Chem. Senses 26: 517–522, 2001

Vomeronasal Epithelial Cells of Human Fetuses Contain Immunoreactivity for G Proteins, Go_α and $Gi_{\alpha 2}$

Shigeru Takami, Maiko Yukimatsu, George Matsumura[1] and Fumiaki Nishiyama

Department of Anatomy, School of Health Sciences, Kyorin University, Hachioji, Tokyo 192-8508 and [1]Department of Anatomy, School of Medicine, Kyorin University, Mitaka, Tokyo 181-8611, Japan

Correspondence to be sent to: S. Takami, Department of Anatomy, School of Health Sciences, Kyorin University, 476 Miyashita, Hachioji, Tokyo 192-8508, Japan. e-mail: takamis@kyorin-u.ac.jp

Abstract

Two G protein subfamilies, Go_α and $Gi_{\alpha 2}$, were identified and localized immunohistochemically in the vomeronasal organ (VNO) of 5-month-old human fetuses. Immunoreactivity for Go_α and $Gi_{\alpha 2}$ was present in a subset of vomeronasal epithelial cells. Prominent immunoreactivity was observed in apical processes and their apical terminals facing onto the vomeronasal lumen. Nerve fibers associated with the VNO exhibited intense immunoreactivity for Go_α and weak immunoreactivity for $Gi_{\alpha 2}$. Since Go_α and $Gi_{\alpha 2}$ are characteristically expressed and coupled with putative pheromone receptors in rodent vomeronasal receptor neurons, the present results suggest the possibility that vomeronasal epithelial cells containing Go_α and $Gi_{\alpha 2}$ in human fetuses are chemosensory neurons.

Introduction

Most terrestrial vertebrates possess a vomeronasal system (VNS) that is anatomically separated from the main olfactory system. The VNS is known to be a pheromone-detecting system in many species. The sensory organ of the VNS, the vomeronasal organ (VNO), is a blind sac, situated bilaterally at the base of the nasal septum, and a liquid-filled lumen of the sac communicates with the nasal or oral cavity (Wysocki, 1979; Halpern, 1987; Meredith, 1991). Sensory cells of the VNS, vomeronasal receptor neurons (VRNs) (Takami *et al*., 1994, 1995a), are located within the sensory epithelium (SE) of the VNO. The VRNs are the first-order neurons of the VNS and bipolar in shape; every functional VRN has a single dendrite and an axon originating from its soma. The dendrite reaches the surface of the lumen of the VNO to form a dendritic terminal that bears microvilli (Graziadei, 1977; Halpern, 1987; Meredith, 1991). The axon leaves the SE, forming a group with neighboring axons, an axon bundle. All axons of VRNs make axon bundles, which are called the vomeronasal nerve (VNN), traveling toward the brain, and terminate on dendrites of second-order neurons of the VNS, in the accessory olfactory bulb (AOB) (Halpern, 1987).

Several chemical characteristics of VRNs have been reported using immunohistochemical (Mori, 1987; Schwarting and Crandall, 1991; Taniguchi *et al*., 1993; Johnson *et al*., 1994b; Takami *et al*., 1994, 1995b; Jia and Halpern, 1996) and lectin histochemical (Takami *et al*., 1992, 1994, 1995a; Ichikawa *et al*., 1994; Nakajima *et al*., 1998) techniques. Among these characteristics, the presence of immunoreactivity for two G protein subfamilies, Go_α and $Gi_{\alpha 2}$ (Jia and Halpern, 1996), is most directly related to the function of VRNs because these G proteins are expressed by VRNs and coupled with putative pheromone receptor molecules (Herrada and Dulac, 1997; Matsunami and Buck, 1997). In fact, VRNs are physiologically excited by pheromones/pheromone candidates (Inamura *et al*., 1999; Leinders-Zufall *et al*., 2000).

In the fetal life of humans, the VNO is recognizable as early as gestational week 8 (Kreutzer and Jafek, 1980). After that, the tubular-shaped VNO, which opens into the nasal cavity, the VNN and the AOB become well developed for many weeks (Pearson, 1942; Bossy, 1980; Kreutzer and Jafek, 1980). The VNO and VNN have been documented in 6-month-old fetuses (McCotter, 1915) and even at 28 weeks old (Nakashima *et al*., 1985). Several reports have demonstrated that neuron-specific molecules are immunolocalized in VRN-like cells that are present in the epithelium of fetal VNO (Boehm and Gasser, 1993; Johnson *et al*., 1994a; Kjaer and Hansen, 1996), called the vomeronasal epithelium (VE) (Takami *et al*., 1993). The above anatomical and immunohistochemical findings suggest that human fetuses contain a functional VNS, although no direct evidence exists to confirm this. To clarify that the VE contains VRNs, the present study aimed to examine whether vomeronasal

epithelial cells (VECs) in 5-month-old human fetuses display immunoreactivity for Go_{α} and $Gi_{\alpha 2}$. Results of our study demonstrate that VRN-like cells in the VE of 5-month-old human fetuses contain Go_{α}- and $Gi_{\alpha 2}$-immunoreactive cells.

Materials and methods

We utilized tissues of nine Japanese 5-month-old fetuses (mean crown–rump length: 13.4 cm). These tissues were taken from human tissue specimens kept and authorized in the Department of Anatomy I, Kyorin University School of Medicine. They had been initially fixed in Formalin-based fixatives and stored in 70% ethanol for many years, so that their fixation/preservation condition seemed not to be perfect. The tissue blocks including the VNOs were dissected out from these specimens, and re-fixed in ice-cold Zamboni's fixative overnight, cryoprotected in sucrose (15, 30%) in phosphate-buffered saline, and embedded in Tissue-Tek®, O.C.T. Compound (Sakura Finetechnical Co., Tokyo, Japan) as previously described (Takami *et al*., 1994, 1995a,b; Yamagishi *et al.*, 1996). Fourteen-micrometer-thick cryostat sections were made by an electrical cryostat (Microm HM 500 OM, Carl Zeiss Co., Jena, Germany) in the frontal and sagittal planes.

The protein gene product 9.5 (PGP), which was originally isolated from human brains (Thompson *et al*., 1993), is a soluble protein of 27 kDa that is widely distributed in neurons and neuroendocrine cells (Wilson *et al*., 1988). In the olfactory system, PGP is contained in olfactory receptor neurons and VRNs (Takami *et al*., 1993; Taniguchi *et al*., 1993; Johnson *et al*., 1995; Takami *et al*., 1995b). Furthermore, PGP is contained in VECs of the human VNO (Takami *et al*., 1993; Kjaer and Hansen, 1996). Since the specimens used in this study were stored in Formalin/ethanol solutions for many decades, we first examined whether these VNOs were appropriate for further immunohistochemical studies by use of an antiserum to PGP.

As a method for immunohistochemical labeling, we utilized a peroxidase-labeled streptavidin–biotin (PLSAB) method that is one of the most sensitive detection systems. Its details were previously described (Takami *et al*., 1995b). Briefly, primary antibodies used in this study were polyclonal antibodies against PGP raised in rabbits (Ultra Clone, Ltd, Wellow, UK) at a dilution of 1:2000, polyclonal rabbit IgG against Go_{α} (Santa Cruz Biotech., Inc., Santa Cruz, CA) at a dilution of 1:1000, and two polyclonal rabbit IgGs against $Gi_{\alpha 2}$ (Santa Cruz Biotech., Inc., and Wako Pure Chem., Osaka, Japan) at a dilution of 1:500. As a chromogen, aminoethyl carbazol was used throughout this study. Omission of the primary antibodies from the experimental protocols and preadsorption controls for two antibodies from Santa Cruz were carried out as negative controls. As positive-tissue controls, cryostat sections of VNOs of rats (Sprague–Dawley strain) were utilized.

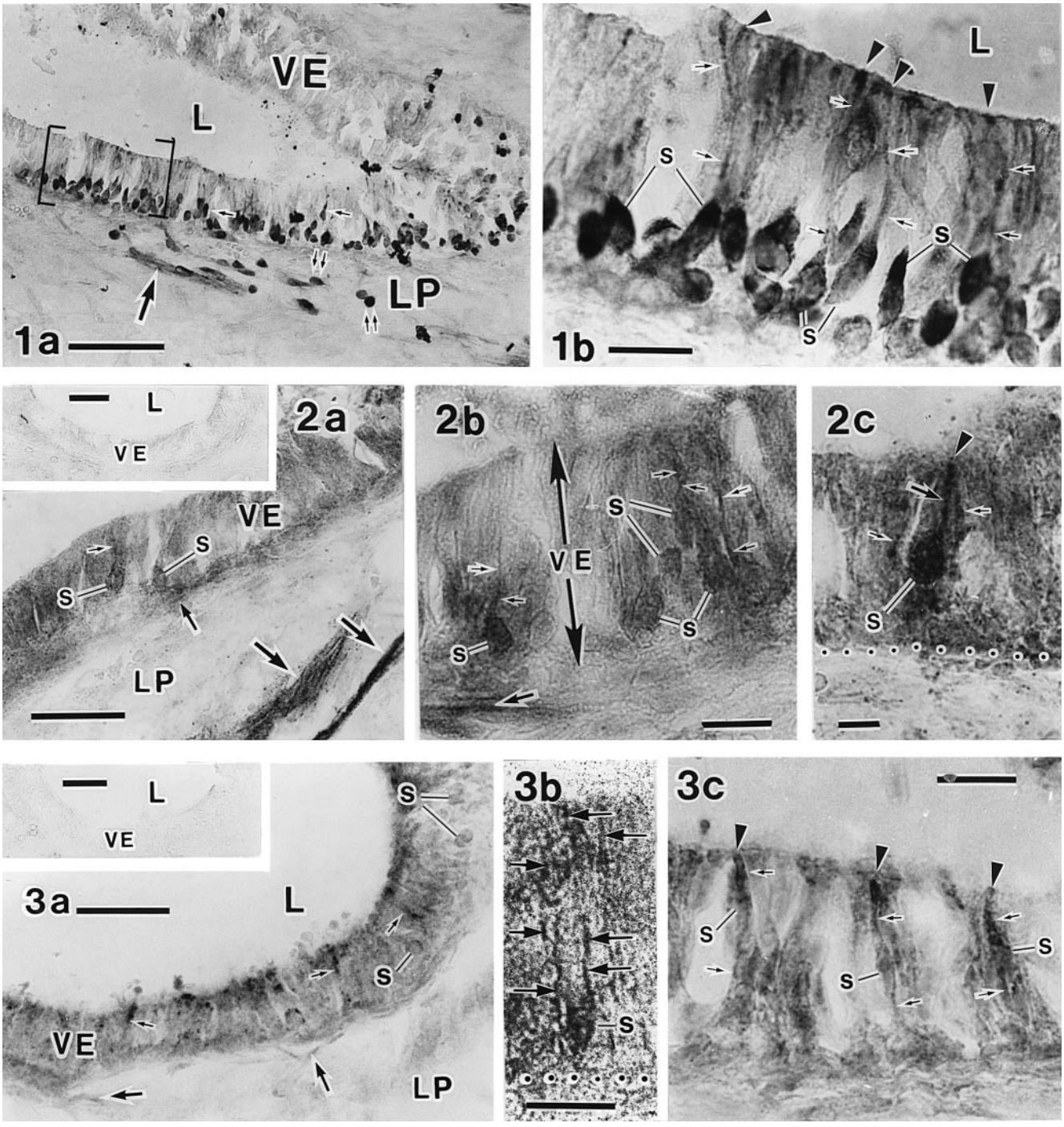
Results

The PLSAB method was successful to visualize PGP immunoreactivity in all specimens examined. In the VNOs, plenty of VRN-like VECs were PGP-immunoreactive (Figure 1a). PGP immunoreactivity was distributed throughout their somata and apical processes, some of which reached the luminal surface to form terminals (Figure 1b). Nerve fibers associated with the VNO were PGP-immunoreactive as well. Omission of the PGP antiserum resulted in no reaction products. These observations thus indicate that the specimens used here are liable for further immunohistochemical analyses, although the condition of fixation/preservation of these tissues seemed not be perfect. Neighboring sections to those labeled with the PGP antiserum were immunolabeled with Go_{α} and $Gi_{\alpha 2}$ antisera.

Figure 1 PGP immunoreactivity in the VNO of a 5-month-old fetus. **(a)** Low magnification of a sagittal section. Immunoreactivity for PGP is present in somata (small arrows) in the vomeronasal epithelium (VE), nerve fiber bundles (large arrow) and cells in the lamina propria (double arrows). Enlargement of the area enclosed by brackets are shown in **(b)**. L, vomeronasal lumen. Bar = 100 μm. (b) The enlarged area in (a) showing PGP-immunoreactive cells in the VE. Immunoreactivity is distributed in somata (s), processes (small arrows) and their terminals (arrowheads) at the luminal surface. Bar = 20 μm.

Figure 2 Go_{α} immunoreactivity in the VNO of a 5-month-old fetus. **(a)** Medium magnification of a sagittal section showing Go_{α}-immunoreactive somata (s) and process (small arrow) in the vomeronasal epithelium (VE). A small (medium arrow) and large (large arrows) nerve fiber bundles are present in the lamina propria (LP). Bar = 50 μm. Inset shows a preadsorption control for Go_{α} immunoreactivity, frontal section. No specific reaction is observed in the VE. L, vomeronasal lumen. Bar = 50 μm. **(b)** Higher magnification of the VE, cut in a sagittal plane. Go_{α}-immunoreactive somata (s) and processes (small arrows), some of which are continuous from the somata, are seen. A small nerve fiber bundle (medium arrow) just below the basement membrane is present in the lamina propria. Bar = 20 μm. **(c)** Vomeronasal epithelial cell showing a Go_{α}-immunoreactive soma (s), an apical process (medium arrow) and its terminal (arrowhead). Neighboring processes belonging to other cells are also immunoreactive (small arrows). Bar = 20 μm.

Figure 3 $Gi_{\alpha 2}$ immunoreactivity in the VNO of 5-month-old fetuses. **(a)** Medium magnification of a frontal section showing $Gi_{\alpha 2}$-immunoreactive somata (s) and processes (small arrows) in the vomeronasal epithelium (VE). Some immunoreactive processes can be traced to the epithelial surface facing the vomeronasal lumen (L). $Gi_{\alpha 2}$ immunoreactivity is also detected in nerve fibers (medium arrows) in the lamina propria (LP). Bar = 50 μm. The inset shows a negative control where the primary antiserum is omitted from the staining protocol, frontal section. No specific staining is observed in the VE. L, vomeronasal lumen. Bar = 50 μm. **(b)** $Gi_{\alpha 2}$-immunoreactive VEC whose soma (s) is located in the basal layer of the VE. Immunoreactive processes (small arrows), one of which is derived form the soma apically, are also seen. Dotted lines indicate the relative location of the basement membrane of the VE. Bar = 20 μm. **(c)** Sagittal section containing cells with a spindle-shaped soma. Immunoreactivity for $Gi_{\alpha 2}$ is present in apically located somata (s), processes (small arrows), their terminals (arrowheads) and basal processes (small arrows). Bar = 20 μm.



Go_α immunoreactivity was present in a subset of VECs (Figure 2a). In general, the morphology of Go_α-immunoreactive cells was similar to that of PGP-immunoreactive VECs. Go_α-immunoreactive ovoid somata were distributed from the basal to the apical regions of the VE. Apical processes derived from the somata were also immunoreactive for Go_α (Figure 2a,b). Terminals of apical processes of VECs were recognizable at the luminal surface of the VE (Figure 2c). Although we could not determine whether immunoreactive basal processes originated from the Go_α-immunoreactive somata, Go_α-immunoreactive fibers were present in the vicinity of the basement membrane (Figure 2a,b). Preadsorption control experiments resulted in no specific labeling in the above VECs, which were neuron-like cells, as well as nerve fibers in the lamina propria (Figure 2a, inset).

$Gi_{\alpha 2}$ antisera labeled a small subset of VECs (Figure 3a–c) and nerve fiber bundles in the lamina propria (Figure 3a). There far fewer Gi_α-immunoreactive VECs was than Go_α-immunoreactive ones. Omission of the antisera from

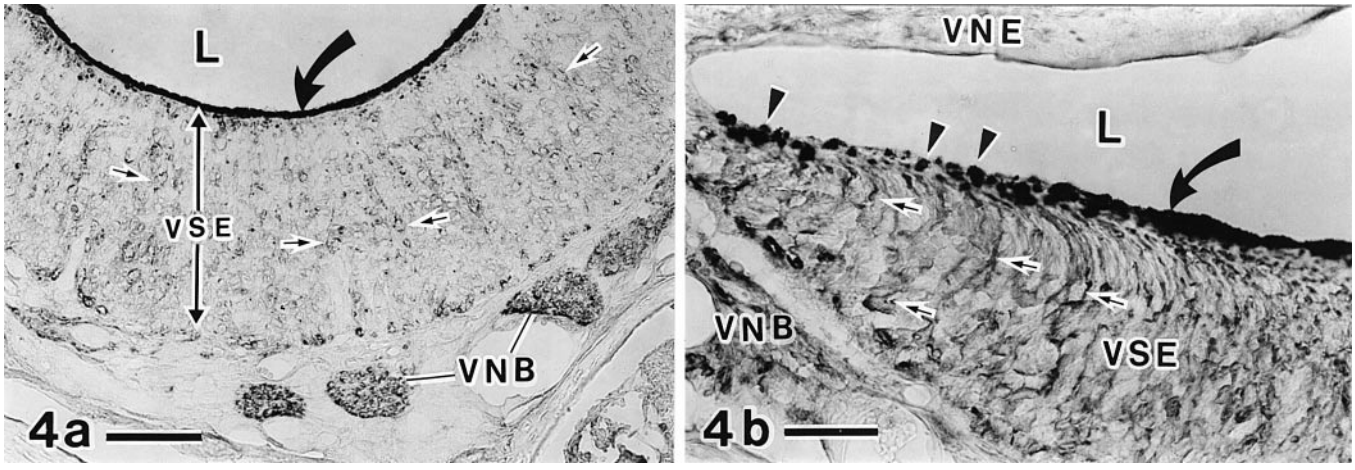


Figure 4 Frontal sections of a rat VNO as positive tissue controls. **(a)** Go_{α} immunoreactivity. The mucomicrovillar complex (curved arrow) of the vomeronasal sensory epithelium (VSE) contains the most intense immunoreactivity, and the somata and dendrites of VRNs exhibit weak immunoreactivity (arrows). Vomeronasal nerve bundles (VNB) in the lamina propria contain moderate immunoreactivity. Bar = 100 μm. **(b)** $Gi_{\alpha 2}$ immunoreactivity. The mucomicrovillar complex (curved arrow) and isolated single dendritic terminals of vomeronasal receptor neurons (arrowheads) contain the most intense immunoreactivity in the VSE, whereas specific labeling is not detected in the apical surface of the vomeronasal nonsensory epithelium (VNE). Somata and dendrites of vomeronasal receptor neurons exhibit weak immunoreactivity (arrows). Bar = 50 μm.

the experimental protocols and preadsorption control experiments (Figure 3a, inset) resulted in no specific reaction in the VE and lamina propria. Although some $Gi_{\alpha 2}$-immunoreactive VECs were morphologically similar to Go_{α}-immunoreactive VECs (Figure 3a,b), others had a spindle-shaped soma (Figure 3c). In addition to a single apical process, each of them tended to have a relatively thick basal process that could be traced down to the basement membrane (Figure 3c). The most intense immunoreactivity for $Gi_{\alpha 2}$ was observed in apical processes and their terminals at the lumen, whereas moderate immunoreactivity was present in somata and basal processes.

As positive tissue controls, VNOs of adult rats were immunolabeled using Go_{α} and $Gi_{\alpha 2}$ antisera. The most intense immunoreactivity for Go_{α} was present in the 'mucomicrovillar complex' (Takami *et al.*, 1994, 1995a) of the vomeronasal sensory epithelium; intense immunoreactivity was found in the vomeronasal nerve bundles, and moderate immunoreactivity in VRNs (Figure 4a). $Gi_{\alpha 2}$ antisera labeled the mucomicrovillar complex, VRNs and vomeronasal nerve bundles (Figure 4b). By contrast, nonsensory epithelium of the VNO did not exhibit any specific reaction (Figure 4b).

Discussion

The results of the present study have demonstrated for the first time that VRN-like VECs in the human VNO contain immunoreactivity for Go_{α} and $Gi_{\alpha 2}$. Although there were relatively high background readings in the VE sections labeled with antisera to Go_{α} and $Gi_{\alpha 2}$, it is still convincing that immunoreactivity for them is present within the apical processes and their terminals of the VECs. Genes for these G proteins are contained in VRNs of rodents (Herrada and Dulac, 1997; Matsunami and Buck, 1997), and intense immunoreactivity for these G proteins are localized in their dendritic terminals (Jia and Halpern, 1996; Matsuoka *et al.*, 2001). Our positive control experiments using rat VNOs have confirmed that the intense immunoreactivity for Go_{α} and $Gi_{\alpha 2}$ is localized in dendritic terminals of VRNs (see Figure 4). Thus, it is likely that genes for Go_{α} and $Gi_{\alpha 2}$ are also expressed in VECs of human fetuses and these G proteins are utilized within cellular components of these cells, including terminals of apical processes.

Adult rodent VRNs express putative pheromone receptors that are coupled with these Go_{α} and $Gi_{\alpha 2}$ (Herrada and Dulac, 1997; Matsunami and Buck, 1997). Physiological experiments using rats and mice have indicated that Go_{α}- and/or $Gi_{\alpha 2}$-containing VRNs in fact respond to putative pheromones. In adult rats, Go_{α}- and $Gi_{\alpha 2}$-containing VRNs responded differentially to pheromone-containing urine samples (Inamura *et al.*, 1999). Six putative pheromones excited a subset of VRNs of mice which contain $Gi_{\alpha 2}$ (Leinders-Zufall *et al.*, 2000). However, there has been no direct evidence indicating that the VNS plays a role as a chemosensory system and detects pheromones *in utero*. In rodents, it has been proposed that fetal VNS is functional (Pedersen *et al.*, 1983; Coppola and Millar, 1994). In humans, the presence of chemoreception *in utero* has been suggested (Schaal and Orgeur, 1992; Schaal *et al.*, 1998), although neither the chemosensory system in human fetuses nor odorants/pheromones in the amniotic fluid have been identified. Functional VRNs of animals bear microvilli from dendritic terminals (Graziadei, 1977; Halpern, 1987) and recent immunoelectron microscopic studies have demonstrated that putative pheromones (Takigami *et al.*, 1999), and Go_{α} and $Gi_{\alpha 2}$ (Matsuoka *et al.*, 2001), are

localized in dendritic terminals and microvilli of VRNs. Therefore, if one would claim that VECs of 5-month-old fetuses are functional chemosensory neurons, it is necessary to demonstrate that these VECs bear microvilli projecting from their terminals, and that pheromone receptors are identified and localized in these microvilli.

We have described that some $Gi_{\alpha 2}$-immunoreactive VECs had a spindle-shaped soma, indicating that they are different from animal VRNs in that the basal process (axon) is very thin (Graziadei, 1977; Halpern, 1987) and hardly visualized by conventional light microscopy. However, VECs that are similar in shape to the above $Gi_{\alpha 2}$-immunoreactive VECs have been reported in the VE of a 4-month-old baby (Johnson *et al.*, 1994a) and of adult humans (Takami *et al.*, 1993; Johnson *et al.*, 1994a). Thus, it is likely that the spindle-shaped bipolar cells are generally present in human VE throughout life.

This paper has also shown that some nerve fibers associated with the VE are $Go_{\alpha}/Gi_{\alpha 2}$-immunoreactive. Since vomeronasal nerve fibers of rodents exhibit $Go_{\alpha}/Gi_{\alpha 2}$ immunoreactivity (Jia and Halpern, 1996), it is probable that the $Go_{\alpha}/Gi_{\alpha 2}$-immunoreactive fibers demonstrated here are axons of VECs and a part of the VNN that is connected with the AOB (Humphrey, 1940; Bossy, 1980). In rodents, segregated projections of $Go_{\alpha}/Gi_{\alpha 2}$-immunoreactive VNN to the AOB (Jia and Halpern, 1996) were present. To clarify the function of the VNS in humans, it is important to examine whether any topographical pattern of projection of $Go_{\alpha}/Gi_{\alpha 2}$-immunoreactive VNN fibers is present human fetuses. Also, their AOB needs to be examined to determine whether synaptic terminals of VECs are identifiable.

In conclusion, the present study has revealed that VECs of 5-month-old human fetuses contained immunoreactivity for Go_{α} and $Gi_{\alpha 2}$. Moreover, it has shown the possibility that fetal VECs of humans are functional chemosensory neurons.

Acknowledgements

This study was partially supported by a Frontier Research Grant from Ministry of Education of Japan (No. 088773), and a research grant for Science and Engineering 1997 from Shiseido Co. Ltd, Japan.

References

Boehm, N. and **Gasser, B.** (1993) *Sensory receptor-like cells in the human fetal vomeronasal organ.* NeuroReport, 4, 867–870.

Bossy, J. (1980) *Development of olfactory and related structures in staged human embryos.* Anat. Embryol., 161, 225–236.

Coppola, D.M. and **Milar, L.C.** (1994) *Stimulus access to the accessory olfactory system in the prenatal and perinatal rat.* Neuroscience, 60, 463–468.

Graziadei, P.P.C. (1977) *Functional anatomy of the mammalian chemoreceptor system*. In Müller-Schwarze, D. and Mozell, M.M. (eds), Chemical Signals in Vertebrates. Plenum Press, New York, pp. 435–454.

Halpern, M. (1987) *The organization and function of the vomeronasal system.* Annu. Rev. Neurosci., 10, 325–362.

Herrada, G. and **Dulac, C.** (1997) *A novel family of putative receptors in mammals with a topographically organized and sexually dimorphic distribution.* Cell, 90, 763–773.

Humphrey, T. (1940) *The development of the olfactory and the accessory olfactory formations in human embryos and fetuses.* J. Comp. Neurol., 13, 431–468.

Ichikawa, M., Takami, S., Osada, T. and **Graziadei, P.P.C.** (1994) *Differential development of binding sites of two lectins in the vomeronasal axons of the rat accessory olfactory bulb.* Devl Brain Res., 78, 1–9.

Inamura, K., Matsumoto, Y., Kashiwayanagi, M. and **Kurihara, K.** (1999) *Laminar distribution of pheromone-receptive neurons in rat vomeronasal epithelium*. J. Physiol. (Lond.), 517, 731–739.

Jia, C. and **Halpern, M.** (1996) *Subclasses of vomeronasal receptor neurons: differential expression of G proteins (Go_{α} and $Gi_{\alpha 2}$) and segregated projections to the accessory olfactory bulb.* Brain Res., 719, 117–128.

Johnson, E.W., Eiller, P.M. and **Jafek, B.W.** (1994a) *Calbindin-like immunoreactivity in epithelial cells of the newborn and adult human vomeronasal organ.* Brain Res., 638, 329–333.

Johnson, E.W., Eller, P.M. and **Jafek, B.W.** (1994b) *Protein gene product 9.5 in the developing and mature rat vomeronasal organ.* Brain Res., 78, 259–264

Johnson, E.W., Eiller, P.M. and **Jafek, B.W.** (1995) *Distribution of OMP-, PGP 9.5- and CaBP-like, immunoreactive chemoreceptor neurons in the developing human olfactory epithelium.* Anat. Embryol., 191, 311–317.

Kjaer, I. and **Hansen, B.F.** (1996) *Luteinizing hormone-releasing hormone and innervation pathways in human prenatal nasal submucosa; factors of importance in evaluating Kallmann's syndrome.* APMIS, 104, 680–688.

Kreutzer, E.W. and **Jafek, B.W.** (1980) *The vomeronasal organ of Jacobson in the human embryo and fetus.* Otolaryngol. Head Neck Surg., 88, 113–123.

Leinders-Zufall, T., Lane, A.P., Puche, A.C., Ma, W., Novothy, M.V., Shipley, M.T. and **Zufall, F.** (2000) *Ultrasensitive pheromone detection by mammalian vomeronasal neurons.* Nature, 405, 792–796.

Matsunami, H. and **Buck, L.B.** (1997) *A multigene family encoding a diverse array of putative pheromone receptor in mammals.* Cell, 90, 775–784.

Matsuoka, M., **Yoshida-Matsuoka, J., Iwasaki, N., Norita, M., Constanzo, R.M.** and **Ichikawa, M.** (2001) *Immunocytochemical study of Gi2α and Goα on the epithelium surface of the rat vomeronasal organ.* Chem. Senses, 26, 161–166.

McCotter, R.E. (1915) *A note on the course and distribution of the nervus terminalis in man.* Anat. Rec., 9, 243–246.

Meredith, M. (1991) *Sensory processing in the main and accessory olfactory systems: comparisons and contrasts.* J. Steroid Biochem. Mol. Biol., 39, 601–614.

Mori, K. (1987) *Monoclonal antibodies (2C5 and 4C9) against lactoseries carbohydrates identify subsets of olfactory and vomeronasal receptor cells and their axons in the rabbit.* Brain Res., 408, 215–221.

Nakajima, T., Shiratori, K., Ogawa, K., Tanioka, Y. and **Taniguchi, K.** (1998) *Lectin-binding patterns in the olfactory epithelium and vomeronasal organ of the common marmoset.* J. Vet. Med. Sci., 60, 1005–1011.

Nakashima, T., **Kimmelman, C.P.** and **Snow, J.B.** (1985) *Vomeronasal*

organs and nerves of Jacobson in the human fetus. Acta Otolaryngol. (Stockh.), 99, 266–271.

Pearson, A.A. (1942) *The development of the olfactory nerve, the nervus terminalis, and the vomeronasal nerve in man.* Ann. Otol. Rhinol. Laryngol., 51, 317–332.

Pedersen, P.A., Steward, W.B., Greer, C.A. and **Shepherd, G.M.** (1983) *Evidence for olfactory function* in utero*.* Science, 221, 478–480.

Schaal, B. and **Orgeur, P.** (1992) *Olfaction* in utero*: can the rodent model be generalized?* Quart. J. Exp. Psychol. B, 44, 245–278.

Schaal, B., Marlier, L. and **Soussignan, R.** (1998) *Olfactory function in the human fetus: evidence from selective neonatal responsiveness to the odor of amniotic fluid.* Behav. Neurosci., 112, 1438–1449.

Schwarting, G.A. and **Crandall, J.E.** (1991) *Subsets of olfactory vomeronasal sensory epithelial cells and axsons revealed by monoclonal antibodies to carbohydrate antigens.* Brain Res., 547, 239–248.

Takami, S., Graziadei, P.P.C. and **Ichikawa, M.** (1992) *The differential stainings of two lectins in the accessory olfactory bulb of the rat.* Brain Res., 598, 337–342.

Takami, S., Getchell, M.L., Chen, Y., Monti-Bloch, L., Berliner, D.L., Stensaas, L.J. and **Getchell, T.V.** (1993) *Vomeronasal epithelial cells of adult human express neuron-specific molecules.* NeuroReport, 4, 375–378.

Takami, S., **Getchell, M.L.** and **Getchell, T.V.** (1994) *Lectin histochemical localization of galactose,* N*-acetylgalactosamine, and* N*-acetylglucosamine in glycoconjugates of the rat vomeronasal organ, with comparison to the olfactory and septal mucosae.* Cell Tissue Res., 277, 211–230.

Takami, S., **Getchell, M.L.** and **Getchell, T.V.** (1995a) *Resolution of sensory and mucoid glycoconjugates with terminal* α*-galactose residues in the mucomicrovillar complex of the vomeronasal sensory epithelium by dual confocal laser scanning microscopy.* Cell Tissue Res., 280, 211–216.

Takami, S., **Getchell, M.L.**, **Yamagishi, M., Albers, K.M.** and **Getchell, T.V.** (1995b) *Enhanced extrinsic innervation of nasal and oral chemosensory mucosae in keratin 14-NGF transgenic mice.* Cell Tissue Res., 282, 481–491.

Takigami, S., Osada, T., Yoshida-Matsuoka, J., Matsuoka, M., Mori, Y. and **Ichikawa, M.** (1999) *The expressed localization of rat putative pheromone receptors.* Neurosci. Lett., 272, 115–118.

Taniguchi, K., Saito, H., Okamura, M., and **Ogawa, K.** (1993) *Immunohistochemical demonstration of protein gene product 9.5 (PGP9.5) in the primary olfactory system of the rat.* Neurosci. Lett., 156, 24–26.

Thompson, R.J., Doran, J.F., Jackson, P., Phillon, AP. and **Rode, J.** (1983) *PGP9.5—a marker for vertebrate neurons and neuroendocrine cells.* Brain Res., 278, 224–228.

Wilson, P.O.G., **Barber, P.C., Hamid, Q.A., Power, B.F., Dhillon, A.P., Rode, J., Day, I.N.M., Thompson, R.F.** and **Polak, J.M.** (1988) *The immunolocalization of protein gene product 9.5 using rabbit polyclonal and mouse monoclonal antibodies.* Br. J. Exp. Pathol., 69, 91–104.

Wysocki, C.J. (1979) *Neurobehavioral evidence for the involvement of the vomeronasal system in mammalian reproduction.* Neurosci. Biobehav. Rev., 3, 301–341.

Yamagishi, M., Takami, S. and **Getchell, T.V.** (1996) *Ontogenetic expression of spot 35 protein (calbindin-D28k) in human olfactory receptor neurons and its decrease in Alzheimer's disease patients.* Ann. Otol. Rhinol. Laryngol, 105, 132–139.

Accepted February 1, 2001