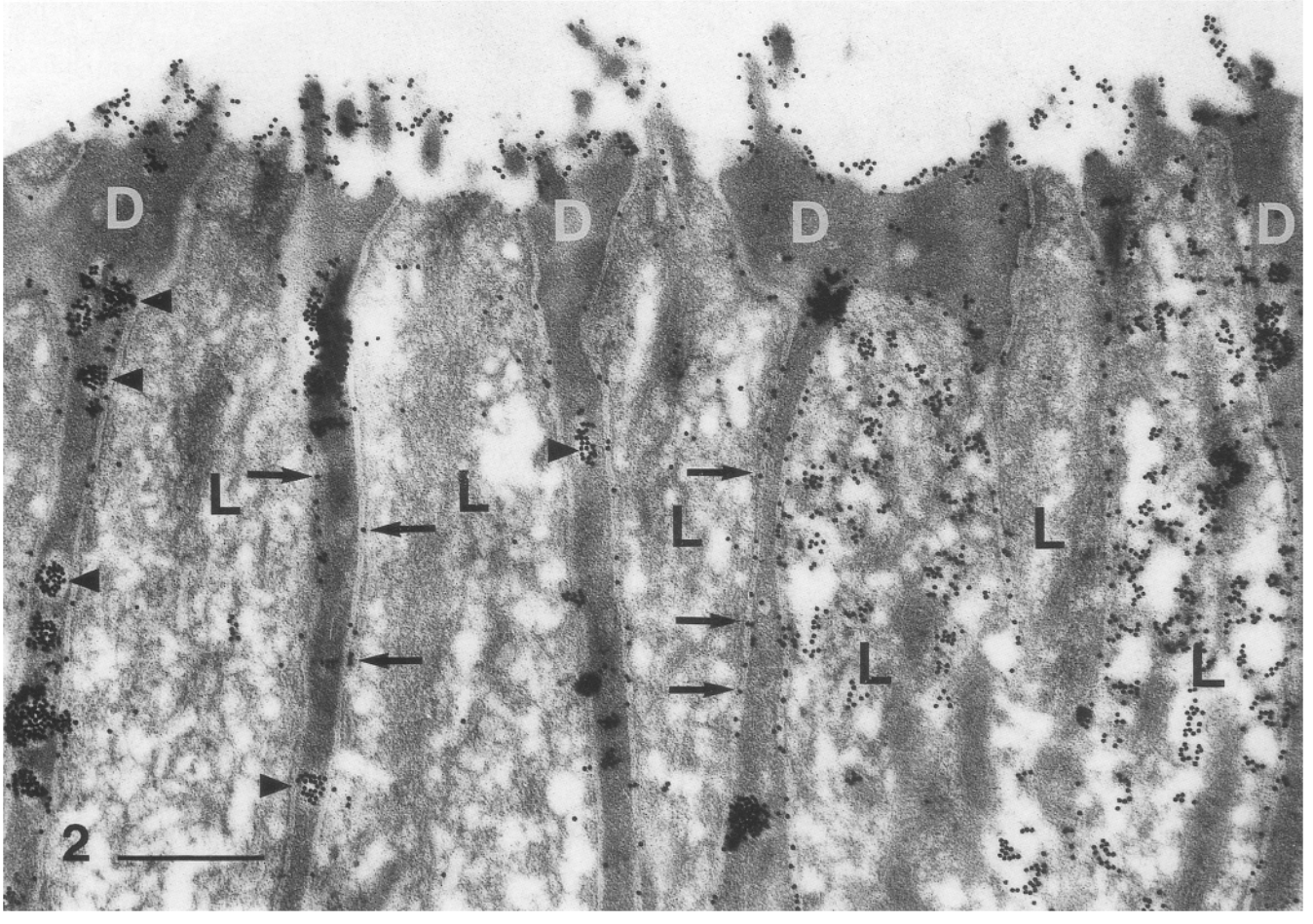
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Table 2. Carbohydrate residues in cells and mucus of the taste bud and adjacent epithelial cells of *Silurus glanis*

Post-embedding studies

Lectin	TB cells			Surface mucus of TB receptor field
	light	dark	marginal	
HPA-*Au*	+	+++	+++	++
DBA-*Au*	−	−	−	−
PNA-*Au*	−	−	−	−
CON A-*Au*	−	−	−	−
SBA-*biot*	?	?	+++	++
DBA-biot	+(?)	+(?)	+++	+++
PNA-*biot*	−	−	−	−
S-WGA-*biot*	?	+(?)	++	?
UEA I-*biot*	−	−	−	−
CON A-*Fe*	−	+	+	+
WGA-*Fe*	+	++	++	+

Pre-embedding studies

Lectin	Nonsensory epith.	Surface mucus of marginal cells	TB receptor field
HPA-*Au*	(+)	(+)	−
HPA-*biot*	+	+	+
DBA-Au	+	+	+
PNA-*Au*	−	−	−
PNA-*biot*	−	−	−
RCA-*biot*	−	−	−
CON A-*Au*	+	−	−
CON A-*Fe*	+	−	−
WGA-*Fe*	+	+	+

Au=gold-labeled; *biot*=biotin-labeled; *Fe*=ferritin-labeled; − = no reaction, +++=maximal reaction intensity

product was visualized with 3′-3′-diaminobenzidine (DAB, Serva, Heidelberg, FRG; passed through a Millipore filter, 0.22 µm) (10 min). Contrasting of the sections was omitted. In some sections, gold-labeled streptavidin (diluted 1:5–1:40, Sigma) instead of the ABC reagent was used.

In order to detect sialic acid residues, some sections were incubated with a 0.3–1 U/ml solution of neuraminidase (E.C.3.2.1.18, Behring, Berlin, FRG) for 1–18 h at 37° C. Controls were incubated with acetate buffer (pH 5.5; 37° C, 1–18 h).

Pre-embedding studies

Tissue preparation and treatment with lectins. Immediately after being cut off the fish, the barbel pieces were incubated for 30 min. in the respective *lectin-gold* resp. *lectin-ferritin* conjugates, then 3 × 10 min washed first in PBS, then in 0.17 *M* cacodylate buffer. Fixation was done for 4 h at 4° C in a solution of 2.5% glutaraldehyde, 2% paraformaldehyde and 0.1% picric acid dissolved in 0.1 *M* cacodylate buffer (pH 7.2). The barbel pieces were postfixed with 1% OsO_4 in cacodylate buffer for 2 h and contrasted with a saturated uranyl acetate solution in 70% ethanol overnight at 4° C (Wohlfarth-Bottermann 1957). Then the samples were embedded in a mixture of Epon-Araldite. Barbel pieces which were destined for incubation with *biotinylated lectins* were treated as follows: After being washed in PBS, the specimens were incubated in a drop of biotinylated lectin (10–20 µg/ml), washed in PBS and fixed as described above. Then the specimens were washed three times in PBS, immersed in a drop of streptavidin-biotin/peroxidase complex and washed again in PBS. The reaction product was visualized with DAB-H_2O_2 in PBS. After washing, the samples were processed for electron microscopy (see above). Osmification and contrasting procedures were omitted.

Some of the tissue specimens were first fixed, then incubated with gold- or ferritin-labeled lectins and then embedded in Epon-Araldite. In this case, osmification and block contrasting were also omitted. The lectins used in this study are summarized in Table 1.

Controls. Controls for lectin cytochemistry included: 1. Binding of the lectin to the respective specific sugar molecule prior to incubation. 2. Incubation of the section with the unlabeled lectin prior to treatment with conjugated lectins. Controls for biotinylated lectins included: 1. Omission of lectin, 2. Omission of ABC, 3. Binding test (corresponding to 1.).

Electron microscopy equipment. Ultrathin sections (60–80 nm thick) were cut on an Ultracut microtome (Reichert, Vienna, Austria) with a diamond knife and mounted on formvar-coated nickel (post-embedding) or copper grids (pre-embedding). The sections were examined under a Philips EM 300 electron microscope.

Results

In *Silurus*, the TB consists of elongate sensory cells. These are gathered into a bulb-like sensory epithelium which is situated at the top of a corium papilla. The sensory cells are surrounded by nonsensory marginal and epithelial cells. Ultrastructurally, sensory cells are classified into light and dark ones, the latter of which possess many vesicles, especially in the apical cytoplasm. A few small microvilli protrude from dark cells into the receptor field, each light cell having only one large receptor villus. The base of a TB is formed by the basal cells; the TBs nerve fiber plexus is located between basal cells and sensory cells.

The results of both the post- and pre-embedding lectin binding studies are summarized in Table 2.

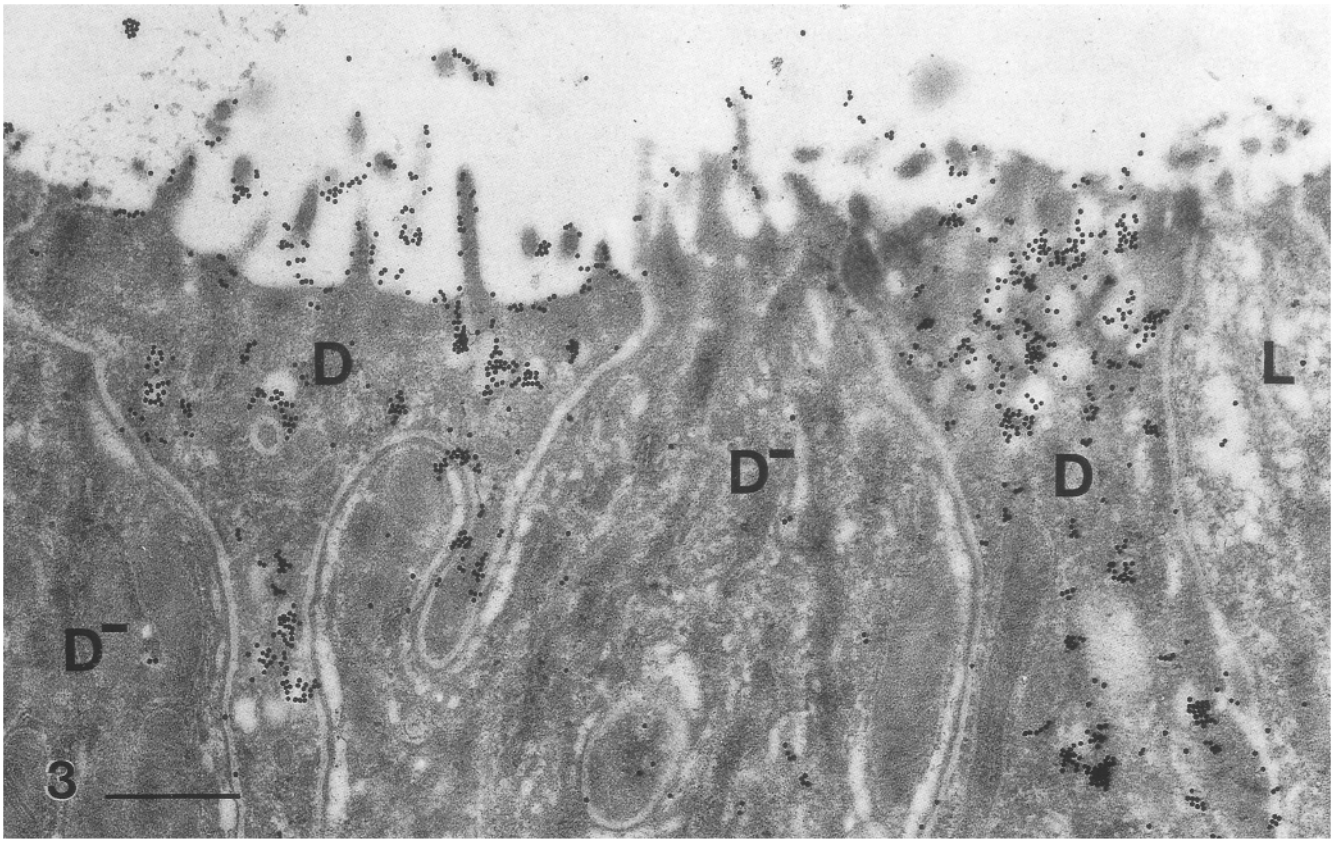
Post-embedding studies

Biotinylated DBA, SBA and S-WGA exhibit a strong positive reaction in the supranuclear parts of marginal

Figs. 3–5. Taste buds of *Silurus* barbels which were fixed, embedded in LR White, cut and then labeled with lectins (post-embedding). **Fig. 3.** HPA-*gold*: TB receptor field. Two dark sensory cells (D^-) without lectin binding are surrounded by slender dark sensory cells (*D*) with numerous vesicles containing GalNAc. *L*=light sensory cell. Contrast agents: UA and Pb. *Bar*=0.5 µm, ×35800

Fig. 4. HPA-*gold*: *Cis*-Golgi area (*arrows*) labeled with HPA. There are considerably fewer GalNAc residues on the enlarged *trans*-side. The rough endoplasmic reticulum (*arrowheads*) lacks HPA binding sites. *MV*=mucous vesicles. Contrast agents: UA and Pb. *Bar*=0.1 µm, ×81300

Fig. 5. HPA-*gold*: TB receptor field. A light sensory cell (*L*) penetrates the surface mucous layer with its large microvillus (*outlined*). The large microvillus shows fewer lectin binding sites than the small microvilli of dark sensory cells (*D*) do. Contrast agents: UA and Pb. *Bar*=0.5 µm, ×36100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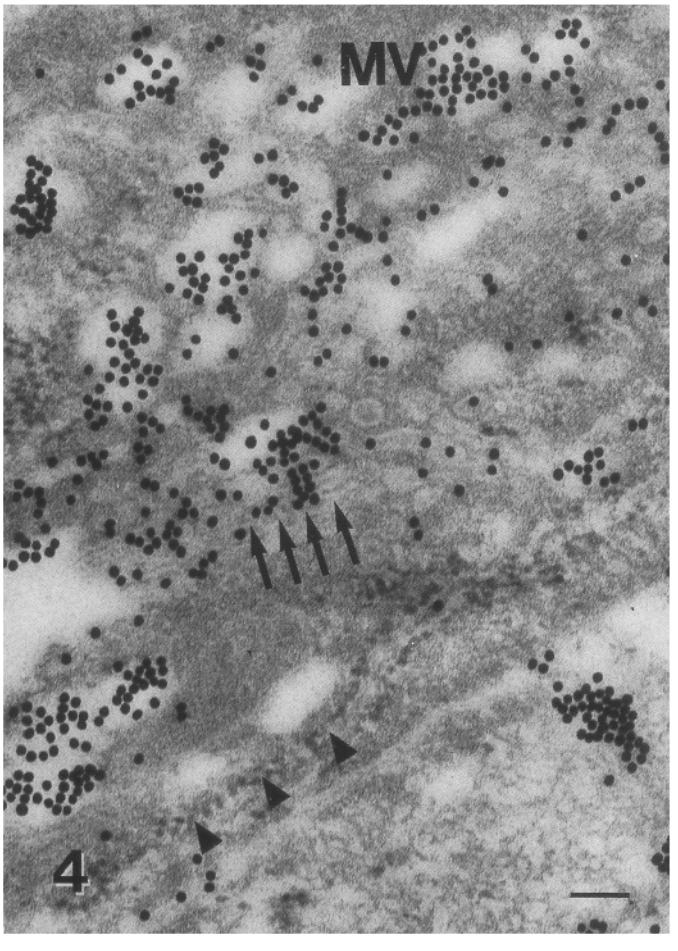
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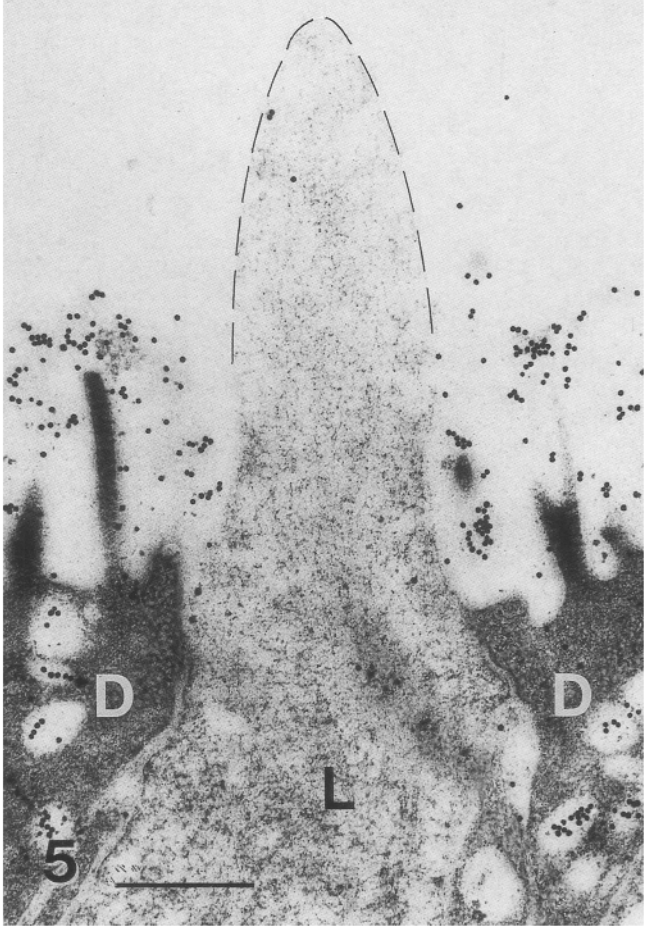
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cells (Fig. 1). However, especially at higher magnifications the ABC-method does not permit differentiation between the reaction product and electron-dense tissue components. For this reason, also gold- and ferritin-labeled lectins were used. Whereas the ABC method gave positive results in LR white-embedded tissues, carbohydrate residues which react with biotinylated lectins could not be detected when gold-labeled streptavidin was used.

From all gold-labeled lectins applied in this study only *Helix pomatia agglutinin* (HPA) reacts with TB sensory cells and with marginal cells: Numerous vesicles in dark sensory cells contain GalNAc residues, whereas light sensory cells are usually lectin negative. There are, however, light sensory cells that show a positive lectin binding pattern, as well as somewhat darker sensory cells which lack lectin binding sites (Figs. 2 and 3). Moreover, HPA-*gold* particles are bound to *cis* cisternae of Golgi fields, more than to their *trans* sides. The endoplasmic reticulum does not contain GalNAc residues (Fig. 4). Both the applied ferritin-labeled lectins, WGA and Con A, react with the vesicular content of dark sensory cells and marginal cells.

The TB's receptor field is covered by a thin layer of mucous substances which is only perforated by the large microvilli of light sensory cells (Figs. 5, 6 and 7). In preparations which were less thoroughly washed prior to fixation and lectin incubation, the remaining surface mucus reacts strongly with HPA-*gold* and WGA-*Fe*. Lectin binding is more associated to small microvilli belonging to dark sensory cells than to the large microvilli of light sensory cells (Fig. 5).

Pre-embedding studies

The results obtained after lectin incubation of barbel pieces for 30 min demonstrate that no lectin or lectin-carbohydrate complexes are absorbed by TB sensory cells or any of the other epithelial cells mentioned. After incubation with WGA-*Fe*, a strong binding reaction of the surface mucus of unspecialized epithelium, marginal cells and the receptor field is to be seen (Fig. 6). In preparations in which the tissues were first fixed and then incubated with lectins, WGA-*Fe* binds directly to the cell surface (Fig. 7). Both DBA-*gold* and Con A-*Fe* bind only to surface mucus of the adjacent nonsensory epithelium (Figs. 8 and 9). In most of the pre-embedding preparations, HPA binds distinctly only to the surface mucus of nonsensory and marginal cells (Fig. 10), but in preparations which were first fixed and then incubated with lectins, HPA-*gold* particles are to be demonstrated also within the receptor field (Fig. 11). The mucus which is situated directly above the goblet cells of the nonsensory epithelium does not react with WGA-*Fe* (Fig. 12).

In contrast to the findings we obtained by the use of gold-labeled HPA, a positive HPA binding reaction to the TBs and epithelial surface mucus takes place when the biotinylated conjugate has been used (Fig. 13). Biotinylated RCA I and PNA do not bind.

Discussion

Glycoproteins consist of protein chains to which one or more oligosaccharide units are attached. These are linked either *N*-glycosidically (from *N*-acetyl-glucosamine) to asparagine, for instance in the glycoprotein hormones, or O-glycosidically (from *N*-acetyl-galactosamine) with serine and/or threonine, which occur in most mucins (Kornfeld and Kornfeld 1980). Two examples are given in Table 3.

In the present study several lectins specific to galactosyl and glucosyl residues were used in order to detect the sugar compound of taste sensory cells. Especially the lectin from the edible snail *Helix pomatia* (HPA) gives information about the carbohydrate distribution and the secretory function of various TB cells. In ultrastructure histochemistry, this lectin has been used for the detection of terminal GalNAc-residues in secretory cells in various organs, e.g. the gastric mucous neck cells, intestinal goblet cells and tracheal epithelial cells (Suganuma et al. 1985; Roth 1984; Ellinger and Pavelka 1985; Wasano et al. 1988). HPA binds not only to mucous granules of dark sensory cells of the TB, to marginal cells and to the surface mucous layer, but also to compounds of the basolateral plasmalemma of TB cells. This supports similar observations of Wasano et al. (1988). HPA and PNA binding activities did not increase after treatment of the section with neuraminidase. These lectins indicate the penultimate position of galactosyl residues which are masked by sialic acids. This finding confirms previous light microscopic studies in *Silurus* (Witt and Reutter 1988), but is in contrast to findings of other authors who detected mucopolysaccharides containing

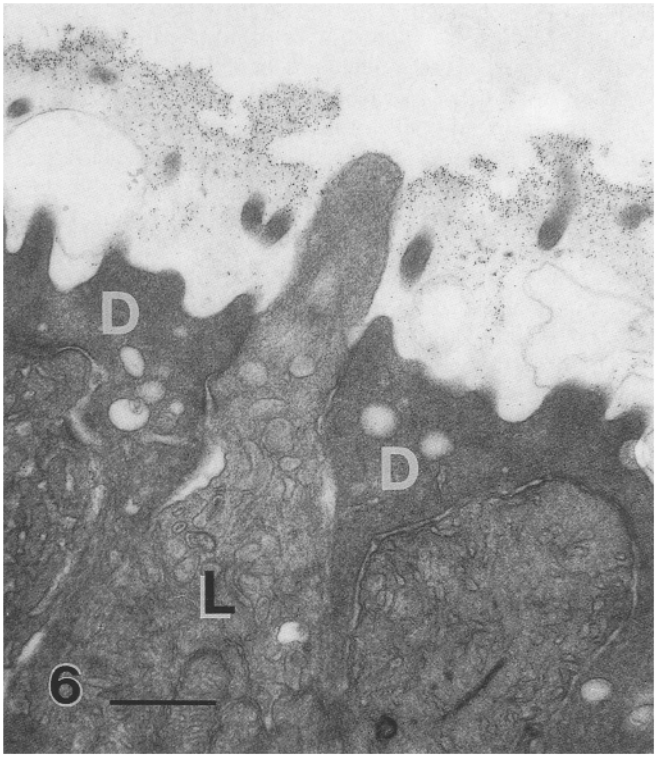
Figs. 6–10. Sections from barbels which were first incubated with lectins, then fixed and embedded in Epon-Araldite (pre-embedding). **Fig. 6.** WGA-*Fe*. TB receptor field. A large microvillus belonging to a light sensory cell (*L*) penetrates the mucous layer which is strongly marked with WGA. Note the diminuishing intensity of lectin binding in the mucus from apex to base. Almost no lectin conjugate reaches the cell surface membranes. No cellular uptake of lectin is to be seen. Contrast agents: UA and Pb. *Bar* = 0.5 μm, × 28200

Fig. 7. WGA-*Fe*. In this preparation the fixation preceded the lectin incubation. WGA binds strongly to the cell surfaces of dark sensory cells (*D*). The large microvillus is not marked. Contrast agents: UA and Pb. *Bar* = 0.5 μm, × 42800

Fig. 8. DAB-*gold*. Surface of a nonsensory epithelial cell. The surface-mucus is apically marked with DBA. Contrast agents: UA and Pb. *Bar* = 0.5 μm, × 53200

Fig. 9. Con A-*Fe*: Surface of a nonsensory epithelial cell. The lectin binds to the surface of the mucus layer. Contrast agents: UA and Pb. *Bar* = 0.5 μm, × 48300

Fig. 10. HPA-*gold*. Transition from the TB receptor field (*left*) with dark (*D*) and light (*L*) sensory cells to the marginal cells (*M*) zone. Only the surface mucus above marginal cells contains irregularly distributed HPA binding sites (*arrows*). Contrast agents: UA and Pb. *Bar* = 1 μm, × 19500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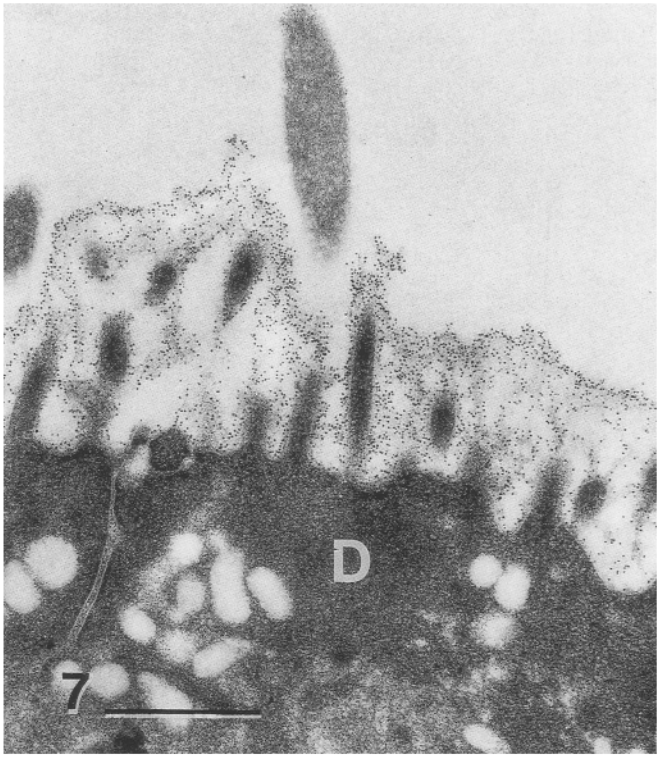
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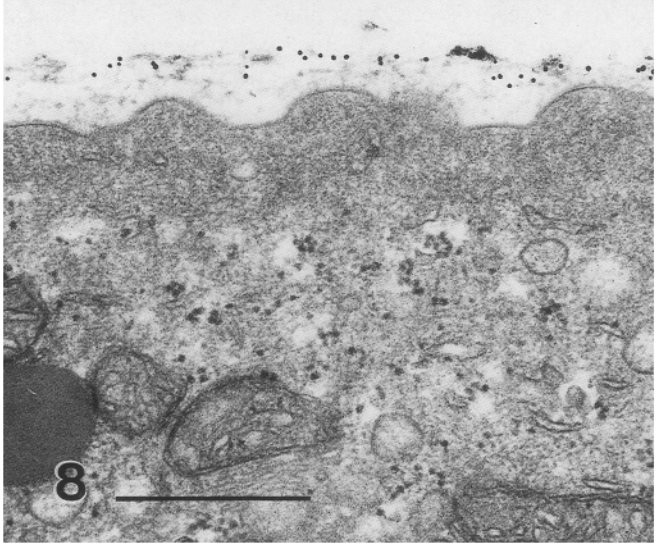
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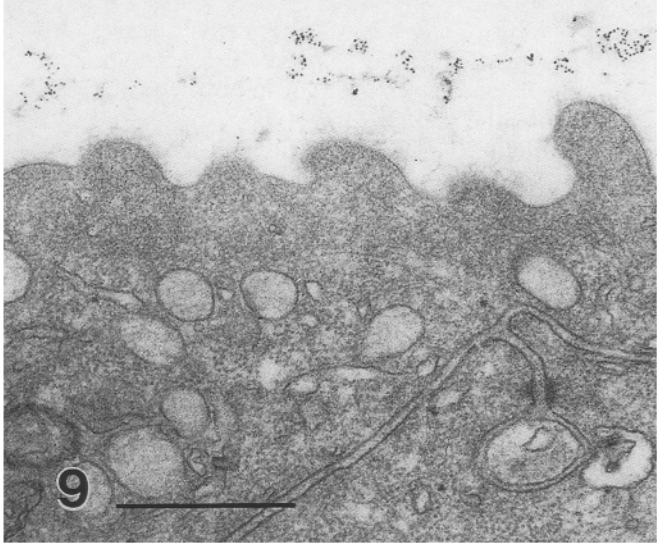
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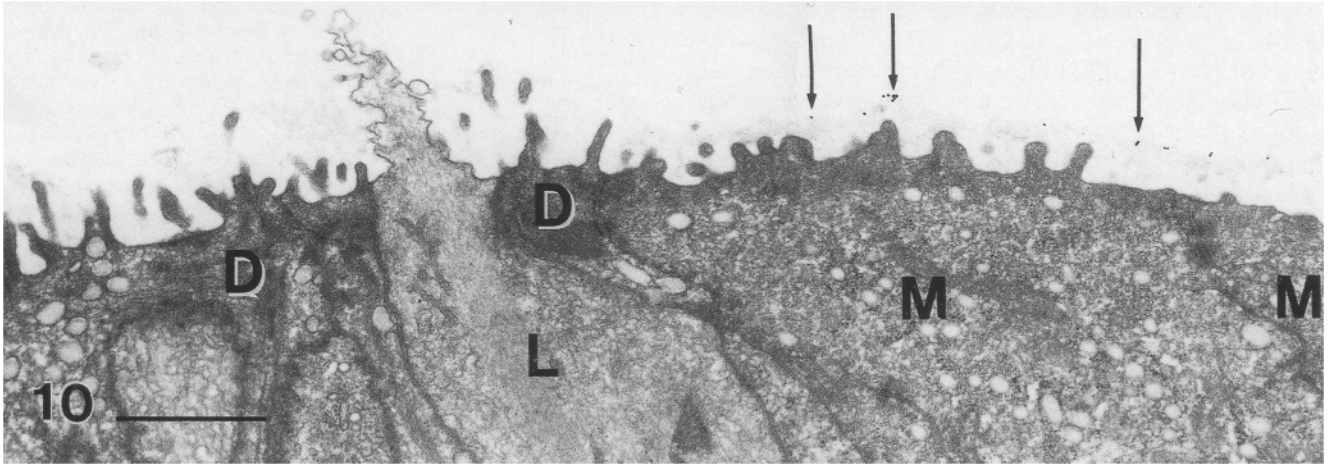
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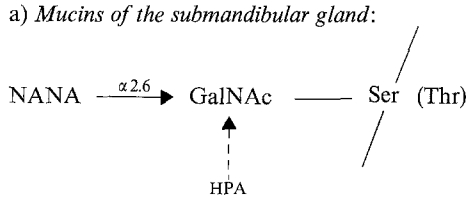
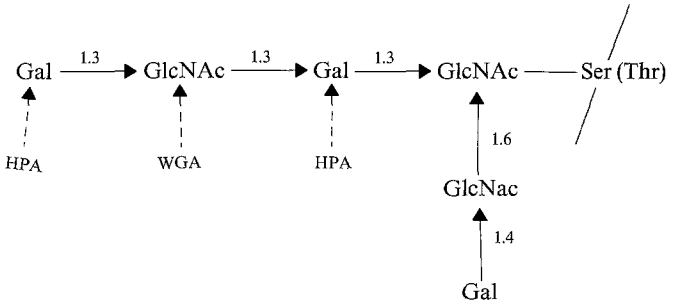
10

Table 3. Two examples of common oligosaccharide units linked to a protein chain via Serine/Threonine (Ser/Thr). HPA and WGA are shown to bind to galactosyl and glucosyl residues, respectively. NANA = *N*-acetyl-neuraminic acid (Kornfeld and Kornfeld 1980).

a) *Mucins of the submandibular gland*:



b) *Human gastric mucin*:



sialic acids in the epidermis of other catfish species (*Heteropneustes fossilis*; *Ictalurus nebulosus*: Zaccone et al. 1985; Zuchelkowski et al. 1985) or in bovine taste receptor cells (Lum and Henkin 1976).

Both the other GalNAc-specific gold-labeled lectins, PNA and DBA, showed almost no binding reaction. In the case of PNA, this is in accordance with our earlier report (Witt and Reutter 1988). PNA has been used to detect the disaccharide D-Gal-β(1–3)-D-GalNAc (Gal/GalNAc) and D-Gal in various tissues (Lotan et al. 1975; Schulte and Spicer 1985). The reason for the lack of PNA binding even after pretreatment with neuraminidase may be due to missing or inaccessible specific sugar residues in *Silurus* TB structures and the adjacent epithelia. In this regard the composition of mucins in *Silurus* TB differs from that of mucins observed in other organs (e.g. submandibular glands; Kornfeld and Kornfeld 1980). The interpretation of the different binding activities of HPA and DBA is difficult, as both lectins are specific for the same disaccharide: GalNAc-α(1–3)-GalNAc (Baker et al. 1983). The relatively low molecular weight (D = 79000) of HPA could explain the good accessibility to the receptor and therefore the high reactivity of HPA in our studies compared to that of PNA (D = 120000) and DBA (D = 140000). The negative results of gold-labeled DBA in post-embedding studies were possibly provoked by the acrylic resin we used, which caused the steric hindrance of the DBA-gold conjugate.

Pre-embedding studies

In preparations in which the lectins were applied prior to fixation and embedding of the tissue specimen (pre-embedding), DBA binds to GalNAc residues situated within the surface mucous layer of nonsensory epithelial cells, but not to the mucus and membranes of marginal and sensory cells. We observed a comparable binding pattern by the use of gold- and ferritin labeled Con A indicating mannosyl and glucosyl residues (Goldstein and Hayes 1978; Debray et al. 1981). Thus, the pre-embedding binding patterns of DBA, HPA and Con A demonstrate that the mucous secretions on and near the TB are not homogenous at all. It is likely that the different binding is caused by a different sugar composition of the mucins, but theoretically it also could be due to a different conformation of the highly polymerized mucous substances. In general, the mucus situated above nonsensory epithelial cells allows more lectins to bind than does the mucus which covers the receptor field of the TB and the marginal cells. The different binding behavior of the mucins to lectins points to their possibly highly specialized functions within compartments above the TB surface.

Post-embedding studies

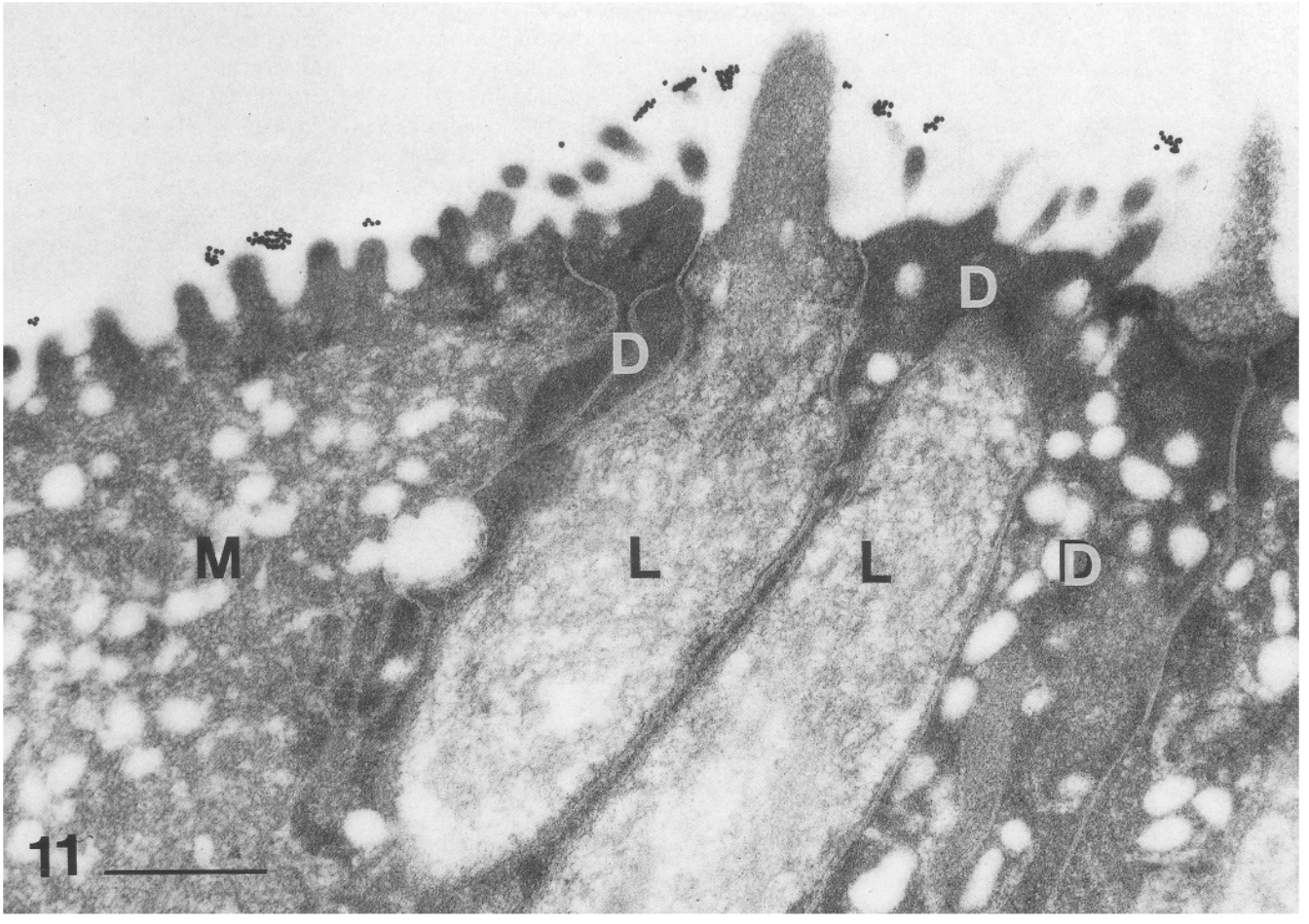
The results of post-embedding investigations demonstrate distinct differences in the composition of mucins of dark sensory cells, marginal cells and the adjacent nonsensory epithelial cells. In experiments performed on a section, HPA is suitable for a functional differentiation between the TB cells themselves. In Silurids there are up to five different TB cell types under discussion (Desgranges 1965), but besides the basal cells, only two, the "dark" and the "light" sensory cells, are generally accepted (Reutter 1978, 1986). The relatively electron-dense dark sensory cells contain numerous GalNAc- and GlcNAc-rich vesicles. These sugars are obviously bound to mucins which are released into the receptor field region. Therefore a secretory function can be attributed to these cells.

In contrast, most of the less electron-dense light sensory cells exhibit no lectin binding sites. However, some

Figs. 11–13. Sections from barbels, which were first incubated with lectins, then fixed and embedded in Epon-Araldite (pre-embedding). **Fig. 11.** HPA-*gold*. Transition from the TB receptor field (*right*) to the marginal zone (*left*). In contrast to Fig. 10, the barbel was first fixed and then exposed to the lectin. After fixation, more lectin binding sites become detectable, also above the receptor field. *M* = marginal cell, *L* = light sensory cell, *D* = dark sensory cell. Contrast agents: UA and Pb. *Bar* = 0.5 µm, × 44300

Fig. 12. WGA-*Fe*. Surface of an epidermal goblet cell (*G*). WGA binds almost exclusively to the surface of surrounding nonsensory epithelial cells (*E*). Contrast agents: UA and Pb. *Bar* = 0.5 µm, × 37700

Fig. 13. HPA-*biot*. Taste bud receptor field. Biotinylated HPA was processed with ABC and visualized with DAB-H_2O_2. The reaction product marks the mucus and the surface of small microvilli of dark sensory cells (*D*). *L* = Light sensory cell. Contrast agents: UA and Pb. *Bar* = 0.5 µm, × 33500


D
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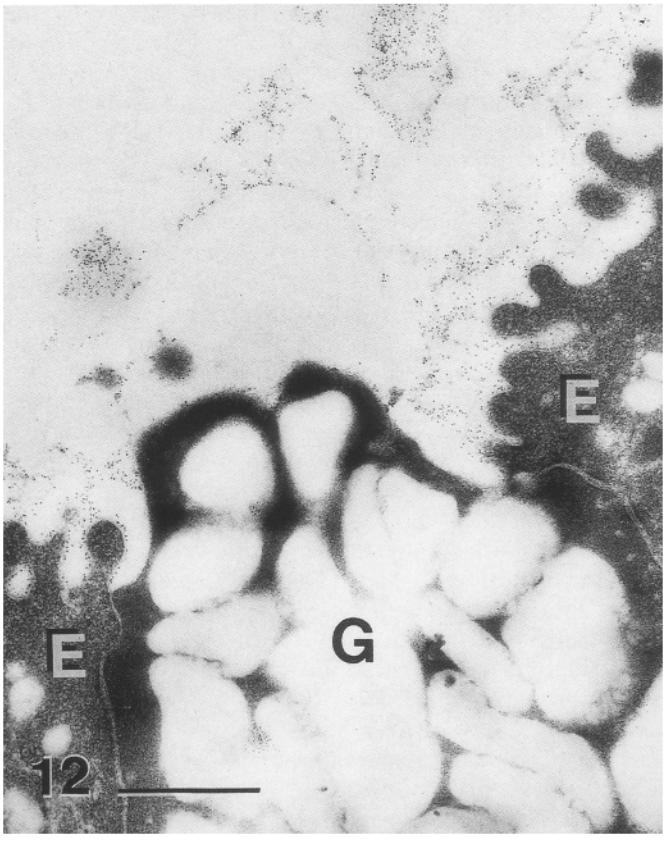
11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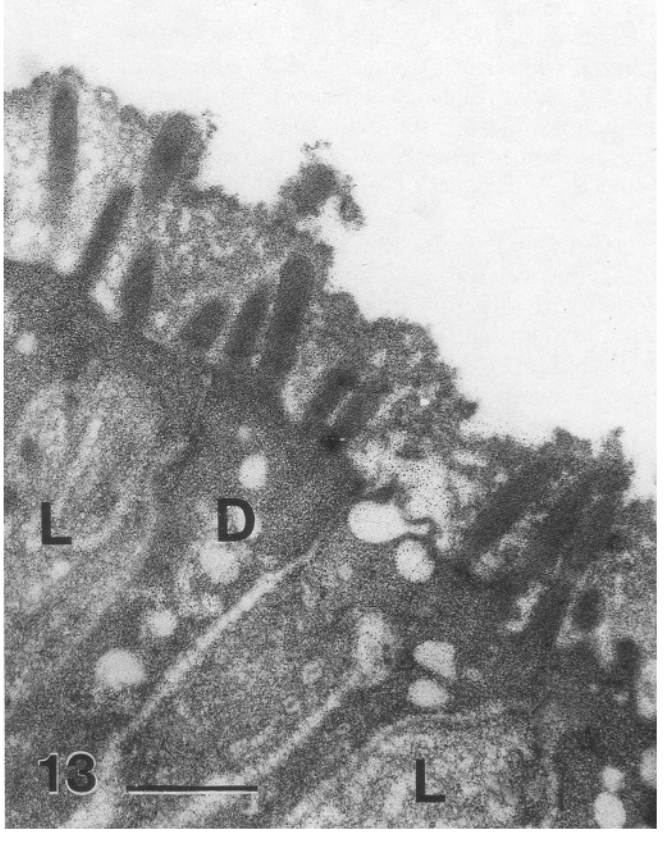
L
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of the light sensory cells contain GalNAc residues that are located rather diffusely in the supranuclear cytoplasm. The significance of this finding remains unclear. Since the light sensory cells do not contain vesicles or secretory granules, the question arises whether HPA binding in these cells indicates the presence of mucins or other O-glycosidically-linked glycoproteins. The second possibility of interpretation would be supported by Wasano et al. (1988), who identified in tracheal secretory cells three major HPA binding glycoproteins, one of which is not a mucin, but an integral membrane constituent. This seems to be responsible for HPA binding to the basolateral plasmalemmata of sensory cells in our experiments. Moreover, the different lectin binding pattern in distinct light sensory cells could be explained by a different procedure of glycosylation of oligosaccharide chains, according to Roth (1984): During an early stage of glycosylation, it is possible to expose GalNAc residues temporarily to HPA, whereas during advanced glycosylation, as is the case in most of the other light sensory cells, the former terminal GalNAc residues could be masked by other monosaccharides. Therefore, HPA would no longer have access to GalNAc residues. Cell lineage studies of Delay et al. (1988) suggest the presence of only one cell type in the vertebrate taste bud, from which all other morphologically different cells arise. Though our histochemical approach clarifies not the highly dynamic process of the cellular takeover and development in the taste bud, we believe for above-mentioned reasons that lectin-binding light sensory cells represent an immature or precursor light sensory cell. However this does not exclude a temporary specialized function of these cells.

The dark sensory cells also show different HPA staining patterns. Besides the predominant slender (HPA-positive) sensory cells, some bottle-shaped, dark, mitochondria-rich sensory cells are lectin-negative (Fig. 3). Since these cells show no signs of secretory activity, these dark cells could be specialized light sensory cells.

HPA binding to the apical plasmalemmata also differs between dark and light sensory cells: Dark sensory cells with secretory functions exhibit more HPA binding sites than light sensory cells. It is not clear whether lectin binding on the surface of these cells is due to integral membrane glycoproteins or to membrane-associated mucins, or to both. In preparations in which the mucous layer was rigorously washed away, considerably less lectin binding on the surface of superficial cells was observed. In any case, light sensory cells penetrate the surface mucous layer of the receptor field by means of large microvilli (Reutter 1987). They are certainly exposed to other gustatory information than the numerous small receptor villi of dark sensory cells which are situated within the surface mucus layer of the receptor field.

Physiological significance of lectin binding sites in taste buds

Finally, based on both on-section and pre-embedding studies, the interaction between lectins and carbohydrates allows speculations about the significance of these compounds for taste perception. Lectin-carbohydrate interactions play an important role in normal recognition phenomena (reviewed by Sharon and Lis 1972), but also in the pathogenesis of infection and metastasis of cancer cells (reviewed by Gabius et al. 1986). The interaction between a circulating free compound and a membrane-bound constituent might take place in the following way: Freely circulating (macro)molecules which possess exposed lectins are bound by respective glycoconjugates situated on a cell surface (lectin receptors). This binding process has been found to be essential for the pathogenesis of viral (Luther et al. 1989) and bacterial (Winberg 1984; Uhlenbruck 1987) infections and also for the elimination of bacteria via phagocytosis (lectinophagocytosis: Ofek and Sharon 1988). In the respiratory and the olfactory epithelium, lectin binding sites are discussed as essentials for selectivity in virus attacks (Lundh et al. 1989; Luther et al. 1989). Although a similar function for the taste epithelium has not been yet proven, we suggest that the mucous layer above the receptor field and the adjacent epithelium work as a selective barrier against different taste substances.

As our pre-embedding results with HPA and WGA show, most of the respective lectin is bound by GalNAc (HPA) and GlcNAc (WGA) containing mucous substances. Relatively few particles reach the surface of the epithelium directly. Therefore the mucus might be a selective barrier against potent lectin-containing agents.

Lectins have been shown to be useful reagents for characterization of olfactory receptors (Chen et al. 1986; Kalinoski et al. 1987). We suggest that taste transduction is at least partly dependent on the activity of lectins. Several authors postulate a glycoprotein receptor for carbohydrates (i.e. endogenous lectin), for instance in the taste epithelium of the cow (Lum and Henkin 1976) and the catfish (Holland and Teeter 1981; Caprio and Byrd 1984). In the taste cells of Rhesus monkeys, sugar binding sites for gold-labeled thaumatin, a sweet tastant, have also been detected histochemically (Farbman et al. 1987). The inverse model of lectin-sugar interaction was described in channel catfish by Kalinoski et al. (1989). They found that DBA could inhibit the taste response to alanine (Ala), an effective taste stimulus in catfish. The putative receptor for Ala is a membrane-associated glycoprotein (Bryant et al. 1986). In our *Silurus* experiments the positive HPA binding at the apical plasmalemma of sensory cells could point to such a glycoprotein. It is as yet unclear whether exogenous lectins are to be considered as blocking agents for taste substances or as parts of taste substances themselves, or both.

In conclusion, the lectin from *Helix pomatia* is a suitable tool for the characterization of TB sensory cells, especially to determine the secretory status of the cells. The GalNAc-rich vesicles in dark sensory cells are of secretory nature. The mucus covering the receptor field obviously allows only a small fraction of the applied lectins to reach the surface of the sensory cells. Although there is too little information about the functions of these secretions, it can be assumed that the mucus presents a kind of filter system to lectins or lectin-sugar com-

plexes in order to select particular substances prior to the chemoreception processes which take place on the plasmalemmata of the microvilli on the sensory cells.

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