Ultrasound of the Olfactory Epithelium in a Flatfish, Barfin Flounder (Verasper moseri)

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Abstract. In this study, we examined the olfactory epithelium (OE) of the barfin flounder by transmission electron microscopy. As in the case of the ordinary teleost, the OE of the barfin flounder had 3 types of olfactory receptor cells (ciliated olfactory receptor cell, microvillous olfactory receptor cell and crypt cell), 3 types of supporting cells (ciliated, microvillous and crypt supporting cells) and basal cells. Each type of OE cells in the barfin flounder had similar ultrastructure to that of the ordinary teleost. Crypt cell is the third type of olfactory receptor cell unique to fish, whose function is unclear. The barfin flounder may be a suitable material to study crypt cells because it has relatively abundant crypt cells in the OE.

Key Words: fish, olfactory epithelium, ultrastructure.

NOTE Anatomy

Many vertebrates have anatomically separated two olfactory organs: the olfactory epithelium (OE) and the vomeronasal organ (VNO). They detect general odorants and pheromones, respectively [1, 3]. Although the OE is observed in all vertebrates, the VNO first appeared in amphibians during phylogeny. Since fish do not possess VNO, the OE of fish has been supposed to detect both general odorants and pheromones [9]. As in the case of other vertebrates, the OE of fish consists of the olfactory receptor cells (ORCs), supporting cells (SCs) and basal cells (BCs). In fish, there are three types of ORCs: the ciliated ORCs (cORCs), microvillous ORCs (mORCs) and crypt cells (CCs) [9]. In goldfish, the cORCs and mORCs have been shown to express genes homologous to the mammalian odorant receptors and vomeronasal receptors, respectively [2, 10, 11]. They are bipolar ORCs. On the other hand, CCs are the third type of ORCs unique to fish. Although CCs have been found in the OE of various kind of fish [4, 8], their function in the olfaction is still unclear. In addition, SCs have been supposed to help ORCs in their olfactory function and BCs have been supposed to be the precursor cells of ORCs [9].

The barfin flounder is a suitable material to study fish olfaction, because it is one of the macrosmatic fish, depends mainly on the olfaction for feeding, and is convenient to obtain as it is cultivated in a local research institute. Previously using the barfin flounder, we investigated the morphology of olfactory system by scanning electron microscopy, lectin histochemistry and immunohistochemistry [12–14]. In the present study, we examined the OE of the barfin flounder by transmission electron microscopy (TEM) to reveal the possible relationship between their ultrastructure and function.

Three adult barfin flounders, about 30 cm in length, were obtained from the Iwate Fisheries Technology Center (Kamaishi, Japan). After euthanasia by decapitation, the olfactory epithelium was dissected out and fixed in 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) overnight at 4°C. Subsequently, it was postfixed in 1% osmium tetroxide for 2 hr at 4°C, dehydrated in a graded series of ethanol, displaced with propylene oxide and embedded in epoxy resin. Ultrathin sections were cut at 1 μm and stained with toulidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope H-800 (Hitachi, Tokyo, Japan).

In the nasal sac of the barfin flounder, there were many lamellae covered with the OE (Fig. 1A and 1B). As in the case of the ordinary teleost, the OE of barfin flounder consisted of bipolar ORCs, CCs, SCs and BCs. Most CCs were situated in the upper portion of the OE with the exception of a small number of CCs in its lower portion (Fig. 1B and 1C). At the apical part of the OE, the cytoplasm of the SCs were alternately arranged with the dendrites of bipolar ORCs. Each ORC protruded its dendrite slightly above the apical end of the SCs (Fig. 2). Since bipolar ORCs were more electron-lucent than the neighboring SCs, they were easily distinguishable from the SCs. Desmosomes were observed between bipolar ORC and SC, as well as between SC and SC (Fig. 2). The somata of bipolar ORCs were situated in the lower half of the OE and contained well-developed rough endoplasmic reticulum (rER) and Golgi apparatus around their elliptical nuclei (Fig. 3A). On the free border of the OE, two types of bipolar ORC, cORC and mORC, were distinguished (Figs. 2, 3B and 3C). The former was equipped with cilia about 300 nm in diameter on the tip of its dendrite. Basal bodies were observed at the base of the cilia. The dendrite of cORC contained numerous...
microtubules and mitochondria (Fig. 3B). On the other hand, mORC was equipped with microvilli about 100 nm in diameter on the tip of its dendrite. The dendrite of mORC also contained numerous microtubules and mitochondria. In addition, a small number of centrioles were present in the dendrite of mORC (Fig. 3C). CCs were ovoid cells, scattered mainly in the upper third of the OE and surrounded by the crypt SCs (CSCs). Both CC and CSC were more electron-lucent than their neighboring cells (Fig. 4A). CC had apical invagination lined with microvilli. At the bottom of this invagination, cilia were observed. In the cytoplasm of CC, microtubules and mitochondria were observed (Fig. 4A). The nucleus of CC was oval and situated in the lower third of the somata. The cytoplasm of CC abruptly tapered below the nucleus to form the axon (Fig. 4B). CSCs were equipped with microvilli at their free border and contained numerous mitochondria in their cytoplasm (Fig. 4A). Desmosomes were found between CC and CSC, as well as between CSC and adjacent ordinary SC (Fig. 4A). The nuclei of CSCs were oval and situated deeper than those of CCs (Fig. 4B). On the other hand, the nuclei of SCs were spindle-shaped and situated randomly in the OE. SCs contained well-developed rER in the perinuclear cytoplasm (Fig. 5A). On the free border of the OE, two types of SCs were distinguished: cSC equipped with cilia about 300 nm in diameter and mSC equipped with microvilli about 100 nm in diameter (Fig. 5B). There was no distinction in cell organelles between cSC and mSC. Two types of SCs contained secretory granules, mitochondria, rER and Golgi apparatus in common in their cytoplasm (Fig. 5B). The cytoplasm of SCs extended to the basal membrane and surrounded the axon bundles of ORCs and the BCs (Fig. 6). BCs had irregularly shaped nucleus and poorly developed organelles in their cytoplasm. Their cytoplasm also extended to the basal membrane to surround the axon bundles of ORCs (Fig. 6).
Among ORCs, the cORCs and mORCs were typical bipolar neurons and had cilia and microvilli at the tip of their dendrites, respectively. In most vertebrates, cORCs in the OE express the odorant receptors and mORCs in the VNO express the vomeronasal receptors [1, 3]. As fish lacks the VNO, cORCs and mORCs are intermingled in the fish OE. In the barfin flounder, cORCs and mORCs had similar ultrastructure to those in the ordinary teleost [9]. Centrioles were often observed in the dendrites of mORCs in the barfin flounder. This characteristic is common to fish mORCs [9], as well as to the vomeronasal receptor cells of many terrestrial vertebrates. In some fish, the somata of cORCs and mORCs are situated in the lower and upper portions of OE, respectively [9]. In our previous study, immunohistochemical analysis for PGP 9.5 and calretinin revealed that two types of ORCs with distinct properties distributed separately in the OE of the barfin flounder [12, 14]. In the present study, however, we could not find any structural differences by TEM examination between ORCs in the upper and lower portions of the OE. Therefore, it is unknown whether the somata of cORC and mORC are distributed separately in the OE of barfin flounder as revealed by the immunohistochemical analysis for PGP 9.5 and calretinin.

Although we examined CCs in the barfin flounder by lectin histochemistry and immunohistochemistry [12, 14], ultrastructure of CCs in the barfin flounder remained unclear. In the present study, we revealed that CCs of barfin flounder have similar ultrastructure to those of the ordinary teleost [8]. The most striking feature of CCs is the apical invagination equipped with both cilia and microvilli. As the morphology of CCs is very different from that of cORCs or mORCs, the olfactory function of CCs may also be different from them. Although the function of CCs is still unclear, it has been suggested by lectin histochemistry and immunohistochemistry that CCs in the barfin flounder may perceive wider range of odorants than cORCs and mORCs [12, 14]. Several authors suggested that CCs are different from cORCs and mORCs with respect to their projection area in the olfactory bulb [11] and that CCs may be associated with the perception of some chemical stimuli [5] or sex pheromones [6, 7, 10]. Further investigation is required to reveal the precise function of CCs in the olfaction.

In summary, this study revealed that the OE of barfin flounder consists of three types of ORCs (cORCs, mORCs and CCs), three types of SCs (cSCs, mSCs and CSCs) and the BCs. The barfin flounder may be a suitable material to study CCs because it has relatively abundant CCs in the OE.

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REFERENCES
2. Cao, Y., Oh, B. C. and Stryer, L. 1998. Cloning and localiza-


