Chem. Senses 25: 369–380, 2000

The Vomeronasal Cavity in Adult Humans

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Abstract

We observed the surface of the anterior part of the nasal septum of living subjects using an endoscope. In ~13% of 1842 patients without pathology of the septum, the vomeronasal pit was clearly observed on each side of the septum, and in 26% it was observed only on one side. The remaining observations indicated either the presence of putative pits or no visible evidence of a pit. However, repetitive observations on 764 subjects depicted changes over time, from nothing visible to well-defined pits and vice versa. Based on 130 subjects observed at least four times, we estimate that ~73% of the population exhibits at least one clearly defined pit on some days. By computer tomography, the vomeronasal cavities were located at the base of the most anterior part of the nasal septum. Histological studies indicated that the vomeronasal cavities consisted of a pit generally connected to a duct extending in a posterior direction under the nasal mucosa. Many glands were present around the duct, which contained mucus. There was no sign of the pumping elements found in other mammalian species. Most cells in the vomeronasal epithelium expressed keratin, a protein not expressed by olfactory neurons. Vomeronasal epithelial cells were not stained by an antibody against the olfactory marker protein, a protein expressed in vomeronasal receptor neurons of other mammals. Moreover, an antibody against protein S100, expressed in Schwann cells, failed to reveal the existence of vomeronasal nerve bundles that would indicate a neural connection with the brain. Positive staining was obtained with the same antibodies on specimens of human olfactory epithelium. The lack of neurons and vomeronasal nerve bundles, together with the results of other studies, suggests that the vomeronasal epithelium, unlike in other mammals, is not a sensory organ in adult humans.

Introduction

Frederic Ruysch discovered the vomeronasal cavities in humans in 1703 (Figure 1A). He described a 'canalibus nasalibus' on each side of the anterior part of the nasal septum of a young cadaver (Figure 1A) (Ruysch, 1703; Hollnagel-Jensen and Andreasen, 1948)]. Von Sömmering (Von Sömmering, 1809) confirmed these observations on adult cadavers and Kölliker (Kölliker, 1877) (Figure 1B) made a detailed study of the position of the vomeronasal cavities in the nasal septum of dead foetuses, children and adults. In 18 adults he found these cavities 8.5 mm (range 6–13 mm) above the floor of the nasal cavity and 24 mm (range 21–29 mm) from the nostril. The opening of the cavity, visible as a pit at the surface of the septum, had a diameter of 1.1 mm (range 1–1.6 mm) and the length of the internal duct was 3.6 mm (range 2–7 mm). Potiquet (Potiquet, 1891) extended these observations to living adults. By inserting a fine stylet into visible pits (Figure 1C), he estimated the length of the cavity to be 3–4 mm. He observed both sides of the nasal septum of 200 living subjects and found only 100 pits, i.e. they were present in only 25% of the observed nasal cavities. Ludvig Jacobson described in great detail the vomeronasal organ in a number of mammalian species [Jacobson, 1813 in (Trotier and Døving, 1998)]. He also noted the lack of development of the vomeronasal structure in humans and said that the vomeronasal organ could be 'a sensory organ which is a sense about which human beings have no conception'. More recently, Johnson *et al.* observed 100 living adults and found a vomeronasal pit either on both sides (nine subjects) of the nasal septum or only one side (30 subjects) (Johnson *et al.*, 1985). In histological sections from cadavers they identified at least one vomeronasal cavity in ~70% of nasal septa. Gaafar *et al.* observed vomeronasal pits in ~76% of subjects (Gaafar *et al.*, 1998). Other studies (Garcia-Velasco

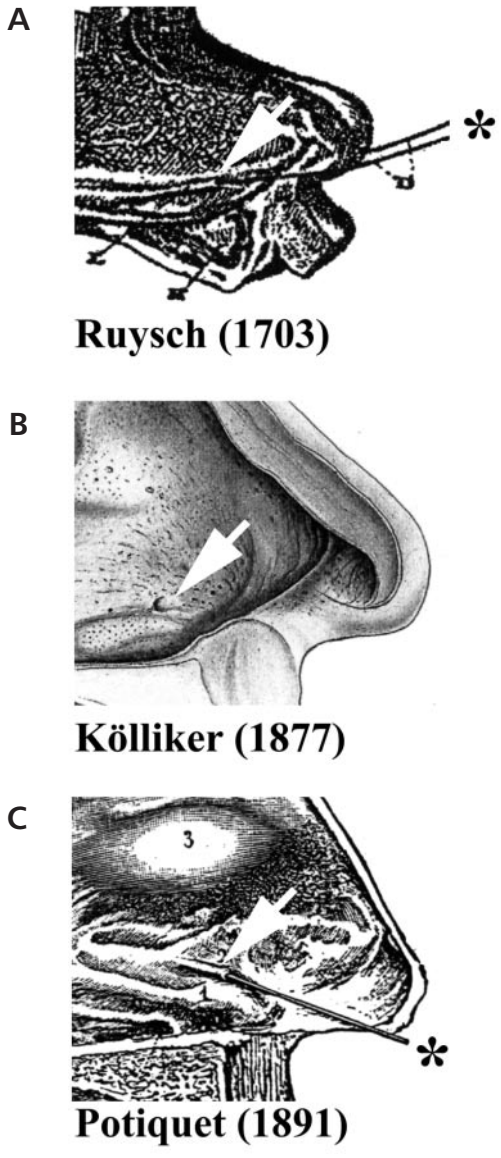

Figure 1 Localization of the vomeronasal cavities (indicated by arrows) in humans, as they were illustrated in the last centuries. **(A)** Frederic Ruysch, in 1703 (Ruysch, 1703; Hollnagel-Jensen and Andreasen, 1948), was the first to indicate, with two bristles (*), the existence of these 'canalibus nasalibus'. He wrote, in Latin: 'On both sides of the anterior and inferior part of the nasal septum appears the opening of a duct. I have not read about the existence and utility of that in authors: I consider it serves for the secretion of mucus' (translation by courtesy of Annick Le Guerer). **(B)** Anton Kölliker, in 1877, gave the exact position of these cavities in the nasal septum of adult cadavers. **(C)** Potiquet, in 1891, was the first to study the position and length of these cavities in living adult subjects. He inserted a stylet (*) to estimate the length of the vomeronasal cavity that extended towards the back.

and Mondragon, 1991; Moran *et al.*, 1991; Stensaas *et al.*, 1991) claim that most adults have visible bilateral vomeronasal pits, but do not give many details about the criteria used for identification. In a previous study (Trotier *et al.*, 1996), we observed the vomeronasal pits in a few subjects.

Table 1 Age structure of the population of 2031 subjects observed by endoscopy

Age (years)	Women	Men
15–24	79	103
25–34	182	154
35–44	186	151
45–54	268	170
55–64	238	164
64–74	156	99
>74	45	36
Total	1154	877

One aim of the present study was to obtain a better estimate of the prevalence of vomeronasal pits using a larger population.

A central question concerning the vomeronasal structure in adult humans is its functionality. If the vomeronasal cavity were to act, like in other mammals, as a sensory organ bringing information to the brain it must contain receptor neurons. However, to our knowledge, the evidence for functional vomeronasal receptor neurons connected to the brain is very inconclusive in adult humans. Attempts to demonstrate the existence of neurons, in adult vomeronasal epithelia, were negative (Jordan, 1973; Johnson *et al.*, 1985; Moran *et al.*, 1991; Johnson, 1998; Smith *et al.*, 1998), except in one study (Takami *et al.*, 1993) where a few epithelial cells, having a bipolar shape, were stained with an antibody against neuron-specific enolase. However, the density of these putative sensory neurons did not exceed a few immunoreactive cells per 200 μm of vomeronasal luminal surface. Electron microscopy studies of adult vomeronasal ducts (Stensaas *et al.*, 1991; Gaafar *et al.*, 1998; Jahnke and Merker, 1998) also suggest that some epithelial cells could be considered as putative neurons, but arguments are indirect and not conclusive. It seems essential that more information must be obtained before drawing a definite conclusion about the sensory function of the vomeronasal epithelium in adult humans.

Materials and methods

Observations with the endoscope

We observed both sides of the nasal septum of 1154 women and 877 men. All age groups (Table 1) were represented for both genders (15–94 years; mean age = 46 ± 17 years for men and 49 ± 16 years for women). The endoscope used was a Storz Foreward Endoscope 0°, 4 mm external diameter, supplied with a cold light. We estimated the optic resolution to be ~0.2 mm. The tip of the endoscope was gently introduced through the nostril to observe the surface of the nasal septum. When necessary, we carefully removed the mucus with a cotton pad. In some cases we applied

a nasal spray of vasoconstrictor (Aturgyl, oxymetazoline, Synthélabo, France) to reduce the turgescence of the nasal mucosa and facilitate the observation of a larger surface of the septum. After reaching an agreement on the criteria of decision (presence of a well-defined pit, presence of a putative pit, nothing visible), one of us (C.E.) systematically made all the observations. Photographs of the anterior part of the nasal septum and videotape recordings were routinely taken, particularly when no pit could be seen, for further evaluation by others observers. In a few attempts we applied around the pit a spray of carbon particles, which does not involve irritation of the tissue, to visualize the mucus flow.

For each subject the diagnostic of a possible pathology of the nasal mucosa was established using appropriate clinical methods. Subjects were gathered into the following four etiological groups: (i) no pathology (n = 773); (ii) presence of nasal polyps (n = 402); (iii) inflammation of the mucosa due to allergy or rhinitis (n = 667); and (iv) altered nasal septum due to perforation or surgical modification of the anterior part of the nasal septum (n = 189). Subjects from groups 1, 2 and 3 did not have an altered nasal septum (n = 1842). Statistical analysis was performed using the χ^2 test. Means are given ± 1 SD.

Computer tomography

The nasal cavities of seven patients (five males and two females; mean age 48 years, range 15–63 years) were examined using computer tomography after filling of the vomeronasal cavity with Iopamiron 370® (a tri-iodine water-soluble contrast substance commonly used in radiology; Sherring-Plough, UK). For these subjects nasal computer tomography was prescribed for medical reasons not related to the pathology of the nasal septum. All subjects were informed and gave their consent to this observation. The contrast substance was injected using a sterile catheter (Venflon, 100 μm in diameter, 32 mm in length) positioned under endoscopy at the entrance of either one pit or both pits when present.

Consecutive 1 mm CT scans were acquired by a tomodensitometer with helicoidal acquisition (CT High Speed Scanner, General Electric Medical System, France; 120 kV, 80 mA/slice). The irradiation was limited to the inferior part of the nasal cavity, from the palate bone to the inferior edge of the middle turbinate.

Histology

Specimens were collected from four fresh cadavers of subjects who have consented to post-mortem scientific studies and from 11 patients undergoing a surgical procedure that required a large ablation of the tissues at the anterior part of the nasal septum. The ablation, performed by a surgeon on patients under general anaesthesia, was necessary for medical reasons not related to the present study. According to the recommendations from the Declaration of Helsinki, subjects were informed about the procedure and gave their consent. Specimens of nasal epithelium were fixed in buffered 3% formaldehyde or Bouin's fixative for 2 days, dehydrated and embedded in paraffin. Serial sections (4 μm thick) were made and stained with haematoxylin–eosin and saffron. Of the 15 specimens, nine contained a vomeronasal structure. Seven of these were subsequently processed using various antibodies.

Immunohistochemistry

Antibodies specific for keratin (Immunotech KL1, Marseille, France, dilution 1/50), neuron-specific enolase (Dako H14, Glostrup, Denmark, dilution 1/100), olfactory marker protein (OMP; a gift of Prof. Frank L. Margolis, University of Maryland, Baltimore, MD; dilution 1/600), S-100 protein (Dako Z311, polyclonal; dilution 1/500), chromogranin A (Dako DakA3; dilution 1/100), synaptophysin (Dako SY 38; dilution 1/20; after microwave pre-treatment of sections), tau protein (Dako A024, polyclonal; dilution 1/200), neurofilaments (Immunotech 1065; no dilution) and vimentin (Immunotech V9; dilution 1/50) were used. After incubation, the fixation of the antibody was revealed using an avidin–biotin complex peroxidase method (Vectastain ABC kit, Vector Lab, Burlingam, CA). Several slides at different levels of each vomeronasal structure were incubated with each antibody. All the slides were counterstained with haematoxylin. For all antibodies, simultaneous processing of serial sections of adult human olfactory mucosa was performed. Omission of the primary antibody was used as a negative control in each case.

Results

Endoscopy

Well-defined pits

In some cases the vomeronasal pit was found almost immediately when looking at the antero-inferior one-third of the nasal septum with the endoscope. It appeared as an obvious depression into the nasal mucosa (Figure 2A–D). The size of these pits ranged from ~1 mm to ~2.5 mm. In most of these cases, the posterior edge (p in Figure 2) of the pit dropped much more rapidly into the nasal mucosa than the anterior edge (a in Figure 2) and made a well-defined ridge, with a crescent or rounded shape. For over half of these observations the opening of the internal duct (see below) was clearly seen. Carbon particles, when applied around the pit, moved over the pit, transported by the flow of mucus that moved in an antero-posterior direction. Suggestively, the mucus did not specifically converge towards the pit.

In other cases (Figure 2E) the pit was also well defined but smaller, down to ~0.3 mm. Most of the time, these small pits became apparent only after clearing the mucus layer with a cotton pad.

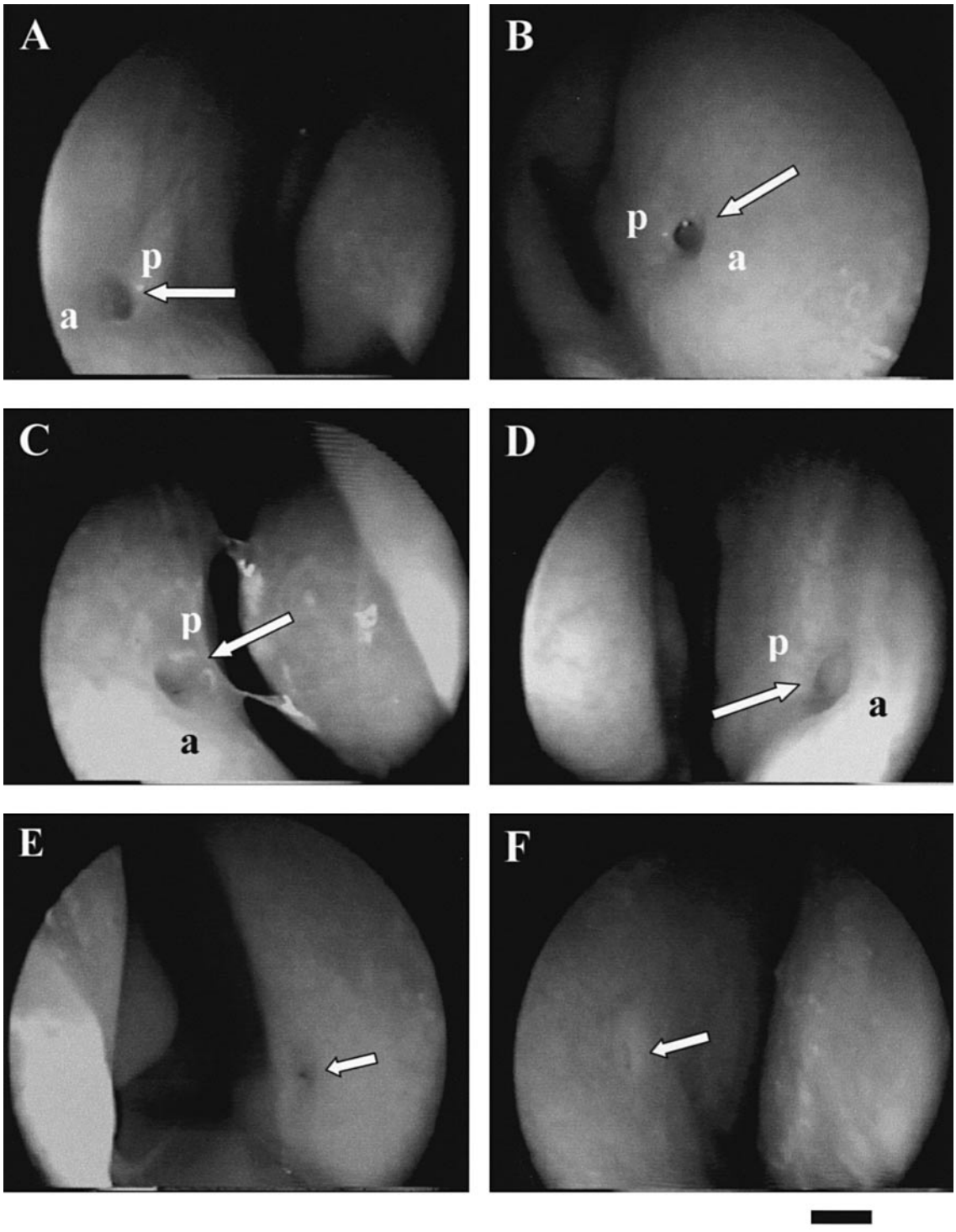

Figure 2 Vomeronasal pits (indicated by arrows) observed by endoscopy of the nasal septum of adult humans. Micrographs were taken from videotape recordings. **(A–D)** Large pits. Scale: ~1 mm. The anterior edge (a) and posterior edge (p) of the pits are indicated. **(E)** The pit was visible as a marked depression of small size. Scale: ~0.7 mm. **(F)** Example of a putative pit, nearly flat with the surface of the nasal mucosa. Scale: ~1 mm.

Putative pits

Sometimes, well-defined pits were not found but the presence of putative pits (Figure 2F) was suspected in the expected region of the septum. These putative pits, of ~1–2 mm in diameter, appeared as being nearly flush with the surface. No clear depression into the nasal mucosa could be seen and the posterior edge was not visible.

Table 2 Aspects of the vomeronasal pits at the first observation of the left and right sides of the nasal septum of 2031 subjects

Left side	Right side	Group 1 (no pathology)	Group 2 (polyposis)	Group 3 (allergy/rhinitis)	Total (groups 1–3)	Group 4 (altered septum)
1	1	104 (13.5%)	64 (15.9%)	76 (11.4%)	244 (13.2%)	3 (1.6%)
1	0	69 (8.9%)	31 (7.7%)	37 (5.5%)	137 (7.4%)	3 (1.6%)
1	?	43 (5.6%)	27 (6.7%)	40 (6.0%)	110 (6.0%)	2 (1.1%)
0	1	59 (7.6%)	39 (9.7%)	44 (6.6%)	142 (7.7%)	7 (3.7%)
?	1	33 (4.3%)	22 (5.5%)	32 (4.8%)	87 (4.7%)	7 (3.7%)
0	0	320 (41.4%)	163 (40.5%)	307 (46.0%)	790 (42.9%)	149 (78.8%)
0	?	54 (7.0%)	27 (6.7%)	46 (6.9%)	127 (6.9%)	6 (3.2%)
?	0	56 (7.2%)	20 (5.0%)	57 (8.5%)	133 (7.2%)	10 (5.3%)
?	?	35 (4.5%)	9 (2.2%)	28 (4.2%)	72 (3.9%)	2 (1.0%)
Total		773	402	667	1842	189

Four etiological groups were considered. '1' stands for the observation of a well-defined pit. '?' indicates the presence of a putative pit. '0' designates that no pit was visible. No statistical difference (χ^2 test) being found between groups 1, 2 and 3, they were combined to give the prevalence of the pits in 1842 subjects observed once.

Absence of visible pit

In many cases nothing similar to a pit or a putative pit could be seen at the surface of one or both sides of the nasal septum, despite a thorough cleaning of the mucus and a careful observation of the said region of the septum. In these cases the area of observation was extended to all the observable surface of the septum.

Prevalence of pits and putative pits

For each subject we noted, for each side of the nasal septum, '1' when a pit was present, '?' when a putative pit was present and '0' when no vomeronasal pit could be observed. Considering the left and the right sides of the septum and the three possibilities (presence of a pit, presence of a putative pit or nothing visible), nine classes of subjects were observed. Table 2 indicates the number of subjects in each of these classes for 773 subjects without nasal pathology, 402 subjects having nasal polyps and 667 subjects showing an inflammation of the nasal mucosa due to allergy or rhinitis. Analysis of the results described in Table 2 also indicates that these two pathological groups and the reference group are homogeneous ($\chi^2 = 0.15$). Therefore we pooled these three groups to give an estimation of the prevalence of the pits in 1842 subjects. It is evident from Table 2 that less than half of the subjects (42.9%) had no visible pit on either side of the septum at a single observation.

In the first step of analysis, we considered only the prevalence of clearly defined pits (such as in Figure 2A–E). Putative pits were not considered. In that case, one can observe that 244 subjects (13.2%) had a pit on each side of the septum, 247 subjects (13.4%) had a pit only on the left and 229 subjects (12.4%) had a pit only on the right. About 39% of our sample showed at least one well-defined vomeronasal pit.

In the second step, we included putative pits. In that case, 513 subjects (27.9%) had bilateral pits, 270 subjects (14.7%) had a pit only on the left and 269 subjects (14.6%) a pit only on the right. About half of the population (57%) had at least one well-defined pit or one putative pit. No statistical difference (χ^2 test) was observed when we considered the sex of the subjects. Analysis made after repartitioning the subjects into classes of 10 years age range revealed that there were no changes in frequency of the vomeronasal pit with age.

The probability of observing a pit, or a putative pit, in 189 patients with an altered septum due to either nasal perforation or surgery of the nasal septum was significantly lower ($\chi^2 < 0.01$) than for the group of 1842 reference subjects (Table 2). Most of them (73.4%) had no visible vomeronasal pit on either side of the septum, 6.9% had bilateral pits and the remaining (19.7%) had only one pit either on the left or on the right side.

Repetition of the observations

We had the opportunity to repeat the observation of the nasal septum on 764 subjects without any pathology of the nasal mucosa. The time between the observations ranged from a few days to a few months. No statistical difference ($\chi^2 = 0.30$) was observed between the frequencies observed in the nine classes of Table 2 in the first and the second series, indicating a global stability of the observations at the level of the whole population of subjects. However, there was a variability of the observations for some subjects. Two features were noted. A vomeronasal pit observed on the first inspection could not be observed on the second inspection. Conversely, a vomeronasal pit could be observed on the second inspection where nothing could be seen on the first inspection. More precisely, only 65.3% of the observations remained constant between the two observations. Of 414 well-defined pits visible in the first series, 52 (12.6%) became putative pits and 54 (13.0%) were no longer visible in the second series. One hundred and sixty pits appeared in the second series from 255 putative pits and from no detectable

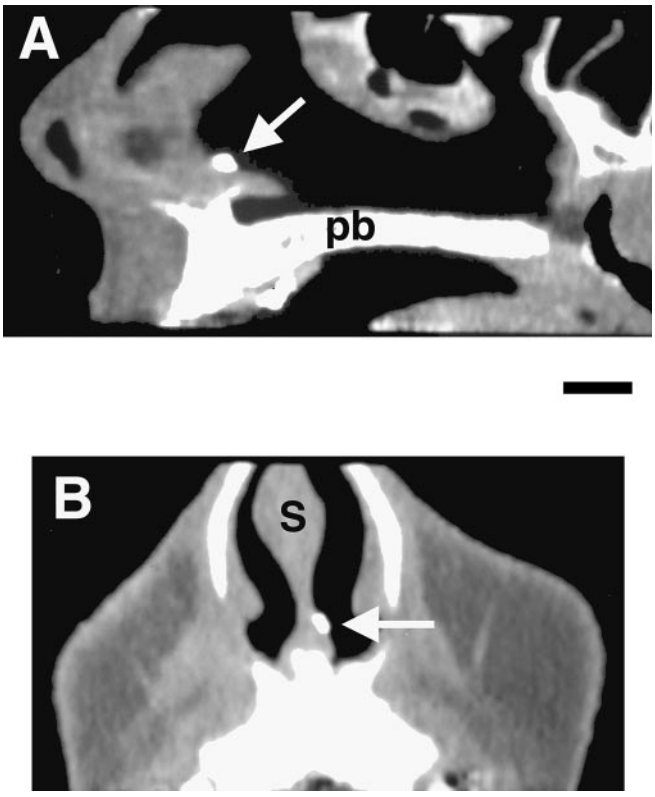

Figure 3 Computer tomograms of the nasal cavity of a subject, after injection of a contrast substance into the vomeronasal pit. **(A)** Sagittal section. The vomeronasal cavity (arrow) is located approximately at the apex of the nasal spine. The nostril is at left and the palatine bone (pb) is indicated. **(B)** Coronal section. The vomeronasal cavity (arrow) is located in the basal enlargement of the nasal septum (s). Scale: 1 cm.

pit (n = 859) in the first series. Of the 255 putative pits seen in the first series, 194 were no longer visible and 69 became well-defined pits. Ninety-one pits appeared from no detectable pit in the first inspection. Following these observations, it is clear that the appearance of the vomeronasal pit could change over time for a given subject. In 130 subjects seen four times or more, at least one vomeronasal pit could be observed in 95 subjects (73.1%) and at least a putative pit could be observed on at least one side in an additional 24 subjects (18.5%). No pit or putative pit could be detected on either side of the septum in only 11 subjects (8.5%).

Computer tomography

To get more detailed information about the position of the vomeronasal cavities in the nasal septum, we injected a water-soluble contrast substance into clearly visible pits of seven subjects. Computer tomography scans were performed ~15 min after the injection, and coronal, sagittal and axial sections of the nasal cavities were reconstructed (Figure 3). The location of the opaque spot indicated the position of the vomeronasal cavity. Its length ranged from 2 to 5.7 mm (mean 3.5 ± 1.2 mm) (Figure 3A) in different subjects. The vertical position of the stained region was 9.1 ± 1.3 mm (range 6.2–10.7 mm) above the crest of the palatine bone. Its antero-posterior position was 3.3 ± 4 mm (range 4.3–10 mm) anterior to the ascendant branches of the maxillary bone. In all cases the opaque spot was located in the enlargement seen at the base of the nasal septum (Figure 3B). In three subjects the vomeronasal cavities were injected with the contrast substance on both sides of the septum (not shown). Opaque spots were found at the same vertical position on each side of the nasal septum, the antero-posterior positions differing by <0.7 mm except in one subject. The lengths of the stained cavities differed by <0.2 mm.

Connection of the pit with the vomeronasal duct

One of the longest vomeronasal ducts that we observed is presented in Figure 4. In this preparation the sections were made along a plane parallel to the long axis of the duct and perpendicular to the surface of the nasal mucosa. Therefore, both the medial side and the lateral side of the duct were observed simultaneously. In other mammals, the epithelium of the medial side contains vomeronasal neurons and the lateral side is lined with ciliated epithelium on a structure containing erectile tissue and blood vessels, which are involved in the active pumping of the stimuli into the lumen.

In Figure 4, the vomeronasal pit, in direct contact with the nasal cavity, appeared as a funnel. The diameter and the depth of the pit was ~600 μm. The posterior edge (p in Figure 4) of the pit deepened rapidly into the nasal mucosa. The vomeronasal duct started at the base of this posterior edge. Adjacent serial sections indicated that the duct was closed, at its posterior end, after a length of ~2 mm. The lumen of the duct had a diameter of ~100 μm and contained mucus. Many glands (arrows in Figure 4) were present on both sides of the duct, below the epithelium lining the lateral and medial sides of the lumen. The duct of some of these glands clearly reached the surface of the epithelium (g in Figure 4).

In contrast to other mammals (see Døving and Trotier, 1998), there was no sign of any erectile tissue, large blood vessels or encapsulating cartilage around the duct.

Five other specimens contained a similar vomeronasal structure although the length of the duct was sometimes smaller than in Figure 4. In three additional specimens the pit was present but the duct was very short or absent. In six other specimens nothing similar to a vomeronasal structure could be found.

Immunohistochemistry

Anti-keratin

In the olfactory epithelium (Figure 5, O) keratin was found in supporting cells, located in the upper part of the epithelium, and in cells located near the basal lamina. Bipolar olfactory receptor neurons were not reactive and therefore their cell bodies made a distinctive unstained layer in the lower half of the epithelium. No such clear layer

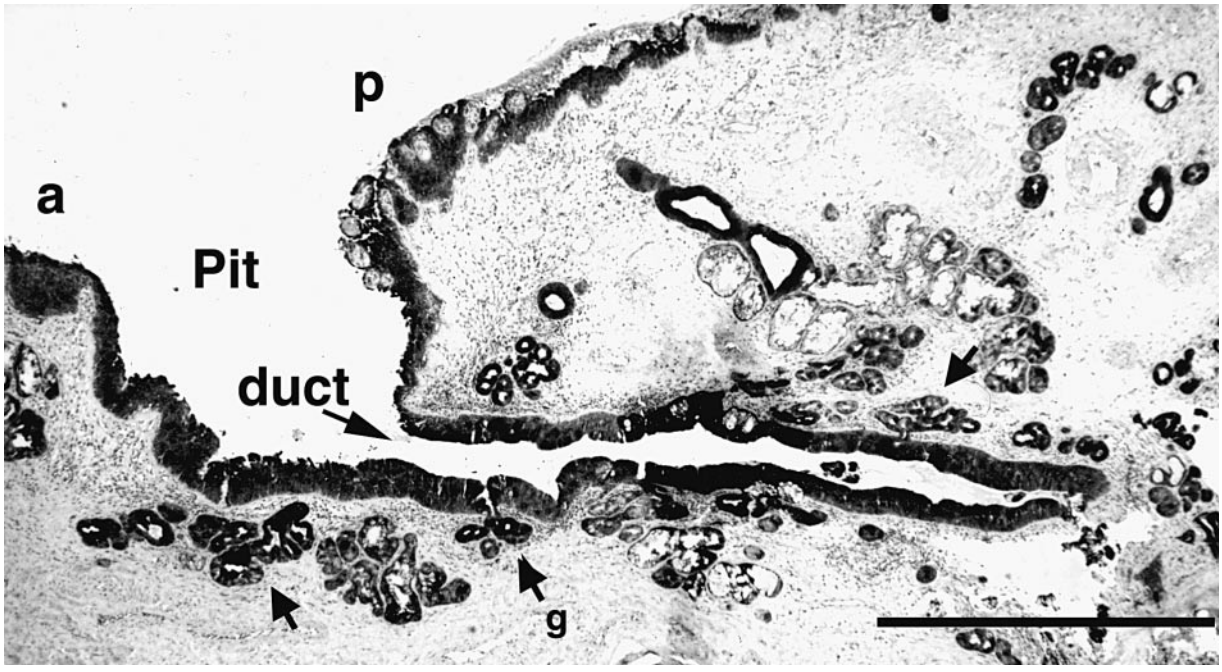

Figure 4 Histological section through the pit and the vomeronasal duct in a specimen collected from of an adult man (45 years) during nose surgery. As in Figure 2, a and p denote the anterior and posterior edges of the pit. The duct starts at the back of the pit and extends over ~2 mm under the nasal mucosa. Sub-epithelial glands are indicated by arrows. g indicates a gland that communicates with the lumen of the vomeronasal duct. This section was stained for keratin by immunohistochemistry and counterstained with haematoxylin. Scale: 1 mm.

could be observed in sections of vomeronasal ducts (Figure 5, V). The majority of cells were stained with no obvious difference between the epithelium covering the lateral side of the lumen and the epithelium covering the medial side. Some epithelial cells were not stained, but they did not form a clear layer in the epithelium. Among them, very rare cells had a morphology that could evoke the typical bipolar shape of vomeronasal neurons observed in all other species. It should be emphasized that the density of these 'bipolar cells' was extremely low. For example, only one cell, indicated by the arrow in Figure 5 (V) and shown in negative at higher magnification in the picture on the right, was found in this section of the epithelium covering the medial side of the lumen.

The same observations were made from five out of the six other specimens of vomeronasal structures; the last one was not tested.

Anti-OMP

The antibody against the olfactory marker protein stained the cytoplasm of olfactory receptor neurons in the olfactory epithelium (Figure 6, O). These cells had a typical bipolar shape with a long dendrite reaching the surface of the epithelium. The same antibody applied in the same conditions failed to stain any cell in the vomeronasal epithelium, either in the medial epithelium or in the lateral epithelium (Figure 6, V).

We tested this antibody on the six other vomeronasal specimens. In none of them could we reveal the presence of OMP-expressing cells in the epithelia. In two specimens, glands below the epithelium were stained.

Anti-protein S-100

The S-100 protein is a marker expressed in glial and Schwann cells wrapping axon fascicles. Myoepithelial cells and some duct cells of normal seromucous glands also express S-100. In the olfactory epithelium (Figure 7, O), the antibody stained Schwann cells enwrapping olfactory axons and revealed olfactory nerve bundles leaving the olfactory epithelium in the direction of the olfactory bulb. These olfactory nerve bundles are indicated by arrows in Figure 7 (O). No such nerve bundles could be observed around the vomeronasal duct, below the epithelium lining the medial side of the lumen (Figure 7, V) or the lateral side (not shown). S-100 expression was observed in glands (such as in the lower left corner of Figure 7, V) and some background activity was found in the mucus filling the lumen.

This antibody was tested on five out of the six other vomeronasal specimens (one specimen was not tested). In none of them could staining of nerve bundles be observed.

Anti-neuron-specific enolase

This antibody stained a number of receptor neurons in the olfactory epithelium (not shown). In four out of the seven vomeronasal structures no staining was observed. In the remaining three specimens, a very few epithelial cells were reactive to the antibody (not shown).

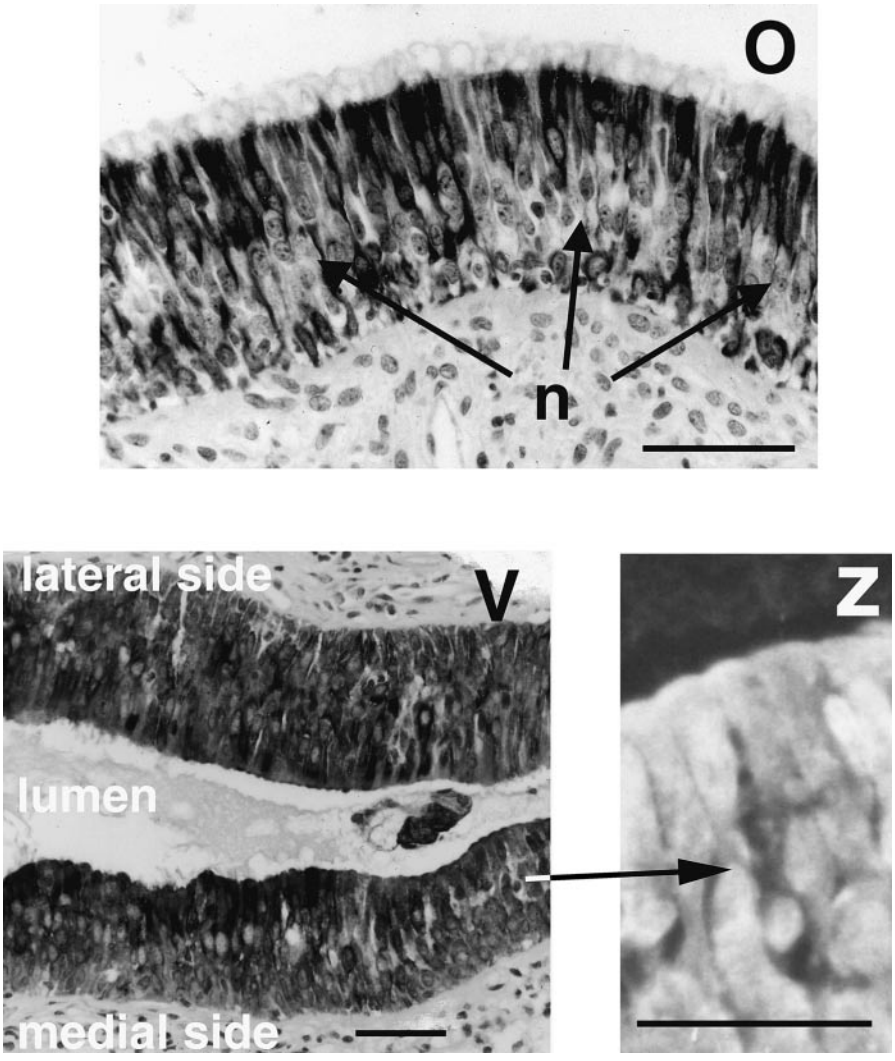

Figure 5 Staining of human olfactory epithelium and adult vomeronasal epithelium with an antibody for keratin. (O) In the olfactory epithelium receptor neurons (*n*) do not express keratin and make a clear layer below the stained layer of supporting cells. Scale: 50 μm. (V) In the vomeronasal cavity, most cells in the epithelium on the medial and on the lateral side of the lumen were stained. Same specimen as in Figure 4. Scale: 50 μm. (Z) An example of an unstained cell observed in (V), shown at a higher magnification after inversion of colours. This cell presents a bipolar shape reminiscent of the morphology of vomeronasal receptor cells in other species. Scale: 30 μm.

Other antibodies

Other antibodies, against vimentin, neurofilaments, glial fibrillary acidic protein, synaptophysin, chromogranin A and tau-protein, were not reactive either in olfactory epithelium or in the seven vomeronasal structures.

Discussion

Anatomy of the vomeronasal pits

In the present study, we confirm that the opening of the vomeronasal structure can be observed in many subjects. In some cases it made a clearly defined depression in the nasal mucosa. The injection of a contrast substance, followed by computer tomography, indicated that the vomeronasal cavity was located in the enlargement seen at the base of the nasal septum. This is exactly the position of the vomeronasal organ in human embryos (Kjær and Fischer Hansen, 1996a). This observation is significant because Johnson *et al.* showed that many small pits observed by endoscopy to be distributed across large areas of the septum were, in fact, the openings of glands (Johnson *et al.*, 1985).

We found that the antero-posterior position of the vomeronasal duct, in computer tomographies, corresponds well with previous observations (Figure 1) and more recent findings (Jordan, 1973; Johnson *et al.*, 1985), ~2 cm from the nostril.

Originally discovered by Ludvig Jacobson (Trotier and Døving, 1998), a cartilaginous capsule separates, in all other mammals, the vomeronasal organ from the nasal cavity. We did not observe such cartilage around the duct in adult humans, which agrees with previous observations (Potiquet, 1891; Johnson *et al.*, 1985). In addition, other mammals use

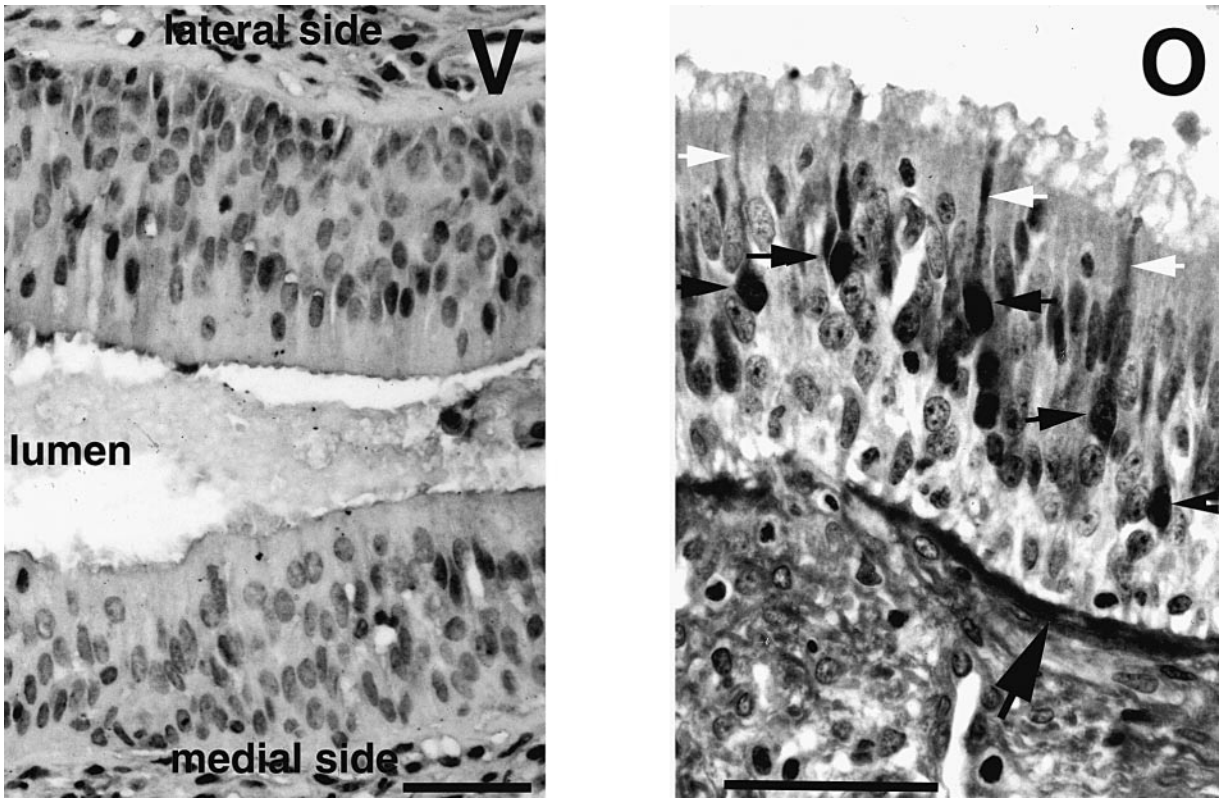

Figure 6 Staining with antibody for olfactory marker protein. (O) In the olfactory epithelium, cell bodies (small black arrows) and dendrites (small white arrows) of mature olfactory neurons are stained. Olfactory axons (large black arrow) below the epithelium are also stained. (V) No staining is found in the lateral and medial epithelium of the vomeronasal duct. Same specimen as in Figure 4. Scales: 40 μm.

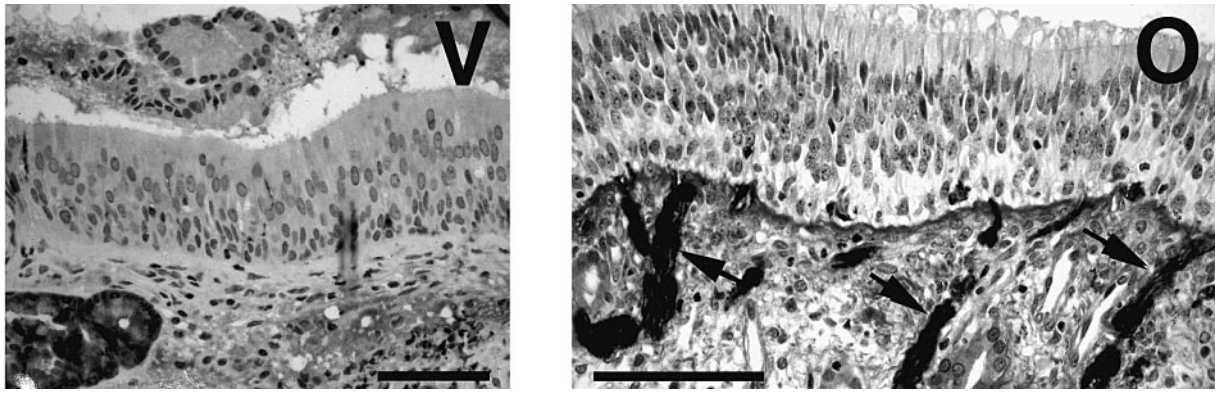

Figure 7 Staining with antibody for S-100 protein. (O) Schwann cells in olfactory nerve bundles (arrows) are stained in the olfactory epithelium. (V) No staining is observed in the vomeronasal epithelium, except in the gland located in the lower left corner. Same specimen as in Figure 4. Scales: 70 μm.

a special device, consisting of a turgescent tissue irrigated by large and small blood vessels, to pump in and out the stimulus present in the nasal cavity (Døving and Trotier, 1998). These elements were not discernible in our histological material. This lack of pumping elements has already been emphasized (Johnson *et al.*, 1985; Jordan, 1973). We agree with Jacobson (Trotier and Døving, 1998), who considered that the vomeronasal structure is rudimentary or regressive in adult humans. In a few attempts we observed, during a few minutes, that the flow of nasal mucus was not obviously directed towards or from the pit. Therefore one should consider that substances in the nasal mucus might reach the vomeronasal duct only by passive diffusion.

Inspection of the nasal septum of a given individual led to three possible observations concerning the vomeronasal pit: the presence of a well-defined pit, the presence of a putative

pit or no pit visible. An intriguing outcome of the present study is that the appearance of the pit may change for a given individual depending on the time of observation. In some cases, repetition of the observation indicated that well-defined pits appeared where only putative pits or even no visible pits were observed in the first inspection. Therefore one cannot conclude the absence of a pit when nothing is visible from a single observation; repeated observations at different times are required. If we consider the 130 subjects observed at least four times, we can conclude that ~73% of them presented a well-defined pit on one day or another. This percentage increases to ~91% if we consider putative pits.

A well-defined pit may also change into a 'putative' pit or 'no visible' pit during successive inspections. These observations suggest the existence of an unknown mechanism that may change the appearance of the pit and therefore reduces the possibility of seeing it by endoscopy. In this context it is of interest that Pearlman says: 'Seeing the facility with which the opening of the organ can be found in the cadaver, it is astonishing how difficult it is to find it in the living subjects' (Pearlman, 1934). Johnson *et al.* made similar observations: in living subjects they observed that 39 nasal septa out of 100 presented at least one visible pit, whereas histological observation from cadavers indicated that ~70% of nasal septa presented at least one vomeronasal pit (Johnson *et al.*, 1985).

The probability of finding a pit or a putative pit does not depend on obvious pathology of the nasal mucosa, such as polyposis, rhinitis or allergy. It is only when the anterior part of the septum was altered by perforation or surgical septoplasty that the probability of finding pits was significantly lower. No significant effect of age or sex was observed.

Histology of the vomeronasal epithelium

There have been few electrophysiological studies aimed at revealing nervous activity of the vomeronasal cavity in humans. A negative shift of the surface potential of the pit was recorded following application of putative human pheromones (Monti-Bloch and Gosser, 1991; Monti-Bloch *et al.*, 1994). By analogy with the slow voltage change evoked by odorants at the surface of the olfactory epithelium, this electrical signal has been considered by the authors as the receptor potential induced by activation of vomeronasal receptor neurons. According to this interpretation, the activation of the vomeronasal pit may trigger autonomic responses and modifications of the blood level of some hormones (Berliner *et al.*, 1996; Monti-Bloch *et al.*, 1998a,b). However, to our knowledge, the existence of functional vomeronasal receptor neurons that connect to the brain is doubtful in adult humans (Jordan, 1973; Johnson *et al.*, 1985; Moran *et al.*, 1991; Johnson, 1998; Smith *et al.*, 1998). The immunohistochemistry shown here demonstrates the absence both of OMP and of glial elements essential for the wrapping of the unmyelinated vomeronasal axons.

It has been demonstrated that OMP is a protein that is found in mature neurons in the olfactory epithelium (Buiakova *et al.*, 1994; Krishna *et al.*, 1995; Walters *et al.*, 1996) and in the vomeronasal organ of other species (Johnson *et al.*, 1993; Berghard *et al.*, 1996; Liman and Corey, 1996). Therefore if mature vomeronasal neurons exist in adult humans it should be possible to demonstrate OMP. However, in the present study no staining was found in the vomeronasal epithelia using an antibody against OMP. In other species, new vomeronasal receptor neurons grow out from progenitor cells (Barber and Raisman, 1978; Wang and Halpern, 1988). If this renewal process exists in adult humans, some new immature receptor neurons may appear from time to time. That could explain why some intraepithelial cells, having a typical bipolar shape, can be observed when histological sections are stained with an antibody against neuron-specific enolase (Takami *et al.*, 1993) or remain unstained with anti-keratin antibody (Figure 5, V). However, these putative immature neurons are present at a very low density that seems quite problematic for eliciting any surface potential change during chemical stimulations.

The presence of a few neuron-like cells in the adult vomeronasal epithelium does not imply that a message is sent to the brain. For doing so, vomeronasal neurons must be connected to the accessory olfactory bulb. Ensheathing glial cells expressing S-100 are present around vomeronasal nerve fibres in other species (Astic *et al.*, 1998). In the present study we did not find vomeronasal nerve bundles using a specific antibody against S-100 protein whereas olfactory nerve bundles were stained. In this context, it is difficult to assign a sensory function to the vomeronasal epithelium of human adults.

From embryos to adults

The vomeronasal ducts develop in human embryos (Bossy, 1980; Kreutzer and Jafek, 1980). According to Boehm and Gasser (Boehm and Gasser, 1993), at 12 and 23 weeks of gestation the vomeronasal epithelium contains clusters of neuron-specific enolase-positive cells looking like olfactory receptors; at 36 weeks the organ is lined by a respiratory epithelium and does not show any receptor-like cells. Ortmann (Ortmann, 1989) found receptor cells in four out of seven foetal vomeronasal organs (11–18 weeks). Luteinizing hormone-releasing hormone (LHRH)-immunoreactive cells are detected in the bilateral vomeronasal organs at 8–12 gestational weeks (Kjær and Fischer Hansen, 1996a) and in the nerve fascicles arising from the organ to the olfactory bulb (Boehm *et al.*, 1994; Kjær and Fischer Hansen, 1996b). As in other mammals, LHRH-secreting neurons migrate from the olfactory placode to the brain during the early stages of foetal life, following vomeronasal and terminal nerve fibres (Schwanzel-Fukuda *et al.*, 1996). In some foetuses (10–12 weeks) the vomeronasal organ is clearly

dissolving; in 17- to 19-week-old foetuses the vomeronasal organ may not be found (Kjær and Fischer Hansen, 1996a). The development of the vomeronasal structures seems to be limited to the embryonic stage, when they play a role for the migration of LHRH-secreting cells towards the brain.

In all other species, vomeronasal receptor axons make synaptic contact with secondary neurons in the accessory olfactory bulb. In human foetuses, the accessory olfactory bulb is present at 8 weeks (Bossy, 1980), 18 weeks (Humphrey, 1940; Meisami and Bathnagar, 1998) and 26 weeks (Humphrey, 1940). However, in older foetuses the accessory olfactory bulb regresses and, indeed, is *not* found in the adult human [for a review see (Meisami and Bathnagar, 1998)].

Conclusion

The present study has given anatomical, histological and immunohistochemical data that all indicate that in adult humans the vomeronasal structure is a remnant of the vomeronasal organ found in mammals. This statement is in accordance with findings and opinions of previous authors as discussed above. In our opinion, a viable function of receptor neurons has never been convincingly demonstrated. We consider that the vomeronasal structure does not function as a sensory organ in adult humans. In conclusion, the vomeronasal structure might have a function only during human foetal life in contributing, together with the terminal nerve and other structures, to the migration of neurosecretory cells containing LHRH to their proper sites in the brain.

Acknowledgements

We sincerely thank Prof. Frank Margolis for the generous gift of the antibody for OMP, Marie-France Bouvet, Isabelle Levesque and Carole Sanchez for their excellent technical assistance, Prof. Patrick MacLeod for critical reading of the manuscript and Prof. Jean Pierre Lassau for giving us access to post-mortem specimens.

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Accepted January 27, 2000