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Taste Modulators are Tools to Gain a Better Insight into Specific Sensitivity of Chemoreceptors in Blowflies

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Introduction

One of the questions arising in studying taste transduction is whether natural sweets are distinguished from sweeteners as the former are the major source of metabolic energy. Blowflies are a convenient preparation to study chemoreception, given their relatively simplicity, availability and the vast body of related work published. The labellar chemosensory system of blowflies consists of sensilla housing four chemoreceptive neurons, named 'salt', 'sugar', 'water' and 'deterrent' cells after their best recognized stimuli. While spike activity from these sensory neurons is readily recordable, dendrite membranes remain difficult to access (Murakami and Kijima, 2000), making the mechanism(s) of transduction little understood at the membrane level. However, information can be obtained by studying the effects on the spike activity of specific pharmacological modulators used in association with chemical stimuli.

The sugar receptor cell is capable of detecting a broad variety of substances, such as pyranose or furanose sugars, as well as amino acids and proteins. On the other hand, Ahamed *et al.* (2001) found that the sweetener glycyrrhizin activates the 'pyranose site' of the 'sugar' cell membrane in *Phormia regina*, while Na-saccharine stimulates the 'deterrent cell' in *Protophormia terraenovae* (Liscia *et al.*, 2004).

Calcium ions appear to be in many ways involved in the transduction mechanisms of various tastants. We therefore decided to investigate the role of calcium in the chemoreception mechanism(s) of sugars and sweeteners. In the present study we have specifically investigated the reception mechanism of a natural pyranose sugar (sucrose) and a commonly used sweetener for humans (Na-cyclamate) by addressing such issues as the involvement of Ca^{2+} cascade and/or of Ca^{2+} channels in the sugar transduction mechanism.

Materials and methods

The spike activity from the chemosensory cells was recorded by means of the tip-recording technique (Hodgson *et al.*, 1955) in labellar chemosensilla of *Protophormia terraenovae*. The electrophysiological recordings were pass-band filtered (10–1000 Hz), digitized through a Metrabyte DAS-16 A/D converter (10 000 points/s) and stored on disk for computer analysis. Spikes in the discharges were sorted out by means of the S.A.P.I.D. Tools software (Smith *et al.*, 1990). Spike analysis was applied to the first second of the discharges skipping the first 30 ms from the onset of stimulation.

Taste stimuli

Concentrations of 1–100 mM Na-cyclamate were used in the dose–response relationship, while 200 mM sucrose and 50 mM Na-cyclamate were applied alone or added with the following modulators.

Modulators

The following compounds were added to the stimulating solutions: amiloride (inhibitor of the ENaC superfamily of ion channels); W-7 (calmodulin antagonist and inhibitor of Na-activated cation channels); EGTA (Ca^{2+} chelator); Mibefradil (blocker of the Ca^{2+} T-type channel); SK&F-96365 (inhibitor of receptor mediated Ca^{2+} influx).

All stimuli were administered in a blind sequence at the concentration of 0.1 mM except for EGTA (1 mM).

Statistical analysis

Significant differences were calculated by means of Student's *t*-test (Statistical software package) with a 95% confidence level.

Results

Mean spike firing frequency values ± SE recorded from labellar chemosensilla in response to 1–100 mM Na-cyclamate are shown in Figure 1. A clear dose-response is seen for the 'sugar' cell and an inverse correlation for the 'water' cell. Since at the highest concentration tested (100 mM) the response of the 'salt' cell was relatively high, 50 mM was adopted as the test concentration to further study the stimulating effect of Na-cyclamate on the 'sugar' cell.

Figure 2A shows that both amiloride and EGTA decrease the response to Na-cyclamate but not to sucrose; in contrast, W-7 decreases the response to sucrose but not to Na-cyclamate. Mibefradil decreases the responses to both stimuli, particularly affecting the tonic portion of the discharge (200–1000 ms; Figure 2B). SK&F-

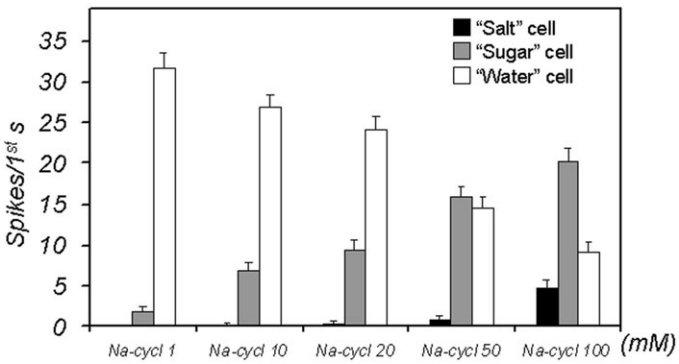

Figure 1 Dose–response relationship following stimulation with 1, 10, 20, 50 and 100 mM Na-cyclamate of the labellar taste chemosensilla of *P. terraenovae*. Spike firing frequency mean values of the 'salt', 'sugar' and 'water ' cell within the first second of the discharges ± SE (vertical bars). Number of sensilla tested: 80–98.

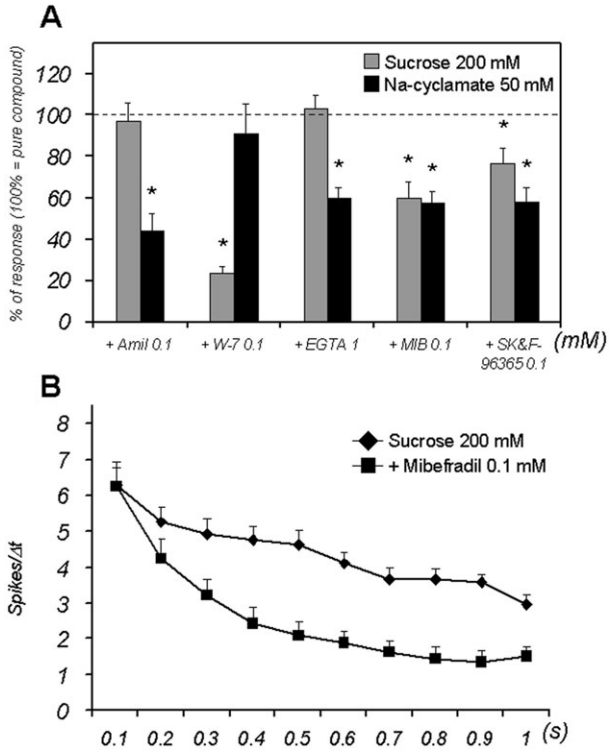

Figure 2 Normalized spikes frequencies ± SE (vertical bars) from the 'sugar' cell following addition to 200 mM sucrose or 50 mM Na-cyclamate of (A) amiloride (0.1 mM), W-7 (0.1 mM), EGTA (1 mM), Mibefradil (0.1 mM) and SK&F-96365 (0.1 mM) in the labellar taste chemosensilla of *P. terraenovae*. Broken line is the spike frequency value in response to pure sucrose or Na-cyclamate set = 100%. Number of sensilla tested: 70–90. Asterisks indicate significant differences ($P \leq 0.05$) with respect to pure compounds. **(B)** Time course (spikes/100 ms intervals) of the 'sugar' cell in response to sucrose and sucrose + mibefradil.

96365 inhibits the stimulating effectiveness of both sucrose and Na-cyclamate.

Discussion

Our results show that Na-cyclamate predominantly stimulates the same sensory cell ('sugar') as sucrose. However, evidence is provided that the two stimuli act through different transduction mechanisms: in fact, differences in spike firing frequency were found between sucrose and Na-cyclamate, when various pharmacological modulators were added to these stimuli in the test solution. The response to Na-cyclamate seems to be largely mediated by an Na^+ influx across amiloride-sensitive channels (ENaC) and, to a lesser extent, by an amiloride-insensitive component (AIC) which could account for the relative sensitivity to EGTA, Mibefradil and SK&F-96365, thus implying the involvement of Ca^{2+} in the transduction mechanism.

Instead, the sucrose response is amiloride insensitive, as previously reported (Liscia *et al.*, 1997), but is inhibited by W-7 (Liscia *et al.*, 2002), thus suggesting a different pathway. A Na^+-activated cation channel could be in fact opened by an early Na^+ influx through sugar-activated ionotrophic channels such as those described by Murakami and Kijima (2000) in the fleshfly. W-7 interacts either from the outside of the membrane and/or from the inside by inhibiting Ca^{2+}-modulated cascades (Laver *et al.*, 1997; Zhainanarov *et al.*, 1998). Ion influx would mainly be made of Na^+ and Ca^{2+}, both of which reinforce channel activation, thus sustaining spread of depolarization.

Similar Na^+-activated W-7-inhibited cation channels have been reported in the antennal olfactory dendrites of lobsters (Zhainanarov *et al.*, 1998, 2001). Mibefradil strongly reduced the tonic portion of the discharge (200–1000 ms; Figure 2B), and this suggests an effect on the Na^+-activated cation channel but no influence on the early Na^+ current entering via the ionotrophic channel.

In conclusion, we found that sucrose and Na-cyclamate activate separate transduction pathways on the same chemosensory 'sugar' cell in the labellar sensilla of the blowfly *P. terraenovae*. Besides, the different effects exerted by the various modulators tested point to an involvement of Ca^{2+} in the transduction mechanism of both sucrose and Na-cyclamate.

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