Chem. Senses 24: 469–472, 1999

α-Gustducin-immunoreactive Solitary Chemosensory Cells in the Developing Chemoreceptorial Epithelium of the Rat Vallate Papilla

Andrea Sbarbati, Caterina Crescimanno, Paolo Bernardi and Francesco Osculati

Institute of Human Anatomy and Histology, University of Verona, Verona, Italy

Correspondence to be sent to: Andrea Sbarbati, Institute of Anatomy and Histology, University of Verona, Medical Faculty, Strada Le Grazie 8, I-37134, Verona, Italy. e-mail: sbarbati@borgoroma.univr.it

Abstract

The presence of solitary chemosensory cells was studied in rat vallate papillae during the first week of post-natal life by α-gustducin immunocytochemistry. In 1- to 3-day-old rats, isolated α-gustducin-immunoreactive cells were found within the epithelium of the vallate papilla. These cells, mainly located in the basal layer, were scattered among keratocytes and wrapped in α-gustducin-negative epithelial cells in a glia-like fashion. The α-gustducin-immunoreactive cells were usually round and some of them gave rise to short, large processes directed towards the lumen of the oral cavity or the basal lamina. Rarely, some cells showed an evident bipolar shape. Small taste buds containing either α-gustducin-immunoreactive or α-gustducin-negative cells appeared in the vallate papillae of 4-day-old rats in which isolated, bipolar-shaped α-gustducin-immunoreactive cells were also found. After the first week of post-natal life, the taste buds appeared basically similar to those of adult animals. In conclusion, the present study demonstrates that the presence of epithelial cells with characteristics of solitary chemosensory cells precedes the development of the taste buds.

Introduction

In a previous study, we demonstrated that during the first days of post-natal life the epithelia of rat vallate papillae contain isolated cells with a bipolar shape, nerve contacts and neuroendocrine-type granules (Sbarbati *et al*., 1999). On the basis of ultrastructural characteristics, we suggested that these elements could be homologous to the solitary chemosensory cells (SCCs) described in aquatic vertebrates. In these latter animals, feeding and exploration of the chemical environment is performed using, in addition to the olfactory and to the gustatory systems, a further kind of epithelium-nerve organization based on the presence of SCCs. The elementary unity of this chemoreceptorial structure differs from the taste buds of the gustatory apparatus, and consists of a single epithelial cell contacted by nerves and lacking a specialized connective bed. In fishes, SCCs form a system of differentiated sensory epithelial cells which is not organized into discrete end organs and may occur in the epithelia of the oropharynx, the gills and the skin (Whitear, 1992). In the lamprey, the corresponding cells have been called oligovillous cells (Whitear and Lane, 1983). To date, SCCs are considered to be typical for aquatic vertebrates and seem to be absent in the skin of mammals, although some data suggest that cells with characteristics of SCCs may be present in the digestive apparatus of rodents (Hofer and Drenckhahn, 1996; Hofer *et al*., 1996; Luciano and Reale, 1997).

In the rat, a demonstration of elements with the morphological characteristics of SCCs in an early stage of development of the gustatory epithelium may be relevant to understanding the ontogeny of the chemoreceptive system of the oral cavity. We have not found a description of similar cells in previous studies on taste bud development (Farbman, 1965; Miller and Chaudhry, 1974; Hoseley and Oakley, 1987; Miller and Smith, 1988; Farbman and Mbiene, 1991; Oakley *et al.*, 1991; Barlow *et al.*, 1996; Wakisaka *et al.*, 1996; Witt and Reutter, 1996; Northcutt and Barlow, 1998). Therefore, further data are necessary to assess the origin, eventual functional role and fate of these cells.

To further characterize these cells, we have studied their immunoreactivity for α-gustducin during early post-natal development. Taste-specific G protein was first demonstrated in rats (McLaughlin *et al.*, 1992) and then confirmed in man (Takami *et al.*, 1994). α-Gustducin is expressed specifically in taste cells of the circumvallate, foliate and fungiform papillae of rat lingual tissue. In rat vallate taste buds, α-gustducin has been found in cells with characteristics of Type II (light) cells (Boughter *et al.*, 1997). Other authors have suggested that α-gustducin is also expressed in the cytoplasm of Type III cells and probably in microvilli of Type I cells of the vallate taste buds (Menco *et al.*, 1997). Therefore, α-gustducin is considered to be a potent marker of cells which are chemosensitive, and the visualization of an eventual α-gustducin immunoreactivity (IR) in the

developing gustatory epithelium could provide information about the relationship between SCCs and the taste organs.

Material and methods

The study was performed on Wistar rats at different ages, ranging from immediately after birth to 11 days old, and maintained at the departmental animal facility. Animals were anesthetized with ether and killed by dislocation of cervical vertebrae. Ten rats were perfused i.a. with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Following the perfusion fixation, the lingual tissue was postfixed by immersion for 1 h in the fixative. Afterwards, the tissue was transferred in a 10% sucrose solution in phosphate buffer. Blocks of lingual tissue containing the vallate papillae, as well as adjacent epithelium, muscles and glands, were dissected. For immunohistochemistry, tissue blocks were sectioned on a sliding, freezing microtome. Parallel free-floating sections (30 μm) were collected in phosphate buffered saline (PBS) (pH 7.4) and washed in several changes of this solution before further processing.

In another seven rats the tongues were removed after death and fixed by immersion in 4% neutral buffered formalin for 2–6 h at 4°C, rapidly dehydrated by alcohol steps, transferred to xylene and embedded in paraffin (melting point 52°C; Merck, Darmstadt, Germany). Paraffin sections (6–8 μm) were cut and stretched at 45°C, allowed to dry and stored at 4°C until use.

For the immunohistochemical experiments free-floating sections were processed without pre-treatment, while paraffin sections were deparaffinized in xylene and dehydrated in a graded series of ethanol.

Free-floating as well as paraffin sections were incubated for 10 min with 3% hydrogen peroxide in methanol to inhibit endogenous peroxidases. Sections were then incubated for 15–20 min with 3% normal swine serum diluted in PBS/bovine albumin serum (PBS/BSA) 1.5% (pH 7.4). Afterwards, the sections were incubated for 2 h with the primary antibody anti-α-gustducin (Santa Cruz Biotechnology Inc., Heidelberg, Germany, Cat. No. sc-395, dilution 1:200). Sections were washed in PBS and then incubated with a secondary antibody (biotinylated swine anti-rabbit, Code No. E353) (Dako, Glostrup, Denmark, dilution 1:400). An avidin–biotin complex (ABC) technique was used to reveal sites of antigen–antibody reaction. For the ABC method a commercial kit (ABComplex/HRP code no. K0355, Dako) was used. Kit instructions were followed with regard to dilution and incubation times.

Peroxidase activity was revealed by diaminobenzidine (Sigma; St Louis, MO). The sections were then dehydrated through an ethanol series, cleared in xylene and coverslipped with Entellan.

Parallel to the above immunohistochemical procedures, controls were performed by replacing the primary antibody with 10% non-immune serum or with PBS/BSA 1.5%. Further controls were carried out by omitting the secondary antibody.

Results

In 1- to 3-day-old rats, vallate papillae were already evident. The epithelium of the vallum was rather thin and, indeed, was composed of five or six layers of cells. In this epithelium, isolated α-gustducin-immunoreactive (IR) cells were found (Figure 1a,b). These cells, mainly located in the basal layer of the epithelium, were scattered among keratocytes and were often in contact with the basal lamina (Figure 1a,b). They were wrapped in α-gustducin-negative cells in glia-like manner. A clear extracellular space was often visible around the latter. The α-gustducin-IR cells were round, but often gave rise to short, large processes directed toward the lumen or the basal lamina (Figure 1a). Rarely, a bipolar shape was evident.

During the first 3 days after birth, we rarely found more than one α-gustducin-IR cell in the same bud-like structure. In 4- to 5-day-old rats, the pattern of the chemosensory epithelium was more pleomorphic, and we observed taste buds composed of both α-gustducin-IR and α-gustducin-negative cells (Figure 1c,d). Isolated, bipolar-shaped α-gustducin-IR cells were also found.

After the first week of post-natal life, the taste buds appeared basically similar to those of adult animals, being composed of numerous α-gustducin-IR cells separated by α-gustducin-negative cells (Figure 1e,f).

The controls were always negative.

Conclusion

The present work demonstrates that single cells present in the vallate papillae of the rat during the first days of extrauterine life are α-gustducin-IR, similar to clustered taste cells located in the buds of adult rodents (McLaughlin *et al.*, 1992; Boughter *et al.*, 1997). Studies in α-gustducin-knockout mice implicate this protein subunit in the transduction of both bitter- and sweet-tasting substances (Wong *et al.*, 1996) and therefore the present work is consistent with the hypothesis that these elements are related to the chemoceptive cell line. This result is also consistent with our previously reported electron microscopic findings which demonstrated nerve fibers contacting isolated bipolar cells in the developing chemoreceptive epithelium (Sbarbati *et al.*, 1999). The present work also provides further data on the morphology and location of these cells, as we have demonstrated that during the first days of post-natal life, these cells are round elements which do not seem to reach the free surface of the epithelium. A relationship with the basal membrane does not seem to be constant, although it is difficult to evaluate this relationship at the light microscopic level. The finding that in the posterior portion of the tongue, the presence of α-gustducin-IR cells is limited to the vallate papilla suggests that the future gustatory epithelium is

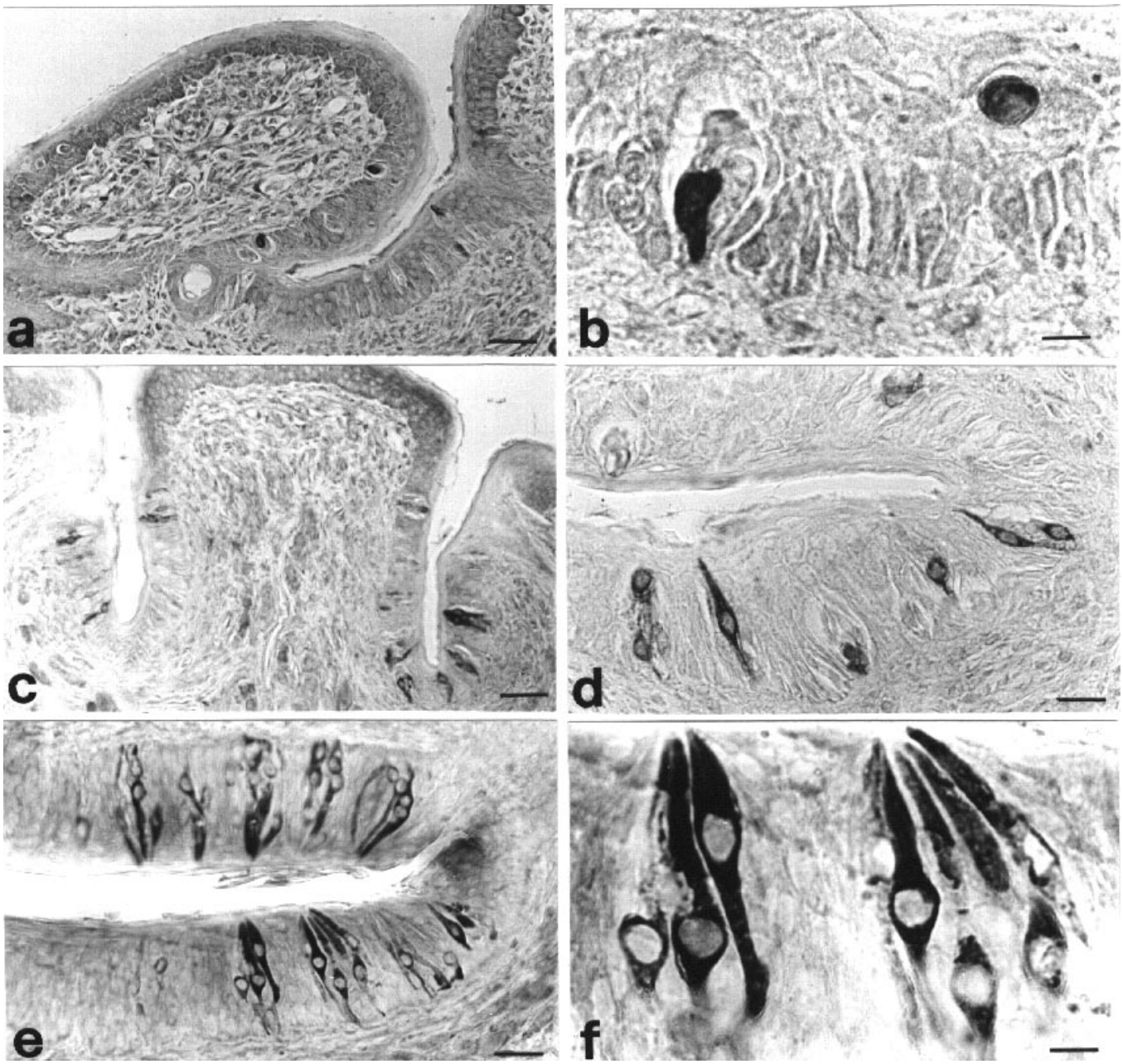

Figure 1 α-Gustducin-IR in developing gustatory epithelium of rat circumvallate papilla. **(a)** Isolated immunoreactive cells are clearly visible in a 1-day-old rat. Bar = 60 μm. **(b)** A higher-power view of α-gustducin-IR cells in a 2-day-old rat. Some cells give rise to short processes directed towards the lumen or the basal lamina (bar = 7 μm). In **(c)**, a 4-day-old rat, small taste buds composed both of α-gustducin-IR and gustducin negative cells are visible (bar = 45 μm). In **(d)**, a 6-day-old rat, isolated, bipolar-shaped α-gustducin-IR cells are present (bar = 18 μm). In **(e)**, numerous α-gustducin-IR cells are contained in adult rat taste buds (bar = 29 μm). **(f)** A high-power view of two taste buds within adult circumvallate papilla (bar = 8 μm).

determined before the appearance of the taste buds. The immunocytochemical and ultrastructural features (tonofilaments, desmosomes) of the α-gustducin-IR cells demonstrate that they are epithelial cells. This conclusion seems to be in agreement with recent studies on the ontogeny of the gustatory epithelium (Stone *et al.*, 1995; Barlow *et al.*, 1996). In addition, the present work demonstrates that the cells wrapping the putative chemoceptive cells in a glia-like manner are α-gustducin-negative. Further studies seem to be necessary to evaluate an eventual relationship between these cells and α-gustducin negative cells present in taste buds.

Taken together with our previous ultrastructural data, the present immunocytochemical findings suggest that SCCs may be present in the oral cavity of mammals. This may be a parallel to the findings in aquatic vertebrates. Further data

are necessary to determine if a relationship exists between the SCCs present in the developing gustatory epithelium and the α-gustducin-IR isolated cells which are located in other portions of the digestive system, such as the epithelia of the pancreatic ducts or gallbladder (Hofer and Drenckhahn, 1996; Hofer *et al.*, 1996). It has been suggested that these latter cells may be also solitary chemoreceptors. At the present phase of study, to speculate about possible functional roles of the cells that we have described in the vallate papilla is difficult. However, in our opinion, the demonstration of the presence of these cells could be important because it suggests that oral chemoreception in the suckling rats could be mediated by an SCC system. The cells that we have described precede the development of taste buds and, since similar elements have not been described in adult mammals, it is possible that their importance is limited to a short period of post-natal life. In the following days, the SCCs could be removed by apoptosis or be incorporated in the taste buds. The latter hypothesis seems to be in accordance with the suggestion that taste buds may be compound sensory organs containing several cell types, one of which may be an SCC (Sbarbati *et al.*, 1990; Osculati and Sbarbati, 1995; Finger, 1997).

References

Barlow, L.A., Chien, C.B. and **Northcutt, R.G.** (1996) *Embryonic taste buds develop in the absence of innervation*. Development, 122, 1103–1111.

Boughter, J.D., Pumplin, D.W., Yu, C., Christy, R.C. and **Smith, D.V.** (1997) *Differential expression of α-gustducin in taste bud populations of the rat and hamster*. J. Neurosci., 17, 2852–2858.

Farbman, A.I. (1965) *Electron microscope study of the developing taste bud in rat fungiform papilla*. Dev. Biol., 11, 110–135.

Farbman, A.I. and **Mbiene, J.P.** (1991) *Early development and innervation of taste bud-bearing papillae on the rat tongue*. J. Comp. Neurol., 304, 172–186.

Finger, T.E. (1997) *Evolution of taste and solitary chemoreceptor cell systems*. Brain Behav. Evol., 50, 234–243.

Hofer, D. and **Drenckhahn, D.** (1996) *Cytoskeletal markers allowing discrimination between brush cells and other epithelial cells of the gut including enteroendocrine cells*. Histochem. Cell Biol., 105, 405–412.

Hofer, D., Puschel, B. and **Drenckhan, D.** (1996) *Taste receptor-like cells in the rat gut identified by expression of α-gustducin*. Proc. Natl Acad. Sci. USA, 93, 6631–6634.

Hoseley, M.A. and **Oakley, B.** (1987) *Development of the vallate papilla and taste buds in rats*. Anat. Rec., 218, 216–222.

Luciano, L. and **Reale, E.** (1997) *Presence of brush cells in the mouse gallbladder*. Microsc. Res. Tech., 38, 598–608.

McLaughlin, S.K., McKinnon, P.J. and **Margolskee, R.F.** (1992) *Gustducin is a taste-cell-specific G protein close related to the transducins*. Nature, 357, 563–569.

Menco, B., Yankova, M.P. and **Simon, S.A.** (1997) *Freeze-substitution and post-embedding immunocytochemistry on rat taste-buds: G-proteins, calcitonin-gene-related peptide (CGRP), and choline acetyl transferase (ChAT).* Microsc. Microanal., 3, 53–69.

Miller, R.L. and **Chaudhry, A.P.** (1974) *An ultrastructural study on the development of vallate taste buds of the golden Syrian hamster*. Acta Anat., 95, 190–206.

Miller, I.J., Jr and **Smith, D.V.** (1988) *Proliferation of taste buds in the foliate and vallate papillae of postnatal hamsters*. Growth Dev. Aging, 52, 123–131.

Northcutt, R.G. and **Barlow, L.A.** (1998*) Amphibians provide new insights into taste-bud development*. Trends Neurosci., 21, 38–43.

Oakley, B., LaBelle, D.E., Rilay, R.A., Wilson, K. and **Wu, L.H.** (1991*) The rate and locus of development of rat vallate taste buds*. Dev. Brain Res., 58, 215–221.

Osculati, F. and **Sbarbati, A.** (1995) *The frog taste disc: a prototype of the vertebrate gustatory organ*. Prog. Neurobiol., 46, 351–399.

Sbarbati, A., Zancanaro, C., Franceschini, F., Balercia, G., Morroni, M. and **Osculati, F.** (1990) *Characterization of different micro-environments at the surface of the frog's taste organ*. Am. J. Anat., 188, 199–211.

Sbarbati, A., Crescimanno, C., Benati, D. and **Osculati, F.** (1999) *Solitary chemosensory cells in the developing chemoreceptorial epithelium of the vallate papilla*. J. Neurocytol., 27, 631–635.

Stone, L.M., Finger, T.E., Tam, P.P. and **Tan, S.S.** (1995*) Taste receptor cells arise from local epithelium, not neurogenic ectoderm*. Proc. Natl Acad. Sci. USA, 92, 1916–1920.

Takami, S., Getchell, T.V., McLaughlin, S.K., Margolskee, R.F. and **Getchell, M.L.** (1994) *Human taste cells express the G protein α-gustducin and neuron-specific enolase*. Mol. Brain Res., 22, 193–203.

Wakisaka, S., Miyawaki, Y., Youn, S.H., Kato, J. and **Kurisu, K.** (1996) *Protein gene-product 9.5 in developing mouse circumvallate papilla: comparison with neuron-specific enolase and calcitonin gene-related peptide*. Anat. Embryol., 194, 365–372.

Whitear, M. (1992) *Solitary chemoreceptor cells*. In Hara, T.J. (ed.), Fish Chemoreception. Amsterdam, Elsevier Press, pp. 103–125.

Whitear, M. and **Lane, E.B.** (1983) *Oligovillous cells of the epidermis: sensory elements of lamprey skin*. J. Zool. Lond., 199, 359–384.

Witt, M. and **Reutter, K.** (1996) *Embryonic and early fetal development of human taste buds: a transmission electron microscopical study*. Anat. Rec., 246, 507–523.

Wong, G.T., Gannon, K.S. and **Margolskee, R.F.** (1996) *Transduction of bitter and sweet taste by gustducin*. Nature, 381, 796–1003.

Accepted May 17, 1999