Morphological Characteristics of the Vallate Papillae of the One-Humped Camel (Camelus dromedarius)

A. A. El Sharaby1*, M. A. Alsafy2, S. A. El-Gendy2 and S. Wakisaka3

Addresses of authors: 1 Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt; 2 Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt; 3 Department of Oral Anatomy and Developmental Biology, Osaka University Graduate School of Dentistry, Osaka, Japan

*Correspondence:
Tel.: +2 0106 4191 119;
fax: +2 045 3591 017;
e-mail: elsharaby@yahoo.com

With 7 figures

Received September 2011; accepted for publication February 2012
do: 10.1111/j.1439-0264.2012.01149.x

Summary

In this study, the morphology of the vallate papillae of camel was investigated using gross, light and scanning electron microscopy as well as immunohistochemistry. Vallate papillae were arranged along an identical line on each side of the lingual torus and revealed remarkable individual differences. However, each papilla – round or flat, small or large, single or paired – was surrounded by a prominent groove and an annular pad. Based on our findings, postnatal development and formation of new papillae occur in camel. Microscopically, taste buds were constantly observed along the medial wall epithelium, and in the papillary wall epithelium on both sides of the secondary groove apparently separating the vallate papillae. In addition, an aggregation of taste buds was occasionally observed at the bottom of the lateral wall epithelium. Using SEM, we observed several pits and microplicae on the surface of papillae as well as distinct taste pores on the peripheral parts of the dorsal surface. We demonstrated immunoreactivity of α-gustducin only in mature taste buds. We conclude that the morphological features and microstructure of vallate papillae are a characteristic feature in camel compared to other ruminants. These features might have evolved to assist the camel in the manipulation and tasting of thin organic stiff plants that grow in its environment and therefore might have related to the feeding habits of the animal.

Introduction

The morphologies of papillae on the dorsal tongue surface have previously been researched across numerous animals, enabling differentiation of papillae type and morphology according to animal food habits (Kubota, 1988). The one-humped camel (Camelus dromedarius) is a native animal to the dry climates, where thorny plants with rough and hard stems grow. Thus, the camel’s mouth is very sturdy and is developed to maintain efficient feeding of these plants and is rubbery so that thorns and branches won’t damage it (Sui et al., 1983). Up to our knowledge, few reports are available on the morphological features of the camel tongue (in adult: Qayyum et al., 1988; Erdunchaolu et al., 2001; Peng et al., 2008; and during the fetal period: Doughbag, 1988; Salehi et al., 2010). Likewise other ruminants, the foliate papillae are absent in camel and in place there are plenty of vallate papillae on the lingual torus. About 12–16 large flattened circumscribed papillae are arranged in two lines on both rims of the lingual torus, and each papilla is separated from a surrounding thick annular pad by a prominent gustatory groove. The vallate papillae on the outer line of papillae appeared to be larger than those in the inner line. Nevertheless, the nature and/or morphologies of the vallate papillae have not been previously investigated. According to Kubota (1988), there is an intimate relationship between the habitat, the feeding habits and the development of vallate papillae. In the process of species evolution, the number of vallate papillae increased like occurred in ruminants (Qayyum and Beg, 1975; Prakash and Rao, 1980; Chamorro et al., 1986; Scala et al., 1995; Kumar et al., 1998; Emura et al., 2000b; Tadjalli and Pazhoonand, 2004; Kurtul and Atalgin, 2008). The purpose
of the present study is to examine and compare with those in other ruminants the morphological and microstructural characteristics of the vallate papillae in the one-humped camel. As well, the relationship between these features and the feeding behaviour in ruminant animals is discussed.

Materials and Methods

Material collection and tissue preparation
The tongues of 15 clinically healthy camels of both sexes and different ages (2.5–7 years) were used in this study. Tongues were collected from Cairo slaughterhouses directly after slaughtering the animals and rinsed with 0.1 m phosphate buffer saline (PBS, pH 7.4). Regions of lingual torus containing vallate papillae were carefully dissected out and kept in neutral buffered formalin for at least a week prior to the subsequent processing. The gross morphology of all the tongues was carried out on both fresh and fixed specimens.

Light microscopy
For histological observation, tissue blocks were fixed in Bouin’s solution at room temperature for 2–3 weeks and processed in an automatic tissue processor (Histomatic Tissue Processor Y, Model 166MP, Fischer Scientific) to dehydrate them in ascending grades of ethanol, and clearing with xylene. These specimens were oriented transversely and serially sectioned using a microtome at 10 μm. The serial sections were collected on glass slides with 4–6 sections per slide, dried, and stained with hematoxylin and eosin. The sections were examined at various magnification levels on the light microscope (Zeiss Photomicroscope III).

Scanning electron microscopy (SEM)
The whole tongues were rinsed with 0.1 M phosphate buffer (pH 7.4), and all the vallate papillae were dissected out and fixed in a modified Karnovsky’s solution (Karnovsky’s, 1965) for overnight at 4°C. Samples were then post-fixed with 2% tannic acid and 1% OsO4 solution. After dehydration through a graded ethanol series at room temperature, specimens were substituted with 100% ethanol: t-butanol (1:1) for 1 h and t-butanol for 1–1.5 h at about 30°C and then kept overnight in t-butanol below 4°C (Inoue and Osatake, 1988; Kobayashi et al., 2003). The specimens were thereafter dried in t-butanol freeze dryer (JFD-300; Jeol, Akishima, Tokyo, Japan) and mounted on metal stubs and sputter-coated with platinum/carbon (plasma multicoater, PMC-5000; Meiwa, Osaka, Japan). The mounted specimens were observed at various angles under a scanning electron microscope (JSM-5310 LV SEM, Jeol) at accelerating voltages of 5 ± 10 kV.

Immunohistochemistry
We used polyclonal anti α-gustducin (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) to estimate the maturation of taste buds. Preparation of the antibodies and the procedures of avidin-biotin complex (ABC)
method we used in this study were achieved according to El Sharaby et al. (2006).

Results

Gross anatomy

The tongue of camel was elongated and dorsoventrally flattened along its cranial two-thirds with a rounded apex and a well-developed torus (Fig. 1a). There were 4–6 large vallate papillae arranged on each side closer to one another forming two lines almost parallel to the rim of lingual torus. The shape and size of the papillae revealed remarkable differences; they were not identical or symmetrical in the two lines in the same specimen (Fig. 1b–g). The cranially situated papillae were found most frequently smaller in size as compared to the caudal papillae of the same side. Accessory papillae, smaller in size, were frequently observed medial to the main papillae. Their number, size and shape as well as their relations to the larger papillae were also variable. In some specimens, two papillae were observed while being surrounded by a common annular pad and primary grooves. These papillae were separated by secondary grooves. Several profiles of vallate papillae were observed in young (Fig. 1b–e) and old animals (Fig. 2a–h): (1) Flattened papilla either rounded or oval in shape was centrally concave and surrounded by a complete groove and distinctly prominent annular pad (Fig. 2a). This was the most commonly observed papilla having 80–160 mm length and 50–85 mm width. (2) A prominent small-sized papilla was oval or rounded in shape and surrounded by a narrow shallow groove and annular pad (Fig. 2b). It was observed most frequently at the rostral or caudal aspect of the papillary line. (3) Flattened papilla was small in size and projected beyond the annular pad (Fig. 2c). (4) Centrally depressed papilla with peripheral edges at the same level with the annular pad (Fig. 2d). (5) Flattened papilla at early stage of developing an additional annular pad (Fig. 2e). (6) Two papillae very close to each other shared in part the annular pad, but each was surrounded by independent groove (Fig. 2f). Sometimes, a small or accessory papilla might be associated. (7) Two papillae were enclosed together by a common annular pad (Fig. 2g). (8) Two papillae were apparently fused and enclosed by a common annular pad (Fig. 2h).

Scanning electron microscopy

Small-sized papillae were observed in both the young and old animals. SEM observations revealed this papilla with a visibly elevated central-shaped doughnut region surrounded by a deep groove and annular pad. The surface of the papilla had micropapillae but lacked the taste pores (Fig. 3a,b). Medium-sized papillae were flattened oval in shape and separated from a prominent annular pad by a shallow groove. The peripheral parts of the surface were smooth, while the central parts had shallow grooves and micropapillae (Fig. 3c,d). Numerous, wide orifices of lingual glands, with diameters reaching about 50–100 μm,
were located on the dorsal surface of the papilla. Some taste pores were found at the peripheral areas close to the grooves (Fig. 3e). In old specimens, two flattened papillae were enclosed by a prominent annular pad and distinct primary and secondary grooves (Fig. 4a). The papillary surface had micropapillae and showed openings of the lingual glands (Fig. 4b). It was wrinkled and divided by shallow grooves and having micropapillae and desquamated cells (d). Some taste pores were found at the peripheral areas of the papilla close to the annular groove (e, arrows). The head arrows (c & e) refer to the orifices of lingual glands, with diameters reaching about 50–100 μm, which were frequently located toward the peripheral parts of the dorsal surface of the papilla.

Light microscopy

Histological observations showed that the vallate papillae were generally lined with keratinised stratified squamous epithelium, which was composed by basal, spinosum, granulosum and corneum layers (Fig. 5a). Lingual glands located in the deeper parts of the papillae, and they opened into the annular groove. The surface epithelium was less keratinised compared to that of the surrounding annular pad except the peripheral parts of the papilla. Dermal inter-digitation of variable sizes into the epidermis was observed along the whole surface. Few taste buds were observed along the medial papillary wall epithelium of the small-sized papillae, which was surrounded by shallow groove (data not shown). In the typical papillae, several taste buds with prominent taste pores were found along the whole length of the medial papillary wall epithelium. However, they were concentrated at the deeper parts of the medial wall (Fig. 5b, c). The taste buds were spindle- or columnar-shaped, 50 μm in width, and 50–150 μm in height. A taste pore was approximately 4 μm in diameter. In some specimens, an aggregation of taste buds was occasionally observed deeply at the bottom of the lateral groove wall close to the duct of Ebner glands (Fig. 5d). In some old specimens, two vallate papillae became very close to each other (separated or apparently fused) and surrounded by a common groove and annular pad (Fig. 6a).

The papillae were separated from the surrounding lingual epithelium by primary groove; meanwhile the two papillae were separated from each other by secondary groove (Fig. 6a). In some specimens, the latter led to a sinus deep to the two papillae. Taste buds were constantly observed in the entire length of the medial wall of the primary groove (Fig. 6b, d), and in the two side epithelium of the secondary groove (Fig. 6c). Meanwhile, we did not observe taste buds along the dorsal surface epithelium of the vallate papillae in the investigated specimens.

Immunolabelling of α-gustducin

The immunoreactivity for α-gustducin (−IR) was not observed in the small-sized or accessory papillae. In the medium-sized papillae, α-gustducin-IR was recognised within the taste buds only (Fig. 7a, b). The number of −IR cells per taste bud was variable according to the size of the papilla. Each −IR cell was spindled-shaped, longitudinally oriented having ovoid nuclei and a smooth regular outline throughout their length with a larger diameter at the nuclear region tapering to a smaller diameter at either ends. A pronounced −IR was evenly distributed throughout the cytoplasm from the apex, where apical processes converged at the taste pore to the basal region of the taste bud.

Discussion

In agreement with the previous studies (Qayyum et al., 1988; Erdunchaolu et al., 2001; Peng et al., 2008), the
present results demonstrated that the morphological features and microstructure of the vallate papillae are a characteristic feature in camel.

Spatial distribution and microstructure of the vallate papillae

It is well known that the number and distribution of vallate papillae varied between species from entirely absent, as in cape hyrax (Emura et al., 2008), single in mouse, rat and hamster (Iwasaki et al., 1997) to abundant as in ruminants (Qayyum and Beg, 1975; Prakash and Rao, 1980; Chamorro et al., 1986; Scala et al., 1995; Kumar et al., 1998; Emura et al., 2000b; Tadjalli and Pazhoohmand, 2004; Kurtul and Atalgin, 2008), and about 60 in the black rhinoceros (Emura et al., 2000a). In the present study, we found between 8 and 12 vallate papillae were lined up on the both rims of the lingual torus, which were frequently associated on their medial aspect with smaller papillae. This formation, which coincides with the findings of Qayyum et al. (1988) and Erdunchaolu et al. (2001), is the unique characteristic of the camilidae family, seems to compensate the absence of foliate papillae as suggested by Kubota (1988).

We observed annular pad in close association with each vallate papilla, which is said to regulate the access and retention of the saliva in the trench (Chamorro et al., 1986). The surfaces of vallate papillae were very irregular showing micropapillae and grooves and were not smooth as mentioned by Qayyum et al. (1988). We also found deep pits closely associated with taste pores and openings of the lingual glands, Ebner’s glands, on the peripheral
parts of the surface epithelium. These micropapillae and the grooves on the surface must protect the superficial cells keeping them moist by the secretion of lingual glands. They also direct the fluid toward primary and secondary grooves to wash away the gustatory materials from the taste buds that face the groove. Then, the taste buds can receive fresh stimulation by gustatory materials.

The distribution of taste buds shows some differences. Our results demonstrated numerous taste buds constantly along the entire length of the medial papillary wall, which coincide with those reported in camel (Qayyum et al., 1988; Erdunchaolu et al., 2001), cattle (Tabata et al., 2003; Karadag et al., 2010) and deer (Adnyane et al., 2011). In addition, we also observed taste buds
on both sides of the secondary groove and within the epithelial lining of deep sinuses in the lateral papillary wall, which has not been reported before. In contrast, Doughbag (1988) and Salehi et al. (2010) found taste buds on the dorsal surface of the vallate papillae of the embryonic tongue. It is not clear whether the taste buds also exist or not in the papillary side epithelium. It seems likely that taste buds appear first in the surface epithelium then in the papillary wall in the embryonic period. Mbiene and Roberts (2003) reported that a drastic shortfall occurs in taste buds in the surface epithelium and only the taste buds in the side wall epithelium which remain in the adult vallate papilla of rat.

Vallate papillae of camel continue development after birth?

In this study, we demonstrated several profiles of vallate papillae apparently at different stages of development in the tongue of camel. Definitive papillae are flattened, centrally concave and surrounded by a continuous groove and a distinctly prominent annular pad. Up to this stage, there were three stages: (1) Prominent small rounded papilla surrounded by a shallow groove and prominent annular pad, which had few taste buds. (2) Small flattened papilla projected beyond the annular pad. (3) Centrally concave papilla with peripheral edges at the same level with annular pad. Doughbag (1988) examined the prenatal development of vallate papillae in camel and reported that the papillae develop gradually in a caudo-rostral direction forming one column on each side of the lingual torus. We also found in older specimens of camel tongue, various degrees of fusion of two papillae and lay down of small or accessory papillae arranged constantly medial to the larger or main papillae. This formation has not been recorded in other animals including ruminants and may be characteristic only for camel. It seems that the camel vallate papillae may have a degree of development and growth during life, which seems to be correlated with seasonal variations or feeding habits.

Immunoreactivity of taste buds and gustatory functions of camel vallate papillae

In this study, we used $\alpha$-gustducin to estimate the maturation of taste buds (i.e. presence of $\beta$-IR type II cells). We did not demonstrate $\alpha$-gustducin in the taste buds in the small-sized and accessory papillae. The taste buds in these papillae are apparently immature. Meanwhile, taste buds in the medium-sized and larger papillae showed $\alpha$-gustducin-IR. The demonstrated $\beta$-IR cells are typically similar to those observed in cattle and referred as mature type II cells (Tabata et al., 2003).

Finally, the present results of the vallate papillae in camel exhibited some different characteristics from other domestic ruminants. This supports the hypothesis of Kubota (1988) that morphology of the vallate papillae have evolved in correlation with the habitat and feeding behaviour. These unique morphological characteristics assist the camel in the prehension and manipulating of inorganic stiff plants that grow in its favourable environment.

References


