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THE VOMERONASAL (JACOBSON'S) ORGAN IN MAN: ULTRASTRUCTURE AND FREQUENCY OF OCCURRENCE*

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Summary—These investigations address three major questions: (1) What is the frequency of occurrence of the vomeronasal (Jacobson's) organ (VNO) in man? (2) what is the ultrastructure of the human VNO? and (3) does the VNO contain sensory receptor cells? Macroscopic and microscopic intranasal clinical examinations of over 200 persons revealed paired bilateral vomeronasal pits on the anterior 1/3 of the nasal septum in all cases. Biopsies of the vomeronasal pits and surrounding tissues were examined by light and electron microscopy. These studies showed that the vomeronasal pit leads to a closed tube, 2–8 mm long, lined by a unique pseudostratified columnar epithelium unlike any other in the human body. The anterior end of the tube is lined by tall, columnar cells with a sparse population of short microvilli. The posterior end of the VNO is lined by an epithelium that contains three morphologically distinct cell types: (1) basal cells; (2) "dark cells—tall, slender cells with heterochromatic nuclei and electron-dense cytoplasm that often contain mucigen-like granules; and (3) "light" cells—large, clear cells, extending from the basement membrane to the organ's lumen. Each "light" cell has a round, euchromatic nucleus and a clear cytoplasm that often contains many Golgi stacks and membrane-limited vesicles filled with material of modest electron density. The cell apex is tipped by a few short microvilli. Whether these cells subserve any sensory function awaits further investigation.

INTRODUCTION

The vomeronasal system, also called the accessory olfactory system, is an important component of the chemosensory apparatus of many vertebrate animals. In a variety of mammals, for example, the vomeronasal organ (VNO)—also called Jacobson's organ—responds to odorants [1, 2] and plays several important roles in reproductive behavior [3–6]. As Wysocki [7] has put it:

"As a periscope from the diencephalon, the vomeronasal system may monitor exogenous hormones, 'pheromones' ".

Like the main olfactory system, the vomeronasal system centers its function around chemosensitive bipolar neurons—primary sense cells, located in a neuroepithelium, that send a dendrite to the site of stimulus reception and an axon to the brain. Like the olfactory receptor neurons, the vomeronasal receptor neurons turn over during the life of the animal, and—following injury or cell death—can be replaced by new neurons derived from mitotically-active stem cells called basal cells [8].

Where studied, the olfactory and vomeronasal systems are separate and parallel [9]. The olfactory receptors, located in the olfactory neuroepithelium of the nasal cavity, send axons to the olfactory bulb of the brain via the olfactory nerve. The vomeronasal receptors, located within the VNO associated with the nasal cavity, send axons to the accessory olfactory bulb of the brain via the accessory olfactory nerve.

For years, the vomeronasal system in humans has been neglected, and has commonly been regarded as absent or vestigial in adults [10]. Recently, however, the occurrence of a VNO in adult humans has been shown to be far more common than previously believed [11–13]. Furthermore, the vomeronasal pits visible in the anterior 1/3 of the septum of the human nose have been shown by light microscopy [11] and electron microscopy [12, 13] to lead to a blind-ended tube lined by a unique pseudostratified columnar epithelium.

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Given the facts outlined above, three major questions arise:

(1) What is the frequency of occurrence of vomeronasal pits in adult humans?
(2) What is the fine structure of the human VNO?
(3) Are sensory receptors present in the human VNO?

It is to these three questions the present investigations are addressed.

METHODOLOGY

Inspection of the nasal cavity for vomeronasal pits

The incidence of occurrence of the VNO of Jacobson has been investigated grossly, without surgical intervention, in 200 persons in the Otolaryngology Clinic of the Rocky Mountain Taste and Smell Center at the University of Colorado Health Sciences Center (UCHSC). Macroscopic and microscopic intranasal examinations were performed upon consenting patients and volunteers from the student body, faculty and staff of UCHSC. Macroscopic investigations were done with the naked eye by a trained surgeon (Dr Jafek); microscopic examinations were performed with a Zeiss binocular stereo operating microscope with a long working distance. In both macroscopic and microscopic examinations, no local anesthesia was required, as inspection involved no patient discomfort and took <30 s. The inner surface of the nostril was wiped clean, the outer rim of the nostril was expanded slightly with a nasal speculum, and the vomeronasal organ was visible as a small pit on the anterior 1/3 of the nasal septum, usually located about 1 cm dorsal to the columella and 2 mm above the floor of the nose.

Tissue preparation for electron microscopy

In most cases, the human VNO is bilateral; one pit is visible on either side of the nasal septum. After receiving informed, written consent, one biopsy of tissue containing the vomeronasal pit and the presumptive VNO was removed from each of 2 patients under general anesthesia during the course of surgical septohinoplasty. After surgical removal from the nasal cavity, tissues were fixed in a paraformaldehyde–glutaraldehyde mixture [14], rinsed in buffer and photographed *in toto* at low magnification to reveal the orientation of the vomeronasal pit in the surrounding tissue. Next, the tissue was dissected, and pieces of the VNO were prepared for scanning electron microscopy (SEM) and conventional transmission electron microscopy (TEM). For SEM, tissues were critical-point dried in a Tousimis Samdri device, coated with gold, and photographed with a Cambridge Stereoscan SEM. For TEM, tissues were post-fixed in buffered 2% osmium tetroxide, dehydrated in a graded acetone series, embedded in Spurr's [15] low-viscosity epoxy resin and sectioned with a Porter-Blum MT-2B ultramicrotome. For light microscopy, thick (1 μm) sections were placed on glass slides, stained with toluidine blue and photographed with a Zeiss universal microscope using planapochromatic optics. For electron microscopy, thin sections were collected on Formvar-coated slot grids using the Domino Rack technique of Moran and Rowley [16], doubly contrasted with uranyl acetate and lead citrate, and photographed on a Philips CM-10 electron microscope at 80 kV.

RESULTS

Frequency of occurrence of vomeronasal pits

During the initial phases of these investigations, subjects' nasal cavities were examined with the naked eye. In these cases, vomeronasal pits were observed in 1/10 of the subjects studied. Recently, however, small VNO pits not apparent to the unaided eye have been detected with the aid of a 40 × binocular stereo operating microscope. Since we have been examining subjects both macroscopically and microscopically, the frequency of observation of paired bilateral pits signifying the presence of the VNO has risen to 100/100; i.e. the VNO seems to be present in all persons examined. The present study, based on over 200 persons, included adult men and women of all ages. In addition, paired bilateral VNO pits have been observed in persons of Oriental, Negro, Caucasian and mixed racial backgrounds.

Location and size of vomeronasal pits

Inspection of the interior of the nasal cavity shows the VNO presents itself as a pair of bilateral pits—one on either side of the anterior 1/3 of the nasal septum. Although the location varies somewhat between individuals, each vomeronasal pit is usually observed about 1 cm dorsal to the columella and 1 mm above the

floor of the nose. The larger pits, readily visible to the naked eye, can measure up to 2 mm dia (Fig. 1). Smaller pits, which require magnification to see, can measure as little as 0.2 mm dia. A small piece of the nasal septum containing a vomeronasal pit and its surrounding tissues has been dissected free and photographed whole in Fig. 1. Here, the pit appears as a round hole in a field of nasal mucosa. The same piece of tissue was cut in half through the center of the pit, laid on its side and photographed at low magnification by SEM in Fig. 2. In this image, the wall of the vomeronasal pit (P) extends upward from the nasal mucosa (M) lining the interior of the septum, which faces downward in this illustration. The duct of the VNO (D), from which one wall was removed during dissection, courses up and to the right in this specimen. The fine structure of the epithelium lining the duct is discussed below.

The fine structure of the human VNO

The surface of the cells lining the same duct shown in Fig. 2 are shown at higher magnification by SEM in Fig. 3. Here, the apical poles of the cells are rounded, of uniform size and decorated by a sparse population of short microvilli. When the same epithelium (from the other half of the same VNO) is photographed by TEM (Fig. 4), most of the cells are seen to be tall, thin, columnar cells. In this specimen, a single ciliated cell, thinner than those seen in the

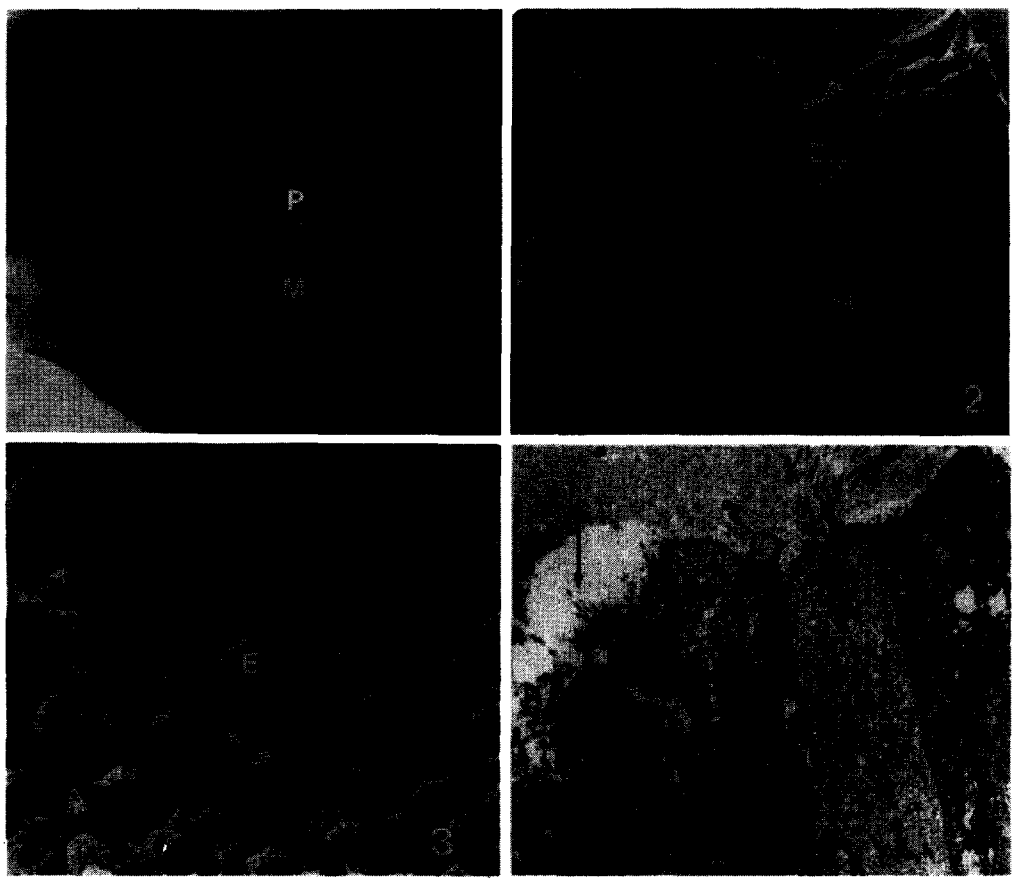

Fig. 1. "Macro" photograph of a biopsy specimen from a human nasal septum containing a vomeronasal pit (P) and surrounding nasal mucosa (M). 25 × .

Fig. 2. Low-magnification SEM of half of the specimen shown in Fig. 1 after being cut vertically through the center of the pit and nearly parallel to the long axis of the vomeronasal duct. The opening of the vomeronasal pit (P) is at the bottom of the specimen, as is the nasal mucosa (M) lining the septum. The vomeronasal duct (D) extends upwards and to the right, where it exits the plane of section. 25 × .

Fig. 3. Higher-magnification SEM of the surface of the epithelium lining the vomeronasal duct shown in Fig. 2. Note the sparse population of short microvilli projecting from epithelial cells (E). 2000 × .

Fig. 4. TEM of the pseudostratified columnar epithelium lining the VNO of Figs 1–3. Tall columnar cells (E) with rounded cell apices tipped by short microvilli comprise most of the epithelium. A single ciliated epithelial cell (C) is present. 3000 × .

typical respiratory epithelium that lines the non-olfactory part of the nasal cavity, is present.

Figures 5–7 illustrate light and electron images of a VNO from a different individual than that shown in Figs 1–4. This specimen was cut in cross section at various levels from its anterior end at the vomeronasal pit to its posterior limit at the closed end of the duct. Figure 5 is a light micrograph of a cross section through the VNO taken 2/3 of the distance along the length of the vomeronasal duct (1/3 from its blind-end). Here, the VNO apears as a flattened tube whose lumen is lined by a pseudostratified columnar epithelium consisting of three morphologically distinct kinds of cells. Tall, columnar cells with densely-stained cytoplasm are evident; these resemble the cells shown in Figs 3 and 4. Interspersed amongst these "dark" cells are lightly-stained cells that extend from the basement membrane to the free surface of the epithelium. These "light" cells contain prominent, euchromatic nuclei, many mitochondria and have rounded cell apices. Below both of these light and dark cells are basal cells—small cells that sit atop the basement membrane. The basement membrane, in turn, rests upon a highly vascular lamina propria of connective tissue.

When viewed by TEM, the light and dark cells of the VNO epithelium are seen to contain significant differences in ultrastructure. The dark cells are long, thin and span the distance between the basement membrane and the epithelial surface. The nuclei, which are heterochromatic, dark-staining and elliptical in shape, vary in location, and may be found in the lower and upper 1/3 of the epithelium. The cytoplasm of the dark cells is often filled with electron-lucent inclusions reminiscent of the mucus droplets of goblet cells seen in normal respiratory epithelium. The light cells, which also extend from the basement membrane to the epithelial surface, are quite different. Their nuclei are almost perfectly round, euchromatic and have a prominent nucleolus located near the nuclear envelope. The light cells' nuclei are arranged in a uniform band in the lower 1/3 of the epithelium. Beneath the nucleus, the basal pole of the cell thins out and sends a narrow process to the basement membrane. The apical

Fig. 5. Light micrograph of a cross section through the posterior 1/3 of a different VNO from that of Figs 1–4. The lumen (LU) is lined by a pseudostratified columnar epithelium with three distinct cell types: basal cells (B); "dark" cells (D); and "light" cells (L). LP, lamina propria. 600 ×.

pole of the cell is rounded, and has short microvillar projections. The apical cytoplasm has many mitochondria, and contains a rich population of membrane-limited inclusions of moderate electron density. These inclusions, shown at higher magnification in Fig. 6, are often seen in close association with stacks of the Golgi apparatus. The presence of many well-developed Golgi stacks, taken together with the euchromatic nature of the nucleus, suggest that light cells are transcriptionally active and engaged in biosynthesis and packaging of materials for external and/or internal use.

DISCUSSION

These investigations were designed to answer several questions:

(1) What is the frequency of occurrence of the VNO (Jacobson's organ) in man?
(2) What is the fine structure of the human VNO?

Fig. 6. TEM of the VNO epithelium shown in Fig. 5. The "dark" cells (D) have elliptical, heterochromatic nuclei. The cytoplasm in the apical domain of the cells contains mucigen-like granules (M). The "light" cells have round, euchromatic nuclei (N). Note the membrane-limited vesicles (V) with contents of moderate electron density in the supranuclear cytoplasm. Golgi stacks (G) are abundant. Several slender microvilli (arrow) extend from the cell surface into the lumen (LU) of the VNO. Small basal cells (B) sit atop the basement membrane (BM). 2100 × .

(3) Does the ultrastructure of the human VNO reveal the presence of cells that appear to be sensory receptors?

These questions will be discussed in order below.

Frequency of occurrence of the VNO in man

The present studies clearly show that paired, bilateral vomeronasal pits are consistently present in all 200 subjects studied. Furthermore, the presence of bilateral vomeronasal pits is independent of age, gender and race. Although further studies should be done to confirm these observations, the present data—combined with those of Johnson *et al.* [11] and Stensaas *et al.* [13]—suggest most, if not all, humans contain paired, bilateral vomeronasal pits in the anterior 1/3 of the nasal septum. Many of the pits are small; the lower limit of their size (0.2 mm) coincides with the limit of resolution of the human eye. Since most studies to date have been done without magnification, it is possible that the subjects in whom vomeronasal pits were not seen actually possessed pits that could have been detected with a binocular operating microscope, such as the one employed in the present study.

The fine structure of the VNO in man

The present study shows the anterior end of the human VNO is lined by a pseudostratified columnar epithelium that contains mostly tall, columnar cells with rounded cell apices decorated by a sparse population of short microvilli. These cells resemble those observed by Stensaas *et al.* [13] in VNOs taken from human cadavers. In addition, they resemble cells from the non-sensory epithelium of the VNO of mammals such as the rat [17]. The VNO of the rat, a chemoreceptive organ that modulates reproductive behavior (see Refs [6, 7] for reviews), has a crescent-shaped lumen. The lateral (concave) side contains a non-sensory epithelium containing tall, thin, densely-staining cells similar to those described above. The sensory epithelium, which lines the medial, convex, side of the

Fig. 7. Higher-magnification TEM of "light" cells in the human VNO epithelium. Note the vesicles (V) near the Golgi stacks (G) above the round, euchromatic nuclei (N). 6500 ×.

organ, contains both supporting cells and sensory cells. The sensory cells are bipolar chemoreceptor neurons with their cell bodies are located in the vomeronasal neuroepithelium. Each bipolar neuron sends a dendrite to the epithelial surface and an axon to the accessory olfactory bulb of the brain via the accessory olfactory nerve. At the site of stimulus reception on the epithelial surface, the cell surface of the dendrite tip contains many long, slender microvilli that greatly amplify the area of membrane surface available for contact with chemostimulatory molecules.

Does the human VNO contain sensory receptors?

It is interesting to note that observations on the fine structure of the human VNO both in this study and that of Stensas *et al.* [13] have not revealed any cells that are ultrastructurally identical to the chemoreceptive bipolar neurons described in other mammals [1]. The present study, however, shows the presence of two morphologically distinct cell types in the human vomeronasal epithelium. One cell type, called the "light" cell, is unlike any other cell in the human body, and is of unknown function. Therefore, whether or not it functions as a sensory receptor is unknown. It has some of the features associated with nerve cells in that it has a large, round, euchromatic nucleus, a generally electron-lucent cytoplasm and large numbers of Golgi stacks in the parykaryon. Consequently, it is interesting to speculate the "light" cells might represent some sort of neuronal element in the vomeronasal epithelium. This is, however, simply speculation; the real answer to the question "Does the human VNO contain sensory receptors?" remains unknown, and should be vigorously pursued in future research programs.

In conclusion, then, the present study has shown that: (1) most, if not all, humans contain bilateral vomeronasal pits on the anterior 1/3 of the septum; and (2) behind this pit lies a tube, some 2–8 mm long, lined by an interesting epithelium, quite unlike any epithelial lining described to date in the human body. These investigations, then, have answered several questions, and focus attention on a very important question that needs to be answered: "Does the human VNO, once dismissed as absent or vestigial in adults, contain functional neuronal chemoreceptive units? If so, what are they, and where are they located?"

If this paper has stimulated interest in pursuing the quest for the structure and function of the human VNO, it has served its purpose. We encourage the scientific community to vigorously pursue investigations of the human VNO—an organ about which precious little is known, and one which may be of functional significance.

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