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Histological and lectin histochemical studies of the vomeronasal organ of horses

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ABSTRACT

The morphological characteristics and glycoconjugate composition of the vomeronasal organ (VNO) of the horse was investigated using histological, immunohistochemical, and lectin histochemical methods. The VNO is bilaterally located at the base of the nasal septum, has a tubular structure surrounded by cartilage, and consists of sensory and non-sensory epithelia. Immunohistochemical examination showed that the vomeronasal sensory epithelium (VSE) consisted of receptor cells positive for both olfactory marker protein (OMP) and protein gene product 9.5 (PGP 9.5), supporting cells, and basal cells. VNO receptor cells were positive for G protein $G\alpha_{i2}$ (vomeronasal receptor type 1 marker), but not $G\alpha_o$ (vomeronasal receptor type 2 marker). Lectin histochemical studies using 21 biotinylated lectins showed that the free border of the VSE was positive for 20 lectins. The receptor and supporting cells reacted with 16 lectins while the basal cells reacted with 15 lectins, with varying intensities. In the vomeronasal non-sensory epithelium, the free border was positive for 19 lectins. The cilated cells were positive for 17 lectins and the basal cells were positive for 15 lectins. The vomeronasal glands, positioned in the lamina propria, were stained with both periodic acid Schiff (PAS) and alcian blue (pH 2.5). Eighteen lectins stained the acinar cells of the vomeronasal glands with various binding patterns. These findings suggest that horse VNO receptor cells express vomeronasal receptor type 1, and the VNO glands have mucous to seromucous characteristics. Moreover, each lectin differentially binds each cell type in both the VNO sensory and non-sensory epithelia.

1. Introduction

The vomeronasal organ (VNO) is the receptor organ of the vomeronasal system, which perceives various molecules related to social and reproductive communication and transmits signals to the accessory olfactory bulb (Brennan, 2001; Park et al., 2014; Takami, 2002; Yokosuka, 2012; Zufall et al., 2002). The lumen of the VNO is covered by the vomeronasal sensory epithelium (VSE) medially and the vomeronasal non-sensory epithelium (VNSE) laterally (Lee et al., 2003). The VNO glands are located in the lamina propria and secrete mucous and/or seromucous elements into the luminal surface of the VNO epithelium, playing a role in the detection of odorous molecules by the receptor cells of the VSE (Carmanchahi et al., 2000). VNO receptor cells have been classified into two types expressing vomeronasal receptor type 1 or vomeronasal receptor type 2, which are known as positive $G\alpha_{i2}$ and $G\alpha_o$, respectively (Hagino-Yamagishi, 2008; Yokosuka, 2012). While rodent VNO receptor cells are positive for both $G\alpha_{i2}$ and $G\alpha_o$, those of other domestic animals, including goats, are only positive for $G\alpha_{i2}$ (Takigami et al., 2004).

Lectins are carbohydrate binding proteins that protrude from glycolipids and glycoproteins (Bies et al., 2004) and can differentiate macromolecules in mammalian tissues (Spicer and Schulte, 1992). They are involved in biological activities through the process of glycoconjugation, including cell-to-cell self-recognition, extra-

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cellular matrix interactions, embryonic development, cell growth, cell differentiation, cell signaling, cell adhesion, apoptosis, and inflammation (Nimrichter et al., 2004; Opdenakker et al., 1993).

In olfactory systems, glycoconjugates detected by lectin histochemistry are considered to discriminate the subpopulation of olfactory receptor cells (Plendl and Sinowatz, 1998). In the accessory olfactory system (Plendl and Sinowatz, 1998), both the sensory and non-sensory mucosa of the VNO contain exogenous and endogenous carbohydrate moieties that play crucial biological roles in mice (Salazar and Sanchez Quinteiro, 2003), rats (Lee et al., 2012), sheep (Ibrahim et al., 2013), pigs (Park et al., 2012b), and the common marmoset (Nakajima et al., 1998).

With regards to the horse VNO, Taniguchi et al. investigated the fine structure of the horse VSE (Taniguchi and Mikami, 1985), while Salazar et al. studied the vomeronasal cartilage (Salazar et al., 1995). In terms of analyzing the cell phenotypes of the VNO, Garcia-Suarez et al. studied the expression of the Trk A-like and epidermal growth factor receptors (Garcia-Suarez et al., 1997). In line with previous reports, we examined the histological characteristics and cellular localization of signals including protein kinase C and nitric oxide synthase (Lee et al., 2001), and also examined the lectin binding patterns of the VSE using 6 lectins (Lee et al., 2003). However, our previous study was insufficient to fully understand the characteristics of carbohydrate moieties in the horse VNO, including the VSE and the VNSE.

The aim of the present study is to evaluate the morphological characteristics of the horse VNO, with special reference to lectin histochemistry, to clarify the characteristics of carbohydrate specificity, as well as to confirm the VNO receptor type in horses.

2. Materials and methods

2.1. Tissue preparation

Three horse samples (mature, Table 1) were obtained from the Korea Horse Racing Association and local abattoir. Animals were sacrificed through the administration of over-dosed of succinylcholine. For light microscope evaluation, the VNOs were removed immediately after death and fixed in 10% buffered formalin for 48 h. All experimental procedures were conducted in accordance with the Jeju National University Guidelines for the Care and Use of Laboratory Animals.

2.2. Histological examination

Formalin-fixed VNOs were trimmed and decalcified in a sodium citrate–formic acid solution. The solution was changed several times, until the bony pieces softened, as described in our previous study (Park et al., 2012a, b). The decalcified VNOs were dehydrated in a graded ethanol series (70%, 80%, 90%, 95%, and 100%), cleared in xylene, embedded in paraffin, and sectioned at a thickness of 5 μm. After deparaffinization, the serial sections were stained with hematoxylin-eosin (HE), periodic acid Schiff (PAS), and alcian blue (pH 2.5). The morphological evaluation of the VNO focused on male horses, as only one VNO sample from a female horse was obtained and was histologically similar to that of the males.

Table 1
Description of the horses used in this study.

Age	Weight (kg)	Sex
3 years	510	Male
3 years	500	Male
4 years	530	Male

2.3. Antibodies

To confirm the presence of receptor cells in the VSE, immunohistochemistry was performed using a goat polyclonal antibody against the olfactory marker protein (OMP, Santa Cruz Biotechnology, CA, USA) and a rabbit polyclonal antibody against protein gene product 9.5 (PGP 9.5, Bio trend, Köln, Germany). To identify vomeronasal receptors types, a rabbit polyclonal anti-Gαi2 antibody and a rabbit polyclonal anti-Gαo antibody (Santa Cruz Biotechnology) were used. The primary antibodies are listed in Table 2.

2.4. Immunohistochemistry

Sections (5 μm) of paraffin-embedded tissue were deparaffinized and heated in a microwave (800 W) in citrate buffer (0.01 M, pH 6.0) for 5 min. After cooling, the sections were exposed to aqueous 0.3% hydrogen peroxide for 20 min to block endogenous peroxidase activity. Then, non-specific binding was blocked with 10% normal goat serum (ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA), washed in phosphate-buffered saline (PBS, pH 7.4) for 1 h, and allowed to react with the goat anti-OMP antibody (1:400), rabbit anti-PGP 9.5 antibody (1:800), rabbit anti-Gαi2 antibody (1:200), or rabbit anti-Gαo antibody (1:200) for 1 h at room temperature. After washing in PBS, the sections were reacted for 45 min with biotinylated rabbit anti-goat IgG (1:100; Vector Laboratories) or biotinylated goat anti-rabbit IgG (1:100; Vector Laboratories). The sections were washed in PBS and incubated for 45 min with the avidin–biotin peroxidase complex (ABC Elite Kit; Vector Laboratories) prepared according to the manufacturer's instructions. After washing in PBS, the peroxidase reaction was developed for 3 min using a diaminobenzidine substrate kit (DAB Kit; Vector Laboratories), prepared according to the manufacturer's instructions. The sections were counterstained with hematoxylin for 30 s, washed in running tap water for 20 min, dehydrated through a graded ethanol series, cleared with xylene, and mounted with Canada balsam (Sigma-Aldrich, St. Louis, MO, USA).

2.5. Lectin histochemistry

Three lectin screening kits (I–III) were purchased from Vector Laboratories (Burlingame, CA, USA). Lectins were classified according to the binding specificity and inhibitory sugars, including *N*-acetylglucosamine, mannose, galactose/*N*-acetylgalactosamine, complex type *N*-glycan (PHA-E and PHA-L), and fucose groups (Table 3) (Kaltner et al., 2007). The follwing sugars were obtained from Sigma-Aldrich and used for competitive inhibition (See Table 3): α-methyl mannoside/α-methyl glucoside (Cat#, M6882, Sigma-Aldrich), ß-D-galactose(Cat#, G6637, Sigma-Aldrich), lactose (Galß1, 4Glc) (Cat#, L0100000, Sigma-Aldrich), melibiose (Galα1, 6 Glc) (Cat#, M5500, Sigma-Aldrich), *N*-acetyl-D-galactosamine (α-D-GalNAc) (Cat#, A2795, Sigma-Aldrich), *N*-acetyl-D-glucosamine (ß-D −GlcNAc) (Cat#, A8625, Sigma-Aldrich). Chitin hydrolysate (Cat#, SP-0090, Vector) was purchased from Vector Laboratories.

Lectin histochemistry was performed as described in previous studies (Lee et al., 2012; Park et al., 2014, 2012a, b). Briefly, the paraffin-embedded VNOs were sectioned at a thickness of 5 μm using a microtome. The sections were mounted on glass microscope slides, the paraffin was removed, and the sections were rehydrated. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 30 min. After three washes with PBS, the sections were incubated with 1% bovine serum albumin to block non-specific activity. The sections were rinsed with PBS and incubated using the ABC method with 21 biotinylated lectins (Table 3) at 4 °C overnight. Signals were developed using a diaminobenzi-