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Characterisation of glycoconjugate sugar residues in the vomeronasal organ of the armadillo *Chaetophractus villosus* (Mammalia, xenarthra)

P. D. CARMANCHAHI[1], C. C. FERRARI[1], H. J. ALDANA MARCOS[1,2], J. M. AFFANNI[1,2], C. A. SONEZ[3] AND D. A. PAZ[1]

[1] *Instituto de Neurociencia* (*INEUCI-CONICET*), *Universidad de Buenos Aires.* [2]*Facultad de Medicina. Universidad de Morón, and* [3]*Facultad de Agronomia y Veterinaria, Universidad de Río Cuarto, Córdoba, Argentina*

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ABSTRACT

Conventional carbohydrate histochemistry and the binding patterns of 21 lectins were analysed to characterise the glycoconjugate content in the components of the vomeronasal organ of the armadillo *Chaetophractus villosus*. The mucomicrovillous complex of the sensory epithelium bound most of the lectins studied. No reaction was observed with Con A, PSA, S-Con A and SBA, and the sustentacular cells were stained with UEA-I, DSL, LEL, STL and Con A. The vomeronasal receptor neurons were labelled with S-WGA, WGA, PNA, UEA-I, STL, Con A, S-Con A, ECL and RCA_{120}. The basal cell layer reacted with S-WGA, WGA, LCA, UEA-I, DSL, LEL, STL, Con A, JAC and VVA. The nonsensory epithelium exhibited a differential staining in relation to the different components. The mucociliary complex stained with ECL, DBA, JAC, RCA_{120}, STL, LCA, PHA-E, PHA-L, LEL, BSL-I and VVA. However, SJA and UEA-I stained the mucus complex lining a subpopulation of columnar cells. The cytoplasm and cell membranes of columnar cells was labelled with DBA, DSL and LCA. The apical region of these cells exhibited moderate reactivity with LEL and SJA. None of the lectins bound specifically to secretory granules of the nonsecretory cells. Basal cells of the nonsensory epithelium were labelled with DSL, LEL, LCA, BSL-I and STL. The vomeronasal glands showed a positive reaction with WGA, DSL, LEL, LCA, DBA, PNA, RCA_{120} and SBA. Subpopulations of acinar cells were observed with ECL, S-WGA, Con A, S-Con A and DBA. PNA and RCA_{120} stained the cells lining the glandular ducts. In comparison with previous results obtained in the olfactory mucosa of the same group of armadillos, the carbohydrate composition of the vomeronasal organ sensory epithelium differed from the olfactory sensory epithelium. This is probably related to the different nature of molecules involved in the perireceptor processes.

Key words: Lectins; olfactory mucosa; mucus; vomeronasal organ; armadillo.

INTRODUCTION

The receptor organ of the accessory olfactory system is the vomeronasal organ (VNO), which receives pheromonal molecules related to social and reproductive behaviour, and transmits the information to the accessory olfactory bulb (Farbman, 1992). The VNO is located in the anterior part of the base of the nasal septum. In the majority of species the mucosa is lined with a sensory epithelium (SE) containing vomeronasal receptor neurons, sustentacular cells and basal cells, and a nonsensory epithelium (NSE) with ciliated, nonciliated secretory and basal cells. Vomeronasal glands are present in the submucosa. The ducts of these glands reach the lumen in the dorsolateral region between NSE and SE (Loo & Kanagasuntheram, 1972; Carmanchahi et al. 1999). Both epithelia are covered by a mucus layer, which may influence the access of stimulating molecules to receptor neurons and affect the threshold for mole-

Correspondence to Dr Pablo D. Carmanchahi, Instituto de Neurociencia (CONICET), FCEyN, Ciudad Universitaria, Pab. II, 4 piso, Lab. 102, (1428) Buenos Aires, Argentina. e-mail: pdc@bg.fcen.uba.ar

cular recognition (Bal & Anholt, 1993). The glycocalyx is also considered a site of the air/mucosa interaction. It represents the final barrier between the plasma membranes of the epithelial cells and the external medium, where it acts as a 'molecular sieve' regulating access to the cell membranes (Foster et al. 1992). Several properties of the mucus and the glycocalyx depend on the glycoconjugate (GC) contents.

GCs in the mucus are major determinants of mucus viscosity and of diffusional permeability, ion binding and bacterial entrapment (Foster et al. 1991). Cell surface GCs were shown to participate in differentiation, degeneration and regeneration (Etzler, 1985; Breer, 1991). Lectins are nonimmune glycoproteins of plant or animal origin, which bind carbohydrate residues on GCs. They are useful tools for (1) recognition of the mucus and glycocalyx components (Foster et al. 1992; Menco & Farbman, 1992; Bal & Anholt, 1993), (2) intracellular localisation of sugar residues (Ihida et al. 1991; Danguy et al. 1994), and (3) characterisation of distinct cellular populations (Accili et al. 1992; Spicer & Schulte, 1992; Shapiro et al. 1995).

Reports of lectin-binding patterns in the VNO are scarce. They have revealed the presence of specific sugar residues expressed in vomeronasal receptor neurons and glands (Barber, 1989; Takami et al. 1994; Shapiro et al. 1995). However, the results were inconsistent. The binding patterns are different even in closely related species (Salazar & Sánchez Quinteiros, 1998). On the other hand, most of the studies of lectin histochemistry in mammals were made in only a few species (Loo & Kanagasuntheram, 1972; Lundh et al. 1989; Taniguchi et al. 1992; Saito et al. 1994; Takami et al. 1994; Nakajima et al. 1998).

The mammalian order Xenarthra is a small but morphologically varied neotropical group of about 15 genera (Wetzel, 1985). Its living representatives include sloths, anteaters and armadillos. Armadillos are suitable models for morphological (Affanni et al. 1969; Benitez et al. 1994; Ferrari et al. 1998) and physiological (Affanni & García Samartino, 1970, 1984) studies on the olfactory system on account of the extensive rhinencephalic development with large main and accessory olfactory bulbs and olfactory tubercles. A well-developed VNO was described in *Chaetophractus villosus*, with the GCs of the mucus layers presumably synthesised by 2 cellular populations, the columnar cells of the NSE and the acinar cells of the vomeronasal glands (Carmanchahi et al. 1999).

The aims of this study were to identify and localise specific GC sugar residues using 21 biotinylated lectins in the VNO of the armadillo *Chaetophractus villosus*, and also to study the lectin-binding patterns of the olfactory mucosa and VNO of the armadillo *Chaetophractus villosus* to reveal similarities and differences. The latter goal was suggested by a previous study from our laboratory with the same biotinylated lectins, showing the complex characterisation of GCs in the olfactory mucosa components of this species of armadillo (Ferrari et al. 1999).

MATERIALS AND METHODS

Five males and 3 females sexually mature armadillos *Chaetophractus villosus* (Dasypodidae, Xenarthra) were used. Their weight ranged from 2.5 to 3.7 kg. They were housed in individual cages and were supplied with tap water and dog chow ad libitum. The dark–light cycle consisted of 12 h light–12 h dark.

The animals were anaesthetised with ketamine hydrochloride (40 mg/kg) and sodium thiopental (60 mg/kg) and perfused via the aorta with saline followed by fixative. Three fixatives were used: Bouin's fluid, neutral buffered saline formalin and B4-G (6% HgCl and 0.1% glutaraldehyde in 1% sodium acetate). The latter mixture was considered to be a good fixative for tissues containing GCs (Foster et al. 1991; Spicer & Schulte, 1992). VNO were removed from the nasal cavity, embedded in paraffin and sectioned at 5 μm.

Conventional histochemistry

The following procedures were used: (1) periodic acid-Schiff (PAS) reaction for neutral or weakly acidic GCs (Pearse, 1985); (2) Alcian blue 8GX at pH 2.5 (AB 2.5) used for testing simultaneously sulphate esters and carboxyl groups in GCs; (3) AB at pH 1.0 (AB 0.1) for the characterisation of sulphated GCs (Lev & Spicer, 1964; Spicer & Schulte, 1992); (4) a combination stain AB at pH 2.5 and PAS (AB/PAS) (Mowry, 1956) to allow acidic and neutral GCs differentiation; (5) sulphated and carboxylated GCs differentially observed using AB pH 0.5 and Alcian yellow pH 2.5 (AB/AY) (Cook, 1990).

Lectin histochemistry

Lectin binding sites were demonstrated by means of biotin-labelled probes. Paraffin sections were hydrated and washed in 0.05 M phosphate buffer saline (PBS)

Table 1. *Carbohydrate binding specificities used*

Lectin	Source	Binding specificity	Inhibitory sugar
N-acetylglucosamine			
DSL	*Datura stramonium*	(β-1,4) N-acetylglucosamine	N-acetylglucosamine
ECL	*Erythrina cristagalli*	(β-1,4) N-acetylglusamine > β galactose	N-acetylglucosamine
LEL	*Lycopersicon esculentum*	β-N-acetylglucosamine	α and β N-acetylglucosamine
STL	*Solanum tuberosum*	β-N-acetylglucosamine	N-acetylglucosamine
S-WGA	*Triticum vulgaris*	β-N-acetylglucosamine	N-acetylglucosamine
WGA	*Triticum vulgaris*	N-acetylglucosamine	N-acetylglucosamine
Mannose			
Con A	*Canavalia ensiformis*	α-D-mannose > α-glucose	Mannose
LCA	*Lens culinaris*	α-D-mannose	Mannose
PSA	*Pisatum sativum*	α-D-mannose	Mannose
S-Con A	*Canavalia ensiformis*	α-D-mannose	Mannose
Galactose/N-acetylgalactosamine			
BSL-I	*Bandeiraea simplicifolia*	α-N-acetylgalactosamine, α-galactose	N-acetylgalactosamine
DBA	*Dolichos biflorus*	α-N-acetylgalactosamine	D-galactose
JAC	*Artrocarpus integrifolia*	α-galactose	D-galactose
PHA-E	*Phaseolus vulgaris*	complex oligosaccharides	D-galactose
PHA-L	*Phaseolus vulgaris*	complex oligosaccharides	D-galactose
PNA	*Arachis hypogea*	terminal galactose, β 1,3 N-acetylgalactosamine	D-galactose
RCA_{120}	*Ricinus communis*	β-galactose, β-N-acetylgalactosamine	D-galactose
SBA	*Glycine max*	α-N-acetylgalactosamine	N-acetylgalactosamine
SJA	*Sophora japonica*	β-N-acetylgalactosamine	α-N-acetyl-D-galactosamine
VVA	*Vicia villosa*	β-N-acetylgalactosamine	N-acetylgalactosamine N-acetylgalactose
Fucosa			
UEA I	*Ulex europaeus*	α-fucose	Fucose

(pH 7.4) for 15 min. Subsequently, the slides were incubated with the biotin-labelled lectins (see Table 1) for 60 min at room temperature. Then, the slides were treated with FITC-conjugated streptavidin 1:50 (Vector Labs. Burlingame, CA) washed in PBS and mounted in PBS/glycerol. The concentrations needed to give optimal staining intensity were determined for each lectin and are summarised in Table 1.

Controls for the lectin staining procedure included (1) omission of lectin from the medium, and (2) preincubation of the lectins with the appropriate competing sugars (0.2–0.5 M in Tris buffer) for 1 h at room temperature (Table 1).

The localisation of lectin staining was similar with the 3 fixatives used. However, even if the staining was more intense in tissues fixed in B4-G, some lectins obtained better images with Bouin's fluid as fixative.

RESULTS

The SE consists of mucomicrovillous complex, sustentacular cells (SCs), vomeronasal receptor neurons (VRNs) and basal cells (BCs). The mucomicrovillous complex was defined according to Rama Krishna et al. (1992) as being composed of microvillous dendritic terminals of VRNs, microvilli of the sustentacular cells and a mucoid component over the free surface.

The nonsensory epithelium is composed of a mucociliary complex and a pseudostratified columnar epithelium with 3 cell types: columnar ciliated cells, columnar nonciliated secretory cells, and basal cells. The mucociliary complex includes the cilia and microvilli of columnar cells and mucoid component over its free surface. Numerous infiltrating neutrophils among the epithelial cells were frequently observed as previously described by Carmanchahi et al. (1999).

The lamina propria contains loose connective tissue with vessels and vomeronasal nerves. An outstanding and unusual presence of vomeronasal glands with secretory ducts piercing the SE was observed (not shown). The submucosa shows loose connective tissue with large venous sinuses, nerves and vomeronasal glands. They are compound-branched tubuloacinar glands. The ducts of these glands reach the lumen in the dorsolateral region between NSE and SE (Carmanchahi et al. 1999).

Conventional carbohydrate histochemistry

The results obtained with conventional carbohydrate histochemistry are summarised in Table 2. The mucomicrovillous complex was stained with PAS; AB 2.5, AY and AB/PAS. The mucociliary complex

Table 2. *Histochemical characteristics of the vomeronasal organ of* Chaetophractus villosus[1]

	Sensory epithelium		Nonsensory epithelium		
	Mucomicrovillous complex	Cell bodies	Mucociliary complex	Cell bodies	Vomeronasal glands
PAS	++	−	+	−	+++
AB pH 0.5	−	−	−	−	−
AB pH 2.5	++	−	++	−	++*
AY/AB	++ AY	−	++ AY	−	++ AY*
AB+PAS	++ AB/PAS	−	++ AB/PAS	−	++ PAS +++ AB/PAS

[1] − no staining; + weak staining; ++ moderate staining; +++ intense staining.
* See text.

Table 3. *Lectin binding in the sensory epithelium of the vomeronasal organ of* Chaetophractus villosus[1]

Lectin	Micromicrovillous complex	Receptor neurons		Sustentacular cells		Basal cells
		Dendrite	Body	Basal	Apical	
N-acetylglucosamine						
DSL	+++	−	−	+	+	++
ECL	+++	−	+	−	−	−
LEL	+++	+	++*	+	+	++
STL	++	+	−	+	+	++
S-WGA	+++	++	++*	−	−	+++
WGA	+++	++*	++*	−	+	++
Mannose						
Con A	−	−	+++*	+	+	++
LCA	++	−	++	−	−	+++
PSA	−	−	−	−	−	−
S-Con A	−	−	++	−	−	−
Galactose/N-acetyl Galactosamine						
BSL-I	+++	−	++*	−	++*	++
DBA	++*	−	−	−	−	−
JAC	++	−	−	−	−	+
PHA-E	++	−	−	−	−	−
PHA-L	+++	−	−	−	−	−
PNA	+++	++*	++*	−	−	−
RCA_{120}	++	−	+	−	−	−
SBA	−	−	−	−	−	−
SJA	+	−	−	−	−	−
VVA	++	−	−	−	−	++
Fucosa						
UEA-I	+++	+++	+++	+++	+++	+++

[1] − no staining; + weak staining; ++ moderate staining; +++ intense staining.
* See text.

showed the same histochemical reaction. The cells of the vomeronasal glands exhibited good stainability with PAS, AB 2.5, AY and AB/PAS, although glandular cells without any reaction were frequently observed. The AB pH 2.5 positive reaction suggests the carboxylic nature of the acidic GCs. Positive staining with AY in AB/AY technique supports these results. The combined AB/PAS technique showed acinar cells with neutral and weakly acidic GCs (bright rose cells). Cells with acidic and neutral GCs (purple-blue cells) were also observed.

Lectin histochemistry

The lectin binding pattern in the VNO and its associated structures are summarised in Table 3 and Table 4.

Lectin-binding pattern in the sensory epithelium

Mucomicrovillous complex. The majority of the biotin labelled lectins appeared to bind to the mucomicrovillous complex. Intense reactivity for DSL (Fig.

Table 4. *Lectin binding in the nonsensory epithelium, glands and nerves of the vomeronasal organ of* Chaetophractus villosus[1]

Lectin	Nonsensory epithelium			Vomeronasal glands	Vomeronasal nerve
	Mucociliary complex	Sustentacular cells	Basal cells		
N-acetylglucosamine					
DSL	−	+++	+++	+++	++
ECL	+++*	−	−	++*	−
LEL	++	++*	++	+++	−
STL	++	−	+	−	++
S-WGA	−	−	−	+++*	−
WGA	−	−	−	+++	++
Mannose					
Con A	−	−	−	++*	−
LCA	++	+++*	++	+++	−
PSA	−	−	−	+	−
S-Con A	−	−	−	+*	−
Galactose/N-acetyl galactosamine					
BSL-I	+*	−	++	−	−
DBA	+++*	+++*	−	+++*	−
JAC	+++	−	−	−	−
PHA-E	++	−	−	−	−
PHA-L	++	−	−	−	−
PNA	−	−	−	+++	−
RCA_{120}	+++*	−	−	+++	−
SBA	−	−	−	+	−
SJA	+++*	++	−	−	−
VVA	+	−	−	−	−
Fucosa					
UEA-I	++*	−	−	−	−

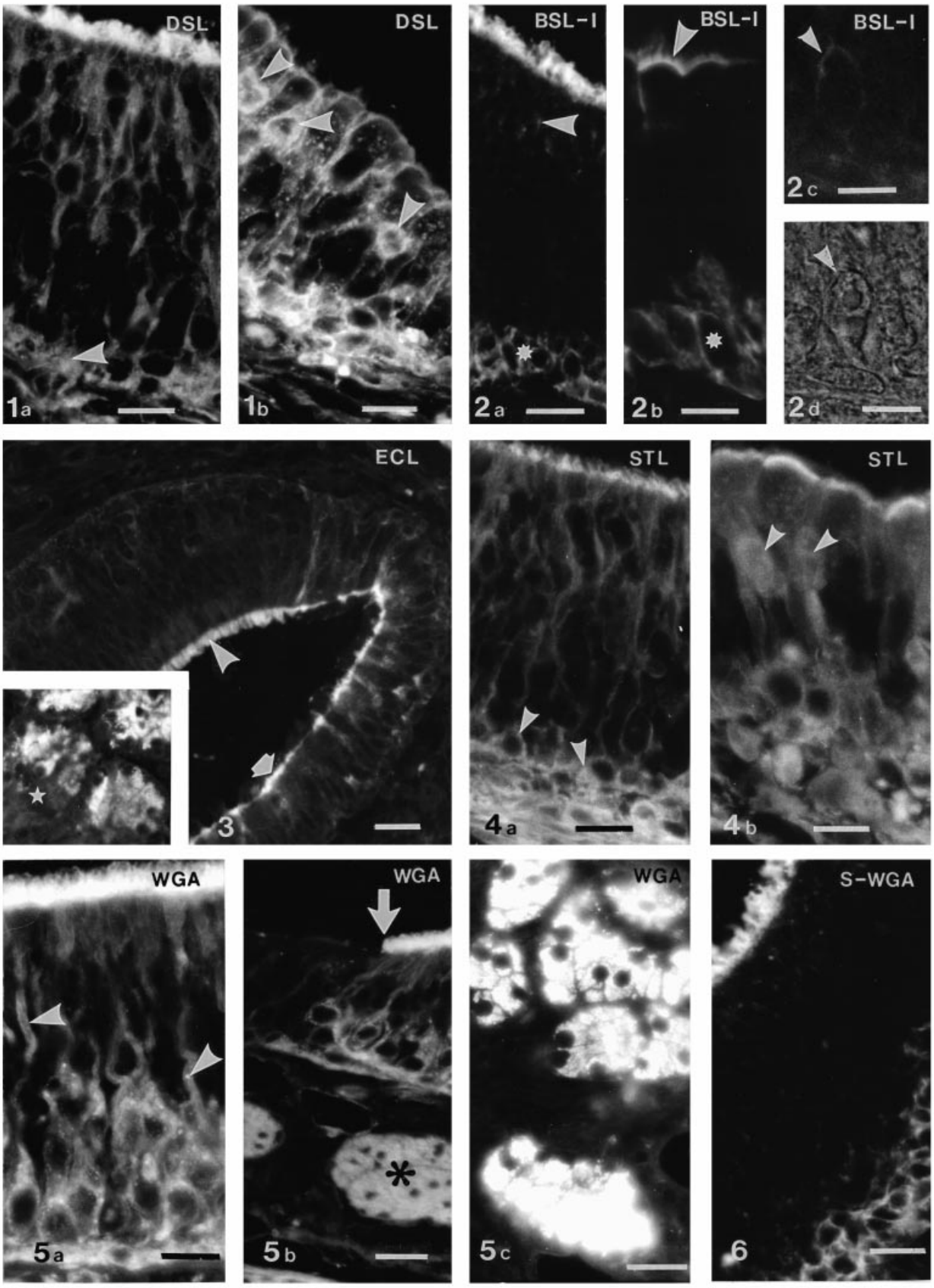
[1] − no staining; + weak staining; ++ moderate staining; +++ intense staining.
* See text.

1*a*), ECL (Fig. 3), LEL (Fig. 7*a*), S-WGA (Fig. 6), WGA (Fig. 5*a*, *b*), BSL-I (Fig. 2*a*), PHA-L (Fig. 12*a*), PNA (Fig. 13) and UEA-I (Fig. 19) were observed throughout the entire complex. Moderate reactivity was observed for STL (Fig. 4*a*), LCA (Fig. 8*a*), JAC (Fig. 17), PHA-E (Fig. 11*a*), RCA_{120} (Fig. 14*a*) and VVA (Fig. 16). DBA showed moderate fluorescence, associated with patchy regions with weaker fluorescence or nonfluorescence (Fig. 18). Weak reaction was seen with SJA (Fig. 15). The boundaries between the SE and NSE were sharply demarcated when S-WGA, WGA and UEA-I were used. No reaction was obtained with Con A, PSA, S-Con A and SBA.

Sustentacular cells. Cell membranes and cytoplasm, but not the nucleus were intensely labelled by UEA-I (Fig. 19). Weak fluorescence with the same pattern was observed with DSL (Fig. 1*a*), LEL (Fig. 7*a*), STL (Fig. 4*a*) and Con A (Fig. 9). The apical region showed moderate granular fluorescence for BSL-I (Fig. 2*a*) and weak fluorescence for WGA (Fig. 5*a*)

Vomeronasal receptor neurons. All the receptor cell bodies, but not their nuclei showed moderate reactivity for S-WGA (Fig. 6), WGA (Figs 5*a*, *b*) and PNA (Fig. 13). The dendrites were seen scattered throughout the SE. Intense granular label with S-WGA, WGA and PNA distributed in the supranuclear region and proximal dendritic processes were seen at higher magnification. Furthermore, dendritic processes and cell bodies, but not nuclei were strongly labelled with UEA-I (Fig. 19). Weak fluorescence in the dendritic processes was obtained with STL (Fig. 4*a*). Con A strongly labelled the perinuclear cytoplasm, but the label was stronger in the supranuclear region (Fig. 9). The supranuclear cytoplasm exhibited moderately punctate labelling with LCA (Fig. 8*a*) and S-Con A (Fig. 10). Weak labelling with ECL (Fig. 3) and RCA_{120} (Fig. 14*a*) was observed. Interestingly, BSL-I moderately stained the neurons located close to the basal layer (Figs 2*c*, *d*).

Basal cells. Intense reactivity in the cytoplasm, but not in the cell nuclei, was obtained with S-WGA (Fig. 6), LCA (Fig. 8*a*) and UEA-I (Fig. 19). Moderate fluorescence was observed with DSL (Fig. 1*a*), LEL (Fig. 7*a*), STL (Fig. 4*a*), WGA (Figs 5*a*, *b*), Con A (Fig. 9), BSL-I (Fig. 2*a*), JAC (Fig. 17) and VVA (Fig. 16).



Figs 1–6. For legend see opposite.

Lectin-binding pattern in the nonsensory epithelium

Mucociliary complex. ECL (Fig. 3), DBA (Fig. 18), JAC (Fig. 17), RCA_{120} (Fig. 14*b*) and SJA (Fig. 15) showed a strong reaction in the mucociliary complex. Moderate intensity was observed for STL (Fig. 4*b*), LCA (Fig. 8*b*), PHA-E (Fig. 11*b*) and PHA-L (Fig. 12*b*). Weak staining was obtained with LEL (Fig. 7*b*), BSL-I (Fig. 2*b*), VVA (Fig. 16) and UEA-I (Fig. 19). LEL, BSL-I, SJA and UEA-I stain the mucociliary complex covering a subpopulation of columnar ciliated cells. PHA-E and RCA_{120} apparently failed to stain the complex covering the columnar secretory cell.

Columnar cells. Strong labelling by DBA binding was distributed in cell membranes and cytoplasm but not in nuclei (Fig. 18). DSL strongly stained the cell membranes of ciliated cells. A granular profile was obtained with this lectin in the supranuclear region (Fig.1*b*). This lectin also stained the granules of the infiltrating neutrophils (Fig. 1*b*). LCA showed strong reactivity in the supranuclear region (Fig. 8*b*). LEL (Fig. 7*b*) and SJA (Fig. 15) exhibited moderate reactivity only in the apical region of ciliated cells. None of the biotinylated lectins bound to secretory granules of nonciliated secretory cells.

Basal cells. Intense reaction with DSL was observed (Fig. 1*b*). LEL (Fig. 7*b*), LCA (Fig. 8*b*) and BSL-I (Fig. 8*b*) showed moderate staining. Weak fluorescence was obtained with STL (Fig. 4*b*).

Vomeronasal glands. Acinar cells showed intense reactivity for S-WGA, WGA (Fig. 5*c*), DSL, LEL, LCA (Fig. 8*c*), DBA, PNA (Fig. 13) and RCA_{120} (Fig. 14*c*). Moreover, PNA and RCA_{120} also labelled the cells lining the ducts. However, moderate intensity for ECL (Fig. 3) and Con A (Fig. 9), and weak staining with PSA (not shown), S-Con A (Fig. 10), and SBA (not shown) were seen. All the biotinylated lectins except ECL, S-WGA, Con A, S-Con A and DBA stain the whole glandular endpieces. Those latter lectins showed reactivity in some glandular cells.

Vomeronasal nerve. An intense reaction was observed with LEL, STL and WGA (Fig. 5*b*).

DISCUSSION

Conventional histochemistry showed that the mucous lining of both vomeronasal epithelia in *Chaetophractus villosus* contains neutral and carboxylated acidic GCs.

The vomeronasal glands are composed of cells with either neutral or neutral and carboxylated acidic GCs contents. Acinar cells without labelling were frequently observed.

In contrast, vomeronasal glands of the common marmoset, tree shrew and slow loris showed neutral GCs (Loo & Kanagasuntheram, 1972; Taniguchi et al. 1992). These results indicate that armadillos possess different acinar cell populations. This agrees with a previous ultrastructural study in which we showed glandular cells with different secretory granules. We classified those cells as serous in terms of their electron microscopic appearance (Carmanchahi et al. 1999). However, these histochemical results and our previous ultrastructural observations lead us to rename them, according to Pinkstaff (1993) and Tandler et al. (1994), as seromucous glandular cells.

The identification of sugar residues was improved by lectin histochemistry in comparison with conventional histochemistry.

Fig. 1. (*a*) DSL. Sensory epithelium. A strong reaction in the mucomicrovillous complex is seen. Plasma membranes, cytoplasm of sustentacular cells and basal cells (arrowhead) are stained. Bar, 15 μm. (*b*) DSL. Nonsensory epithelium.The plasma membrane of columnar and basal cells is strongly labelled. Infiltrating neutrophils (arrowheads) between the columnar cells can be observed. Bar, 15 μm.

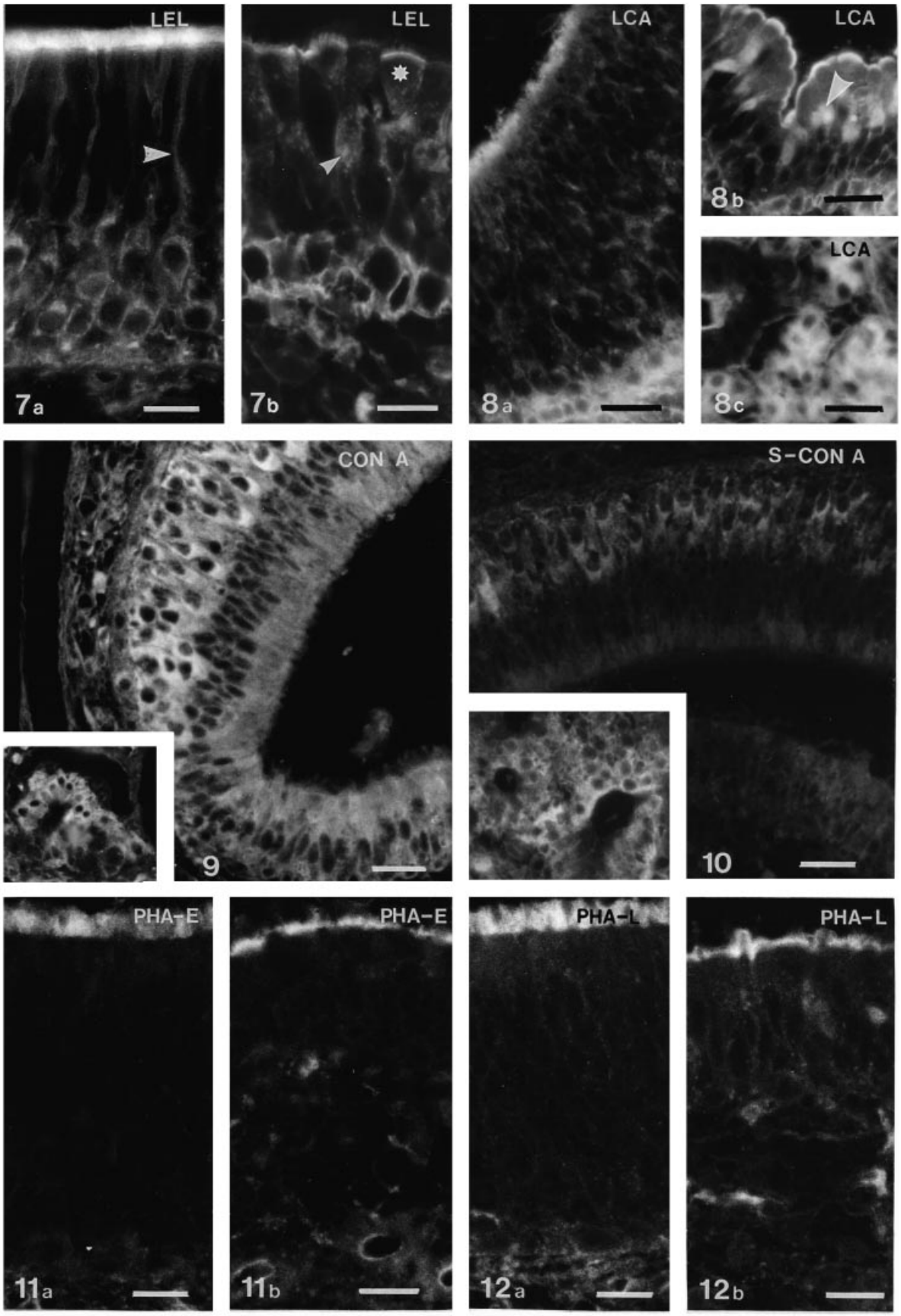
Fig. 2. (*a*) BSL-I. Sensory epithelium. Positive reaction in the mucomicrovillous complex and in the apical region of sustentacular cells (arrowhead) can be seen. Basal cells also show a positive reaction (asterisk). Bar, 15 μm. (*b*) BSL-I. Nonsensory epithelium exhibits a positive reaction in a subpopulation of ciliated columnar cells (arrowhead) and in basal cells (asterisk). Bar, 15 μm. (*c*) Neuron close to the basal layer is also labelled with this lectin (arrowhead) Bar, 15 μm. (*d*) The same neuron (arrowhead) visualised with differential interference technique (Nomarski). Bar, 15 μm.

Fig. 3. ECL. Both mucomicrovillous (arrowhead) and mucociliary (arrow) complexes exhibit a positive reaction with this lectin. The inset shows moderate labelling in a subpopulation of vomeronasal glandular cells. Some nonreacting cells can be seen (asterisk). Bar, 30 μm.

Fig. 4. (*a*) STL. Sensory epithelium. Positive reaction in the mucomicrovillous complex can be seen. A strong reaction is observed in basal cells (arrowheads). Bar, 15 μm. (*b*) STL. Nonsensory epithelium. The mucociliary complex and the basal layer appear moderately labelled. Infiltrating neutrophils (arrowheads) are also stained. Bar, 15 μm.

Fig. 5. (*a*) WGA. Sensory epithelium. A strong reaction is observed in the mucomicrovillous complex. A punctate staining pattern is observed in both supra and perinuclear cytoplasm of vomeronasal neurons. Dendrites are seen scattered throughout the epithelium (arrowheads). The basal layer exhibits moderate stainability. Bar, 15 μm. (*b*) WGA. The abrupt transition (arrow) between the sensory and nonsensory epithelium is observed. A positive reaction is also observed in the vomeronasal nerves (asterisk). Bar, 30 μm. (*c*) Vomeronasal glands. The acinar cells are strongly stained. Bar, 15 μm.

Fig. 6. S-WGA. Sensory epithelium. An intense positive reaction is observed in the mucomicrovillous complex. A less intense label can be seen in the basal cells. Bar, 15 μm.



Figs 7*a*–12*b*. For legend see opposite.

Seventeen out of 21 lectins bound to the mucomicrovillous complex. This fact demonstrates the great complexity of the mucous layer in the VNO, which contains N-acetylglucosamine, α-D-mannose, galactose/N-acetylgalactosamine and α-fucose. This complex pattern of lectin staining was also observed in rats (Saito et al. 1994), mice (Lundt et al. 1989) and common marmosets (Nakajima et al. 1998). In contrast with what was found in rats and mice, we did not see labelling with Con A, PSA, RCA_{120} and SBA. On the other hand, the *C. villosus* olfactory mucus layer lacks α-fucose and α-D-mannose residues (Ferrari et al. 1999). In contrast, the VNO was also labelled with ECL, BSL-I, PNA, RCA, SBA, SJA and VVA. The mucus composition of the armadillo VNO is apparently more complex than the mucus of the olfactory mucosa (Fig. 20). The different composition of both mucus layers might reflect differences in the properties of stimulating molecules. This is suggested by the probability that the mucus composition might influence the access of odorants to the chemosensory membrane. In turn, this might affect the threshold for odorant recognition (Bal & Anholt, 1993). This agrees with previous studies reporting that the olfactory receptor cells are stimulated by volatile chemicals and that the VRNs neurons are activated mainly by non volatile molecules (Wysocki et al. 1982). In the olfactory epithelium, WGA is associated with membrane glycoproteins involved in olfactory transduction mechanisms (Breer, 1991). Likewise, the microvillous dendritic terminals of VRNs containing N-acetylglucosamine sugar residues may also be associated with similar olfactory transduction mechanisms. Carbohydrates in the SE are more complex than those found in the NSE. On the other hand, PHA-E and PHA-L stained the mucus lining the vomeronasal and olfactory epithelia, but not the mucus over the respiratory epithelium (Ferrari et al. 1999) (Fig. 20). These carbohydrate residues are presumably involved in olfactory transduction.

In armadillos the lectin-binding pattern of the vomeronasal SCs differs from that of the olfactory mucosa (Fig. 20). The most interesting feature was the presence of UEA-I binding sites, apparently specific for the vomeronasal SE, since we did not get any label with this lectin in the olfactory mucosa of the same animal. The same pattern was described in mice and rats (Lundh et al. 1989; Saito et al. 1994).

The VRNs showed a variety of terminal sugars greater than the olfactory receptor neurons. The VRNs cell bodies show N-acetyl-glucosamine, α-D-mannose, D-galactose/N-acetylgalactosamine and α-fucose. Our results agree with those reported by Saito et al. (1994) in rat. On the other hand, the common marmoset lacks N-acetyl-glucosamine (Nakajima et al. 1998). Con A stained the supranuclear area of the receptor neurons but not the dendritic processes. This finding demonstrates a transient binding of Con A, LCA, PNA, and WGA to moieties during the reticulum and/or Golgi apparatus transitions (Danguy et al. 1994; Brinck et al. 1995). Furthermore, we have demonstrated the presence of these organelles in the supranuclear region (Carmanchahi et al. 1999). Additionally, the VNO showed a great amount of α-fucose. This sugar residue, in contrast, is not found in the OM of this species (Ferrari et al. 1998). The great amount of α-fucose in the VRNs and its absence in the OM, presumably indicate that α-fucose might play an important role in the action of nonvolatile molecules on VNO.

Our results also indicated a different distribution of binding sites for BSL-I in the VRNs. Their somas, located at the region close to the basal layer, contained perinuclear-binding sites for BSL-I. These results suggest that 2 subsets of receptor neurons could be distinguished. The presence of 2 segregated subsets of vomeronasal neurons has been demonstrated using lectin histochemistry (Takami et al. 1994) and antibodies to G proteins (Halpern et al. 1995). This segregation might indicate 2 functional possibilities: a

Fig. 7. LEL. (*a*) Sensory epithelium. The mucomicrovillous complex exhibits a strong reaction. A punctuate lectin binding pattern is observed in the perinuclear cytoplasm and dendrites of the vomeronasal receptor neurons (arrowhead). Bar, 15 μm. (*b*) LEL. Nonsensory epithelium. A subpopulation of columnar cells (asterisk) and all the basal cells are labelled. Infiltrating neutrophils (arrowhead) are also observed in this epithelium. Bar, 15 μm.

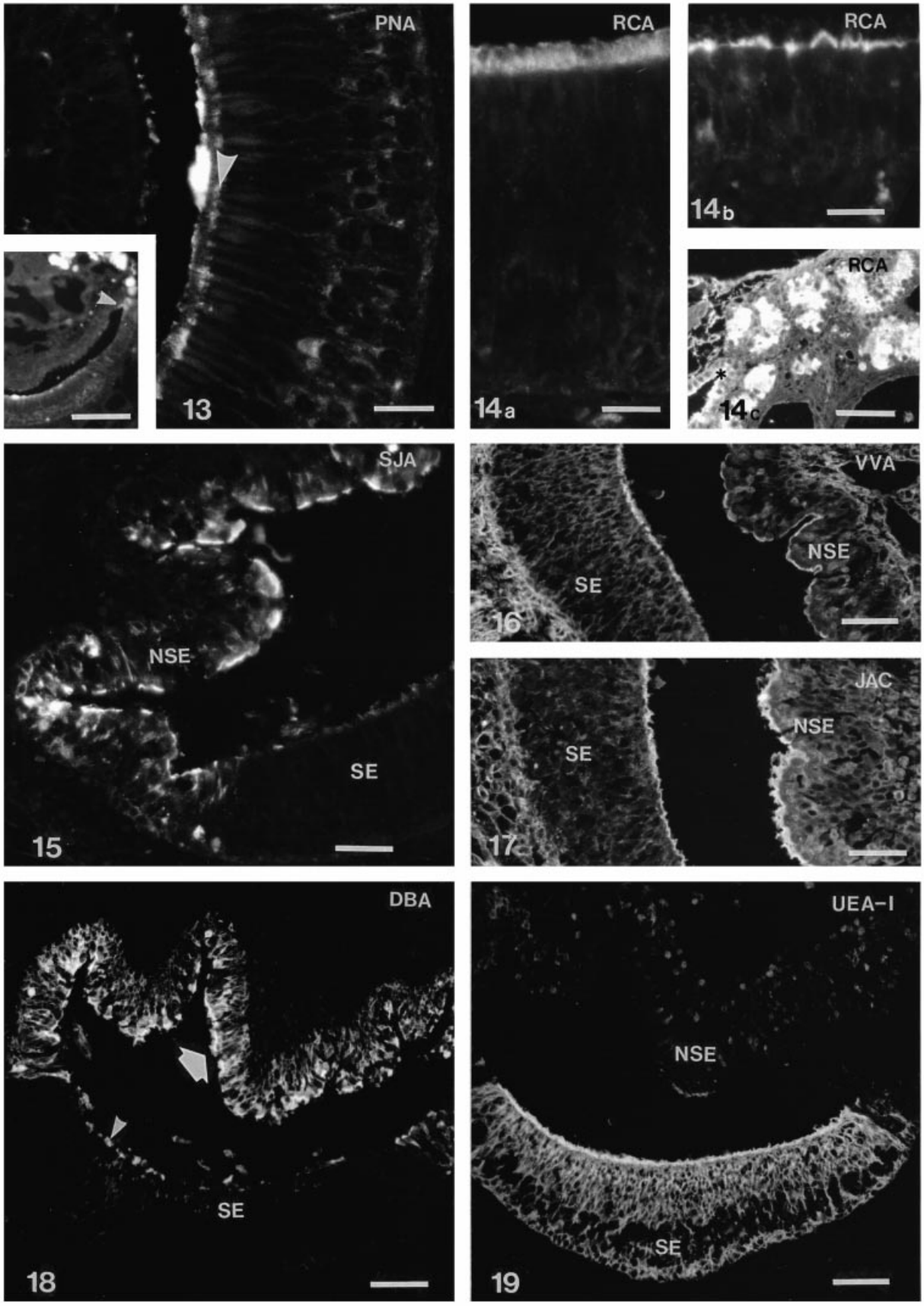
Fig. 8. (*a*) LCA. Sensory epithelium. A positive reaction in the mucomicrovillous complex and basal cells can be observed. Bar, 15 μm. (*b*) LCA. Nonsensory epithelium. The mucociliary complex, the supranuclear cytoplasm of columnar cells (arrowhead) and the basal layer are stained. Bar, 30 μm. (*c*) The vomeronasal glandular cells are strongly stained. Bar, 30 μm.

Fig. 9. Con A. The sensory epithelium shows a strong reaction in the perinuclear cytoplasm of vomeronasal receptor neurons and in the basal region. Inset: most of the acinar cells of the vomeronasal glands are labelled. Bar, 30 μm.

Fig. 10. S-Con A. A moderate reaction is observed in the supranuclear cytoplasm of the vomeronasal receptor neurons. Inset: moderate label in a subpopulation of vomeronasal glandular cells can be observed. Bar, 30 μm.

Fig. 11. *PHA-E*. Both mucomicrovillous (*a*) and mucociliary (*b*) complexes show positive reaction. Bar, 15 μm.

Fig. 12. PHA-L. Mucomicrovillous (*a*) and the mucociliary (*b*) complexes are similarly stained by PHA-E. Bar, 15 μm.



Figs 13–19. For legend see opposite.

subset projection to defined subdivisions of the accessory olfactory bulb (Shapiro et al. 1995) or an immature stage of the VRNs (Jia & Halpern, 1998).

The binding pattern of lectins in VNO BCs differs from that found in olfactory mucosa of *Chaetopractus villosus* (Ferrari et al. 1999) (Fig. 20). This would indicate that VNO BCs have a different composition or dissimilar neuronal differentiation pattern. At the ultrastructural level, we have recently described 2 BC types: globose as for BCs in the junctional area, and flat as for those above the basal membrane (Carmanchahi et al. 1999). However, both BC types could not be differentiated by their lectin-staining pattern.

None of the lectins used in this study permitted us to characterise the secretory granules in columnar nonciliated secretory cells, reported in previous ultrastructural studies (Carmanchahi et al. 1999). Only the apical region of the ciliated cells reacted to LEL and SJA. LEL indicates the presence of oligosaccharides containing poly-N-acetylgalactosamine residues (Danguy et al. 1994), which could be related to glycogen (Spicer et al. 1983). This feature agrees with ultrastructural studies showing the presence of glycogen particles (alpha type) in the apical region of these cells (Carmanchahi et al. 1999).

The VNO glands contain GCs with N-acetylglucosamine, α-D-mannose and galactose/N-acetylgalactosamine sugar residues. Probably, GCs in the mucoid component of the mucomicrovillous complex are derived primarily from those glands. However, the lectin-binding pattern of the glands differs from that found in both mucous complexes of the VNO. LEL, BSL-I, JAC, PHA-E, PHA-L, SJA and VVA labelled the mucomicrovillous complex but failed to stain acinar cells. This result could be attributed to 2 factors. First, modification of carbohydrates after being secreted; second, NSE secretory cells would also collaborate in the composition of the mucous layer. Additionally, ultrastructural studies showed great numbers of secretory granules in those cells (Carmanchahi et al. 1999).

We found an unusual gland with few acini in the SE lamina propria. These acini were observed only with Alcian blue (pH 2.5) and we think that they presumably participate in mucus production. N-acetylglucosamine, α-D-mannose, galactose/N-acetylgalactosamine and α-fucose were reported in vomeronasal glands of rats and common marmosets (Saito et al. 1994; Nakajima et al. 1998). However, the VNO glands of armadillos do not show α-fucose residues. It would be useful to know whether the VNO glands resemble Bowman's glands. Both contain N-acetylglucosamine, α-D-mannose and galactose/N-acetyl galactosamine, but both failed to stain with UEA-I which is specific for α-fucose sugar residues. However, ECL, LCA and RCA only stain the vomeronasal glands whereas LEL, JAC, SBA and SJA label exclusively the Bowman's glands. The differences between the lectin-binding pattern in the VNO and OM glands could be explained by the different nature of the molecules involved in perireceptor processes in both systems.

Our results on the free border, dendrites and cell bodies show that the number of lectins binding to the VNO is greater than those of the OM (Fig. 20). A similar pattern was found in rodents (Saito et al. 1994; Takami et al. 1994), but this is contrary to previous results obtained in the common marmoset (Nakajima et al. 1998). On account of these results, Nakajima et al. (1998) hypothetised that the VNO is less active than olfactory epithelium in some physiological functions, including signal transduction associated with sugar residues. We think that the number of lectins bound to the VNO is not necessarily related to

Fig. 13. PNA. A strong reaction is observed in the mucomicrovillous complex. The supranuclear cytoplasm and dendrites are moderately stained. The apical protrusion of the dendritic process appears labelled (arrowhead). Bar, 30 μm. Inset: low magnification of the vomeronasal organ showing a strong reaction in ducts and vomeronasal glands. Arrowhead indicates the endings of the glandular ducts in the transitional area between NSE and SE. Bar, 210 μm.

Fig. 14. (*a*) RCA_{120}. Sensory epithelium. The mucomicrovillous complex is labelled. Bar, 15 μm. (*b*) RCA_{120}. Nonsensory epithelium. A positive reaction of mucociliary complex can be seen. Bar, 30 μm. (*c*) RCA_{120}. The vomeronasal glands and their ducts (asterisk) are also strongly stained. Bar, 210 μm.

Fig. 15. SJA. A patchy pattern is observed in the mucociliary complex of the nonsensory epithelium (NSE). Weak labelling in the mucomicrovillous complex of the sensory epithelium (SE) is also seen. Bar, 30 μm.

Fig. 16. VVA. The sensory epithelium (SE) shows a positive reaction in the mucomicrovillous complex and the basal cell layer. Weak stainability is observed in the mucociliary complex of the nonsensory epithelium (NSE). Bar, 30 μm.

Fig. 17. JAC. Positive reaction in the mucomicrovillous complex and in basal cells can be observed in the sensory epithelium (SE). The nonsensory (NSE) epithelium exhibits intense labelling in the mucociliary complex. Bar, 30 μm.

Fig. 18. *DBA*. This lectin mainly stains the nonsensory epithelium (arrow). A positive patchy reaction (arrowhead) is observed only in the mucomicrovillous complex of the sensory epithelium (SE). Bar, 30 μm.

Fig. 19. *UEA-I*. This lectin stains all layers of the sensory epithelium (SE), including the mucomicrovillous complex. Weak staining is observed in the mucociliary complex of a subpopulation of columnar ciliated cells of the nonsensory epithelium (NSE) Bar, 30 μm.

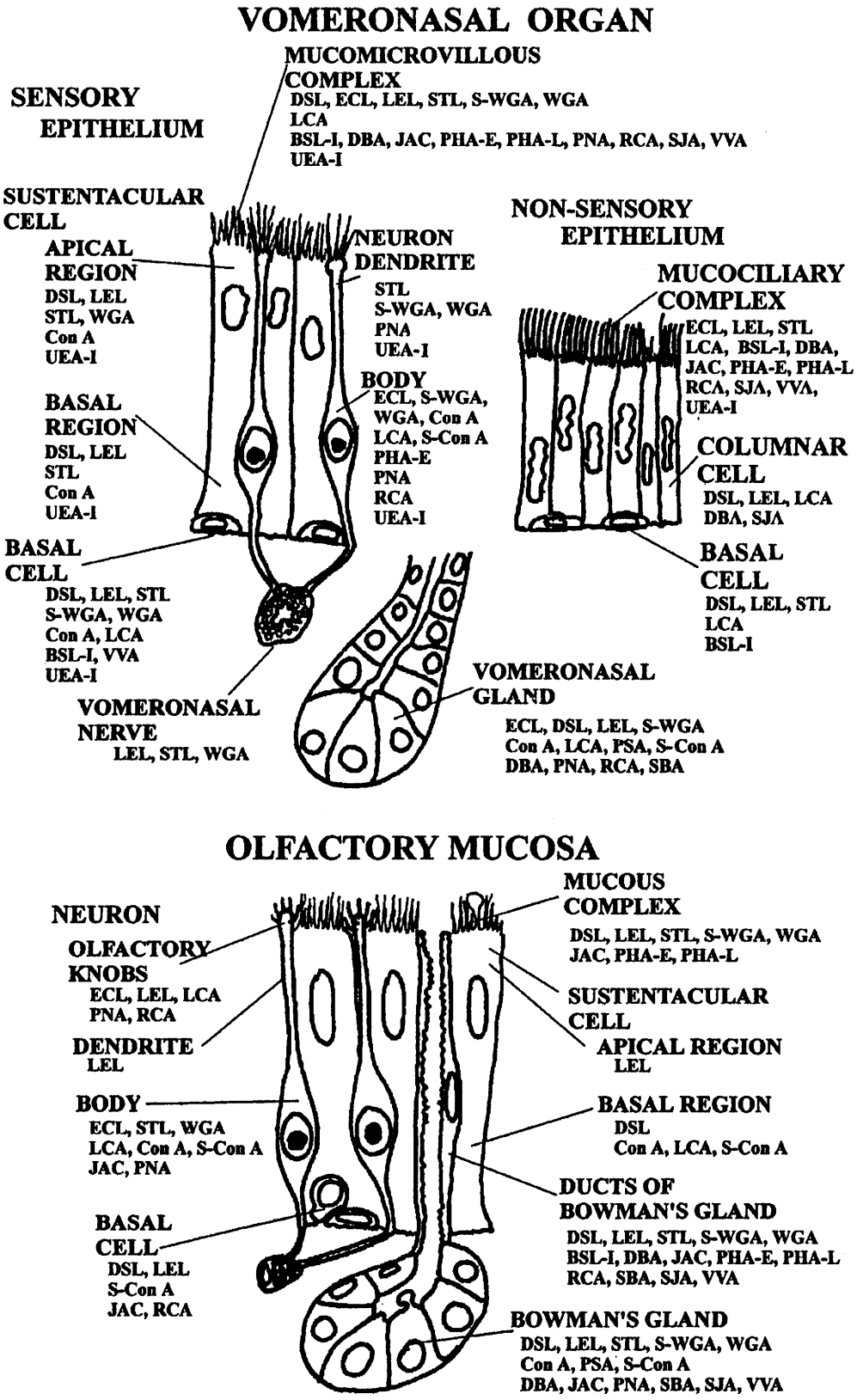


Fig. 20. Diagram illustrating the comparative lectin distribution in the vomeronasal organ and the olfactory mucosa (Ferrari et al. 1999) of the armadillo *Chaetophractus villosus*.

the degree of activity of this organ. The functional significance of most of GCs is still unknown, in spite of the recent advances in glycobiology. However, the histochemical study on the GCs of the VNO could provide a basis for further experimental investigations.

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