

Recovery of Salt Taste Responses and PGP 9.5 Immunoreactive Taste Bud Cells during Regeneration of the Mouse Chorda Tympani Nerve

Keiko Yasumatsu, Noriatsu Shigemura, Ryusuke Yoshida and Yuzo Ninomiya

Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University, Fukuoka 812-8582, Japan

Correspondence to be sent to: Yuzo Ninomiya, e-mail: nino@dent.kyushu-u.ac.jp

Key words: amiloride, fungiform papillae, PGP9.5, regeneration, sodium taste, taste nerve

Introduction

Taste receptor cells are replaced with an average life span of ~10 days in mammals. This turnover is accompanied by continuing synaptic reconnection between newly formed taste cells and gustatory fibers. However, little is known of how sensory information of the fibers is maintained during the synaptic reconnection. Our previous study examined taste responses of regenerated mouse chorda tympani (CT) fibers and revealed that each fiber type classified based on sensitivity to amiloride maintains its characteristics after synapse reformation between regenerated taste axons and receptor cells. That is, we found that there are approximately equal numbers of two types of NaCl-responsive neurons; one type showed strong suppression by amiloride [amiloride-sensitive (AS), N-type fiber], and the other type showed only weak or no suppression by amiloride [amiloride-insensitive (AI), E-type fiber] in intact, regenerated and cross-regenerated taste nerve (Ninomiya, 1998). Our subsequent study investigated the processes of reformation of AI and AS neural systems during the CT regeneration. We postulated and verified whether incoming regenerated CT axons would (i) induce AI and AS properties after synapse formation with identical progenitors; (ii) innervate taste cells randomly followed by elimination of mismatched branches; or (iii) selectively innervate AS or AI taste progenitor cells (Yasumatsu *et al.*, 2003). The results revealed that NaCl responses of the CT recovered from 3 weeks after the nerve crush and most NaCl responsive fibers showed AI (E-type). The number of fibers responding to NaCl after amiloride formed a bimodal distribution from 4 weeks and there were no clusters of fibers with intermediate sensitivity to amiloride (Yasumatsu *et al.*, 2003, figure 6). Moreover, N-type and E-type were clearly different in K_d value and response selectivity (KCl/NaCl response ratios) right from the beginning of their appearance. Thus, these findings from our electrophysiological studies are consistent with the view that regenerating taste axons selectively innervate their corresponding classes of taste progenitor cells. In the present study, to test the possibilities further, we examined PGP9.5, a maker of neurons (Thompson *et al.*, 1983) and sensory paraneurons (Iwanaga *et al.*, 1992), immunoreactive (IR) taste bud cells and the number of taste buds after crushing the mouse CT nerve.

Materials and methods

Experimental manipulation

Subjects were adult male and female C57BL/6N Crj mice (Charles River Japan, Tokyo, Japan), 8–20 weeks of age, ranging in weight from 20 to 32 g. At 8–10 weeks of age, mice were divided into five groups, one intact control group and four nerve-crush groups, respectively 2, 3, 4 and ≥ 5 weeks after bilateral CT nerves were crushed. Animals were anesthetized with pentobarbital sodium (40–50 mg/kg i.p.), and the bilateral CT nerves were exposed at ~5 mm rostrally apart from their entry to the bulla and repeatedly crushed at a single point with number 5 forceps.

Tissue preparation

The animals from the five groups were anesthetized with pentobarbital and perfused through the left ventricle with a physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Tongues were excised and immersed in the same fixative for 3 h at 4°C and then rinsed with 0.1 M PB containing 20% sucrose for 12 h. The tongues were divided longitudinally at the median sulcus into two pieces with equal width, one piece for immunohistochemistry of PGP 9.5 and the other for hematoxylin and eosin staining to measure the number of taste buds. Cryostat sections were cut and thawed onto gelatine-coated slides.

Immunohistochemistry

Sections, 10 μ m thick, were treated with 10% normal donkey serum in PBS for 60 min, having first been immunoreacted with rabbit anti-PGP9.5 polyclonal antibody (Biogenesis) at a dilution of 1:600 in PBS for 12 h at 4°C. The sections were then reacted with fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG (Jackson Labs) at a dilution of 1:600 for 2 h at room temperature. Slides were rinsed with PBS and coverslipped. PGP9.5-IR cells were counted in complete sets of serial sections of randomly selected taste buds. The number of taste buds with taste pores was counted in the remaining halves of the tongues. Complete serial sections with 20 μ m thickness were stained with hematoxylin and eosin. All images were collected with a CCD camera using DP controller (Olympus) software.

Results and discussion

Fibers and bundles in the containing PGP 9.5 were frequently seen in the whole papilla, including the epithelium and the core of the papilla containing connective tissue. Fibers were seen entering epithelium and taste buds when taste buds were present (Figure 1A,C–E). Some taste bud cells were found to exhibit PGP 9.5-IR. The immunoreactive cells are slender and contain round or invaginated nucleus (Figure 1A). At 2 weeks after crushing the CT, dermal indentations into the epithelium, papillae without taste buds, could be seen (Figure 1B). There was no significant difference in the number of papillae among all groups (data not shown). Nerve fibers often penetrate into epithelium of the papillae (Figure 1B), and some fungiform papillae contain one taste bud primordium or a taste bud containing a taste pore. The existence of a taste pore is often used as a criterion of functional maturation of regenerating and developing taste buds (Segerstad and Hellekant, 1989; Harada *et al.*, 2000). The mean number of PGP-IR cells in a bud was 47.6% of the control PGP-IR cells (Table 1). The CT nerve of this time responded to tactile but not to taste stimuli. Cheal *et al.* (1977) reported that it required about three days for axons to re-establish a taste bud with functional receptors in gerbils. It may be plausible that some molecules involved in taste transduction or synapses did not exist. At 3 weeks, the number of taste buds containing a pore increased to 50% of control. The

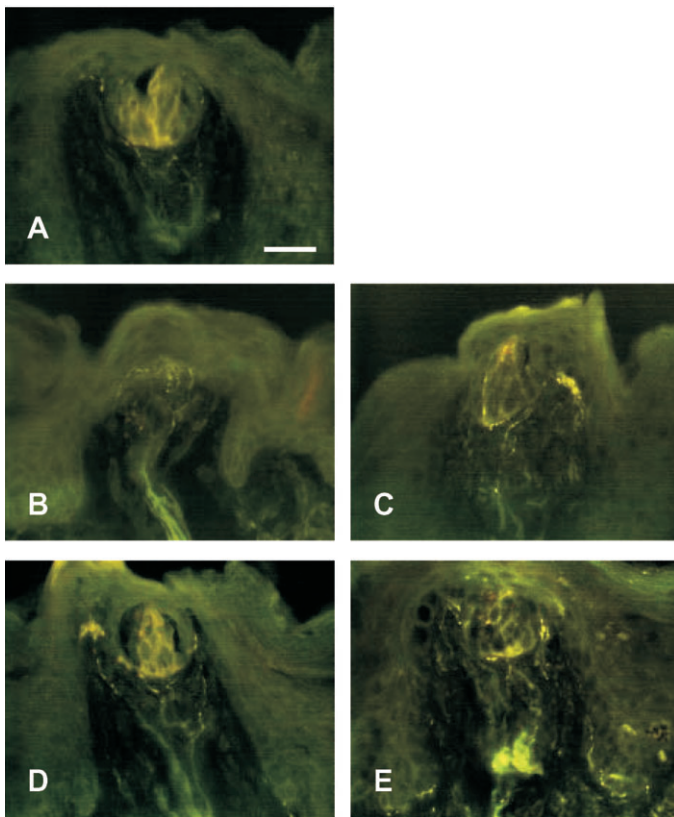


Figure 1 Immunohistochemical detection of PGP9.5 in regenerating mouse fungiform papillae. **(A)** Some taste bud cells of control fungiform papilla were found to exhibit PGP 9.5-IR. **(B)** Fungiform papillae without a taste bud were often seen in 2 weeks after crushing CT. **(C)** No PGP-IR cell was detected in this section of 3 weeks. **(D)** At 4 weeks, some taste bud cells showed PGP-IR. **(E)** At ≥ 5 weeks, the number of PGP-IR cells and taste buds increased.

number of taste buds with a pore in a papilla was 0.43, and PGP-IR cells in a bud were 76.2% of the control (Figure 1C). In 2–3 weeks, although no AS taste responses were observed in the CT, there were taste bud cells expressing α , β , γ subunits of ENaC (Shigemura *et al.*, unpublished observation), suggesting differentiation of taste cells is independent of synapse formation. These results weaken possibility (i); regenerated CT axons would induce AI and AS properties after synapse formation with identical progenitors. At 4 weeks, the proportion of buds with a pore and the number of PGP-IR cells became larger than that at 3 weeks after nerve crush (Figure 1D). At >5 weeks, the number of taste buds with a pore in a papilla and PGP-IR cells increased, suggesting functional recovery of these papillae (Figure 1E). If the number of PGP-IR cells decreased after

Table 1 Percent control number of PGP-IR cells, taste buds with a pore and number of taste buds with pore in a papilla in control and experimental groups

	Percent of control number of PGP-IR cells	Percent of control number of taste buds with a pore	Number of taste buds with a pore in a papilla
Control	100	100	0.84
2 weeks	47.6	17.9	0.19
3 weeks	76.2	50.0	0.43
4 weeks	85.7	76.4	0.66
≥ 5 weeks	90.5	70.8	0.70

increment, possibility (ii) might be supported. Although in fact, the number of PGP-IR cells and taste buds kept increasing during the course of regeneration. This observation is consistent with the finding that impulse discharges of both E-type and N-type fibers kept increasing during regeneration (Yasumatsu *et al.*, 2003). Taken together, in the present study, results suggest that (iii) individual axons in each type may increase branches to their matched type of taste progenitor cells with maintaining the selectivity.

Acknowledgements

This work was supported by Grant-in-Aids 15209061 (Y.N.) and 16791127 (N.S.) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- Cheal, M., Dickey, W.P., Jones, L.B. and Oakley, B. (1977) Taste fiber responses during reinnervation of fungiform papillae. *J. Comp. Neurol.*, 172, 627–646.
- Harada, S., Yamaguchi, K., Kanemaru, N. and Kasahara, Y. (2000) Maturation of taste buds on the soft palate of the postnatal rat. *Physiol. Behav.*, 68, 333–339.
- Segerstad, H.C. and Hellekant, G. (1989) Changes in number and morphology of fungiform taste buds in rat after transection of the chorda tympani or chorda-lingual nerve. *Chem. Senses*, 14, 335–348.
- Iwanaga, T., Han, H., Kanazawa, H. and Fujita, T. (1992) Immunohistochemical localization of protein gene product 9.5 (PGP9.5) in sensory paraneurons of the rat. *Biomed. Res.*, 13, 225–230.
- Ninomiya, Y. (1998) Reinnervation of cross-regenerated gustatory nerve fibers into amiloride-sensitive and amiloride-insensitive taste receptor cells. *Proc. Natl Acad. Sci. USA*, 95, 5347–5350.
- Thompson, R.J., Doran, J.F., Jackson, P., Dhillon, A.P. and Rode, J. (1983) PGP 9.5—a new marker for vertebrate neurons and neuroendocrine cells. *Brain Res.*, 278, 224–228.
- Yasumatsu, K., Katsukawa, H., Sasamoto, K. and Ninomiya, Y. (2003) Recovery of amiloride-sensitive neural coding during regeneration of the gustatory nerve: behavioral-neural correlation of salt taste discrimination. *J. Neurosci.*, 23, 4362–4368.