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Expression of N-cadherin and cell surface molecules in the taste buds of mouse circumvallate papillae

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ABSTRACT

Objectives: Cadherins are a type of adhesion molecule involved in cell–cell recognition and morphogenesis. N-cadherin is predominantly expressed in the nervous system, and is localized at synapses, where it not only plays an adhesive role, but also participates in the regulation of synaptic function and plasticity. Taste cells within taste buds have a limited lifespan, and are replaced on a regular basis. However, little is known regarding the expression pattern of cadherins in taste bud cells. In this study, we examined whether taste bud cells in mouse taste papillae express N-cadherins, and if so, which cell type (s) the N-cadherins are found in.

Methods: We examined the expression of N-cadherins by reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, and *in situ* hybridization.

Results: RT-PCR results demonstrated that N-cadherins are expressed in the taste bud-containing epithelium of the circumvallate papilla in mice. Based on immunohistochemical analyses, N-cadherins were found to be expressed in a subset of taste bud cells of gustatory papillae. Double-labelling studies showed that N-cadherin co-localizes with α-gustducin, aromatic L-amino acid decarboxylase (AADC), carbonic anhydrase IV (CA4), and phospholipase C β2 (PLCβ2).

Conclusions: Our study indicated that N-cadherins are expressed in type II and III taste cells. Taken together with results from previous studies, we propose that N-cadherins might play a functional role in the establishment of nerve terminal connections.

1. Introduction

Cadherins are essential for maintaining multicellular structures, and play a role in vital processes such as embryogenesis, pattern formation, and maintenance of specific tissue architectures. Classic cadherins are Ca^{2+}-dependent adhesion molecules that contain five extracellular subdomains, which are separated from the cytoplasmic domain by a single transmembrane segment [1]. The cytoplasmic domain binds to and interacts with catenins, which transduce signals and serve as bridges to the cytoskeleton [2]. During development, cadherins assist in proper positioning of cells and morphogenesis [3,4]; in nervous tissues, N-cadherin acts as the target for nerve terminals [5,6] and in the formation of synapses [7,8]. N-cadherin, a member of the cadherin superfamily of cell adhesion molecules, mediates Ca^{2+}-sensitive homophilic binding between apposed cell membranes, and is implicated in neurite outgrowth, dendritic arborization, axon guidance, and early stages of synaptogenesis [9,10].

Taste buds are chemoreceptors that function as end-organs for taste. In mammals, most taste buds are located in the stratified squamous epithelium of the dorsal surface of the tongue, and are concentrated in circumvallate, foliate, and fungiform papillae. Gustatory cells found in taste buds have been identified as paraneurons, as they possess characteristics of both neuronal and epithelial cells [11]. Like neurons, these cells form synapses, store and release transmitters, and are capable of generating action potentials [12,13]. Like epithelial cells, mammalian taste cells have a limited lifespan, and are regularly replaced from approximately 10 proliferative basal stem cells per bud [14–17]. Therefore, gustatory nerve terminals need to establish new connections on a regular basis with newly formed cells. However, little is known regarding the mechanisms that allow gustatory nerve fibers to locate appropriate taste cells.

In this study, we examined whether taste bud cells in mouse taste papillae express N-cadherins, and if so, in which cell type(s) the N-cadherins are located. We performed double immunostaining for

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N-cadherin and aromatic L-amino acid decarboxylase (AADC), carbonic anhydrase 4(CA4), α-gustducin, or phospholipase C β2 (PLCβ2). We further examined the expression pattern of N-cadherin mRNA in mouse taste buds.

2. Materials and methods

2.1. Animals

Adult ICR mice (10-weeks-old) of both sexes (male: 4, female: 3) were used for this study. Use of these animals was approved by the Kyushu Dental University Animal Care and Use Committee, and all animal protocols conformed to the National Institute of Health guidelines.

2.2. Tissue preparation

Mice were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg), and were perfused through the left ventricle with 4% paraformaldehyde (PFA) in phosphate buffer (pH 7.4). Tongues of perfused mice were fixed overnight in the same fixative, and were embedded in OCT compound (Sakura, Torrance, CA, USA). Cryostat Section (4–6 μm) were mounted on MAS-coated Superfrost slides (Matsunami Glass Ind., Osaka, Japan), and were stored in airtight boxes at −80 °C.

2.3. Reverse transcription polymerase chain reaction (RT-PCR)

For RT-PCR, dissected tongue tissues containing circumvallate papillae, as well as tongue tissues from non-gustatory regions, were incubated for 60 min at 37 °C with 2% type IV collagenase (Sigma) in supplemented a-MEM (Cosmo Bio. Co., Ltd.). Following the incubation period, the papillary epithelium was manually separated from the underlying connective tissues using fine forceps. Total RNA was isolated from the epithelium of circumvallate papillae, the brain, and tongue epithelium without taste buds; isolated RNA was incubated with DNase I, and was reverse-transcribed using oligo-dT primers and avian myeloblastosis virus (AMV) reverse transcriptase at 42 °C for 4 h to yield single-stranded cDNAs. Following denaturation at 94 °C for 120 s, PCR amplification was performed under the following conditions: 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min for a total of 35 cycles. The reaction was terminated after a 15-min elongation step at 72 °C. The reverse transcriptase step was omitted in negative control samples to confirm the removal of all genomic DNA. Amplification products were analyzed on 2% agarose gels, and were visualized with ethidium bromide. Amplification products were sub-cloned and sequenced for confirmation of identities. The sequences of the primers used were as follows:

N-cadherin: 5′-AGGGTGGACGTCATTGTAGC-3′ (forward) and 5′-CGGTTGATGGTCCAGTTTCT-3′ (reverse); β-actin: 5′-CACCCTGTGCTGCTCACC-3′ (forward) and 5′-GCACGATTTCCCTCTCAG-3′ (reverse).

2.4. In situ hybridization

Tissue sections were processed as previously described [18]. In brief, rehydrated sections were treated for 10 min with 0.2 N HCl, followed by 5 min incubation with proteinase K (1 μg/ml in Tris-ethylenediaminetetraacetic acid (EDTA)). Sections were then washed in phosphate buffered saline (PBS), re-fixed for 20 min in 4% PFA, and treated twice for 15 min with glycine (2 mg/mL in PBS). Sections were pre-hybridized for 1 h at room temperature in hybridization buffer. Digoxigenin-labeled antisense and sense riboprobes were produced from plasmids containing N-cadherins. Hybridization was performed overnight at 60 °C in hybridization buffer containing 0.5–1.0 μg/ml riboprobe. Excess probe was removed by sequential washes, and sections were blocked for 1 h in 1% blocking reagent-containing maleic acid buffer (0.1 M maleic acid and 0.15 M NaCl). Tissue sections were then incubated for 2 h with anti-digoxigenin antibody conjugated to alkaline phosphatase (diluted 1:250 in blocking solution). Antibody binding was visualized with 4-nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP). Prior to imaging or immunohistochemistry, sections were re-fixed in 4% PFA.

2.5. Immunohistochemistry

For immunohistochemical staining, tissue sections were blocked for 2 h in 5% goat serum in PBS, and were incubated with primary rabbit anti-N-cadherin (1:400; Novus Biologicals, NB600-1038, Lot No. 006FDF) overnight at 4 °C in a humidified chamber. Following a PBS rinse, sections were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000; Molecular Probes) overnight at 4 °C. Slides were again rinsed with PBS, and were coverslipped with Vectashield (Vector Laboratories, USA). The specificity of N-cadherin immunoreactivity against mouse tissues was determined by substituting a buffer for the primary antibody.

For double immunohistochemical staining, tissue sections were incubated with anti-N-cadherin (1:400) and anti-carbonic anhydrase IV (CA4) (1:200; R&D system) primary antibodies. Alexa Fluor 488 conjugated donkey anti-rabbit IgG (1:1000) and Alexa Fluor 546 conjugated donkey anti-goat IgG (1:1000) were used as secondary antibodies.

For double immunohistochemical staining using primary antibodies from the same host species, the following procedure was used [19]: sections were first incubated with anti-N-cadherin (1:400) primary antibody and with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:1000) secondary antibody. After rinsing with PBS, sections were sequentially incubated with anti-rabbit Fab fragment (1:100; Jackson laboratory), anti-gustducin (1:1000; Santa Cruz), anti-PLCβ2 (1:1000; Santa Cruz), or anti-AADC (1:200; GeneTex) primary antibodies overnight at 4 °C. Alexa Fluor 568 conjugated-goat anti-rabbit IgG (1:1000) was used as the secondary antibody. Slides were rinsed with PBS, and were coverslipped with Vectashield (Vector, USA). Negative controls were generated by omitting primary antibodies.

All images were obtained by changing the filter cube on a cooled CCD camera (Olympus); no alteration was made to the focus and the x/y coordinates of the section. The contrast and color of the digital images were adjusted, and figure plates were created using the Adobe Photoshop CS5 program for Macintosh.

2.6. Data analysis

For quantification, alternate section was examined to avoid counting the same cells. Immunopositive cells were defined by the presence of nuclear profile.

3. Results

3.1. RT-PCR analysis

We performed RT-PCRs to determine N-cadherin gene expression in mouse taste buds (Fig. 1). RT-PCR was performed using RNAs prepared from brain tissues, circumvallate papilla epithelium, and areas of the tongue epithelium that did not contain taste buds. The expected amplicon size with primer sets specific for mouse N-cadherin was 586 bp; amplified cDNAs were sequenced to confirm identity. As expected, RNAs from tongue epithelium lacking taste buds did not yield amplification products.

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