NOTE *Anatomy*

Immunohistochemical studies for the neuronal elements in the vomeronasal organ of the one-humped camel

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ABSTRACT. The neuronal elements of the vomeronasal organ (VNO) of camel were investigated immunohistochemically. PGP 9.5 labeled the receptor cells in the vomeronasal sensory epithelium, but not the supporting or basal cells. OMP stained some receptor cells, but no immunoreactive signals for OMP were detected in the non-sensory epithelium. PLCβ2 labeled scattered cells in the sensory epithelium and a larger number of cells in the non-sensory epithelium. Double labeling immunohistochemistry revealed that the PLCβ2-positive cells were surrounded by substance P-positive nerve fibers. Collectively, these data suggest that the camel VNO bears, in addition to the mature vomeronasal receptor cells, trigeminally-innervated solitary chemosensory cells which are expected to play a substantial role in the control of stimulus access to the VNO.

KEY WORDS: olfactory system, OMP, PGP 9.5, PLCβ2, SCCs

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The vomeronasal organ (VNO) and the accessory olfactory bulb (AOB) are components of the accessory olfactory system or the vomeronasal system [8, 22, 28]. The VNO houses receptor neurons which detect pheromones and other semiochemical substances [9, 13, 14, 21, 27] and distribute sensory information to the AOB [5, 24].

Besides the vomeronasal receptor cells, the VNO houses several types of sensory cells. They include a population of epithelial cells named the solitary chemosensory cells (SCCs). Distribution of the SCCs has been demonstrated in mice VNO using phospholipase C-β2 (PLCβ2), α-gustducin and transient receptor potential channel M5 (TRPM5) [19, 29]. SCCs have the ability to detect harmful substances in the nasal cavity [6, 7, 15, 18, 26] and in the VNO [12, 29]. In mice, the entrance of the VNO is abundant in SCCs which spread sensory information onto the trigeminal nerve fibers and involved in the control of stimulus access to the VNO [19]. In the shortage of control, the irritants and harmful substances are supposed to achieve a greater approach to the VNO and cause damage to the vomeronasal receptor cells [17].

The camel is an important animal for the Egyptian animal industry, living in desert and is subjected to extreme hot and dry atmosphere. However, as far as we know, no data are available on the immunohistochemical features of the olfactory organs of camel. In this study, the VNO of the one-humped camel, *Camelus dromedarius*, was evaluated immunohistochemically with some neuronal markers to clarify the distribution and degree of differentiation of the receptor cells, in addition to elucidate the existence of other neuronal elements, such as the SCCs, which may play an important role in protecting the vomeronasal receptor cells from over-access of irritant substances.

Heads from three adult, male one-humped camels were obtained from Beni-Adi slaughter house (Assuit, Egypt). The nasal parts were immersed in 10% neutral buffered formalin, then decalcified in 10% ethylenediaminetetraacetic acid (pH 7.4), routinely embedded in paraffin and cut transversally at 5–7 μm. Some sections were stained with hematoxylin-eosin, periodic acid/Schiff (PAS), alcian blue (pH 2.5) or Crossmon's trichrome for histological examinations.

Immunofluorescence: After deparaffinization and rehydration, antigen retrieval was performed in 10 mM sodium citrate buffer (pH 6.0) containing 0.1% Tween 20 using autoclave at 121°C for 10 min. Sections were rinsed in 0.01 M phosphate buffered saline (PBS, pH 7.4) and incubated with 2% normal donkey serum in PBS at room temperature (RT) for 30 min to block nonspecific reactions. After rinsing, sections were incubated with one of the following antibodies: rabbit anti-protein gene product (PGP 9.5) (UltraClone, RA 95101, Isle of Wight, U.K.), goat anti-olfactory marker protein (OMP) (Wako Pure Chemical, 544, Osaka, Japan),

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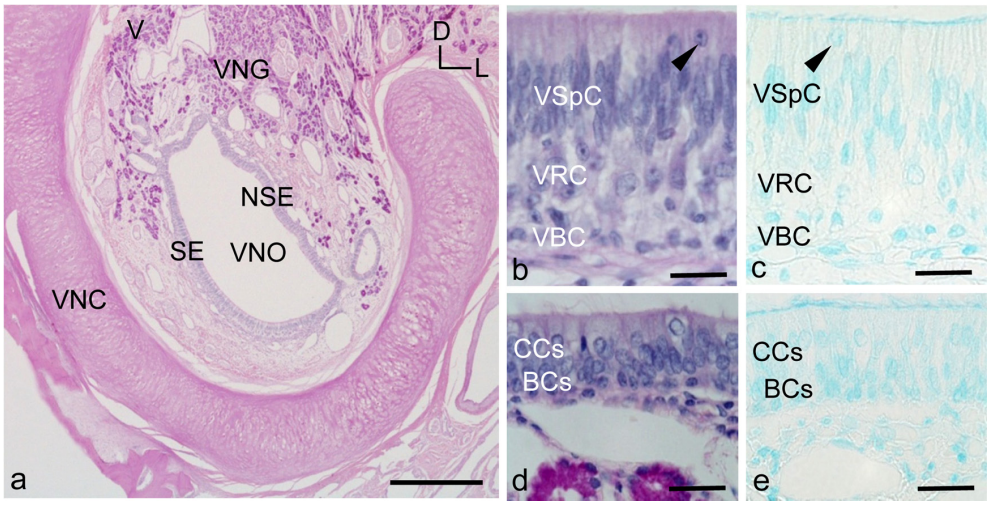

Fig. 1. Histological features of the vomeronasal organ in the one-humped camel. (a) Transverse section of the vomeronasal organ stained with periodic acid Schiff (PAS). Bar: 100 μm. (b) Vomeronasal sensory epithelium stained with PAS and (c) Alcian blue 2.5. Arrowheads indicate superficially-situated cells. (d) Vomeronasal non-sensory epithelium stained with PAS and (e) Alcian blue 2.5. Bars: 30 μm in (b)–(e). BCs, basal cells; CCs, ciliated cells; NSE, non-sensory epithelium; SE, sensory epithelium; VBC, vomeronasal basal cells; VRC, vomeronasal receptor cells; VSpC, vomeronasal supporting cells; VNG, vomeronasal gland; VNO, lumen of the vomeronasal organ.

rabbit anti-PLCβ2 (Santa Cruz Biotechnology, Sc-206, Santa Cruz, TX, U.S.A.), rabbit anti-α-gustducin (Santa Cruz Biotechnology, Sc-395) and goat anti-TRPM-5 antibody (Abcam, ab87642, Tokyo, Japan), at RT for 2 hr. Subsequently, they were rinsed with 0.1% Triton-X in PBS twice each for 5 min, once with PBS for 5 min and incubated with alexa fluor 488-donkey anti-rabbit IgG or alexa fluor 488-donkey anti-goat IgG at RT for 1–2 hr. After rinsing, sections were mounted with Vectashield mounting medium with DAPI (Vector Laboratories, H-1200, Burlingame, CA, U.S.A.). Control staining was made by the use of PBS to replace the primary antibody. No specific staining was detected in the control sections.

Double labeling immunohistochemistry: After deparaffinization, rehydration and antigen retrieval as described above, sections were incubated with 2% normal donkey serum in 1% BSA-PBS to block the non-specific bindings. Sections were incubated with a mixture of the primary antibodies, anti-PLCβ2 together with one of the two antibodies: rat anti-substance P (8450–0505, AbD Serotec, Oxford, U.K.) or goat anti-OMP, for 2 hr at RT. After washing, sections were incubated with a mixture of fluorochrome-conjugated reagents (alexa fluor 488-donkey anti-rabbit IgG and TRITC-donkey anti-rat IgG or TRITC-donkey anti-goat IgG) for 1–2 hr at RT. Sections were rinsed and mounted with Vectashield mounting medium with DAPI. Control staining was made by the use of PBS to replace the primary antibody.

The topographical and histological features of the camel VNO were in agreement with the findings of the previous reports [1, 11]. Briefly, the camel VNO was a pair of epithelial tubes situated at the base of the nasal septum (Fig. 1a). The VNO was surrounded by a J-shaped ring of hyaline cartilage. The lamina propria of the VNO contained vomeronasal glands, connective tissues, nerve bundles and blood vessels. PAS and alcian blue intensely stained the vomeronasal glands as well as the vomeronasal cartilage. The ducts of the vomeronasal glands opened into both the lateral and medial walls of the VNO. The lateral wall of the VNO was lined with the non-sensory epithelium, while the medial wall was lined with the sensory epithelium.

Nuclei of the vomeronasal basal cells were situated at the basal region of the sensory epithelium, while those of the receptor cells were situated at the middle region. In addition, nuclei of the supporting cells were located at the apical region of the epithelium and were arranged in two to four layers (Fig. 1b and 1c). The nuclei of the vomeronasal basal cells were smaller than those of the receptor and supporting cells. The vomeronasal receptor cells were pear-shaped and expanded their dendrites to the free border of the sensory epithelium and their axons in the direction of basal membrane. The nuclei of receptor cells had distinct nucleolus and were arranged in one or two layers, and their cytoplasm was faintly-stained with PAS. A fourth type of cells was situated at the level just below the free border (arrowhead in Fig. 1b and 1c). These superficially-situated cells had small, round or oval, pale nuclei with distinct nucleolus.

In the non-sensory epithelium, the goblet cells were located between the ciliated columnar cells. The number of goblet cells decreased caudally till disappear at the middle of the longitudinal length of the VNO (Fig. 1d and 1e).

To clarify the distribution of neuronal cells, the camel VNO was immunostained with anti-PGP 9.5 antibody, a neuronal marker that is known to be expressed in most

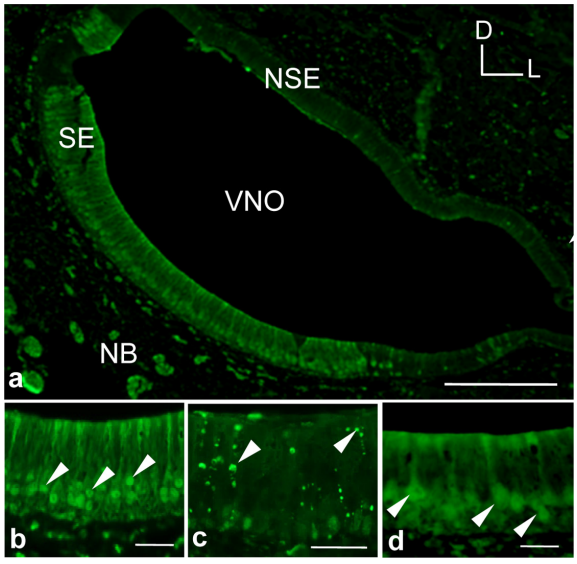

Fig. 2. Anti-PGP 9.5 and OMP immunostaining in the vomeronasal organ. (a) Transverse section of the vomeronasal organ stained with PGP 9.5. Bar: 100 μm. (b) Vomeronasal sensory epithelium stained with PGP 9.5. Arrowheads indicate the vomeronasal receptor cells. (c) Vomeronasal non-sensory epithelium stained with PGP 9.5. Arrowheads indicate PGP 9.5-positive nerve fibers. (d) Vomeronasal sensory epithelium stained with OMP. Arrowheads indicate the mature vomeronasal receptor cells. Bar: 20 μm (b)–(d). NB, nerve bundles.

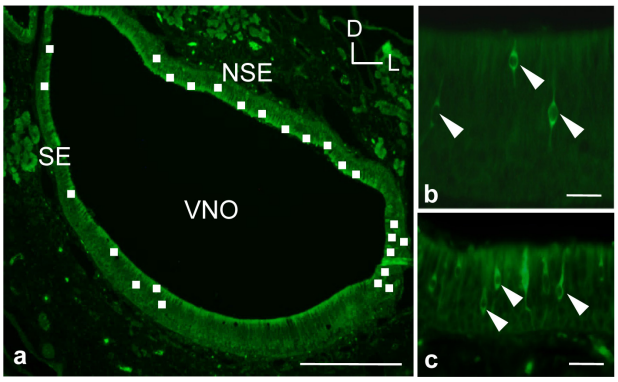

Fig. 3. Anti-PLCβ2 immunostaining in the vomeronasal organ. (a) Transverse section of the vomeronasal organ stained with PLCβ2. White plots indicate the distribution of the PLCβ2-positive cells in the camel VNO. Bar: 100 μm. (b) PLCβ2-positive cells (arrowheads) in the vomeronasal sensory epithelium. (c) PLCβ2-positive cells (arrowheads) in the vomeronasal non-sensory epithelium. Bar: 20 μm in (b) and (c).

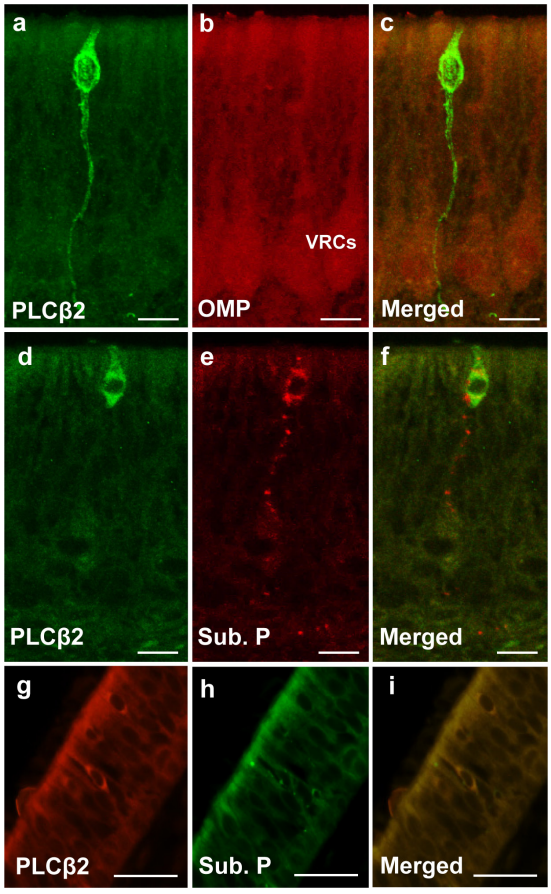

Fig. 4. Double-labeling immunohistochemistry in the vomeronasal sensory (a)–(f) and non-sensory (g)–(i) epithelia. PLCβ2-positive cells are shown in (a), (d) and (g). (b) OMP immunostaining of the vomeronasal receptor cells. (c) Merged picture of (a) and (b). (e) and (h) Substance P-positive nerve fibers surrounding the PLCβ2-positive cells. (f) Merged picture of (d) and (e). (i) Merged picture of (g) and (h). Bar: 10 μm in (a)–(f) and 25 μm in (g)–(i).

neurons of all vertebrates and plays an important role in the ubiquitin regulation [3, 10, 23]. PGP 9.5 immunoreactivities were found in a large number of the vomeronasal receptor cells in a similar manner that were demonstrated in the rat and canine vomeronasal receptor cells [4, 20]. In the sensory epithelium, PGP 9.5 labeled most of the vomeronasal receptor cells, while the supporting and basal cells were negative to PGP 9.5 (Fig. 2a and 2b). PGP 9.5 immunostained the cytoplasm and nucleus of the vomeronasal receptor cells, but not those of the supporting, basal or superficially-situated cells (Fig. 2a and 2b). In the non-sensory epithelium, some PGP 9.5-positive nerve fibers were detected, while all cells were negative to PGP 9.5 (Fig. 2c). In the lamina propria of the VNO, PGP 9.5 intensely labeled the nerve bundles (Fig. 2a).

Subsequently, the camel VNO was immunostained with another neuronal marker, OMP, specific for the mature olfactory receptor cells [2]. OMP stained some vomeronasal receptor cells, but not the superficially-situated cells in the vomeronasal sensory epithelium (Fig. 2d). The OMP-positive cells were located in a lower level of the epithelium, and the number of cells expressed OMP was fewer than those expressed PGP 9.5. Also, cells in the non-sensory epithelium were negative to OMP (not shown).

In order to verify the presence of SCCs in the camel VNO, the expression for PLCβ2, a constituent in the signal transduction pathway of the SCCs, was examined. PLCβ2 immunostained a number of cells in the sensory epithelium

of the VNO (Fig. 3a and 3b). PLCβ2-positive cells were usually detected in the superficial layer that contains the nuclei of supporting cells. Occasionally, few PLCβ2-positive cells were located in the middle layer that contains the nuclei of receptor cells. The shape of PLCβ2-positive cells was mostly spindle-shaped with both apical and basal cell processes. Some PLCβ2-positive cells had a highly-developed basal cell process. The presence of PLCβ2-positive cells with such morphology cannot be detected in mice VNO [19]. In the non-sensory epithelium, PLCβ2-positive cells were numerous and irregular in shape (Fig. 3c). Although we did not make any statistical analyses in this study, the density of PLCβ2-positive cells varied along the anterior-posterior axis of the VNO. PLCβ2-positive cells were most abundant at the entrance of the VNO (vomeronasal duct region), followed by the anterior region of the VNO. Caudal region of the VNO contained the PLCβ2-positive cells less frequently.

In order to confirm that the PLCβ2-positive cells in the sensory epithelium are not belonging to the vomeronasal receptor cells, double labeling experiments were performed using PLCβ2 and OMP. As shown in Fig. 4a-4c, PLCβ2-positive cells were not stained with OMP. Furthermore, in order to see if the PLCβ2-positive cells were innervated or not, the camel VNO was double immnuostained with PLCβ2 and Substance P. Unlike the mice VNO, where detection of the trigeminal nerve endings in the sensory epithelium has not been reported [16, 19], the majority of the PLCβ2-positive cells in the vomeronasal sensory epithelium were surrounded by substance P-positive nerve fibers (Fig. 4d-4f). In the non-sensory epithelium, some PLCβ2-positive cells were surrounded by substance P-positive fibers (Fig. 4g-4i). In order to get more confirmation about the nature of the PLCβ2-positive cells, other markers for SCCs, such as α-gustducin and TRPM5, were examined. But, no positive immnuoreactivities could be demonstrated, probably due to the lack of cross reactivity to the camel.

Finally, we can conclude that the camel VNO owns differentiated, fully-matured receptor cells as well as the SCCs, which are not labeled by PGP 9.5 or OMP, but immunostained with PLCβ2 and innervated by the trigeminal nerve fibers. The SCCs may play an important role in the control of stimulus access to the camel VNO and avoid damage to the vomeronasal receptor cells in the desert environment where they can be subjected to continuous sources of irritant and harmful substances. Functional studies are required to see if these SCCs are involved in safeguarding of the receptor cells in the camel VNO by responding to a variety of compounds including strong odorants or bacterial signaling molecules, as has been shown for the SCCs in mice [19, 25].

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