NOTE  Anatomy

Differential Expression of Histochemical Characteristics in the Developing Olfactory Receptor Cells in a Flatfish, Barfin Flounder (Verasper moseri)

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ABSTRACT. Differentiation of the histochemical characteristics of the olfactory receptor cells (ORC) was examined by immunohistochemistry for protein gene product 9.5 (PGP 9.5) and calretinin (CR) and lectin histochemistry for Phaseolus vulgaris agglutinin-L (PHA-L) in the developing olfactory epithelium (OE) of the barfin flounder. PGP 9.5 immunoreactivity was diffuse and CR immunoreactivity was restricted at day 7, but these immunoreactivities became intense in the OE toward day 91. Crypt cells were first identified at day 56. PHA-L staining was faint at day 28, but became intense toward day 91. These findings suggest that PGP 9.5-immunopositive cells, CR-immunopositive cells, crypt cells and PHA-L-reactive cells differentiate independently in the developing OE and constitute subsets of the ORC in the OE.

KEY WORDS: calretinin, Phaseolus vulgaris agglutinin-L, protein gene product 9.5.

Two types of olfactory receptor organs can occur in vertebrates, that is, olfactory epithelium (OE) and vomeronasal organ (VNO). They perform functional assignments by perception of general odorants and pheromonal molecules, respectively [4, 6]. Phylogenetically, the OE exists in all vertebrates from fish to mammals [5], but the VNO is absent in fish and first appears in amphibians as a diverticulum of the nasal cavity [2, 12]. Therefore, the olfactory receptor organ is solely represented by the OE in fish. On the other hand, since putative pheromones are identified in some fish species [14, 15], the olfactory function of the VNO may be taken charge of by the OE in fish. This presumption may be approved by the presence of three kinds of olfactory receptor cells (ORC) in the fish OE, that is, ciliated cells, microvillous cells and crypt cells [1, 3, 18]. Ciliated and microvillous cells are strongly supposed to differ in the property of odorant perception from each other by histochemical and electrophysiological studies [1, 7, 8, 11]. Since the ORC of the OE are ciliated and the ORC of the VNO are microvillous in general in the tetrapod [9], the general odorants may be perceived by the ciliated ORC and pheromonal molecules by the microvillous ORC in fish. In addition, crypt cells are unique olfactory neurons, and their olfactory function remains obscure [3]. On the other hand, we revealed the presence of four kinds of ORC, that is, protein gene product 9.5 (PGP 9.5)-immunoreactive cells, calretinin (CR)-immunoreactive cells, Phaseolus vulgaris agglutinin-L (PHA-L)-reactive cells and crypt cells, in our previous report according to their difference in the immunohistochemical and lectin histochemical characteristics [13]. Although this difference in such histochemical characteristics may only reflect the current functional mode of the same type of the ORC, there remains a possibility that this difference reflects the presence of some kinds of subsets in the ORC of the fish OE. In the present study, therefore, we examined the development of a fish OE by immunohistochemistry and lectin histochemistry to reveal that the difference in such histochemical characteristics in the ORC persists throughout development of the OE to suggest the presence of subsets in the ORC even in the developing OE. We adopted the barfin flounder (Verasper moseri) as materials, because the barfin flounder is one of the macrosmatic fish and depends mainly on olfaction in the feeding [17]. At least 10 fry of the barfin flounder were obtained every 7 days from day 0 to 91 after hatch in Iwate Fisheries Technology Center, Kamaishi, Japan, as materials. Three adult barfin flounders, about 2 to 3 years of age, were also obtained from the same Center. The materials were processed for immunohistochemistry using antibody against PGP 9.5 (UltraClone, Wight, U.K.) or CR (Chemicon International, Temecula, U.S.A.) or lectin-histochemistry using a biotinylated lectin PHA-L (Vector, Burlingame, U.S.A.). PGP 9.5 and CR are excellent markers for the OE of mammals [10, 16], and PHA-L showed the characteristic staining pattern for the ORC in our previous study [13]. Histochemical procedures were detailed in this study. At day 0 after hatch, the primitive OE consisted of undifferentiated cells. No specific stainings were observed for PGP 9.5, CR or PHA-L in the OE. At day 7, although not
fully differentiated, ORC, supporting and basal cells were barely identifiable in the OE. PGP 9.5 immunoreactivity was diffusely observed in the OE and intense at the free surface of the OE and in the undifferentiated ORC (Fig. 1a). CR immunoreactivity was moderate at the free surface of the OE and in a part of the undifferentiated ORC (Fig. 1e). PHA-L staining was intense at the free surface of the OE and in the undifferentiated ORC (Fig. 1i). At day 28, the differentiation of cells in the OE became obvious and nuclei of the ORC tended to occupy the lower half of the OE, those of the supporting cells upper one third of the OE, and those of the basal cells at the bottom of the OE. PGP 9.5 immunoreactivity was intense at the free surface of the OE and in the ORC (Fig. 1b). CR immunoreactivity was moderate in the ORC (Fig. 1f). PHA-L staining was faint in the OE and moderate only in the distal cytoplasm of a part of the ORC (Fig. 1j). At day 56, the cell configuration in the OE became more similar to that in the adult. Crypt cells were first observed in the upper one third of the OE and showed intense PGP 9.5 and CR immunoreactivities. Intense PGP 9.5 immunoreactivity was also observed in the ORC situated in the upper three fourths of the OE (Fig. 1c). Intense CR immunoreactivity was observed in the ORC situated in the upper two thirds of the OE (Fig. 1g). PHA-L staining was intense in the distal cytoplasm of a part of the ORC and moderate in the basal cells or undifferentiated ORC at the bottom of the OE as in the adult (Fig. 1k). At day 91, the cell configuration in the OE, PGP 9.5 and CR immunoreactivities and PHA-L staining were almost the same as those in the adult. Namely, PGP 9.5 immunoreactivity was observed in the ORC situated in the upper three fourths of the OE (Fig. 1d). CR immunoreactivity was restricted to the ORC situated in the upper two thirds of the OE (Fig. 1h). These cells showed intense immunoreactivity for CR in their somata and dendrites. Intense PHA-L staining was observed in a small number of the ORC (Fig. 1l). Their somata were situated in the upper half of the OE and equipped with relatively slender dendrites. In addition, moderate PHA-L staining was observed in the basal cells or undifferentiated ORC situated at the bottom of the OE. Crypt cells were immunopositive for both PGP 9.5 and CR and situated in the upper one third of the OE.

Fig. 1. Light micrographs of the olfactory epithelium in the fry of the barfin flounder (Verasper moseri) at day 7 (a, e, i), day 28 (b, f, j), day 56 (c, g, k) and day 91 (d, h, l) after hatch. (a-d) Immunohistochemistry for PGP 9.5. (e-h) Immunohistochemistry for calcitonin. (i- l) Lectin staining with PHA-L. An arrow at day 56 and 91 after hatch indicates a crypt cell in each micrograph. Scale bars=20 μm
According to the present results, the intensities of PGP 9.5 and CR immunoreactivities and PHA-L staining changed differently in the developing OE. PGP 9.5 immunoreactivity was diffuse at day 7 and became to be observed in the ORC situated in the upper three fourths of the OE toward day 91. PGP 9.5-immunoreactive cells were distributed widely in the upper three fourths of the OE and equipped with thick dendrites and relatively round somata at day 91. CR immunoreactivity was moderate and restricted to a part of the undifferentiated ORC except the free surface of the OE at day 7 and increased in immunoreactive intensity with increasing number of immunoreactive cells, and became to be observed in the ORC situated in the upper two thirds of the OE toward day 91. CR-immunoreactive cells were less numerous than PGP 9.5-immunoreactive cells, distributed in the upper two thirds of the OE and equipped with very thick dendrites and spindle-shaped somata at day 91. Crypt cells were first identified as cells showing both PGP 9.5 and CR immunoreactivities at day 56. Crypt cells were egg-shaped and distributed sparsely in the upper one thirds of the OE toward day 91. CR-immunoreactive cells were distributed widely in the upper three fourths of the OE and compose at least a part of subsets of the ORC. PHA-L immunoreactivity was intense at the free surface of the OE at day 7, but became faint in the OE and moderate only in the distal cytoplasm of a part of the ORC at day 28. Then, PHA-L staining became intense in a small number of the ORC with thick dendrites and moderate in the basal cells or undifferentiated ORC toward day 91. PHA-L-reactive cells were small in number, distributed in the upper half of the OE and equipped with relatively slender dendrites and elliptical somata at day 91. These findings suggest that changes in PGP 9.5 and CR immunoreactivities reflect the differentiation process of PGP 9.5- and CR-immunoreactive ORC because these cells are present already at day 7 after hatch and change their location with increasing reactivities in the OE to show the cell configuration as almost the same as in the adult at day 91 [5]. On the other hand, changes in PHA-L staining were different from those in PGP 9.5 and CR immunoreactivities. Intense PHA-L staining in the OE at day 7 and faint staining in the OE at successive stages except moderate staining in a part of the ORC suggest that the sugar residue recognized by PHA-L [12] was necessary for the OE at early embryonic stage and became unnecessary as the ontogeny proceeds. Reappearance of intense PHA-L staining in the ORC after day 56 may suggest that the same sugar residue becomes necessary again along with the differentiation of the ORC because PHA-L staining is only moderate in the basal cells and undifferentiated ORC even at day 91. Late appearance of crypt cells at day 56 seems to suggest that these cells are necessary for the sophisticated function of the OE [3], because the first olfactory lamella extends to its full length and wholly covered with the OE at day 56 as reported in our previous study [17].

In summary, four types of the ORC were identified in the developing OE by immunohistochemistry for PGP 9.5 and CR and lectin histochemistry for PHA-L in the present study, that is, PGP 9.5-immunopositive cells, CR-immunopositive cells, crypt cells immunopositive for both PGP 9.5 and CR, and PHA-L-reactive cells. These cells were different with each other in the time of initial appearance, changes in the immunoreactivity or staining intensity, cell morphology, or the location in the OE. These findings suggest that these cells differentiate independently during morphogenesis of the OE and compose at least a part of subsets of the ORC revealed by such histochemical characteristics even in the developing OE. It remains obscure whether such histochemical characteristics correspond to the fine structural properties, that is, ciliated or microvillous.

REFERENCES