Although the peripheral taste system relies on multiple signaling pathways to detect and transmit taste signals to afferent gustatory neurons, each of these pathways depends on increases in intracellular calcium to generate a normal output signal (Akabas et al. 1988; Clapp et al. 2006; Ogura et al. 2002; Richter et al. 2003). Some taste cells have conventional chemical synapses and express voltage-gated calcium channels. Other taste cells detect bitter, sweet, and umami taste qualities but lack the chemical synapses. These taste cells, which are called type II taste cells, depend on calcium release from internal stores to generate an appropriate output signal in response to taste stimuli (DeFazio et al. 2006; Zhang et al. 2003).

Therefore, taste cells, like all cells, must tightly regulate their cytosolic calcium levels. The regulation of calcium signals occurs through the actions of various calcium clearance mechanisms (CCMs) as well as via multiple calcium binding proteins (CBPs) located in the cytoplasm (Braunewell and Klein-Szanto 2009; Burgoyne 2007; Burgoyne and Weiss 2001; Haeseleer et al. 2002; Rogers et al. 1990). However, little is currently known about the CCMs or the CBPs that are important in taste cells. Recent studies have identified that mitochondrial calcium uptake (Hacker and Medler 2008) and sodium-calcium exchangers (Laskowski and Medler 2009) are two important CCMs that regulate calcium in taste cells, but our knowledge about the CBPs expressed in these cells is still lacking.

CBPs are a family of approximately 240 proteins, some of which act primarily as “pure” calcium buffers and temporarily immobilize free calcium in the cytoplasm, whereas others act as calcium sensors and have signaling roles within the cells (Burgoyne et al. 2004; Camp and Wijesinghe 2009; Haeseleer et al. 2002; Schwaller 2009). Calmodulin is one well-characterized calcium sensor, but many other CBPs have also been identified as calcium sensors. These calcium sensors have been divided into two large subfamilies:

Expression of Calcium Binding Proteins in Mouse Type II Taste Cells

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Summary
It is well established that calcium is a critical signaling molecule in the transduction of taste stimuli within the peripheral taste system. However, little is known about the regulation and termination of these calcium signals in the taste system. The authors used Western blot, immunocytochemical, and RT-PCR analyses to evaluate the expression of multiple calcium binding proteins in mouse circumvallate taste papillae, including parvalbumin, calbindin D28k, calretinin, neurocalcin, NCS-1 (or frequenin), and CaBP. They found that all of the calcium binding proteins they tested were expressed in mouse circumvallate taste cells with the exception of NCS-1. The authors correlated the expression patterns of these calcium binding proteins with a marker for type II cells and found that neurocalcin was expressed in 80% of type II cells, whereas parvalbumin was found in less than 10% of the type II cells. Calretinin, calbindin, and CaBP were expressed in about half of the type II cells. These data reveal that multiple calcium binding proteins are highly expressed in taste cells and have distinct expression patterns that likely reflect their different roles within taste receptor cells. (J Histochem Cytochem 59:530–539, 2011)

Keywords
taste cells, parvalbumin, calretinin, calbindin D28k, neurocalcin, CaBP

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Expression of Calcium Binding Proteins in Mouse Type II Taste Cells

The neuronal calcium sensor (NCS) proteins and CaBPs, both of which play important roles in neurons (Braunewell and Klein-Szanto 2009; Burgoyne 2007; Burgoyne et al. 2004; Burgoyne and Weiss 2001; Camp and Wijesinghe 2009; Haeseleer et al. 2000; McCue et al. 2010). A few studies have described the expression of CBPs within the taste system, but most have not focused on peripheral taste cells (Chard et al. 1993; Germana et al. 2007; Ichikawa and Helke 1995, 1997; Ichikawa et al. 1991; Ichikawa and Sugimoto 2004). The studies that have reported on the expression of CBPs in taste receptor cells have primarily been in fish (Barreiro-Iglesias et al. 2008; Diaz-Regueira et al. 2005; Germana et al. 2007; Northcutt 2005) and amphibians (Barlow et al. 1996; Kerschbaum and Hermann 1992). Few studies have characterized the expression patterns of multiple CBPs.

The purpose of this study was to measure the expression of CBPs in the peripheral mouse taste system, specifically in the type II taste cells that detect bitter, sweet, and umami stimuli but do not have conventional chemical synapses. We investigated the expression patterns of the CBPs that are widely expressed in neurons, including parvalbumin, calbindin D28k, and calretinin. The expression of these CBPs has previously been reported in the peripheral taste cells of other species, but no studies have focused on the peripheral taste system in mice. We also analyzed the expression patterns of neurocalcin, a member of the NCS proteins as well as a member of the CaBP family of proteins that have previously been identified in the brain and retina (Haeseleer et al. 2000), but their expression in the taste system has not been measured. Western blot analyses determined that each of our antibodies labeled a single protein band of the predicted size, which indicates the antibodies used were appropriate for our immunocytochemical analyses. Using immunocytochemistry, we determined that multiple CBPs are highly expressed in taste cells, and distinct expression patterns exist in relationship to type II taste cells. These results were confirmed with RT-PCR analysis of mRNA from circumvallate taste buds.

Materials and Methods

Animals

For these experiments, we used either C57Bl/6 mice obtained from Jackson Labs (Bar Harbor, ME) or transgenic mice that express green fluorescent protein (GFP) driven by the IP$_3$R3 promoter to identify cells with the PLCβ2/IP$_3$R3 pathway (Hacker et al. 2008), which is a marker for type II taste cells (Clapp et al. 2004). Both male and female mice ranging in age from 6 weeks to 6 months were used in the experiments. We used 18 C57Bl/6 mice and 23 IP$_3$R3-GFP mice for these experiments. Animals were cared for in compliance with the University at Buffalo Animal Care and Use Committee.

Taste Receptor Cell Isolation

Taste receptor cells were harvested from the circumvallate taste buds of C57Bl/6 mice. Tongues were removed from animals and injected under the lingual epithelium with an enzymatic solution containing 0.7 mg collagenase B (Roche, Indianapolis, IN), 3 mg dispase II (Roche), and 1 mg trypsin inhibitor (Sigma, St. Louis, MO) per milliliter of Tyrode’s solution (140 mM NaCl, 5 mM KCl, 1 mM MgCl$_2$, 3 mM CaCl$_2$, 10 mM HEPES, 10 mM glucose, and 1 mM pyruvic acid, pH 7.4). Tongues were incubated in oxygenated Tyrode’s solution for 20 min before the epithelium was peeled from the connective and muscular tissue. The peeled epithelium was incubated for 30 min in Ca$^{2+}$/Mg$^{2+}$-free Tyrode’s solution (140 mM NaCl, 5 mM KCl, 10 mM HEPES, 2 mM BAPTA, 10 mM glucose, and 1 mM pyruvic acid, pH 7.4) before taste cells were removed with a capillary pipette and placed in cell lysis buffer and frozen.

Reverse Transcription and PCR Amplification (RT-PCR)

Isolated taste buds from the circumvallate papillae were lysed, and RNA was purified using the RNeasy Mini Kit from Qiagen (Valencia, CA) according to the manufacturer’s instructions. Total RNA isolated from mixed brain tissue or retina was used as a positive control. mRNA from isolated taste buds was reversed transcribed using Superscript III (Invitrogen, Carlsbad, CA). PCR analysis for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed on each sample to determine sample quality and to check for genomic contamination. Samples were discarded if any genomic contamination was detected. cDNA from samples that lacked genomic contamination were analyzed for the presence of calbindin, calretinin, parvalbumin, neurocalcin, and CaBP. Previously published primers were used for the detection of mRNA encoding for the calbindin D28k (Liu et al. 1996), calretinin (Chaudhuri et al. 2005), parvalbumin (Belge et al. 2007), neurocalcin (Bruheim et al. 2009), and CaBP (Haeseleer et al. 2000). The identity of all PCR products was confirmed by DNA sequencing.

Western Blot Analysis

Cerebellar brain tissue was removed and homogenized. Bradford assays (Bio-Rad, Hercules, CA) were used to quantify the samples before they were diluted in SDS sample buffer (62.5 mmol/l–1 Tris-HCl [pH 6.8], 12% glycerol, 1.25% SDS, and 1.25% β-mercaptoethanol), heated to 65°C for 15 min, and loaded on PAGE gels. For each experiment, 20 µg of protein was separated on gels under constant voltage (200 V) for 45 min, using a Bio-Rad Mini-Protean 3 gel apparatus. Resolving gels consisted of...
8% polyacrylamide (20:1 acrylamide/N, N'-methylenebisacrylamide), and stacking gels were 4% polyacrylamide (20:1 acrylamide/N, N'-methylenebisacrylamide). Separated proteins were then transferred to PVDF membranes under constant voltage (100 V) for 1 hr at 4C. Blots were probed for each CBP using antibodies that were also used for immunocytochemistry. After protein transfer, membranes were blocked in a solution of 2% non-fat dry milk in a Tris-buffered saline solution (20 mM Tris, 500 mM NaCl, pH 7.5) containing 0.05% Tween detergent (TTBS) for 1 hr. Blots were incubated in the same blocking solution containing antibody (1:1000) for 1 hr and then washed three times (5 min per wash) in TTBS. They were next incubated in anti-rabbit biotin IgG (1:10,000; Vector Labs, Burlingame, CA) or anti-goat biotin IgG (1:5000; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 hr and washed again three times in TTBS. Blots were then incubated in an avidin-biotin complex conjugated to horseradish peroxidase (ABC solution; Vector Labs) for 30 min. Finally, antibody complexes labeling the different CBPs were visualized by chemiluminescence detection (Covi et al. 1999).

Immunocytochemical Analysis

Animals (either C57Bl/6 or IP3R3-GFP mice) were anesthetized with Fatal-Plus (Vortech Pharmaceuticals, Dearborn, MI) and then transcardially perfused with a 0.025% heparin solution in 1% sodium nitrite followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.2. Tongues were removed and postfixed for 1 hr at room temperature before being cryoprotected overnight in a 20% sucrose solution at 4C. The following day, tongues were embedded in OCT (TissueTek, Torrance, CA) and frozen for sectioning. Forty micron sections of the circumvallate papillae were cut and washed in phosphate-buffered saline (PBS) three times for 10 min each at room temperature. Sections were incubated in blocking solution (0.3% Triton X-100, 1% normal goat serum, and 1% bovine serum albumin in 0.1 M PBS) for 2 hr at room temperature and then in primary antibody for 2 hr at room temperature before being placed at 4C overnight. Sections were then washed 3 × 10 min in PBS and incubated in the appropriate secondary antibody for 2 hr in the dark at room temperature. Following incubation with secondary antibodies, sections were washed and mounted on slides using Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL).

Antibodies

The following antibodies were used in these experiments: calbindin D28k polyclonal rabbit IgG (1:250; Abcam, Cambridge, MA), calretinin polyclonal rabbit IgG (1:100; Sigma-Aldrich), neurocalcin rabbit polyclonal IgG (1:250; Enzo Life Sciences, Plymouth Meeting, PA), parvalbumin polyclonal goat IgG (1:500; Swant, Bellinzona, Switzerland), CaBP rabbit polyclonal IgG (1:100; Abcam), and frequenin polyclonal chicken IgG (Millipore, Billerica, MA). Rhodamine-conjugated AffiniPure goat anti-rabbit secondary antibodies were purchased from Jackson ImmunoResearch and used at a dilution of 1:250. For frequenin, a rhodamine-conjugated goat anti-chicken antibody was used at 1:250. To test for antibody specificity, each antibody was preincubated with the antigenic peptide for 1 hr (1 µg control peptide with 1 µg antibody) before being applied to the section containing the taste buds. For all antibodies preincubated with the control peptide, specific staining was eliminated.

Image Analysis

Sections were viewed with a three-channel laser-scanning confocal with krypton-argon lasers on a Nikon Diaphot 200 (Nikon, Tokyo, Japan). Images were sequentially captured with a cooled CCD camera, and AxioVision software (Carl Zeiss, Jena, Germany) was used for data acquisition. Images were processed using Adobe Photoshop CS software (Adobe, San Jose, CA) adjusting only brightness and contrast. Settings for the negative control sections were matched to the immunoreactive sections, both for the initial collection of the images and during the final adjustment for brightness and contrast.

Statistics and Cell Counts

Experiments were repeated at least three times to determine labeling patterns for each CBP. Cell counts were performed on IP3R3-GFP-expressing mice to identify type II cells (Hacker et al. 2008). A sequentially captured Z-series stack of 20 laser-scanning confocal micrographs (0.5 µm apart) was analyzed for each sample. Individual cells were determined by their morphology using the antibody labeling, the GFP expression, and the DIC images. The percentage of IP3R3-GFP taste cells that coexpressed the CBP of interest was determined as well as the percentage of immunoreactive taste cells that also expressed IP3R3-GFP. Cells were classified as double labeled on the basis of visual inspection when labeling exceeded background for both markers. The analysis was independently repeated using Image J 1.42 with a cell counter plug-in (National Institutes of Health, Bethesda, MD). Comparable values were obtained using both analysis methods. Values were analyzed using a χ² analysis (Preacher 2001) to determine if there were any significant differences in the number of type II cells that expressed each of the CBPs. The significance level was p < 0.05.

Results

Multiple CBPs AreExpressed in Taste Cells

In an effort to better understand how calcium is regulated in the peripheral taste system, we analyzed the expression patterns for multiple CBPs that have been found in neurons.
Expression of Calcium Binding Proteins in Mouse Type II Taste Cells

Antibody specificity was initially tested using Western blot analysis. For each CBP, samples from brain tissue were separated on 8% SDS-PAGE gels and transferred to a PVDF membrane for analysis. Results for each of the antibodies are shown in Figure 1. We were unable to successfully use the CaBP antibody in a Western blot analysis using mouse tissues, even with increased dilutions. For this antibody, we had to rely only on the antigen block experiment to ensure that our antibody labeling was specific. All of the other antibodies labeled a single protein product of the appropriate size.

Initial immunocytochemical analyses were performed on C57Bl/6 mice to determine the expression patterns for each of the CBPs: calbindin D28k, calretinin, parvalbumin, neurocalcin, and CaBP (Fig. 2). We also tested for the expression of the calcium binding protein NCS-1 (frequenin), but it was not detected (data not shown). For each antibody, preincubation with the antigenic peptide blocked all labeling (see far-right panels for each, Fig. 2), indicating that each of the antibodies was specific in its labeling for the appropriate CBP. In Figure 2B, some light spotty labeling was detected that was also present when secondary antibody was applied alone to the sections (data not shown). We concluded that this was residual nonspecific labeling from the secondary antibody and not due to the primary antibodies that we used. The expression patterns for each of the CBPs within taste receptor cells tended to be spread throughout the cell. Although there was some variability between experiments, we did not see any systematic differences in the labeling patterns between the two mouse strains that were used. The small variability that we did detect is likely due to the fact that these cytosolic proteins are expressed throughout the cytosol, and there may be some small differences between individual animals. The presence of each CBP was confirmed with RT-PCR analysis of the mRNA from isolated circumvallate taste buds (Fig. 3).

Coexpression of Calcium Buffers with Type II Cells

Because the expression of the CBPs was found to be widespread in the taste buds with little to distinguish the expression patterns of each protein, we correlated their expression with the type II taste cells. We used the IP3R3-GFP mouse to identify taste cells that express the PLCβ2/IP3R3 signaling pathway (Hacker et al. 2008). This allowed us to identify type II cells, which can respond to bitter, sweet, or umami tastants by activating a signaling pathway to cause calcium release from internal stores. These taste cells do not express voltage-gated calcium channels and do not have conventional chemical synapses (DeFazio et al. 2006; Huang et al. 2007; Medler et al. 2003; Romanov et al. 2007). We also performed double-labeling experiments using antibodies raised against the CBPs and SNAP-25 in an attempt to correlate expression of the calcium buffers with type III cells that do express conventional chemical synapses (DeFazio et al. 2006). However, we were not able to resolve the labeling patterns sufficiently to perform cell counts, and those experiments were not included in this study. We did not attempt to identify type I cells in this study.

Representative results from the experiments using circumvallate papillae from the IP3R3-GFP mouse are shown in Figure 4 with the corresponding cell counts shown in Table 1. Data in the table are reported as the total number of cells counted for all the mice analyzed. All taste buds that were analyzed for CBP expression were labeled by the antibodies for each protein. Images were taken from each slice for analysis. For each experiment, one or two antibodies were analyzed per mouse. We did not systematically determine the labeling patterns for all of the CBPs for each mouse. Although there was some intermouse variability in the labeling for a particular CBP, the overall level of co-localization was similar for a given CBP. This suggests that technical rather than biological factors were major contributors to the interanimal variability. All of the calcium buffers had some overlap in their expression with the type II cells, although their labeling patterns varied significantly (Fig. 5). Neurocalcin immunoreactivity (IR) was localized in more than 80% of type II cells, whereas parvalbumin IR was found in less than 10% of the type II cells. Calbindin IR, calretinin IR, and CaBP IR were localized in approximately half of the type II cells.

Discussion

Although several studies have reported on the expression patterns of CBPs in taste receptor cells from other species, to our knowledge, this is the first study to characterize the
expression patterns of any CBPs in mouse taste buds. Of the CBPs identified to date, calbindin D28k has been most studied in other mammalian taste systems (Ichikawa and Helke 1997; Johnson et al. 1992; Miyawaki et al. 1996; Miyawaki et al. 1998; Ohkubo et al. 2007; Yamagishi et al. 1993), where it has been reported to have widespread expression within the posterior lingual papillae of the rat (Miyawaki et al. 1996). Calbindin has also been reported to co-localize with gustducin and IP$_3$R3 in taste receptor cells from guinea pig, where its expression was restricted to the IP$_3$R3-expressing cells (Ohkubo et al. 2007). This differs from the mouse, in which only 50% of the calbindin IR was localized in IP$_3$R3-expressing taste cells. In the rat, calbindin IR was expressed in taste cells that were forming

Figure 2. Immunostaining of circumvallate papillae with antibodies to calcium binding proteins (CBPs). Each panel shows laser-scanning confocal micrographs of circumvallate taste buds from C57Bl/6 mice. For each CBP (A, calbindin 28k; B, calretinin; C, parvalbumin; D, neurocalcin; E, CaBP), immunoreactivity is shown in red on the far left with the corresponding DIC image shown next to it. An overlay of the immunoreactivity and DIC image is also shown for each CBP. On the far right, the negative control consisting of the primary antibody preincubated with the binding peptide for the antigen site is shown for each CBP tested. Scale bar is 20 µm.
Expression of Calcium Binding Proteins in Mouse Type II Taste Cells

Chemical synapses with calbindin IR gustatory neurons (Miyawaki et al. 1998), which are likely separate from the IP<sub>3</sub>R3-expressing taste cells (Clapp et al. 2006; Clapp et al. 2001; Miyoshi et al. 2001). Because only half of the calbindin IR taste cells were type II cells in mice, some of the other calbindin-expressing taste cells may be forming chemical synapses comparable to the rat (Miyawaki et al. 1998).

Few mammalian studies have reported on the expression of the other classical CBPs, parvalbumin and calretinin, in taste cells. In rats, calretinin IR was not found in taste receptor cells (Ichikawa et al. 1992; Yamamoto et al. 2000), although it is not clear how thorough these studies were in addressing this question because their focus was on the expression of calretinin in the surrounding nerve endings. Parvalbumin IR was also not localized in the rat taste cells (Ichikawa and Helke 1997; Yamagishi et al. 1993), which is quite different from our findings in mice. We found widespread expression of both of these CBPs in the mouse circumvallate taste cells.

Our data suggest that significant species differences may exist in the expression profiles of CBPs. These potential species differences are underscored when one includes studies on CBPs in non-mammalian systems. Unlike the rat studies, multiple studies have reported calretinin IR in the taste buds of fish (Barreiro-Iglesias et al. 2008; Diaz-Regueira et al. 2005; Germana et al. 2007; Northcutt 2005) and amphibians (Barlow et al. 1996), which correspond to our findings in mice. In the frog, parvalbumin is also expressed in taste cells, whereas calbindin D28k was only localized in the surrounding support cells and not in the taste cells (Kerschbaum and Hermann 1992). Therefore, parvalbumin IR in mouse taste cells corresponds to its expression in frog taste cells but not to its lack of expression in rat taste cells (Ichikawa and Helke 1997; Yamagishi et al. 1993). However, the calbindin D28k expression profile in the mouse circumvallate papillae is similar to its expression in the rat taste system (Ichikawa and Helke 1997; Miyawaki et al. 1996; Miyawaki et al. 1998; Yamagishi et al. 1993) and not to that of the frog (Kerschbaum and Hermann 1992). Taken together, these data strongly suggest that the expression of CBPs can vary significantly by species, and conclusions about their potential functions in taste cells require careful attention to the particular species being studied.

On the basis of the immunocytochemical experiments in this study, we cannot make any direct conclusions about the functional roles of these CBPs in taste transduction. However, these proteins have been shown to have critical roles in maintaining normal function in other neurons. Traditionally, parvalbumin, calbindin D28k, and calretinin have been classified as calcium buffers that bind and immobilize free calcium ions with their EF hand domains (Babu et al. 1985; Cheung 1980; Kretsinger and Nockolds 1973). These proteins have variable expression patterns both in their localization in different neurons and in their concentration, with estimates in the millimolar

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Table 1. Cell Count Information for Each Calcium Binding Protein (CBP) Analyzed

<table>
<thead>
<tr>
<th>Calcium Binding Protein</th>
<th>Mice Used</th>
<th>No. of Taste Buds</th>
<th>Type II Cells</th>
<th>CBP Cells</th>
<th>Double-Labeled Cells</th>
<th>% Type II Cells</th>
<th>% CBP Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbindin</td>
<td>6</td>
<td>43</td>
<td>195</td>
<td>228</td>
<td>88</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>Calretinin</td>
<td>6</td>
<td>43</td>
<td>145</td>
<td>190</td>
<td>62</td>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>3</td>
<td>44</td>
<td>178</td>
<td>237</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Neurocalcin</td>
<td>5</td>
<td>43</td>
<td>190</td>
<td>267</td>
<td>153</td>
<td>81</td>
<td>57</td>
</tr>
<tr>
<td>CaBP</td>
<td>3</td>
<td>24</td>
<td>100</td>
<td>202</td>
<td>44</td>
<td>44</td>
<td>22</td>
</tr>
</tbody>
</table>

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Figure 3. RT-PCR analysis confirms the expression of calcium binding proteins (CBPs) in mouse circumvallate taste buds. RT-PCR analysis of mRNA isolated from circumvallate (C) taste buds using specific primers for each CBP tested. Brain mRNA (B) was used as a positive control for A through D, whereas retinal mRNA (R) was used as the control for E. The far-left lane for each gel contains the 100-bp molecular weight marker. PCR amplicons of the correct size were amplified from the control and taste samples for all of the CBPs tested: (A) calbindin, 305 bp; (B) calretinin, 370 bp; (C) parvalbumin, 188 bp; (D) neurocalcin, 172 bp; (E) CaBP1, 700 bp. PCR product identity was confirmed using sequence analysis.
Figure 4. Co-localization of calcium binding proteins (CBPs) in type II taste cells. Laser-scanning confocal micrographs from mouse circumvallate papillae showing IP3 R3 promoter-driven green fluorescent protein (GFP) expression and immunoreactivity for the CBPs: (A) calbindin 28k; (B) calretinin; (C) parvalbumin; (D) neurocalcin; (E) CaBP. Red (left panels) denotes antibody labeling for each of the CBPs, whereas green (middle panels) denotes GFP expression. Overlays for each are shown on the right. Asterisks (*) indicate double-labeled cells. Scale bar is 20 µm.
Expression of Calcium Binding Proteins in Mouse Type II Taste Cells

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range for some neurons (Kosaka et al. 1993; Oberholtzer et al. 1988). Functional studies in dorsal root ganglia (DRG) neurons revealed that parvalbumin and calbindin both significantly buffer calcium influx signals, causing a decrease in the overall calcium elevations and slowing down the rate of calcium increases in these cells (Chard et al. 1993). All three of these CBPs have been shown to exert neuroprotective effects by reducing the magnitude of cytosolic calcium elevations. More recent studies suggest that calbindin D28k and calretinin may have additional roles in neurons, whereas parvalbumin appears to be a “pure” calcium buffer (Schwaller 2009). It is interesting that parvalbumin is not highly expressed in the mouse type II cells. These taste cells do not express voltage-gated calcium channels, and their evoked calcium signals tend to be small (average peak size = 199 nM; Hacker et al. 2008). This population of taste cells does not appear to use parvalbumin to buffer cytosolic calcium increases and relies on other calcium buffers to regulate these smaller calcium signals. It is possible that parvalbumin would suppress these calcium signals and inhibit the cell’s ability to respond appropriately to a stimulus.

In the taste system, much less is known about the expression of the CBPs that belong to the neuronal calcium sensor subfamily. Neuronal calcium sensor proteins bind to calcium with an affinity above resting free calcium concentrations and undergo a significant conformational change when they bind calcium. Many of these neuronal calcium sensors are targeted to specific regions within the cell or associate with particular target proteins and affect multiple neuronal functions, including ion channel regulation, enzyme regulation, gene transcription, and neurotransmission (Burgoyne 2007; Burgoyne et al. 2004; Burgoyne and Weiss 2001). One of these calcium sensors, neurocalcin, was not expressed in rat circumvallate taste cells (Ichikawa and Sugimoto 2004). However, in the mouse circumvallate papillae, neurocalcin is present in high levels and was coexpressed in 80% of the type II cells. Neurocalcin has been implicated in the trafficking of neurotransmitter receptors (Coussen and Mulle 2006) and regulates guanylate cyclase activity in the retina (Krishnan et al. 2004) and olfactory epithelium (Duda et al. 2004; Duda and Sharma 2008). Other cell signaling regulators have been shown to affect the signaling pathways in type II taste cells (Hennigs et al. 2008), so given the high expression levels of neurocalcin in type II cells, it is interesting to speculate that neurocalcin may exert similar effects on the cyclic nucleotide transduction pathways in taste cells (Clapp et al. 2008; Trubey et al. 2006).

Our study is the first to report on CaBP expression in taste cells, and it reveals that CaBP is widely expressed in circumvallate taste cells with CaBP IR in about half of the type II cells. Calbindin D28k and calretinin were coexpressed in 45%, 43%, and 44%, respectively, of the type II taste cells. Neurocalcin was expressed in 81% of the type II cells, which was significantly greater than any of the other CBPs analyzed (b, p < 0.05). Parvalbumin expression overlapped with 9% of the type II taste cells, which was significantly smaller compared to the other CBPs (c, p < 0.0001).

Conclusions

This is the first study to report on the expression of any CBPs in mouse circumvallate taste cells. Using a transgenic mouse line to identify type II taste cells, we were able to localize calbindin D28k, calretinin, and CaBP expression in approximately half of the type II cells. CaBP1 has been shown to modulate the calcium-dependent channel gating of IP$_3$ receptors in the brain and retina (Li et al. 2009). Based on the widespread expression of this calcium sensor in the taste cells, CaBP may have similar functions in the taste system. Future studies are needed to identify the functional role of this and other CBPs in taste cells.

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Declaration of Conflicting Interests

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References


Miyoshi MA, Abe K, Emori Y. 2001. IP3(3) receptor type 3 and PLCbeta2 are co-expressed with taste receptors T1R and T2R in rat taste bud cells. Chem Senses. 26:259–265.


Zhang Y, Hoon MA, Chandrashekar J, Mueller KL, Cook B, Wu D, Zuker CS, Ryba NJ. 2003. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell. 112:293–301.