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Lectin histochemical studies on the olfactory gland and two types of gland in vomeronasal organ of the brown bear

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ABSTRACT

Olfaction is mediated by the vomeronasal and main olfactory systems, and the peripheral vomeronasal organ (VNO) processes species-specific chemicals that are associated with various behaviors in mammals. Sensory epithelial surfaces of the olfactory mucosa and VNO are covered by mucosal fluid that contains secretory products derived from associated glands, and glycoconjugates in the mucosal fluid are involved in odorant reception. The VNO of brown bears contains two types of glands; submucosal vomeronasal glands (VNG) and multicellular intraepithelial glands (MIG). The present study determined the labelling profiles of 21 lectins in the olfactory glands (OG), VNG and MIG of young male brown bears. The OG reacted with 12 lectins, and the VNG and MIG were positive for seven and eight lectins, respectively. Six lectins bound only to the OG, while four reacted with both or either of the VNG and MIG, but not the OG. The differences of lectin labelling pattern between the OG and glands in the VNO suggest that glycans in covering mucosal fluids differ between the olfactory mucosa and VNO. In addition, *Bandeiraea simplicifolia* lectin-I, *Sophora japonica* agglutinin and Jacalin reacted with the MIG but not the VNG, whereas *Datura stramonium* lectin and concanavalin A bound to the VNG, but not the MIG. These findings indicate that the properties of secretory substances differ between the two types of glands in the bear VNO, and that the various secretions from these two types of glands may function in the lumen of VNO together.

1. Introduction

Most mammals detect chemical substances in the external environment via the olfactory system, which comprises vomeronasal and main olfactory systems, and the respective peripheral organs comprise a vomeronasal organ (VNO) and olfactory mucosa. The VNO processes various species-specific chemicals from skin glands, urines and feces, such as pheromones that are associated with reproductive behavior (Wysocki, 1979; Halpern, 1987), and its morphology is species-specific and reflects the sniffing-behavioral features of animals (Halpern, 1987). Sensory epithelial surfaces in both the olfactory mucosa and VNO are covered by mucosal fluid that contains secretory products derived from associated glands, and these products play a role in the reception of odorants (Khew-Goodall et al., 1991; Getchell and Getchell, 1992; Plendl and Sinowatz, 1998).

The brown bear, *Ursus arctos*, belongs to the order Carnivora and it is generally solitary, with a wide home range (Dahle and Swenson, 2003). Several studies have indicated that scents derived from the skin of brown bears are season-dependent (Tomiyasu et al., 2018a, 2018b) and associated with their behavior (Sergiel et al., 2017), and that chemical substances detected by the VNO seem to mediate communication among this species. We previously described the detailed morphology of the VNO in the brown bear, which possesses a specific secretory system (Tomiyasu et al., 2017). Although the mammalian VNO generally possesses only submucosal glands (Halpern, 1987), the bear VNO contains submucosal vomeronasal glands (VNG) and the multicellular intraepithelial glands (MIG) (Tomiyasu et al., 2017), in addition to goblet cells that generally function in clearing the airways (Reid et al., 2018). In general, the MIG is a rare secretory unit and it is found in the lining of upper respiratory and male genital systems (Banks, 1993). However, the function of the MIG within the VNO of bears remains unknown.

Abbreviations: AB, Alcian blue; MIG, multicellular intraepithelial gland; OG, olfactory gland; PAS, periodic acid-Schiff; VNG, submucosal vomeronasal gland; VNO, vomeronasal organ

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Table 1
Lectin concentrations and inhibitory sugar residues.

Lectins	Abbreviation	Concentration (mg mL^{-1})	Rough specificity[a]	Inhibitory carbohydrates
Wheat germ agglutinin	WGA	1.0×10^{-2}	GlcNAc	GlcNAc
Succinylated-wheat germ agglutinin	s-WGA	1.0×10^{-2}	$(GlcNAc)_n$	GlcNAc
Lycopersicon esculentum lectin	LEL	2.0×10^{-3}	$(GlcNAc)_{2\text{-}4}$	GlcNAc
Solanum tuberosum lectin	STL	1.0×10^{-2}	$(GlcNAc)_{2\text{-}4}$	GlcNAc
Datura stramonium lectin	DSL	4.0×10^{-3}	$(GlcNAc)_{2\text{-}4}$	GlcNAc
Bandeiraea simplicifolia lectin-II	BSL-II	5.0×10^{-2}	α/βGlcNAc	GlcNAc
Dolichos biflorus agglutinin	DBA	5.0×10^{-2}	αGalNAc	GalNAc
Soybean agglutinin	SBA	1.0×10^{-2}	α > βGalNAc	GalNAc
Bandeiraea simplicifolia lectin-I	BSL-I	5.0×10^{-3}	αGal, αGalNAc	Gal
Vicia villosa agglutinin	VVA	1.0×10^{-2}	GalNAc	GalNAc
Sophora japonica agglutinin	SJA	5.0×10^{-2}	GalNAc	GalNAc
Ricinus communis agglutinin-I	RCA-120	2.0×10^{-3}	Gal	Gal
Jacalin		5.0×10^{-4}	Galβ3GalNAc	Gal
Peanut agglutinin	PNA	4.0×10^{-3}	Galβ3GalNAc	Gal
Erythrina cristagalli lectin	ECL	2.0×10^{-2}	Galβ4GlcNAc	Gal
Ulex europaeus agglutinin-I	UEA-I	5.0×10^{-2}	αFuc	Fuc
Concanavalin A	ConA	3.3×10^{-3}	αMan, αGlc	Man
Pisum sativum agglutinin	PSA	4.0×10^{-3}	αMan, αGlc	Man
Lens culinaris agglutinin	LCA	4.0×10^{-3}	αMan, αGlc	Man
Phaseolus vulgaris agglutinin-E	PHA-E	5.0×10^{-3}	Galβ4GlcNAcβ2Manα6(GlcNAcβ4)(GlcNAcβ4Manα3)Manα4	–
Phaseolus vulgaris agglutinin-L	PHA-L	2.5×10^{-3}	Galβ4GlcNAcβ6(GlcNAcβ2Manα3)Manα3	–

Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose; NeuAc, N-acetylneuraminic acid.
[a] Rough specificity is evaluated according to lectin properties announced by Vector Laboratories (Burlingame, CA, USA).

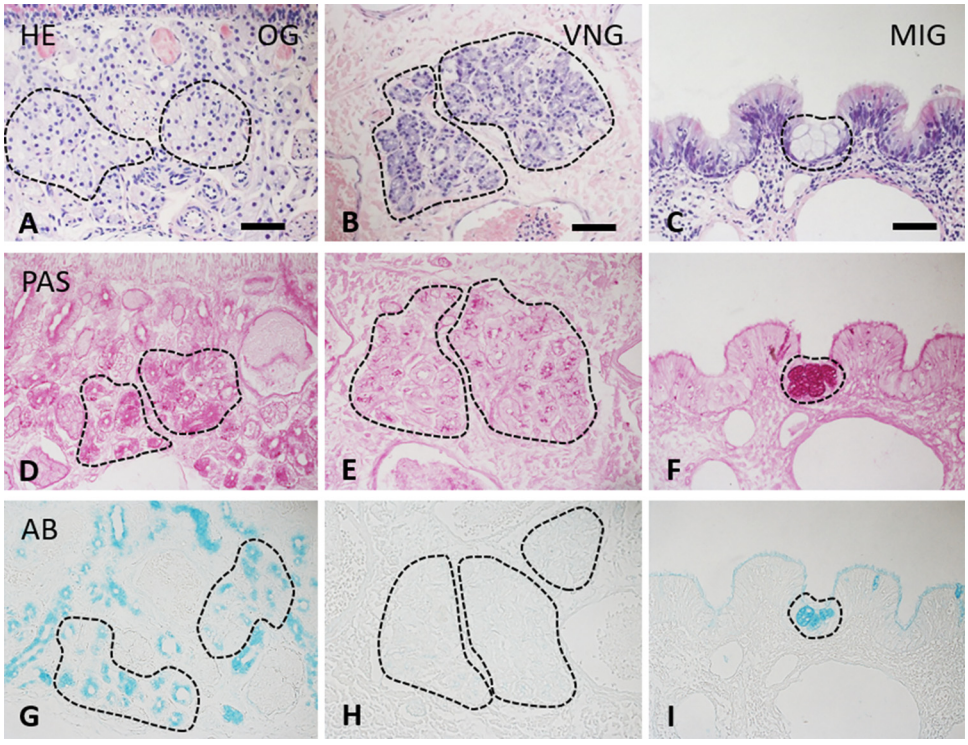

Fig. 1. Histological features of olfactory (A, D, G), submucosal vomeronasal (B, E, H) and multicellular intraepithelial (C, F, I) glands of brown bears.
Staining with (A–C) hematoxylin-eosin; (D–F) periodic acid-Schiff; (G–I) Alcian blue pH 2.5. Dashed lines enclose each gland. Bars = 50 µm. AB, Alcian blue; HE, hematoxylin-eosin; MIG, multicellular intraepithelial gland; OG, olfactory gland; PAS, periodic acid-Schiff; VNG, submucosal vomeronasal gland.

Glycoproteins in the mucosal fluid, as well as in receptor cell membranes, are associated with odorant reception (Plendl and Sinowatz, 1998). Periodic acid-Schiff (PAS) and Alcian blue (AB) stains are traditionally used to detect neutral and acidic mucopolysaccharides, respectively. Lectins are proteins that bind specific terminal carbohydrates on glycans (Damjanov, 1987), and they are extensively used to distinguish cell types (Leathem and Atkins, 1983). Some lectins bind either the olfactory glands (OG) of the nasal mucosa or the VNG of the VNO in rats (Takami et al., 1994), marmosets (Nakajima et al., 1998), armadillos (Ferrari et al., 1999; Carmanchahi et al., 2000) and horses (Lee et al., 2016a, 2016b), indicating that glycoconjugates in secretory granules differ between the OG and VNG of these species. The present study histochemically assessed the binding profiles of 21 lectins in the OG, VNG and MIG of the brown bear to evaluate the difference of secretory substances between these three glands.

2. Materials and methods

2.1. Animals

We examined one 12-month-old captive (ID code: B) and two wild, 3-year-old, sexually-immature male (ID codes: A and F) bears, as described by Tomiyasu et al. (2017). The captive bear lived at Noboribetsu Bear Park (Noboribetsu, Hokkaido, Japan) and died in an