Immunohistochemical detection of neurotrophin-3 and -4, and their receptors in mouse taste bud cells*

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Summary. Neurotrophin-3 (NT3) and neurotrophin-4 (NT4) affect the survival and maintenance of central and peripheral neurons. Using an immunohistochemical method, we examined whether the taste bud cells in the circumvallate papillae of normal mice expressed NT3, NT4, and their respective receptors TrkB and TrkC, and if so, what type of cells in the taste buds expressed them. Double immunostaining for either of them and PGP 9.5, NCAM, or gustducin was used to determine which cell types expressed which neurotrophins and receptors. Normal taste bud cells expressed NT3, NT4, and the TrkB receptor, but not TrkC. The percentage of NT3-immunoreactive cells among all taste bud cells was 89.0%, that of NT4-immunoreactive cells, 58.6%, and that of TrkB-immunoreactive cells, 80.8%. Almost none of the NT4-immunoreactive cells were reactive with anti-PGP 9.5 or the anti-NCAM antibody, but they could be stained with anti-gustducin, revealing that NT4-immunoreactive cells were contained only in the type-II—possibly type-I—cell population. On the other hand, NT3-, and TrkB-immunoreactive cells included type-III cells, together with type-II, -I, and basal cells, because they were positive for PGP 9.5 and gustducin. We conclude that NT4 may exert trrophic actions on all types of taste bud cells by binding to their TrkB receptors, and NT3 may also have a similar, though negligible role.

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Introduction

Taste bud cells have been reported to express specific neurotrophic factors such as NGF (nerve growth factor), BDNF (brain-derived neurotrophic factor), NT3 (neurotrophin-3), GDNF (glial cell line-derived neurotrophic factor), and NTN (neurturin), which promote the development, differentiation, and survival of central and peripheral neurons (Snider, 1994; Nosrat et al., 1996; Takami et al., 1996; Chou et al., 2001; Ganchrow et al., 2003a; Uchida et al., 2003; Yee et al., 2003; Takeda et al., 2004; Kawakoshi et al., 2005).

NGF, BDNF, and NT3, as well as NT4 (neurotrophin-4), form a group of neurotrophins. The functions of these neurotrophins are mediated by their interactions with three high-affinity Trk (tyrosine kinase) receptors. NGF binds to and activates the TrkA receptor, BDNF and NT4 bind to the TrkB receptor, and NT3 binds to the TrkC and weakly to the TrkA and B receptors (Gotz and Scharlt, 1994; Klein, 1994; Snider, 1994; Dechant, 2001; Agerman et al., 2003). NT3 and NT4 have been cloned on the basis of their sequence homologies to NGF and BDNF (Maisonpierre et al., 1990; Jp et al., 1992).

In mutant mice lacking BDNF, TrkB, NT4, or NT3, substantial neuron loss of nodosa-petrosal, geniculate, and vestibular ganglia, in addition to trigeminal ganglion cells, has been observed (Klein et al., 1993; Snider 1994; Liu et al., 1995). In addition, BDNF-, NT4-, or TrkB-deficient mice show reductions in gustatory innervation and numbers of gustatory papillae and taste buds (Nosrat et al., 1997; Zhang et al., 1997; Oakley, 1998; Lieble et al., 1999; Mistretta et al., 1999).

Electron microscopic observations have shown that taste buds possess four distinct types of cells, three of which—types I, II, and III—differentiate from immature basal type-IV cells that originate from the surrounding epithelial basal
cells. The type-I cells contain dense secretory granules that are the secretory precursors of the dense substance in the pores of taste buds. The type-II cells are characterized by an abundance of vesicles and smooth-surfaced endoplasmic reticulum. Type-III cells make afferent synaptic contacts with nerve terminals (Murray et al., 1969; Fujimoto and Murray, 1970; Takeda and Hoshino, 1975; Farbman et al., 1985). By use of an immunoelectron microscopic method, the expression of NCAM (neural cell adhesion molecule) was detected in the cytoplasmic membranes of the type-III cells in mice (Takeda et al., 1992), that of gustducin (a taste bud cell-specific G protein) in the cytoplasm of the type-II cells in rats (Yang et al., 2000), and that of PGP9.5 (protein gene product 9.5) mainly in the cytoplasm of the type-III and to a small level in the type-II cells in rats (Yee et al., 2001). NCAM may be involved in the adhesion of synaptic contacts in the taste buds (Takeda et al., 1992). PGP 9.5 is a soluble protein found in human brain extracts and represents a major component of the neuronal cytoplasm (Thompson et al., 1983). Gustducin is most closely related to the transducins and believed to be a principal mediator of bitter and sweet taste transduction (McLaughlin et al., 1992; Wong et al., 1996). PGP 9.5 or NCAM does not coexist with gustducin within the same taste bud cells; in contrast, PGP 9.5 and NCAM almost always coexist (Yee et al., 2001; Takeda et al., 2004). There have been some reports concerning the relationship between the types of taste bud cells and the neurotrophic factors or their receptors in mice. GDNF was reported to be expressed in Type-II cells, for the reason that GDNF and gustducin coexist in the same taste bud cells (Takeda et al., 2004). Also, it was reported that type-III cells, which are immunoreactive with anti-NCAM or anti-PGP 9.5, contain NGF, TrkA, BDNF, TrkB, GFRα1 (a receptor of GDNF), NTN, and GFRα2 (a receptor of NTN) although other types of cells are also immunoreactive with these antibodies (Uchida et al., 2003; Takeda et al., 2004; Kawakoshi et al., 2005). Until now, there have been no reports concerning the expression of NT4 protein in taste bud cells or whether type-III cells in the taste buds express NT3 and NT4.

We therefore examined immunohistochemically whether the taste bud cells in the circumvallate papillae of normal mice expressed NT3 and NT4 and their respective receptors, TrkC and TrkB, and if so, what type of cells in the taste buds expressed them. Double immunostaining was used to determine which cell types expressed which neurotrophins and receptors.

### Materials and Methods

#### Animals

Adult ddY strain mice (Sankyo Labo Service Co., Tokyo), 6–20 weeks old (30–50 g body weight), were housed in rooms with a controlled temperature (25°C) and humidity (60%). They received food and water *ad libitum* and were kept under a 12-h dark/12-h light cycle before use. All animal procedures were performed in accordance with The Guide to Experiments with Animals of Health Sciences University of Hokkaido, and approved by the Management Committee of the Laboratory Animal Center at the Health Sciences University of Hokkaido.

#### Immunohistochemistry

Twenty untreated normal mice were intraperitoneally anaesthetized with Nembutal (120 mg/kg body weight) and then perfused through the left ventricle with a solution of Zamboni fixative (a mixture of 0.2% picric acid and 2% paraformaldehyde in a phosphate buffer, pH 7.3). Their tongues were excised, and small blocks containing circumvallate papillae were cut, fixed in Zamboni solution overnight at 4°C, and washed sequentially at 4°C for 3 h in serial 0.01M phosphate-buffered saline solutions (PBS) containing 10%, 15%, and 20% sucrose. The tissues were subsequently frozen with liquid Freon 22 spray and sectioned to a 8-μm thickness with a cryostat. After a wash in PBS, the sections were treated with a protein-blocking reagent (DAKO Corporation, CA, USA) for 10 min at room temperature.

For double immunocytochemical staining, the sections were incubated in a solution of four different pairwise combinations of primary antibodies (1:100 dilution in PBS) overnight at 4°C (Table 1): 1) anti-NT3 (a polyclonal rabbit antibody raised against a peptide mapping at the carboxy terminus of the mature form of NT3 of human origin [identical to the corresponding mouse sequence], N-20: sc-547, Santa Cruz Biotechnology, Inc., CA, USA) + anti-PGP 9.5 (a polyclonal sheep antibody raised against 24-residue synthetic peptide 187-210 derived from the human PGP 9.5 sequence; Cosmo Bio., Tokyo); 2) anti-NT4 (a polyclonal rabbit antibody raised against a peptide mapping to an internal domain of the mature form of NT4 of human origin [differing from the corresponding mouse sequence by a single amino acid], N-20: sc-545, Santa Cruz) + anti-PGP 9.5; 3) anti-NT4 + anti-NCAM (a monoclonal rat antibody against the neonatal mouse brain that recognizes a triplet of glycoproteins, with apparent molecular weights of 180, 140, and 120 kDa, at the surface of neurons; Chemicon International, CA, USA); and 4) anti-TrkB (a polyclonal
rabbit antibody raised against a peptide mapping adjacent to the carboxy terminus of the precursor form of TrkB gp 145 of mouse origin, 794: sc-12, Santa Cruz) + anti-PGP 9.5. After a rinse in PBS, the sections were incubated for 2 h at 4°C with a mixture of fluorescence-labeled secondary antibodies (Alexa Fluor 594-labeled donkey anti-rabbit IgG + Alexa Fluor 488-labeled donkey anti-sheep or -rat IgG, 1:100 dilution in PBS; Molecular Probes, OR, USA). Five additional double immunostaining procedures were done in which the primary antibodies were from the same host species, i.e., rabbits (Table 2). In this case the following procedure (Takada et al., 1996) was used (NT3 + gustducin, NT4 + gustducin, NT3 + TrkB, NT4 + TrkB, TrkB + gustducin); the sections were incubated sequentially with the anti-NT3, -NT4, or -TrkB (1:100 dilution) antibody for 2 h at 4°C, protein A conjugated to Alexa Fluor 488 (1:300 dilution, Molecular Probes) for 2 h at room temperature, unconjugated protein A (1:10 dilution; ICN Biomedicals, Inc., Ohio, USA) overnight at 4°C, anti-gustducin (1:100 dilution, a polyclonal rabbit antibody raised against a peptide mapped within a highly divergent domain of Gαgust of rat origin, 1-20; sc-395; Santa Cruz) or -TrkB antibody for 2 h at 4°C, and finally with protein A conjugated to Alexa Fluor 594 (1:300 dilution; Molecular Probes). In some sections, we reversed the order of the primary antibodies against NT4 and gustducin. In controls,

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<td>NT4 Rabbit Santa Cruz + PGP9.5 Sheep</td>
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<tr>
<td>NT4 Rabbit Santa Cruz + NCAM Rat</td>
<td>Alexa Fluor 594 anti-rabbit IgG + anti-rat IgG</td>
<td>Green</td>
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<td>TrkB Rabbit Santa Cruz + PGP9.5 Sheep</td>
<td>Alexa Fluor 594 anti-rabbit IgG + anti-sheep IgG</td>
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Secondary antibodies are from Molecular Probes. All secondary antibodies were made in a donkey.

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All protein A conjugated to Alexa Fluor 488 and 594 are from Molecular Probes. Gustducin is from Santa Cruz. Pro A, protein A.
Fig. 1. a–f: Confocal laser scanning microscopic images after double-labeling of taste bud cells. a–c: NT3-immunoreactive cells in the taste buds are stained with the anti-PGP 9.5 antibody (a, green for PGP 9.5; b, red for NT3; and c, yellow [arrows] for the merged image). Some epithelial cells surrounding taste buds (E) are weakly stained with the anti-NT3 antibody (b). The anti-PGP 9.5 antibody reacts with the nerve fibers in the trench wall and connective tissue (a), and some NT3-immunoreactive fibers in the connective tissue are immunoreactive with anti-PGP 9.5 (c, yellow [N] for merged image). N: nerve fibers; F: furrow. d–f: NT3-immunoreactive cells are stained with the anti-gustducin antibody (d, green for NT3; e, red for gustducin; and f, yellow [arrows] for the merged image). F: furrow. Scale bars = 20 μm

Fig. 2. a–f: Confocal laser scanning microscopic images after a double-labeling of taste bud cells. a–c: NT4-immunoreactive cells are not stained with the anti-PGP 9.5 antibody (a, green for PGP 9.5; b, red for NT4; c, yellow for the merged image). No nerve fibers are immunoreactive with the anti-NT4 antibody in the connective tissue (b). N: nerve fibers; F: furrow. d–f: NT4-immunoreactive cells are not stained with the anti-NCAM antibody (d, green for NCAM; e, red for NT4; f, yellow for the merged image). N: nerve fibers; F: furrow. g–i: The section was first incubated with anti-NT4 and green protein A reagent, and next with anti-gustducin and red protein A reagent. All of the gustducin-immunoreactive cells are stained with the anti-NT4 antibody in the taste bud cells, as indicated by the yellow fluorescence in the merged image (g, green for NT4; h, red for gustducin; and i, yellow [arrows] for the merged image). The remaining cells immunoreact only with the anti-NT4 antibody (i, green). F: furrow. j–l: This section was first incubated with anti-gustducin and green protein A reagent, and next with an anti-NT4 antibody and red protein A reagent. Almost all gustducin-immunoreactive cells are stained with the anti-NT4 antibody in the taste bud cells, resulting in yellow fluorescence in the merged image (j, green for gustducin; k, red for NT4; and l, yellow [arrows] for merged image). Other remaining cells are immunoreactive only with anti-NT4 (l, red). Scale bars = 20 μm
Fig. 2. a–l: Legend on the opposite page.
Fig. 3. a–l: Confocal laser scanning microscopic images after a double-labeling of taste bud cells. a–c: All PGP 9.5-immunoreactive cells are stained with the anti-TrkB antibody, as shown by the yellow fluorescence (a, green for PGP 9.5; b, red for TrkB; and c, yellow [arrows] for the merged image). The remaining taste bud cells have immunoreacted only with the anti-TrkB antibody (c, red). Nerve fibers in the connective tissue under the trench wall (N) are immunopositive for PGP 9.5 and TrkB. F, furrow. d–f: All gustducin-immunoreactive cells are stained with the anti-TrkB antibody, thus giving yellow fluorescence (d, green for TrkB; e, red for gustducin; and f, yellow [arrows] for the merged image). The remaining taste bud cells have immunoreacted only with anti-TrkB antibody (f, green). g–i: Most of the TrkB-immunoreactive cells coexist with NT3-immunoreactive cells (g, green for NT3; h, red for TrkB; and i, yellow [arrows] for the merged image). Nerve fibers in the connective tissue (N) are immunopositive for NT3 and TrkB. j–l: Most of the NT4-immunoreactive cells are positive for TrkB (j, green for NT4; k, red for TrkB; and l, yellow [arrows] for the merged image). The remaining cells have immunoreacted only with the anti-TrkB antibody (l, red). Scale bars = 20 μm
each primary antibody against NT3, NT4, or gustducin was omitted. All sections were washed in PBS, mounted, and examined with a Leica confocal laser scanning microscope.

Other sections were incubated overnight at 4°C with anti-TrkC (a polyclonal rabbit antibody raised against a peptide mapping near the carboxy terminus of TrkC gp140 of porcine origin, 798: se-117; Santa Cruz) at a dilution of 1:100 in PBS. After a wash in PBS, the sections were treated for 2 h at 4°C with Alexa Fluor 594-labeled donkey anti-rabbit IgG at a dilution of 1:50, washed in PBS, and examined with a fluorescence microscope.

Some sections for double staining were incubated with a secondary antibody solution that contained 2 μg/ml DAPI (4′,6-diamino-2-phenylindole dihydro-chloride, MERCK, Darmstadt, Germany) to stain the cell nuclei. The numbers of nuclei in a total of 107 taste buds for three kinds of double-stained sections (NT3 + PGP 9.5, NT4 + PGP 9.5, TrkB + PGP 9.5) were counted in respective photographs obtained with a camera attached to a confocal laser scanning microscope and then the numbers of NT3- and PGP 9.5-, NT4- and PGP 9.5-, and TrkB- and PGP 9.5-positive cells in the taste buds were counted in the same photographs. Then the percentages of PGP 9.5- and NT3-, NT4-, or TrkB-immunoreactive cells in the taste buds of these 3 kinds of groups were calculated.

**Results**

In sections of taste buds from normal mice, the anti-NT3 antibody reacted with the cytoplasm of most of the slender taste bud cells and also perhaps with the basal (I' or type) cells (Fig. 1b, d, 4a). Some epithelial cells surrounding taste buds were weakly stained with the anti-NT3 antibody. The anti-NT4 antibody also reacted with the cytoplasm of some of the slender taste bud cells but not with the basal cells (Fig. 2b, e, g, k, 4c). The anti-TrkB antibody reacted with the cytoplasmic membranes of most taste bud cells, including basal cells, and with the nerve fibers in the connective tissue under the trench wall (Fig. 3b, d, h, k; 4b). On the other hand, the anti-TrkC antibody did not react with either taste bud cells or nerve fibers.

Double immunostaining of the taste buds showed that all of the PGP 9.5-immunoreactive cells were also reactive with the anti-NT3 antibody, as indicated by the yellow fluorescence in the merged image (Fig. 1c) when double-labeled with green (PGP 9.5, Fig. 1a) and red (NT3, Fig. 1b). The remaining taste bud cells immunoreacted only with anti-NT3 (Fig. 1c, red). All of the gustducin-immunoreactive cells were also stained with the anti-NT3 antibody, as indicated by the yellow fluorescence in the merged image (Fig. 1f) after double labeling with green (NT3, Fig. 1d) and red (gustducin, Fig. 1e). The remaining taste bud cells immunoreacted only with anti-NT3 (Fig. 1f, green). On the other hand, the NT4-immunoreactive cells were not stained with anti-PGP 9.5 (Fig. 2a–c, red for NT4 and green for PGP 9.5) or with anti-NCAM (Fig. 2d–f, red for NT4 and green for NCAM). However, all of the gustducin-immunoreactive cells were stained with the anti-NT4 antibody (Fig. 2g–i, green for NT4 and red for gustducin, yellow for a merged image). The remaining cells immunoreacted with only anti-NT4 (Fig. 2i, green). A similar immunoreactivity showing yellow fluorescence in merged images was also found when the order of the
primary antibodies was reversed (Fig. 2j–l), in which case the section was first incubated with anti-gustducin and green protein A and then with anti-NT4 and red protein A reagent.

All of the PGP 9.5-immunoreactive cells were stained with the anti-TrkB antibody (Fig. 3a–c, green for PGP 9.5, red for TrkB, yellow for a merged image). The remaining cells immunoreacted only with anti-TrkB (Fig. 3c, red). Almost all gustducin-immunoreactive cells were also stained with the anti-TrkB antibody (Fig. 3d–f, green for TrkB, red for gustducin, yellow for a merged image). The remaining cells immunoreacted with only anti-TrkB (Fig. 3f, green) or anti-gustducin (Fig. 3f, red).

Double immunostaining for TrkB and NT3 revealed that most of the TrkB was colocalized with NT3 in the same taste bud cells (Fig. 3g–i, green for NT3, red for TrkB, yellow for a merged image). In the staining for TrkB and NT4, most of the NT4-immunoreactive cells were stained with anti-TrkB (Fig. 3j–l, green for NT4, red for TrkB, yellow for a merged image), and the remaining cells immunoreacted with only anti-TrkB (Fig. 3l, red).

Anti-PGP 9.5, -NCAM, and TrkB-antibodies reacted with the nerve fibers in the connective tissue and the trench wall epithelium (Figs. 1a, 2d; 3a, b, d, h, k). None of nerve fibers in the connective tissue or trench wall were reactive with the anti-NT4 antibody (2b, c, e, f, 3j, l); however, the anti-NT3 antibody reacted with some of the nerve fibers in the connective tissue (Fig. 1a–c; 3g–l).

In control reactions, the omission of each primary antibody against NT3, NT4, or TrkB revealed that the protein A reagents did not bind nonspecifically.

The percentages of NT3-, NT4-, or TrkB and PGP 9.5-immunoreactive cells in all taste bud cell nuclei were calculated from confocal laser microscopic images, as shown in Fig. 5. The percentage of NT3-immunoreactive cells was 89.0% ± 5.6 (mean ± S.D.) in the total population of taste bud cells, and that of PGP 9.5-immunoreactive cells, 20.7% ± 7.5 in the same sections. On the other hand, the percentage of NT4-immunoreactive cells was lower, 58.6% ± 12.0, as was that of PGP 9.5-immunoreactive cells, which were NT4 negative, 18.6% ± 10.1. The percentage of TrkB-immunoreactive cells was 80.8% ± 4.5, and that of PGP 9.5-immunoreactive cells, 20.0% ± 7.7.

![Fig. 5. Percentages of NT3-, NT4-, TrkB-, and PGP 9.5-immunoreactive cells in the taste bud cells. The percentage of NT3-immunoreactive cells among all taste bud cells was 89.0%. These cells included PGP 9.5-positive type-III cells (20.7%), gustducin-positive type-II cells, type-I cells, and basal cells. On the other hand, NT4-immunoreactive cells accounted for 58.6% of the cells in the taste buds, and did not include PGP 9.5-positive type-III cells but only type-I cells and gustducin-positive type-II cells. TrkB-immunoreactive cells accounted for 80.8%, and included PGP 9.5-positive type-III cells (20.0%), gustducin-positive type-II cells, type-I cells, and basal cells, as in the case of NT3.](image-url)
Discussion

Normal taste bud cells in mice immunocytochemically expressed NT3, NT4, and TrkB, the latter being the receptor of NT4. On the other hand, TrkC, the receptor of NT3, was expressed in neither taste bud cells nor nerves. However, NT3 in the taste bud cells may weakly bind and activate the TrkB and also TrkA receptors because TrkA is reported to be expressed in the taste bud cells (Germana et al. 2004; Kawakoshi et al. 2005). Ganchrow et al. (2003a) reported that in the mature hamster more than 75% of taste bud profiles showed an immunoreactivity for NT3 but none TrkC in taste bud cells, and that the nerve fibers in the connective tissue were immunopositive for TrkC. In this study, most of the taste bud cells (89%) were similarly NT3-immunoreactive; however, TrkC was not detected in the nerve fibers, as reported by Germana et al. (2004). The remaining 11% of NT3-immunonegative cells are supposed to be degenerative or immature cells in the taste buds.

Some NT3-immunoreactive cells were PGP 9.5-immunoreactive type-III cells and additionally gustducin-immunoreactive type-II cells. The total population of type-III and type-II cells is 56% in the mouse, according to our previous report (Takeda et al., 2004). Most of the taste bud cells (89%) were stained with the anti-NT3 antibody in this study. Therefore, the remaining NT3-immunoreactive cells (89% - 56% = 33%) would be mainly type-I cells because type-I cells correspond to some dark cells, which are observed by the light microscope and account for approximately 50% of all taste bud cells. The few remaining NT3-immunoreactive cells would correspond to the basal cells. NT3 mRNA labeling in rats is mainly found in perigemmal epithelial cells surrounding the taste buds; on the other hand, BDNF mRNA labeling is observed in taste buds, suggesting somatosensory functions for NT3 and a gustatory one for BDNF (Nosrat et al., 1996). However, we definitively detected the NT3 expression in the taste buds in this study, although surrounding epithelial cells also expressed NT3 weakly. Thus, it is likely that NT3 functions as a trophic factor in the taste bud cells of mice, albeit to an inconsiderable degree.

NT4 did not coexist with PGP 9.5 or NCAM in the same taste bud cells but did colocalize with gustducin. Thus, NT4 was not expressed in the type-III cells, but in the type-II cells and perhaps in the type-I cells, which correspond to the NT4-immunopositive and gustducin-immunonegative cells. Also, no NT4-immunoreactive cells were basal cells. In our previous studies, GDNF was expressed in the type-II cells, which contain gustducin and have broader contacts with nerve terminals (Takeda and Hoshino, 1975; Takeda et al., 2004), but not in the type-III cells. Also, type-II cells contained NT3 and NGF, as reported by Kawakoshi et al. (2005). Thus, these neurotrophic factors in the type-II cells might have an important role in taste transduction together with gustducin.

TrkB-immunoreactive cells accounted for 80.8% of the taste bud cells, which included PGP 9.5-positive type-III cells, gustducin-positive type-II cells, and perhaps type-I and basal cells. Also, the percentage of TrkB-immunoreactive cells almost corresponded to that of the NT3-immunoreactive cells, and some of the TrkB-immunoreactive cells were positive for NT4. Therefore, NT3 and NT4 would bind and activate the TrkB receptors of the membranes of all types of taste bud cells, especially those of type-III cells, which have synaptic contacts with nerve terminals. Consequently, they might exert trophic actions to promote the survival of all types of taste bud cells. Such actions of NT3 would be inconsiderable compared with those of NT4 because the morphological changes in the fungiform papillae of NT3 knockout mice are not severe (Nosrat et al., 1997). Both NT4 and BDNF exert their actions through a common receptor, TrkB, but they independently support gustatory sensory neurons (Liebl et al., 1999). Deficiencies in sensory neurons and reductions in gustatory innervation are more severe in TrkB mutant mice than in BDNF mutant ones (Frittsch et al., 1997). Examination of sensory geniculate and nodose petrosal ganglia reveals additive neuronal losses (over 80%) in mice deficient for both BDNF and NT4/5 compared with the approximately 50–60% loss in those deficient in BDNF or NT4/5 (corresponding to NT4) alone (Conover et al., 1995, Liu et al., 1995). Transgenic mice overexpressing BDNF or NT4 in the basal epithelial cells of their tongue show severely reduced numbers of both fungiform papillae and taste buds, which loss appears to be the result of a lack of gustatory innervation; even though excess geniculate ganglion neurons are generated (Ringsstedt et al., 1999; Krimm et al., 2001). The nerve dependency of taste bud cells has long been known (Fujimoto and Murray, 1970; Farbman, 1980; Smith et al., 1994; Takeda et al., 1996, 1999; Oakley, 1998), and several authors reported that BDNF-, TrkB-, NT3-, GDNF-, and GFRα1-immunoreactive taste bud cells after denervation vanished following the disappearance of the taste buds and reappeared at the same time as the taste buds did (Uchida et al., 2003; Ganchrow et al., 2003b; Takeda et al., 2005). Thus, NT4 may exert a trophic action on the taste bud cells together with these other neurotrophic factors.

Lohof et al. (1993) reported that BDNF, but not NT3, could enhance the activity and efficacy of developing neuro-muscular synapses. Furthermore, several authors reported that NGF, GDNF, and NT1 are involved in the transmission at synaptic junctions (Lockhart et al., 2000;
Wang et al., 2002). The cells and synapses in the taste buds are known to be continuously renewed by neural induction (Beidler and Smallman, 1965; Farbman, 1980). Thus, neurotrophic factors—including NT4—in the taste buds may be involved in the formation, activity, and transmission of synapses between the type-III cells and nerves by binding to TrkB receptors on the type-III cells.

In conclusion, we detected NT3, NT4, and TrkB receptors in taste bud cells. We propose that NT4 expressed in the type-II and -1 cells exerts its trophic actions on all types of taste bud cells by binding to their TrkB receptors, and that NT3 expressed in all types of cells also exerts a trophic effect, albeit inconsiderable, by binding to TrkB and TrkA receptors.

References


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