

Histochemical Characterization of the Lectin-binding Sites in the Equine Vomeronasal Organ

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Received February 28, 2003 / Accepted March 28, 2003

Abstract

The binding specificities of various lectins, such as the *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), and the *Bandeiraea simplicifolia* BS-1 (Isolectin B4), *Triticum vulgare* (WGA), *Arachis hypogaea* (PNA), and *Ulex europaeus* (UEA-I) lectins, were studied in the vomeronasal organ of the horse. The microvilli of the vomeronasal sensory epithelium were positive for DBA, SBA, Isolectin B4, WGA, PNA, and UEA-I. The receptor cells showed intense reactivity for DBA and WGA. Lectins were not detected in the supporting cells or basal cells. The Jacobson's glands were positive for WGA and UEA-I, but lectins were absent from the nerve bundles. From these results, we postulate that several lectin-binding carbohydrates on the microvilli and neurosensory cells are associated with chemoreception in the horse. In addition, the differential lectin-binding patterns in the horse suggest that the carbohydrates present in this particular sense organ are species-specific.

Key words: lectin, horse, vomeronasal organ

Introduction

The vomeronasal system plays important roles in mammalian reproduction [5]. The vomeronasal organ (VNO) is a receptor organ, the receptor cells of which project their axons to the accessory olfactory bulb and participate in the perception of con-specific chemical signals (pheromones) [5, 11, 18]. The VNO has a tubular structure, and its lumen is surrounded by two types of epithelium: the vomeronasal sensory epithelium and the vomeronasal nonsensory epithelium [6, 15]. The vomeronasal sensory epithelium is thought to function as a signal detector [14, 16, 18]. Numerous studies

have investigated the importance of the VNO in both reproduction and chemosensory reception in the horse [3]. The equine VNO contains a soft tissue component [3], VNO cartilage [10], and the fine structure of the VNO sensory epithelium [15]. Nevertheless, the carbohydrate-specificity of the sensory epithelium of the equine VNO remains unresolved.

The carbohydrate (lectin-binding) moieties of glycoproteins and glycolipids are important for a variety of biological processes, such as cellular adhesion, cellular recognition, protein folding, and signal transduction [1, 2, 4, 13, 17]. All lectin molecules possess two or more carbohydrate-binding sites, which are essential for the agglutination of cells and reactivity with complex carbohydrates. Each lectin binds to a specific sugar or group of sugars. Many lectins have been characterized, including the peanut agglutinin, wheat germ agglutinin, phytohemagglutinin E, concanavalin A, *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), and the *Bandeiraea simplicifolia* BS-1 lectin (Isolectin B4) [1, 4]. Many studies have focused on the lectin-binding patterns in the VNO neuroepithelia of sheep and pigs [9], rats [7, 12], and marmosets [8]. These studies suggest that carbohydrate distribution on the cell surface of the VNO neuroepithelium is species- and/or cell-type-specific.

The aim of this study is to elucidate the carbohydrate specificity of the sensory epithelium of the horse VNO by examining the content of specific lectins, which included DBA, SBA, Isolectin B4, WGA, PNA, and UEA-I.

Materials and Methods

Tissue sampling

Three 2-year-old male horses (Korea Horse Racing Association, Jeju) were sacrificed. The VNOs were removed from the nasal cavities and fixed in 10% buffered formalin for 48 h, in preparation for histological examination.

Histological examination

The specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5- μ m thickness, and stained with hematoxylin and eosin using routine histological techniques. All of the paraffin-embedded tissue sections from normal horses were stained for lectins.

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Lectins used in this study

The following lectins (all of which were purchased from Sigma Chemical Co., St. Louis, MO) were detected: *Bandeiraea simplicifolia* agglutinin (peroxidase-labeled Isolectin B4); *Dolichos biflorus* agglutinin (peroxidase-labeled DBA); *Glycine max* agglutinin (peroxidase-labeled SBA); *Triticum vulgare* agglutinin (peroxidase-labeled WGA); *Arachis hypogaea* agglutinin (peroxidase-labeled PNA); and *Ulex europaeus* agglutinin I (peroxidase-labeled UEA-I). The specificities of the lectins are listed in Table 1.

Lectin histochemistry

The tissue samples were dehydrated by immersion in a graded ethanol series (70%, 80%, 90%, 95%, and 100%), cleared in xylene, embedded in paraffin wax, and sectioned at 5- μ m thickness using a microtome. The sections were mounted on glass microscope slides, the wax was removed, and the sections were rehydrated. Endogenous peroxidase activity was blocked for 30 min with 0.3% hydrogen peroxide in methanol. After three washes with PBS, the sections were incubated with DBA-peroxidase (diluted 1:10), SBA-peroxidase (1:400), Isolectin B4-peroxidase (1:50), WGA-peroxidase (1:20), PNA-peroxidase (1:10), or UEA-I-peroxidase (1:10) for 3 h at room temperature. The peroxidase was developed with the diaminobenzidine (DAB)-hydrogen peroxidase solution (0.001% 3,3'-diaminobenzidine, 0.01% hydrogen peroxidase, 0.05 M Tris). The sections were counterstained with hematoxylin before being mounted.

Results

The paired tubular structure of the VNO was located at the base of the nasal septum, where it was surrounded by the paraseptal cartilage. The sensory and nonsensory epithelia were located on the medial and lateral walls of the VNO. The sensory epithelium consisted of the receptor, supporting cells, and basal cells. The Jacobson's glands were situated in the lamina propria. Since the ducts of these glands penetrated the epithelium and opened into the VNO lumen, the epithelium was covered with secretions from the Jacobson's glands, as shown in our previous report [6]. Histological examination showed that the VNOs were devoid of inflammatory cells, and all of the tissues were used for lectin histochemistry.

In the vomeronasal sensory epithelium, the microvilli were moderately positive for DBA (Fig. 1, A and B), SBA (Fig. 1, C and D), Isolectin B4 (Fig. 1, E and F), and PNA (Fig. 2, C and D), and strongly reactive for both WGA (Fig. 2, A and B) and UEA-I (Fig. 2, E and F). The receptor cells showed intense reactivity for DBA and WGA, while SBA, Isolectin B4, WGA, and UEA-I were not detected in this cell layer. Lectins were not detected in the supporting cells and basal cells.

The Jacobson's glands showed intense reactivity for WGA (Fig. 2A) and weak reactivity for UEA-I. Lectins were absent from the nerve bundles. The histochemical profiles of the lectins examined in this study are summarized in Table 2.

Table 1. Lectin specificities

Lectin	Abbreviation	Binding specificity
N-acetylgalactosamine group		
<i>Bandeiraea simplicifolia</i> lectin	Isolectin B4	-GalNAc, -Gal
<i>Dolichos biflorus</i> agglutinin	DBA	-GalNAc
<i>Glycine max</i> (soybean agglutinin)	SBA	-GalNAc
N-acetylglucosamine group		
<i>Triticum vulgare</i> (wheat germ)	WGA	-GlcNAc
Galactose group		
<i>Arachis hypogaea</i> (peanut)	PNA	-Gal

Table 2. Histochemical localization of cells that are positive for *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Bandeiraea simplicifolia* BS-1 (Isolectin B4), *Triticum vulgare* (WGA), *Arachis hypogaea* (PNA), and *Ulex europaeus* (UEA-I) in the vomeronasal organ of the male horse

		Lectin					
		DBA	SBA	Isolectin B4	WGA	PNA	UEA-
VNO	Microvilli	+	+	+	++	+	++
	Receptor cell	++	-	-	++	-	-
	Supporting cell	-	-	-	-	-	-
	Basal cell	-	-	-	-	-	-
	Glands	-	-	-	++	-	+

-, No binding; +, infrequent (<33%) binding; ++, >66% binding.

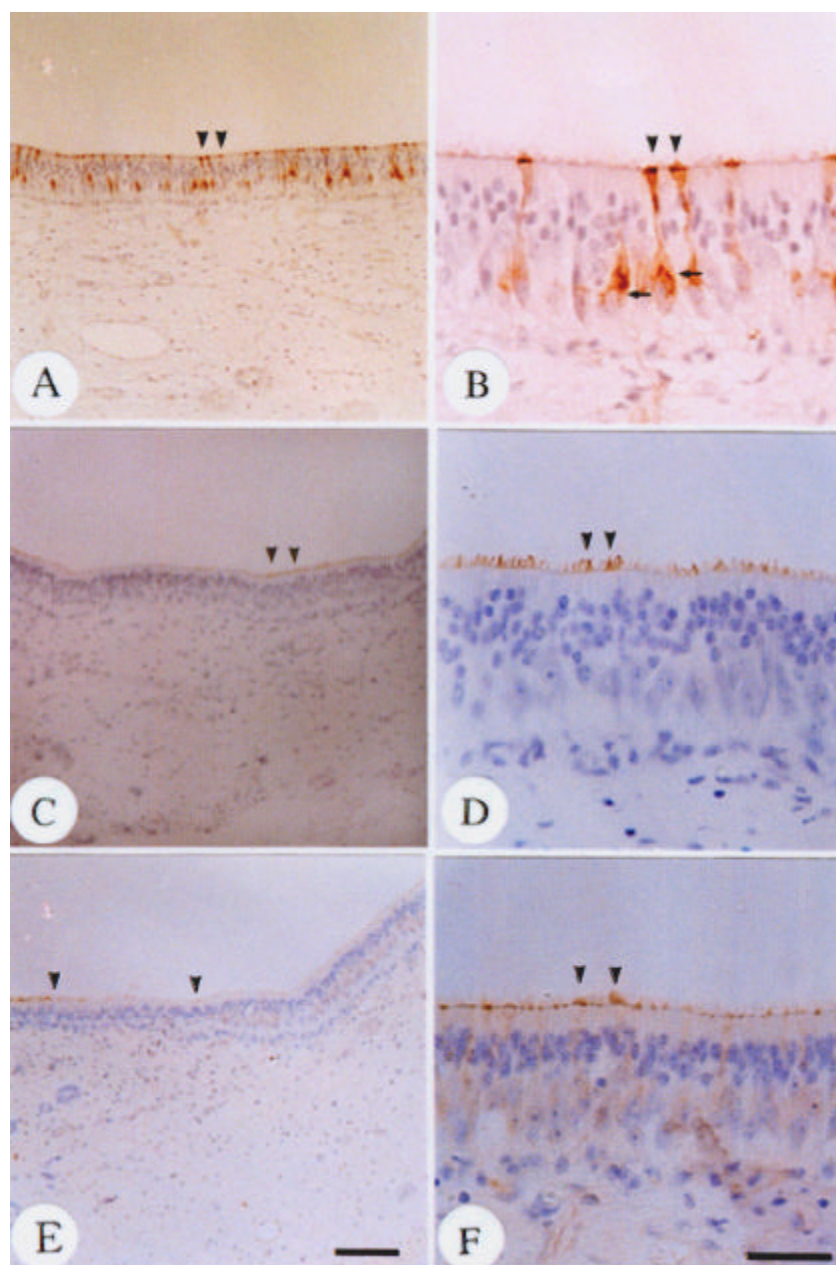


Fig. 1. Histochemical staining of DBA (A and B), SBA (C and D), and Isolectin B4 (E and F) in the vomeronasal sensory epithelium of the horse. The DBA (A and B), SBA (C and D), and Isolectin B4 (E and F) lectins are expressed specifically in the microvilli (arrowheads). DBA (A and B) is expressed in the sensory cells (arrow). The sections were counterstained with hematoxylin. Scale bars = 100 μ m (A, C, and E) or 30 μ m (B, D and F).

Discussion

This is the first study to examine the binding of DBA, SBA, Isolectin B4, WGA, PNA, and UEA-I in the vomeronasal organ of the horse. We found unique characteristics of lectin binding depending cell types. The microvilli of the marmoset vomeronasal sensory epithelium showed intense reactivity for DBA, WGA, PNA, and UEA-I, but were negative for Isolectin B4 [8]. Furthermore, rat microvilli

showed intense reactivity for UEA-I, SBA, and Isolectin B4[12]. In the present study, the equine microvilli showed intense reactivity for WGA and UEA-I, and moderate reactivity for DBA, SBA, PNA, and Isolectin B4. We agree with the hypothesis of Nakajima *et al.*[8] that certain lectins bind preferentially to microvilli and receptor cells, which may be in contact with pheromonal molecules. Pheromonal molecules are thought to bind to the receptor sites on microvilli to induce vomeronasal transduction[16,18]. Part of

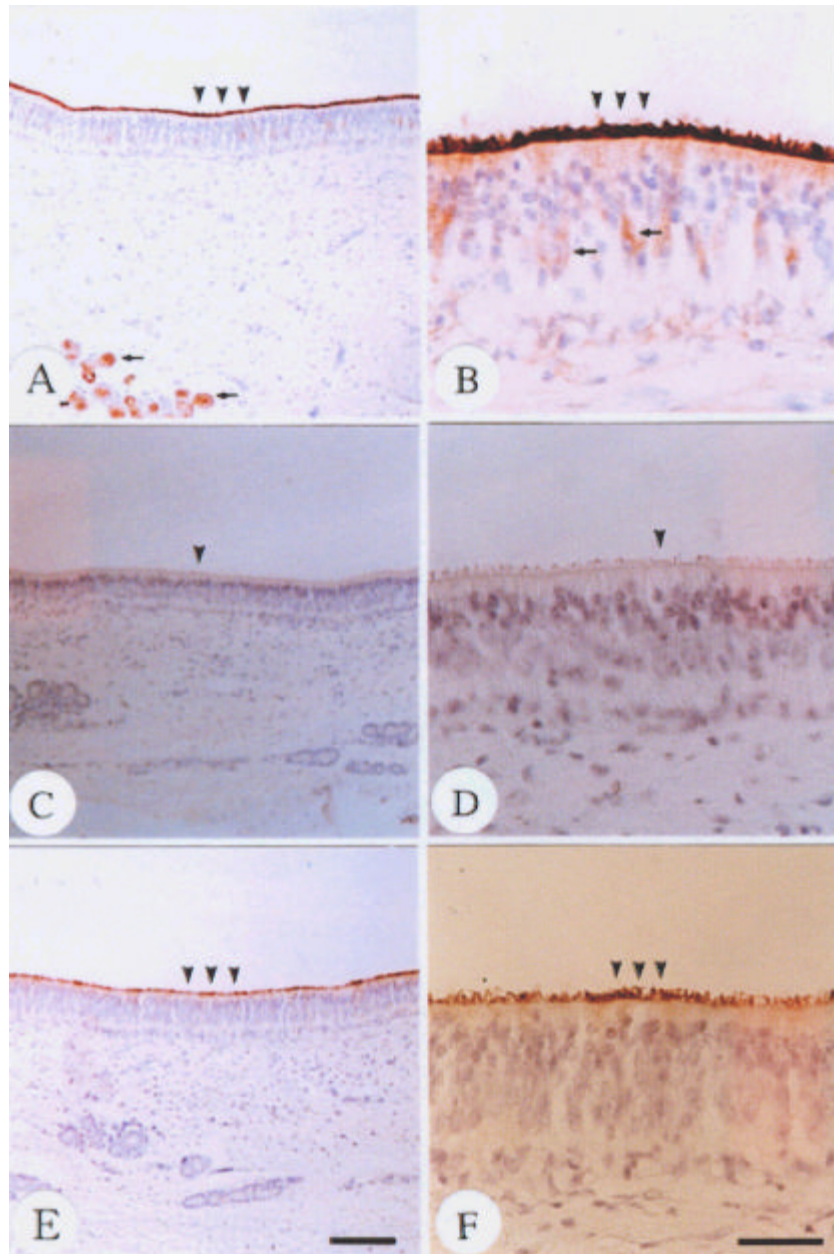


Fig. 2. Histochemical staining of WGA (A, and B), PNA (C and D), and UEA-I (E and F) in the vomeronasal sensory epithelium of the horse. The WGA (A and B), PNA (C and D), and UEA-I (E and F) lectins are expressed specifically in the microvilli (arrowheads). WGA (A and B) is expressed in the sensory cells (B) and glands (A). UEA-I (E and F) is expressed in the glands. The sections were counterstained with hematoxylin. Scale bars = 100 μ m (A, C, and E) or 30 μ m (B, D and F).

the lectin-specific reactivity observed on the VNO microvilli may be due to the presence of glycosylated molecules that are associated with vomeronasal signal transduction. The marmoset receptor cells were positive for WGA and UEA-I, and had weak reactivity for PNA[8]. In the present study, we found a similar result, in that WGA, but not DBA, was detectable in the receptor cells of the horse. We propose that this discrepancy reflects species specificity.

DBA and WGA bound only to a subset of the equine vomeronasal receptor cells. Since the rodent vomeronasal receptor cells may be divided into several subtypes based on lectin histochemistry or immunohistochemistry[7,12], the observed lectin-binding patterns of the equine receptor cells may reflect the various subtypes of the vomeronasal receptor cells in the horse.

The marmoset supporting cells showed positive reactivity

for DBA, Isolectin B4, WGA, and UEA-I, and negative reactivity for PNA, while the basal cells showed positive reactivity for DBA, Isolectin B4, WGA, PNA, and UEA-I[8]. In the present study, the supporting cells and basal cells of the horse showed negative reactivities for the lectins studied. The basal cells differentiate into receptor cells according to the rate of turnover of the receptor cells. Since the lectin-binding patterns of the basal cells were different from those of the receptor cells, it is possible that the sugar residues undergo modifications during the process of maturation into receptor cells[8]. Marmoset glands showed intense reactivity for DBA and WGA, and weak reactivity for PNA[8]. In the present study, the equine glands showed intense reactivity for both WGA and UEA-I.

Our results suggest that several lectin-binding carbohydrates on the microvilli and neurosensory cells of horses are associated with chemoreception. Furthermore, it is likely that the differential lectin-binding patterns in the horse reflect the species-specificity of the carbohydrates in the VNO.

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