S100B-modulated Ca^{2+}-dependent ROS-GC1 transduction machinery in the gustatory epithelium: a new mechanism in gustatory transduction

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Received 21 July 2004; revised 7 September 2004; accepted 9 September 2004

Available online 22 October 2004

Edited by Stuart Ferguson

Abstract Gustatory transduction is a biochemical process by which the gustatory signal generates the electric signal. The microvilli of the taste cells in the gustatory epithelium are the sites of gustatory transduction. This study documents the biochemical, molecular, and functional identity of the Ca^{2+}-modulated membrane guanylate cyclase transduction machinery in the bovine gustatory epithelium. The machinery is a two-component system: the Ca^{2+}-sensor protein, S100B; and the transducer, ROS-GC1. S100B senses increments in free Ca^{2+}, undergoes conformational change, binds to the domain amino acids (aa) Gly962-Asn981 and via the transduction domain aa Ile1030-Gln1041 activates ROS-GC1, generating the second messenger, cyclic GMP. In a recent study, operational presence of this machinery has been demonstrated in the photoreceptor bipolar synapse [Duda et al., EMBO J. 21 (2002) 2547]. Thus, the machinery has a broader role in sensory perceptions, vision in the retinal neurons and gustation in the tongue. The entry of the ROS-GC transduction machinery defines the beginning of a new paradigm of Ca^{2+} signaling in the tongue.

Keywords: Tongue; S100B; ROS-GC membrane guanylate cyclase; Calcium signaling; Taste

1. Introduction

Gustatory transduction is a biochemical process by which the taste receptor cells generate electrical signals in response to the different flavors of taste: sweet, sour, salty, bitter and umami [1–5]. This process occurs in the apical microvilli of the taste cells, which reside in the gustatory epithelium. The taste molecule binds to its specific receptor and initiates a cascade of molecular events, that finally result in the molecule-specific taste perception [6–17]. There are two general types of taste receptors: one, the ion channels and the other, the G-protein coupled seven-transmebrane spanning receptors. The salty-, sour- and umami-taste chemicals work through the ion channels. The active moieties of the salty and the sour chemicals are in their Na^+ and H^+ ion forms. In these forms, they enter the taste cell through two kinds of channels: amiloride-sensitive and amiloride-insensitive. The bitter and the sweet chemicals bind to their G-protein-linked receptors. Several G proteins, G_s, G_i, and G_q, expressed in taste receptor cells have been identified. Through the G-proteins, the taste molecules stimulate the specific second messenger systems of the cyclic nucleotides, or IP_3. However, important mechanistic gaps exist on the details of these processes.

A uniform theme on the gustatory transduction mechanism is that Ca^{2+} is pivotal for its operation. The taste-molecule signal results in the intracellular rise of free Ca^{2+}, depolarization of the plasma membrane, and the transmitter release from the taste cell [3,5,18,19]. That there may be some similarity between the processes of phototransduction and taste transduction is provided by the clues of the presence of two common molecules existing in these processes. Two types of channels from the taste cells have been identified. One is from the frog [20] and the other (CNG_{gust}) is from the rat taste cells [21]. The operational mode of the frog channel is opposite to that of the photoreceptor channel; instead of gating the channel, the cyclic nucleotides, cyclic GMP and cyclic AMP, close the gate [20]. The CNG_{gust}, like the photoreceptor channel and unlike the frog channel, is gated by the cyclic nucleotides. Significantly, both frog and the rat channels are one-order of magnitude more sensitive to cyclic GMP than cyclic AMP [21], suggesting that cyclic GMP may have a role in taste transduction. And a few studies have shown an increase of cyclic GMP level in response to some taste molecules [22,23]. There is also one report on the detection of a membrane guanylate cyclase activity in the apical membrane of the taste receptor cells [24].

The present study is concerned with the biochemical, molecular, and functional identity of a Ca^{2+}-modulated membrane guanylate cyclase transduction machinery in the taste cells of the gustatory epithelium. Its three interlocked signaling components are Ca^{2+}, S100B and ROS-GC1.

2. Materials and methods

2.1. Reagents

GCAP1, GCAP2, and neurocalcin δ were cloned, expressed, and purified as described previously [25–27]. S100B was obtained commercially (Sigma Chemical Co). Characterization of the highly specific antibodies raised in rabbits against ROS-GC1 and S100B has been described previously [27,28]. The ROS-GC1 antibody was purified by passing it through ROS-GC1 antigen coupled onto NHS-activated Sepharose 4B (Pharmacia). S100B antibody was enriched by precipitating the immunoglobulin fraction using ammonium sulfate. ELISA and Western blots were used to determine the titer of the purified antibodies.

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0014-5793/$22.00
doi:10.1016/j.febslet.2004.09.089

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

Fresh bovine tongue was commercially purchased. The epithelial layer was dissected out from the anterior portion of the tongue. Total and poly(A)$^+$ RNA were isolated following the protocol described in [29]. The cDNA library was constructed using Marathon (Invitrogen) or Advantage RT for PCR (BD-Bioscience) kits. The 548 bp ROS-GC1 fragment and the total S100B coding region (279 bp) were amplified by PCR from the cDNA library using specific primers. The amplified fragments were purified on agarose gel and sequenced from the 5′- and 3′-ends to confirm their identities. Amplification of a 238 bp fragment of cDNA for the large ribosomal subunit (L30) served as positive control.

2.3. Preparation of the membrane fraction of the bovine tongue epithelial layer

The epithelial layer containing the fungiform papillae was dissected out from the anterior portion of the bovine tongue, homogenized in buffer containing 250 mM sucrose, 10 mM Tris–HCl (pH 7.4) and protease inhibitors. The 10 000*g* postmitochondrial supernatant was centrifuged at 40 000*g*. The pellet was designated as the membrane fraction.

2.4. Guanylate cyclase activity assay

Membranes were assayed for guanylate cyclase activity as described previously [26,28,30–32]. The amount of cyclic GMP formed was determined by radioimmunoassay [33].

2.5. Western blotting

Western blotting was carried out according to the previously published protocols [26,28,31]. Images of the membranes with the immunoreactive bands were acquired by scanning and processed using software Photoshop 6.0 and Illustrator 9.0.

2.6. Co-immunoprecipitation

Affinity purified anti ROS-GC1 antibody was coupled to the beads (AminoLink® Plus Coupling Gel; Pierce) according to the manufacturer's protocol. Membranes of the gustatory epithelium were solubilized in a buffer containing 20 mM Tris–HCl, pH 7.5, 0.5% Triton X-100, and 2 mM PMSF. The reaction mixture was divided into two equal portions; to the first portion EDTA and EGTA were added to a final concentration of 5 mM each ("$-Ca^{2+}$"), to the second portion Ca^{2+} was added to a final concentration of 100 μM ("$+Ca^{2+}$"). Each portion was incubated with beads-coupled ROS-GC1 antibody (2 μg of anti ROS-GC1 IgG/10 μl of beads). Immunoprecipitation was carried out for 6 h at 4 °C. The beads–antibody–antigen complexes were spun down and washed several times with the respective buffers. Bound antigens were eluted using SDS-sample buffer containing 6 M urea, separated through SDS–polyacrylamide gel electrophoresis (SDS–PAGE; 15% or 6%) and transferred to nitrocellulose membrane. Duplicate samples were probed independently with specific antibodies against ROS-GC1 or S100B. Western blotting was carried out as described above.

3. Results and discussion

3.1. S100B-modulated Ca^{2+}-dependent ROS-GC1 transduction machinery

In the below-described step-by-step analysis, presence of the S100B-modulated Ca^{2+}-dependent ROS-GC1 transduction machinery in the gustatory epithelium was established.

The gustatory epithelium contains a functional Ca^{2+}-dependent membrane guanylate cyclase. The particulate fraction of the bovine gustatory epithelium was prepared from the anterior region of bovine tongue and assayed for the guanylate cyclase activity. Its activity was 7.1 pmol of cyclic GMP formed/min/mg protein. To determine whether the activity is Ca^{2+}-regulated, the membrane fraction was exposed to the increasing concentrations of free $Ca^{2+}$and the guanylate cyclase activity was measured. There was a dose-dependent stimulation of the cyclase activity (Fig. 1A). The maximal

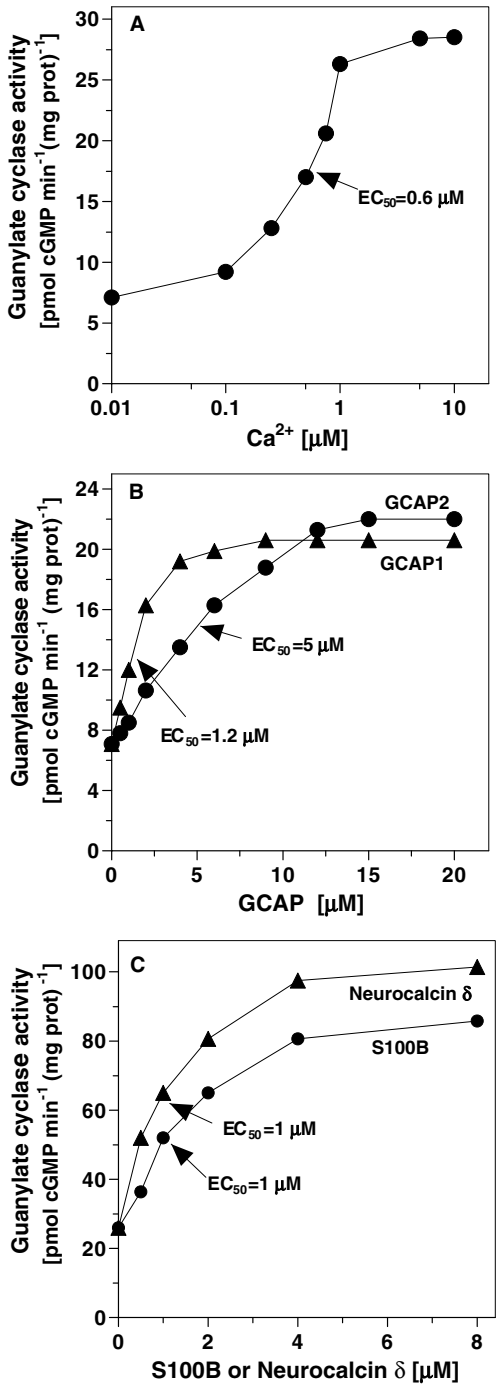

Fig. 1. Ca^{2+} regulation of guanylate cyclase activity in the bovine gustatory epithelium. Membrane fraction of the gustatory epithelium was prepared from the anterior region of the bovine tongue and assayed for guanylate cyclase activity as described in Section 2. (A) Response to Ca^{2+}. Membranes were incubated in the presence of indicated concentrations of Ca^{2+}. (B) Responses to GCAP1 and GCAP2. Membranes were incubated in the presence of 10 nM Ca^{2+} and increasing concentrations of GCAP1 or GCAP2. (C) Responses to S100B and neurocalcin δ. Membranes were incubated in the presence of 10 μM Ca^{2+} and increasing concentrations of S100B or neurocalcin δ. The molecular mass of 24 000 Da was used to calculate the concentration of GCAP1 and GCAP2; of 20 000 Da for S100B; and 22 000 Da for neurocalcin δ. All experiments were carried out in triplicate and repeated at least three times with separate membrane preparations. The results presented are from one typical experiment. Error bars are within the size of the symbols.

activation was ~4-fold over the basal value and was achieved at ~1 μM Ca^{2+}. The EC_{50} value for Ca^{2+} was 0.6 μM. These results show that the gustatory epithelium contains a Ca^{2+}-dependent membrane guanylate cyclase.

The native gustatory epithelium membrane guanylate cyclase mimics the ROS-GC1 activity. There are three known Ca^{2+}-sensitive membrane guanylate cyclases: ROS-GC1, ROS-GC2 and ONE-GC. Both ROS-GCs in the 10–50 nM free Ca^{2+} range are stimulated and in the semimicro to the micromolar range are inhibited by GCAPs (reviewed in: [34,35]). In the semi to the micromolar range of free Ca^{2+}, both are stimulated by S100B, and ROS-GC1 also by neurocalcin δ (reviewed in: [26,28,34]). ONE-GC is only stimulated, and not inhibited, by free Ca^{2+} when accompanied by neurocalcin δ [36,37]. To determine which one of these cyclases is functionally expressed in the gustatory epithelium, individual aliquots of the membranes were incubated with the recombinant forms of GCAP1, GCAP2, S100B, or neurocalcin δ in the presence of the appropriate concentration of free Ca^{2+}. The Ca^{2+}-free GCAP1 and GCAP2 stimulated the guanylate cyclase activity in a dose-dependent manner with respective EC_{50} values of 1.2 and 5 μM (Fig. 1B). The total stimulation was ~3-fold over the basal level (Fig. 1B). The Ca^{2+}-bound S100B and neurocalcin δ also stimulated the membrane guanylate cyclase activity in a concentration-dependent fashion; the EC_{50} of both agents was 1 μM (Fig. 1C) and maximal stimulation of the cyclase in both cases was 3–4 fold (Fig. 1C). These observed EC_{50} values of all these Ca^{2+}-sensor proteins for the gustatory membrane guanylate cyclase are in accord with those established earlier with the reconstituted systems consisting solely of the ROS-GC1 and individual Ca^{2+}-sensor proteins [25,28,30,38–40]. These results show that the anterior portion of the gustatory epithelium contains a guanylate cyclase that mimics the ROS-GC1 activity. And the ability of S100B and neurocalcin δ to stimulate ROS-GC1 in the gustatory epithelial membranes (Fig. 1C) beyond the point achieved through the addition of Ca^{2+} alone (Fig. 1A) indicates that the levels of a Ca^{2+} sensor protein present in these membrane preparations are lower than that needed for the maximal activation of ROS-GC1.

ROS-GC1 transcript is present in the anterior portion of the gustatory epithelium. To show the presence of ROS-GC1 in the gustatory epithelium at molecular level, total RNA and mRNA were isolated from the anterior portion of the gustatory epithelium, and reverse transcribed. This was followed by amplification of the 548 bp fragment corresponding to ROS-GC1 nucleotides 2042–2589 [41]. Amplification of this fragment yielded a single band of the predicted size, as visualized on agarose gel (Fig. 2A, lane 2). Amplification of a 238-bp fragment corresponding to L30, the 30-kDa large ribosomal subunit, served as control (Fig. 2A, lane 3). The amplified ROS-GC1 fragment was purified and sequenced. The nucleotide sequence of the amplified-fragment gave an exact match to the previously determined sequence of bovine ROS-GC1 (GenBank acc# P55203 and Ref. [41]). Thus, the ROS-GC1 transcript is expressed in the gustatory epithelium.

3.2. Biochemical identity of the ROS-GC1 in the membrane portion of the gustatory epithelium

Biochemical identity of the ROS-GC1 in the anterior region of the gustatory epithelium was established by the Western blot analysis, using the ROS-GC1-specific antibody. The experiments were done according to the previously developed protocol [26,28,31]. To validate the specificity of the antibody, Western blot analysis of COS cell membranes expressing ROS-GC1, ROS-GC2 and ONE-GC was performed. As expected, a single immunoreactive band was observed in the membranes of cells expressing ROS-GC1 (Fig. 2B: lane 1, indicated by an arrowhead); there was no immunoreactivity in lanes containing ROS-GC2 (Fig. 2B: lane 2) and ONE-GC (Fig. 2B: lane 3). Having established that the antibody exclusively recognizes

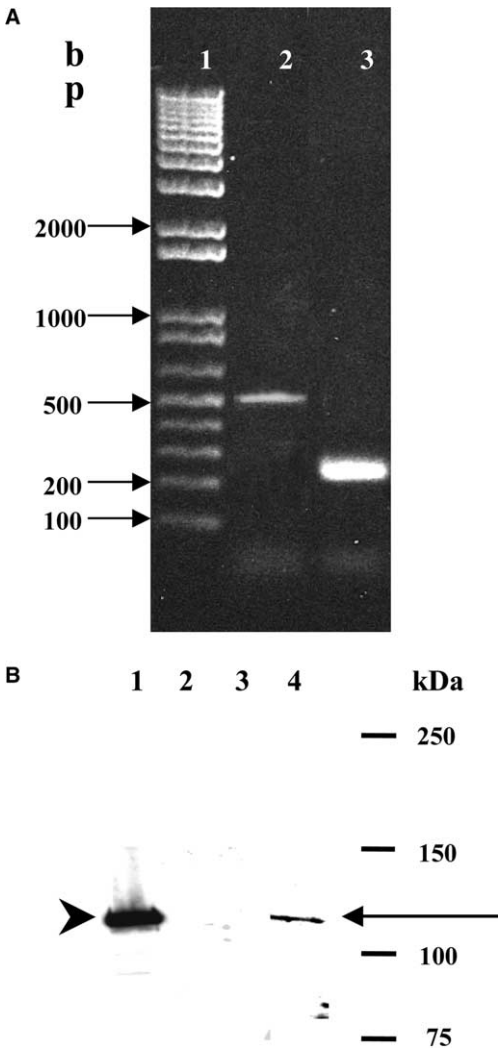

Fig. 2. Expression of ROS-GC1 in the bovine gustatory epithelium. (A) Detection of the ROS-GC1 transcript. RNA was isolated from anterior portion of the bovine tongue and reverse transcribed. From the cDNA, a 548 bp fragment of ROS-GC1 (lane 2) and a 238 bp fragment of L30 (lane 3) were amplified. L30, the control, encodes the highly conserved 30 kDa protein from the large ribosomal subunit. Molecular size markers are provided in lane 1. (B) Detection of ROS-GC protein. Membranes of COS cells expressing ROS-GC1 (100 μg of protein; lane 1), ROS-GC2 (100 μg of protein; lane 2) and ONE-GC (100 μg of protein; lane 3) and of the anterior portion of bovine gustatory epithelium (100 μg of protein; lane 4) were isolated and resolved on 6% SDS–polyacrylamide gel. After the proteins were transferred to nitrocellulose membrane, the blot was incubated with anti-ROS-GC1 antibody and secondary antibody as described in Section 2. The immunoreactive bands were visualized by enhanced chemiluminescence (ECL). The ROS-GC1 immunoreactive band of the anterior portion of the gustatory epithelium (lane 4) is indicated by an arrow and that of the membranes of COS cells expressing ROS-GC1, by an arrowhead. Molecular size markers are given alongside.

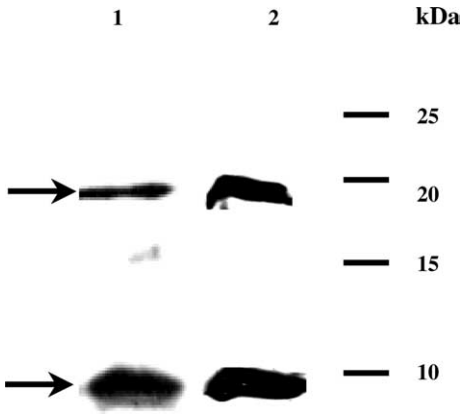

Fig. 3. Expression of S100B in the bovine gustatory epithelium. Membranes of the anterior portion of bovine gustatory epithelium (100 μg of protein; lane 1) were isolated and resolved on 15% SDS-polyacrylamide gel. Commercial S100B (0.3 μg; Sigma Chemical Co.) was used as control (lane 2). After the proteins were transferred to nitrocellulose membrane, the blot was incubated with anti-S100B antibody and secondary antibody as described in Section 2. The immunoreactive bands were visualized by ECL. The bands corresponding to the monomeric and dimeric forms of S100B are indicated by arrows. Molecular size markers are given alongside.

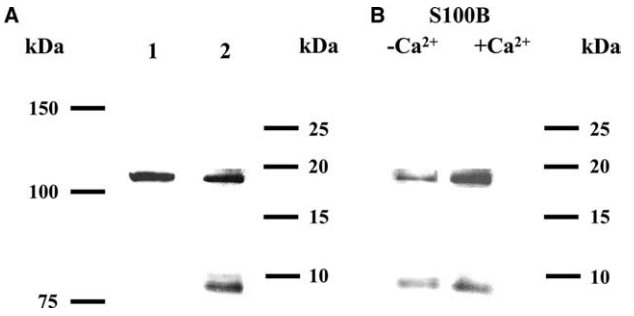

Fig. 4. ROS-GC1 and S100B in the gustatory epithelium – co-immunoprecipitation. (A) ROS-GC1-S100B complex exists in the gustatory epithelium. Membranes of the bovine gustatory epithelium were solubilized and incubated with anti-ROS-GC1 antibody coupled to AminoLink® Plus Coupling Gel (Pierce) as described in Section 2. Duplicate samples were electrophoresed on SDS-polyacrylamide gels and analyzed by Western blotting with anti-ROS-GC1 or anti-S100B antibody. Immunoprecipitated material from 1.5 mg of solubilized membrane proteins has been loaded into each lane. Two μg of antibody was used for each immunoprecipitation. Molecular size markers are given alongside. (B) Formation of the ROS-GC1-S100B complex is Ca^{2+}-dependent. Membranes of the bovine gustatory epithelium were incubated with anti-ROS-GC1 antibody coupled to AminoLink® Plus Coupling Gel (Pierce) in the presence of 5 mM EDTA and 5 mM EGTA (lane "– Ca^{2+}") or 100 μM Ca^{2+} (lane "+ Ca^{2+}") as described in Section 2. Identical aliquots of each reaction mixture were electrophoresed on SDS–polyacrylamide gels and analyzed by Western blotting with anti-S100B antibody. Molecular size markers are given alongside. Intensities of the immunoreactive bands were quantified (ImageMaster, Pharmacia). The relative intensity of the "– Ca^{2+}" vs. "+ Ca^{2+}" band is 1:4.

ROS-GC1, membranes of the anterior epithelium were analyzed. A single immunoreactive band of the apparent mobility of ~116 kDa was detected in the membranes (Fig. 2B, lane 4, indicated by an arrow). The mobility of this band was identical to that observed in the positive control. These results demonstrate the identity of ROS-GC1 in the membranes of the gustatory epithelium. Thus, with these results, the presence of the membrane guanylate cyclase in the gustatory epithelium has been settled by three independent criteria: functional, molecular and biochemical.

The gustatory epithelium expresses S100β both at the mRNA and protein level. A previous study shows the S100 protein immunoreactivity in the fungiform papillae of the tongue [42]. However, its subtype identity is missing. To determine whether S100B is the natural modulator of ROS-GC1 in the gustatory epithelium, responsible for the Ca^{2+}-dependent stimulation of guanylate cyclase activity (Fig. 1A), its presence was investigated at both the mRNA and protein levels. Based on the known sequences of S100B, primers were designed and used to amplify the coding sequence of S100B, from the reverse transcribed gustatory epithelium RNA. The amplified product (279 bp) was purified and sequenced. Sequence analysis confirmed that the amplified product was indeed S100B cDNA.

At the protein level, Western blot analysis showed the presence of two S100B antibody-reacting bands in the membranes of the anterior gustatory epithelium (Fig. 3, lane 1), which were of identical mobility to those of authentic S100B (Fig. 3, lane 2). In both lanes, the apparent mobility of these bands was ~9 and ~19 kDa corresponding, respectively, to the monomeric and the dimeric forms of S100B. It is, therefore, concluded that the Ca^{2+}-sensor component S100B of ROS-GC1 transduction machinery is present in the gustatory epithelium.

ROS-GC1 and S100β physically interact with each other in the gustatory epithelium. In order to form an active functional complex, S100B and ROS-GC1 must physically interact. This was ascertained by co-immunoprecipitation experiments. Solubilized membranes of the gustatory epithelium were incubated with purified ROS-GC1 antibody coupled to commercial beads (AminoLink® Plus Coupling Gel; Pierce). The immunoprecipitated complexes were separated from the beads and analyzed by Western blotting, using antibodies against ROS-GC1 or S100B (Fig. 4A). Both antibodies identified their respective antigens, ROS-GC1 antibody recognized the ROS-GC1 protein (Fig. 4A, lane 1) and S100B antibody, the S100B protein in its monomeric (~ 9 kDa) and dimeric (~19 kDa) forms (Fig. 4A, lane 2). Because both ROS-GC1 and S100B proteins co-precipitated with ROS-GC1 antibody, the results demonstrate that ROS-GC1 and S100B exist as a complex in the membranes of the gustatory epithelium.

There is a notable difference in the apparent ratio S100B-monomer to S100B-dimer in total membranes and in the co-immunoprecipitated fraction (compare Fig. 3 lane 1 and Fig. 4A lane 2). It is known that S100B exists as a dimer in its native state (reviewed in: [43]). The increased ratio of monomer to dimer when S100B is complexed with ROS-GC1 may indicate that the formation of the complex increases the dimer's stability.

The S100β/ROS-GC1 is a Ca^{2+} signal transmission system. To be operative, the S100B/ROS-GC1 system must be Ca^{2+}-sensitve and be able to respond rapidly to the fluctuating Ca^{2+} waves. For this to happen, S100B/ROS-GC1 complex must become more stable in the presence of free Ca^{2+}. This property of the complex in the gustatory epithelium was assessed through the co-immunoprecipitation technique in the presence and absence of Ca^{2+} (Fig. 4B). In the presence of Ca^{2+}, the amount of S100B co-precipitating with ROS-GC1 was 4 times larger than that co-precipitating in its absence (Fig. 4B; compare lanes "+ Ca^{2+}" and "– Ca^{2+}"). Thus, the stability and

formation of the ROS-GC1/S100B complex is Ca^{2+}-dependent. A notable aspect of the complex is that it is present, albeit at a much-reduced level, even without added Ca^{2+} (Fig. 4B, lane "– Ca^{2+}"). This is probably due to the presence of the residual Ca^{2+} concentration in the membranes, as has been observed earlier for the neurocalcin δ/ROS-GC1 complex in the inner plexiform layer of the retinal neurons [26] and for the neurocalcin δ/ONE-GC complex in the cilia of the olfactory neurons [36]. Another possibility is that once S100B binds ROS-GC1, it remains bound to the cyclase even in the absence of Ca^{2+} as has been observed with other intracellular targets of S100B [43,44]. Pre-existent complex will make the system ever ready to receive and transmit Ca^{2+} signals within millisecond time intervals.

Ca^{2+}-modulated S100β/ROS-GC1 signal transduction model. In constructing the Ca^{2+}-modulated ROS-GC1 signal transduction model operative in the gustatory epithelium, the following elements of the transduction machinery established by the previous studies have served as its foundation. (1) The functional form of the ROS-GC1 is dimeric. (2) The S100B-modulated site in ROS-GC1 is at the C-terminal side of the catalytic module [28]. It is noteworthy that such an orientation of the regulatory site is unusual. It has only been observed for the S100B and the neurocalcin δ target sites [26,28]. In all other cases of the membrane guanylate cyclase family, it resides at the N-terminal side of the catalytic module: for ANF-RGC and CNP-RGC the ligand binding site is at the extracellular domain and the ATP-regulatory module at the N-terminal part of the intracellular domain; the GCAP1-modulated site in ROS-GC1 is at the N-terminal part of the intracellular domain (reviewed in: [34]). (3) S100B-modulates ROS-GC1 activity through its two defined domains, binding site amino acid residues (aa) Gly962-Asn981 and the transduction site aa Ile1030-Gln1041 [28]. (4) The binding and the effective activation concentration of Ca^{2+} with S100β ranges from 500 to 750 nM [28].

In response to the taste molecule, there is an elevation of Ca^{2+} range from the nanomolar to the micromolar range. The elevation is detected by the Ca^{2+} sensor S100B, it undergoes conformational change, binds to the domain aa Gly962-Asn981 and via the transduction domain aa Ile1030-Gln1041 activates ROS-GC1, generating cyclic GMP. Cyclic GMP then acts as a second messenger of the parent taste molecule.

The model is simple, straightforward and amenable to its future experimental validation.

The S100B/ROS-GC1 is one of the multiple forms of the Ca^{2+}-modulated ROS-GC1 transduction machinery in the neurosensory and neurosensory-linked neurons. The S100B-modulated ROS-GC1 is one of the multiple forms of the general Ca^{2+}-modulated ROS-GC transduction machinery. Other forms are defined by the composition of its two components: the Ca^{2+}-sensor protein and the transducer enzyme ROS-GC (reviewed in: [26–28,34–36,45]). There are three forms of ROS-GC: ROS-GC1, ROS-GC2, and ONE-GC (also named as ret-GC1, ret-GC2 and GC-D); and four forms of the Ca^{2+}-sensor proteins: GCAP1 [38], GCAP2 [45], S100B [39,40] and neurocalcin δ [26,30,36]. Thus, theoretically, with the individual pairing of a sensor protein with a ROS-GC, the machine can exist in 12 different forms. The complexity of the machine will grow further if the individual neuron contains more than one pairing. In this way, with a defined composition, the machine will show specificity to the Ca^{2+} signal in a particular neuron. An extraordinary biochemical feature of the machine is that in response to a Ca^{2+} spike, it is either stimulated or inhibited. Stimulatory and inhibitory features are bestowed upon it by the nature of the Ca^{2+}-sensor protein and of the transducer enzyme. ROS-GC1 paired with GCAPs is inhibited; paired with S100B or neurocalcin δ, is stimulated. To date, ONE-GC has only been known to pair with neurocalcin δ, and it is only stimulated (and not inhibited) by the Ca^{2+} signals. Each of the guanylate cyclases has a defined target domain for its sensor protein. Analysis of the sensory model systems of vision, olfaction and the pinealocytes has revealed that in the rod outer segments of the photoreceptors, ROS-GC transduction machine's composition is one molecule of GCAP1 and one molecule of GCAP2 with one ROS-GC1 dimer molecule [46]; separately in the two different forms of the pinealocytes it exists as GCAP1/ROS-GC1 or S100B/ROS-GC1 [31]; in the olfactory sensory neurons (cilia), it exists with the pairing of neurocalcin δ/ONE-GC [36,37]; and in the mitral cells of the olfactory bulb it exists as GCAP1/ROS-GC1 [32]. These examples indicate that how with its change in composition, the transduction machinery achieves universality, cellular specificity and complete reversibility in its operation by a wide variety of Ca^{2+} signals generated in the neurosensory and the neurosensory-linked neurons. In keeping with this universality concept, entry of the machinery in gustatory transduction may represent a new paradigm of Ca^{2+} signal transduction and it is envisioned that the machinery may be linked with the multiple events of taste perception.

Acknowledgements: The study was supported by U.S. Public Health Service Grants DC 005349 (R.K.S), EY 10828 and HL 070015 (T.D.). We thank Dr. Ramalingam Krishnan from our laboratory for help in the immunoprecipitation experiments and Joan Sharma for the editorial assistance.

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