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Abstract

It has been known that androgens and estrogens, which are referred to as sex steroids, make many effects on two major nasal chemosensory mucosae such as olfactory mucosa and vomeronasal organ. Our studies conducted in rodents have demonstrated that two of the constituent cells in the olfactory mucosa, sustentacular cells and acinar cells in the associated glands of the olfactory mucosa, Bowman’s glands, express four different enzymes involved in the biosynthesis of estradiol-17β (E2). Furthermore, our ongoing study has shown that olfactory sensory cells contain immunoreactivity for an estrogen receptor (beta-type). In case of vomeronasal organ, vomeronasal sensory cells express two enzymes that catalyze conversion of E2 and estrone, and that of testosterone and androstenedione. In addition, vomeronasal sensory cells contain an estrogen receptor (alpha-type). These results strongly suggest that de novo synthesis of E2 and metabolism of E2 take place in the olfactory mucosa and vomeronasal organ, respectively. With special emphasis of subcellular characteristics of steroid-producing cells, such as presence of large amount of smooth endoplasmic reticulum and vesicular mitochondria, we will introduce our findings and present working hypotheses for E2 functions in the olfactory mucosa and vomeronasal organ.

Keywords: olfactory sensory cell, olfactory sustentacular cell, Bowman’s glands, smooth vomeronasal sensory cell, smooth endoplasmic reticulum, estradiol-17β, estrogen receptor
In addition to the OM, many land-living mammals contain vomeronasal organs (VNOs) that are bilaterally located at the base of the nasal septum [5–8]. Sensory cells of the VNO, vomeronasal sensory cells (VSCs), which are bipolar neurons as well, mainly detect species-specific odorants including pheromones and transmit their odorant information to projection neurons in the accessory olfactory bulb, so-called mitral/tufted cells [9]. Other than the OM and VNO, septal organ and Gruenberg ganglion [8] contain chemosensory cells that have been found at least rodent species [10]. Locations of these four chemosensory mucosae and their primary brain centers are schematically shown in Figure 1A. In this chapter, we will focus on two of them, OM and VNO, to demonstrate experimental data using rats to suggest de novo synthesis of estradiol-17β (E2) in the OM and metabolizing of E2 in the VNO.

By a radioautographic technique, Stumpf and Sar [11] demonstrated the incorporation of 3H-labeled estradiol into cell nuclei of OSCs, duct cells of Bowman’s glands, and mitral cells in the main olfactory bulb where synaptic terminals of OSCs are present. They suggested that these cells contain estrogen receptors and are influenced by estradiol. In ferrets, sex steroids including estradiol increased ferrets’ responsiveness to low concentrations of odors emitted from anal scent glands [12]. Conspecific odor preference by male rats was influenced by estradiol [13]. Bakker et al. [14] demonstrated in aromatase knockout male mice that exogenous estrogens restore
male olfactory investigation of volatile body odors. On the other hands, it has been reported that estradiol makes various effects on the VNO. The volume of VNO in male rats was reported to be significantly larger that of female rats. This sexual dimorphism in rat VNO is established in the perinatal days when estradiol is converted from gonadal testosterone and makes an organization effect on VSCs [15, 16]. Estradiol induced increased immunoreactivity (IR) for immediate early gene in specific population of VSCs in mice [17]. Expression of VNO receptors is sexually dimorphic and influenced by estradiol and testosterone [18].

Steroid hormones are produced and secreted by endocrine cells in the adrenal cortex [19]. Leydig cells or intestinal cells of Leydig in the testis [20], granulosa cells of the ovulatory follicle and cells of the corpus luteum in the ovary [21], and brain cells [22]. One of the subcellular properties of steroid-producing cells is the presence of extremely developed smooth endoplasmic reticulum (SER, [23–25]). Images of rat Leydig cells that were obtained by transmission electron microscope (JEM1230, JEOL, Akishima-shi, Tokyo, Japan) are shown in Figures 1–D. In addition to tubular cisternae of SER (Figure 1C), swollen vesicular mitochondria are seen and associated with cisterna of SER (Figure 1D). In fact, Al-Amri et al. [26] demonstrated in Leydig cells in the house gecko, hemidactylus flaviviridis, that swollen vesiculated mitochondria are associated between SER. Lysosomes, which are described in the cytoplasm of Leydig cells [27], are frequently seen in the rat Leydig cells (Figure 1D).

All of steroid-producing cells express the cholesterol side chain cleavage enzyme (P450scc), which converts cholesterol into pregnenolone [28]. In Leydig cells of the testes, for example, pregnenolone is eventually converted into testosterone, which is the most potent androgen, via metabolic pathways containing 17\(\beta\)-hydroxysteroid dehydrogenases (HSDs). Based on several references [19, 20, 29–31], the main pathway of steroidogenesis from cholesterol to E2 is shown in Figure 2. The terminal enzyme in the sex-steroid-producing pathway is aromatase (CYP19 or P450arom). Granulosa cells of the ovulatory follicle and cells of the corpus luteum in the ovary, for instance, express aromatase (P450arom, [21]). Aromatase converts testosterone

![Biosynthetic pathways of sex-steroids based on [20, 21, 29–31]. Enzymes involved in catalyzed changes are highlighted in blue color. P450scc, cholesterol side-chain cleavage enzyme; HSD, hydroxysteroid dehydrogenase; P450c17, steroid 17alpha-hydroxylase; CYP19, cytochrome P450 family 19.](image-url)
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to E2, an active type of estrogen [20]. The above enzymes are expressed by the cells in the ovary [21]. Mindnich et al. [29] introduced two major roles of 12 17β-HSDs; one, for example, 17β-HSD type 1 (17β-HSD-1) is involved in conversion of an inactivated estrogen, estrone, into an activated estrogen, estradiol, and 17β-HSD type 2 (17β-HSD-2) is involved in its opposite reaction.

Among constituent cells of the OM, olfactory sustentacular cells (SCs) surrounding OSCs are rich in SER [1–3, 32, 33]. Also, Frisch [32] reported in mice as experimental models that acinar cells (ACs) of the Bowman’s glands (BGs), which are associated glands of the OM, contained large amount of SER. Before describing our findings relating to E2 in the OM, we will briefly introduce constituent cells of the rat OM, and ultrastructure of olfactory SCs and ACs.

2. Anatomy of olfactory SCs and ACs of BGs

A schematic drawing showing constituent cells of the rodent OM is shown in Figure 3A. The olfactory epithelium (OE) contains olfactory SCs mature and immature OSCs, globose and horizontal basal cells, and microvillar cells. Olfactory SCs and dendrites of mature OSCs are located side by side. Although duct cells of BGs penetrate into the OE and reach the epithelial surface, ACs are exclusively located in the lamina propria (LP) below the basement membrane. Using a field-emitted scanning microscope (FE-SEM, JSM6700F, JEOL), we were able to understand relationships between olfactory dendrites and SCs three-dimensionally. For example, a cylindrical dendrite between two SCs goes up to the epithelial surface to make a dendritic terminal (DT) can be seen (Figure 3B).

By transmission electron microscopy (TEM), cilia originating from dendritic terminals of OSCs are clearly seen (Figure 3C and D). Olfactory cilia are divided into a short proximal portion (about 400 nm at maximal width/diameter) and long distal one (100–50 nm at diameter). The proximal cilia tend to extend diagonally upward from the dendritic terminal and begin to narrow at around 1–1.5 μm and shift to the distal portion of cilia. By contrast, microvilli (about 100 nm at maximal width) extend substantially straight from the apical membranes of olfactory SCs (Figure 3C). At cross-sectional plate, proximal cilia extend radially from the dendritic terminals and distal cilia run approximately parallel to the epithelial surface (Figure 3D, [10]).

Using mice as experimental models, Makino et al. [33] classified the SER of olfactory SCs into two types, stacked lamellar and reticular-shaped SER. Using adult Sprague–Dawley rats (Rattus norvegicus) as experimental animals, we reported that the supranuclear region of olfactory SCs contained well-developed SER with a reticular form and myeloid bodies (MBs) [34]. The MBs are generally seen in retinal pigment epithelial cells of many species [35–39]. The MBs in olfactory SCs are composed of rows of narrow cisternae bounded by flat membranes, which were narrower than the cisternae of the SERs. They were stacked from 5 to 20 rows, ranging between 200 and 700 nm in width, and arranged mainly parallel to the lateral membrane of the olfactory SCs. The supranuclear region of the OE and SERs and MBs are shown in Figure 4A and B, respectively. Each cisterna of a MB, averaging 11 nm in width, frequently contains electron-dense material. We demonstrated that MBs and SERs are interchangeable (see Figure 5a of [34]). Vesicular mitochondria and associated cisternae, which are shown in Leydig cell (Figure 2C), are seen in the olfactory SCs as well (Figure 4C). In combination with the osmium-DMSO-osmium procedure [40]
and FE-SEM technique, we can see internal structures and relationships between MB, SER, and rough endoplasmic reticulum (RER) three-dimensionally (Figure 4D). Our ongoing study conducted in the OM of Suncus (Suncus murinus) has demonstrated the large amount of SER and characteristic mitochondria (Figure 4E).
Figure 4. TEM images (A, B, C, E) and FE-SEM image (D) of olfactory sustentacular cells (SCs). A. Low-magnified TEM image of the apical part of rat olfactory epithelium (OE), showing olfactory SCs, dendrites (De), cross sectioned olfactory cilia (OC), and microvilli of SC (SC-mv). Enlargement of a box is shown in B. B. Enlargement of the box in A, TEM image. Next to well-developed SER, myeloid bodies (MB) that characterize pigment epithelial cells in the retina are seen. C. TEM image of a rat OE showing mitochondria (Mi). Granular structures near the Mi are glycogen particles. D. FE-SEM image of partially digested cytoplasm of a rat SC. Adjacently located rough endoplasmic reticulum (RER), SER, and MB drawn by different colors are seen. E. TEM image of the Suncus OE showing two olfactory SCs and one dendrite (De) filled in-between them. Well-developed SER and swollen and vesicular Mi characterize the cytoplasm of SCs.
Figure 5.
Light microscopic (A) and TEM (B–F) images of Bowman’s glands of rats. A. Toluidine blue-stained semi-thin Epon-Araldite section showing olfactory epithelium (OE) and underlying lamina propria containing a Bowman’s gland (BG). Acinar cells (ac) of the BG are located below the basement membrane (arrowheads) of the OE. B. Acinar cells that contain nuclei (N) and secretory granules (sg). Note that some of secretory granules line adjacent to the lumen (L). Collagen fibers (cf) are seen near the basal membrane of the acinar cells. C. The cytoplasm of a acinar cell that is close to the cell nucleus (N). Close association between the nucleus and cisternae of rough endoplasmic reticulum (RER) are seen. The cisternae of the RER appear to be continued to those of smooth endoplasmic reticulum (SER). Some of cisternae of the SER are closely associated of the outer membrane of mitochondria (Mi) that contain internal microtubular structures (small arrows). D. Close association of cisternae of SER and mitochondria (Mi). Double or triple-layered cisternae of SER are attached to the outer membrane of Mi. E. Apical region of two acinar cells (AC) showing an aggregation of secretory granules (SG). The enlargement of the box area is shown in F. F. Close association of cisternae of SER, are even mitochondria (Mi), and secretory granules (SG). Note that cisternae of SER are even attached to the membrane of secretory granules.
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BGs (Figure 3A) are called attached glands of the OE and provide serous and mucous products. The secretions from these glands provide most of the mucus covering the olfactory epithelial surface, which protects the epithelial surface from drying and temperature extremes and helps to prevent damage caused by infectious agents and noninfectious particles [3]. Thus, it does make sense that BGs secrete secretory immunoglobulin A and J chain, lactoferrin, and lysozyme [41]. It has been demonstrated that secretory products of rodent BGs contain sulfated carbohydrate substances [42, 43]. By lectin histochemical technique, we demonstrated that secretory granules of rat BGs contain glycoconjugates with internal N-acetyl glucosamine (GlcNAc) residues [44].

Our ongoing study on rat BGs has indicated that ultrastructural characteristics of ACs of BGs are very similar to those in steroid-producing cells [23–26]. For example, light and transmission electron microscopic images of rat BGs are shown in Figure 5. ACs of a BG are located in the lamina propria below the basement membrane of OE (Figure 5A). Secretory granules (SGs) are seen in the supranuclear region of an AC (Figure 5B). The rough endoplasm reticulum (RER) that contains double- or triple-layered cisternae attached with ribosomes is easily seen in the perikaryal region and is connected with SER (Figure 5C). The presence of considerably well-developed SER may be a reflection of GlcNAc-containing glycoconjugates present in SGs of rat BG-ACs [44], because addition of GlcNAc residues to the backbone proteins of GlcNAc-containing glycoconjugates takes place in the RER [45, 46]. In contrast to SER in olfactory SCs, cisternae of SER of ACs appear to be filled with considerably high-dense materials (Figure 5C and D). Also, Mi containing internal microtubular structures are prominently seen (Figure 5C). Furthermore, inner cisterna of double- or triple-layered SER cisternae is attached to the outer membrane of Mi (Figure 5D). SGs are not homogenous in electron density (Figure 5E and F) and appear to be fused in the juxtaluminal region. In addition, cisternae of SER are even attached to the membrane of SGs (Figure 5F). Therefore, we would make a statement that we have extended Frisch’s observation [32] on mouse ACs of BGs in that they contain well-developed SER and provided new ultrastructural characteristics of rat ACs.

3. Expression of P450scc, 17β-HSD-1, 17β-HSD-2, and aromatase in the rat OM

Using RT-PCR analyses, we demonstrated that P450scc, 17β-HSD-1, 17β-HSD-2, and aromatase are expressed in the nasal mucosae of both sexes of adult rats that contain the OM and respiratory mucosa [34]. These results strongly suggest that OM cells express mRNA for P450scc, 17β-HSD-1, 17β-HSD-2, and aromatase. Specific antisera against P450scc, 17β-HSD-1, and 17β-HSD-2 were used for Western blot (WB), a combination of multi-labeling immunofluorescence (IF) and confocal laser-scanning microscopic (CLSM) techniques, and immunoelectron microscopic (IEM) analyses. By WB analyses on nasal tissue specimens of both male and female adult rats, we detected single band at 49 kDa for anti-P450scc antiserum, that at 34.5 kDa for anti-17β-HSD-1, and that at ~43 kDa for anti-17β-HSD-2 antiserum 17β-HSD-2 [34]. These results strongly suggest that the OM cells produce proteins for P450scc, 17β-HSD-1, and 17β-HSD-2.

Antiserum to olfactory marker protein (OMP) equivocally immunostains mature OSCs [47, 48]. Using antiserum to OMP and one of the antisera of the above three enzymes, we determined that both olfactory SCs and cells in BGs contain IF for
P450scc, 17β-HSD-1, and 17β-HSD-2. However, no IF was detected in mature OSCs, immature OSCs, basal cells, and olfactory nerve bundles (Figure 6) [34]. By IEM analyses conducted on OE cells, we demonstrated that immunoreactivity (IR) for P450scc is mainly localized in mitochondria of olfactory SCs, whereas SERs and MBs of these cells contain IR for 17β-HSD-1 and 17β-HSD-2 [34]. Similar subcellular...
immunolocalization of P450scc and HSDs were reported in the adrenal gland and gonads [49–51].

4. Expression of estrogen receptor β (ERβ) in the OM

The above findings strongly suggest that olfactory SCs and ACs of BGs produce E2. It is known that E2 utilizes three types of receptors, such as estrogen receptor alpha (ERα), estrogen receptor beta (ERβ), and G-protein coupled estrogen receptor 1(GPER1). Both ERα and ERβ exist as cell membrane associated form and intracellular form, whereas GPER1 exists as a transmembrane receptor [52]. Based on the results of pilot experiments, we first examined expression and cellular localization of ERβ in adult rats of both sexes. The outline of gene structure of ERβ, which is composed of eight exons, is shown in Figure 7A. Forward and reverse primers of 35 bases for reverse transcription-polymerase chain reaction (RT-PCR) were chosen from cDNA sequences between exon 7and 8. By RT-PCR, ERβ was expressed in nasal mucosae of both sexes as well as ovaries as a positive control (Figure 7B). By WB analysis using a commercially available antiserum against rabbit ERβ, ERβ protein was detected from nasal mucosae of both sexes as well as ovaries (Figure 7C). These results strongly suggest that OM cells in both sexes express and produce ERβ. Using antisera against OMP and ERβ, and 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) that binds to cell nucleus, triple-labeling IF technique was applied to cryostat-cut sections of OMs. Observation by a confocal laser-scanning fluorescence microscope (CLSM, FLUOVIEW FV500-IX-UV, Olympus, Shinjuku-ku, Tokyo, Japan) indicated that mature OSCs contained very intense IF for ERβ in DTs and moderately intense IF in their supranuclear cytoplasm. Furthermore, dendrites of mature OSCs were ERβ-immunoreactive (Figure 8A–C). However, olfactory SCs were immune-negative for ERβ. At subcellular level, post-embedding immunogold TEM technique demonstrated that gold particles that are reflection of ERβ immunoreactivity were localized on cell membranes of both proximal and distal portions of olfactory cilia (Figure 8D–F). The above results [53] strongly suggest that mature OSCs, at least, contain both membranous and cytosolic ERβ.

5. E2 actions in the OM

Based on the above results, we present our working hypothesis in Figure 9. Since our unpublished study have demonstrated that olfactory SCs are immunoreactive for cholesterol receptor (low-density lipoprotein receptor), it is likely that the SCs take up cholesterol via capillary just beneath the basement membrane of the OE. They convert it to pregnenolone using P450scc. Since the SCs produce 17β-HSD-1, and probably aromatase as well, SCs finally produce E2 that makes biological effects on OSCs via the ERs, although the presence and cellular localization of at least two enzymes used from pregnenolone to androstenedione need to be determined (see Figure 2).

6. Morphological features of SER in VSCs

Presence of large amount of SER was described for the first time by Altner et al. [54]. Since then, this ultrastructural feature has been confirmed in many
VNO-possessing animals such as amphibians [55, 56], reptiles [55, 57–60], rodents [61–64], and prosimian primates [65]. However, bioactive molecules produced by SER in VSCs had not been determined.

In each VSC soma located in the basal part of the vomeronasal sensory epithelium (VNSE) of adult rats, the area occupied by SER is very large (Figure 10A). Clusters of SER appear to be present in both supra- and infra-nuclear cytoplasm. An example of
Figure 8. A–C. CLSMF images of a rat OM section triple-labeled with antisera to OMP and ERβ, and DAPI. Somata and dendritic terminals of OSCs that exhibit intense IF for OMP (A) contain intense IF for ERβ (B, C). D–F. TEM images of the apical part of the rat OE. D. Gold particles surrounded in orange, which are reflection of immunoreactivity for ERβ, are localized mainly in membranes of the proximal portion of olfactory cilia (oc-p) that are projected from a dendritic terminal (DT), by a post-embedding immunogold procedure. Gold particles surrounded in orange. E. A result of a negative control experiment in which the primary antiserum was omitted from staining protocol. No gold particles are found in the entire part of this image. F. In the distal portion of olfactory cilia (oc-d), gold particles are present in ciliary membranes and their vicinity.
supranuclear region of a VSC soma is shown in Figure 10B. By TEM tomography (for details see [64]) using 200–300-thick sections, a unit volume of 700 x 700 x 150 nm of each SER area was analyzed to demonstrate microstructures of SER. Some parts of SER cisternae contained arc- and circle-like units that are complicated each other. Furthermore, some of them appeared to form tiny loops (Figure 10B–E).

7. Localization of 17β-HSD-1 in VSCs

By immunohistochemical techniques utilizing three antisera against 17β-HSD-1, specific staining was detected in the VNSE of adult rats (Figure 11A and B). In particular, intense immunoreactivity was seen in the perikaryal region of VSCs and in the apical region of the VNSE (Figure 11C). However, no specific staining was seen when the primary antiserum was omitted from the immunostaining protocol (Figure 11D). Using digoxigenin (Dig)-labeled cDNA antisense and sense probes for 17β-HSD-1, in
Figure 10. TEM images (A–C) of VSCs and results by TEM tomography (D, E). A. Low-magnified TEM image showing longitudinally-cut somata of VSCs. SERs are colored in green, nuclei in blue, and cell boundaries in magenta. Note that the cytoplasm of VSCs is largely occupied by SER. B. Conventional TEM image of a supranuclear region in a VSC. Enlargement of box C within the SER area is shown in C. C. Higher magnification of box C in panel B. Arc-and circle-like units that are numbered 1–6 in box D are clearly seen. Reconstructions of the image in box D by TEM tomography are shown in D and E. D. Reconstruction of the image in box D in panel C by TEM tomography showing units 1–6. E. Reconstruction of image in box D in panel C in partially transparent mode. Complex 3D structures of units 1–6 and their looped characteristics are visible. Adapted from [64] with permission.
situ hybridization (ISH) technique demonstrated that some of VSCs express 17β-HSD-1 gene (Figure 11E and F; [66]).

After confirming the presence of 17β-HSD-1 protein in the VNO by WB analyses (Figure 11G, insert), subcellular localization of its immunoreactivity was examined by a post-embedding immunogold TEM technique [64]. We demonstrated that gold particles, which are reflection of 17β-HSD-1 immunoreactivity, were localized in narrow cisternae of SER. Two representative images are shown in Figure 11G and H. The region shown by these images is equivalent to that analyzed by TEM tomography (Figure 10B–E).
In summary, it is most likely that VSCs of adult rats express 17β-HSD-1 that catalyzes conversion from androstenedione to testosterone, and that from estrone to E2. Since 17β-HSD-1 immunoreactivity seems to be more intense in basally located somata of VSCs, it would be important to assess if regional difference for expression of 17β-HSD-1 is present in the VNSE.

8. VSCs express estrogen receptor α (ERα)

The VNO is known to be a pheromone-detecting sensory apparatus in rodents [5–8, 10, 67]. Hatanaka and Kiura [68] demonstrated that sensitivity of VSCs to pheromones was much more decreased in castrated mice, when compared to control mice. This data indicate that gonadal sex steroids modify physiological activities of VSCs. Setting a hypothesis that modification of sex steroids is mediated by steroid receptor, we first examined the presence of ERα in the VNO of adult rats.

Representative images from an immunohistochemical study are shown in Figure 12. Intense immunoreactivity for ERα is seen in the surface of the VNSE, whereas moderately intense immunoreactivity is seen in the inside of the epithelium (Figure 12A and B). Uterine glands, which are positive control tissues, contain intense immunoreactivity in cell nucleus (Figure 12C). It is interesting to point out...
that immunoreactivity is also seen in the apical part of vomeronasal non-sensory epithelium (VNNE, Figure 12B). No specific staining is seen both in the VNSE and VNNE, when the primary antiserum was omitted from the immunostaining protocol (Figure 12D).

Representative CLSFM images from an IF study in which antisera against OMP and ERα, and DAPI were used are shown in Figure 13. Since dendrites and dendritic terminals (DTs) of VSCs exert intense IF for OMP (Figure 13A), it is apparent that DTs contain IF for ERα (Figure 13B). Optical sections containing apical dendrites show that intense IF for ERα is seen in DTs and apical dendrites just below the DTs (Figure 13C–H).

Representative data from a WB analysis is shown in Figure 14. Bands at 66 kDa that is equivalent to the size of ERα protein are detected both pellet and supernatant (sup.) specimens of VNOs and uteri that contain uterine glands.

9. Actions of sex steroids in the VNO

TEM and FE-SEM images of VSCs are shown in Figure 15. In contrast to OSC, many microvilli extend from the DT (Figure 15A and B). Microvilli extending from the apical membrane of vomeronasal sustentacular cells (SCs) are thicker than those of VSC (Figure 15B). Since intense IF for ERα was detected the apical surface of VNSE (Figure 13), it is most likely the both membranes and cytoplasm of microvilli.
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Using digoxigenin Dig-labeled cDNA antisense and sense probes for 17β-HSD-2, ISH technique demonstrated that some of VSCs express 17β-HSD-2 gene (Figure 16 [66]). Thus, it is most likely that VSCs of adult rats produce 17β-HSD-2.

Also, we have examined immunolocalization of E2 in the rat VNSE. Antiserum to protein gene product 9.5 (PGP) is an excellent marker for both mature and immature VSCs (iVSC) [5, 69]. Using antiserum to E2, PGP, and DAPI, triple-labeling IF technique was applied to cryostat-cut sections of VNOs, and then, these sections were observed by the CLSFM. As demonstrated in Figure 17, intense IF for E2 is present in somata and dendrites of VSCs in a granular fashion. By contrast, moderately intense IF was diffusely seen in the supranuclear region of vomeronasal SCs. These findings would provide supporting evidence that E2 per se is present in both VSCs and SCs.

A hypothetic scheme showing origins and actions of E2 in the rat VNSE is presented in Figure 18. Since rodent VNSE contains intraepithelial capillaries [5, 62], a mature VSC (mVSC) can uptake E2 or estrone from intraepithelial capillary and give E2 to immature VSC (iVSC) via ERα. Conversion between E2 and estrone takes place in

Figure 14.
Representative data by WB analysis using antiserum to ERα. A band at 66kDa, which is corresponding to molecular weight of ER protein, is detected from VNO and uterus pellet specimens that contain cell nuclei, and from their supernatants (sup.) that contain cell membranes and internal unit membrane structures, as well.

Figure 15.
A. TEM image showing the apical part of the VNSE. A dendrite (De) of a VSC reaches a lumen (L) to form a dendritic terminal (DT) from which microvilli (vmc) are extended. Adjacently located sustentacular cells (SC) have microvilli (SC-mv), as well. B. FE-SEM image of the surface of the VNSE showing DT, vm, and SC-mv. Note that short microvilli are extending radially from the DT. Adapted from [10] permission (slightly changed).

A. TEM image showing the apical part of the VNSE. A dendrite (De) of a VSC reaches a lumen (L) to form a dendritic terminal (DT) from which microvilli (vmc) are extended. Adjacently located sustentacular cells (SC) have microvilli (SC-mv), as well. B. FE-SEM image of the surface of the VNSE showing DT, vm, and SC-mv. Note that short microvilli are extending radially from the DT. Adapted from [10] permission (slightly changed).
SER where both 17β-HSD-1 and 17β-HSD-2 work as catalytic enzymes. It would happen that E2 from mVSC bind to growing dendritic terminal. Since brain centers of the vomeronasal system express aromatase and other steroidogenic enzymes [70], E2 may be retrogradely transported to the mVSC via neural connections.

Figure 16. Expression of 17β-HSD-2 in the VNSE of an adult rat by ISH histochemistry. VSC perikarya containing intense staining are seen in the peripheral region of the VNSE.

Figure 17. CLSMF images of a section triple labeled with antisera to protein gene product 9.5 (PGP) and E2, and DAPI. A. Optical section by opening the channels for both PGP and DAPI. Intense IF for PGP are seen in the perikaryal regions of VSCs (VSC-P), dendrites (De) above the layer of sustentacular cell nuclei (N-SC), and dendritic terminals (arrows). B. Optical section by opening the channels for both E2 and DAPI. Both SCs and VSCs contain E.
Although our studies suggest that both OM and VNO are greatly influenced by E2, relationships between cell types and enzymes/estrogen receptors are different from each other (Table 1) and further experiments remain to be carried out to identify cells expressing other E2-related molecules. However, at least two conclusions could be proposed here; (1) both OM and VNO are capable for producing E2, and (2) it is most likely that OSCs and VSCs contain both membrane-bound and cytosolic E2 receptors. Thus, it is most likely that cross-talk between membrane- and nuclear-initiated estrogen signaling takes place in the OM and VNO, as demonstrated in hypothalamus [71].

OSC and VSC are very special sensory cells, because they are bipolar neurons, but replaced by newly developed cells throughout life [3–5, 72, 73]. It is known that E2 promotes neurogenesis [74, 75], axonal [74–76], and dendritic growth [77, 78], rescues neurons [79], and promotes neuroregeneration [74, 75]. Therefore, rodent OM and VNSE are excellent experimental models for studying actions of E2, because differentiation from neuroblasts, dendritic and axonal growth, and neuronal death continuously occur in these neuroepithelia even at adult years.

Rodent OSC share many cytochemical properties with those of human OSCs [80–84]. Thus, it is likely that E2-related enzymes and E2 receptors are expressed...
by human OM cells, although new studies need to be initiated to examine if these molecules are present in human OM tissues. A common but striking feature in adult human OE is that patches of respiratory epithelium often appear in what was thought to be a purely olfactory region [85, 86]. Nakashima et al. [85] reported that the size of these patches varied case to case. Differential sizes of these respiratory patches may depend on differential supply of E2 on human OSCs. Parallel studies utilizing both rodent and human OMs would give us new knowledge about E2 functions, as well as new questions to be solved.

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