The Nitric Oxide–Cyclic GMP Cascade in Sugar Receptor Cells of the Blowfly, *Phormia regina*

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Introduction

It is well known that nitric oxide (NO) is produced by NO synthase (NOS) in postsynaptic nerves and diffuses through membranes into presynaptic nerves where NO activates soluble guanylyl cyclase (sGC) to produce cGMP, resulting in the feedback control of presynaptic nerve activity. However, in taste sensory systems, the NO–cGMP cascade might act differently. In the vertebrate taste receptors, evidence for expression of NOS (Kretz *et al.*, 1998; Zaccone *et al.*, 2002), function of cGMP as a second messenger (Okada *et al.*, 1987; Tonosaki and Funakoshi, 1988; Krizhanovsky *et al.*, 2000) and the regulation of the cGMP level via NO (Rosenzweig *et al.*, 1999) have been reported, which suggest that NO could function for the signal transduction in the taste receptor cells. In the blowfly, *Phormia regina*, there have been reports that suggest the importance of cGMP in the taste transduction. Wieczorek and Schweikl (1985) reported a high concentration of cGMP and high activity of guanylyl cylclase in the taste sensillum-rich region. Amakawa *et al.* (1990) reported that membrane permeable cGMP induces impulses from sugar receptor cells. If cGMP works as a second messenger in the sugar receptor cells, it may be possible that cGMP is produced by sGC activated by NO in the blowfly sugar receptor cells.

In this paper, we review recent progresses in our studies on the taste transduction in *P. regina*. Our study includes analyses by the tip recording (Murata *et al.*, 2004), patch clamping (Satoh *et al.*, 2003) and Ca^{2+} monitoring in the isolated receptor cells (Murata *et al.*, 2003). Throughout these studies, animals were reared under 12 h light/12 h dark cycles.

Pharmacological examination of the NO signaling system in sugar receptor cells

We performed pharmacological investigation to test whether NO participates in the transduction of the sugar receptor cells using the tip recording method. Impulses were induced from taste receptor cells in the largest chemosensilla of animals aged 5–7 days after emergence with a stimulating solution (100 mM sucrose/10 mM NaCl solution) in a glass capillary electrode. The intensity of the taste response was represented by the number of impulses during 150–350 ms after the beginning of the stimulation. To address whether NO is involved in sugar-mediated activation of the neurons in these sensilla, we introduced NO scavenger or NOS inhibitor into these cells by incubating the tip of the chemosensilla with a solution of each reagent plus 0.03% deoxycholate (DOC) for 2 min followed by 5 min of recovery time (DOC method: Ozaki and Amakawa, 1992). Taste responses were recorded before and after the introduction of those reagents into the cells. The ratio of the impulse numbers of the latter record to that of the former record was calculated (= relative response) to indicate the effect of each reagent.

We first examined if the sugar response decreased by reducing the NO in the cell. Reducing the intracellular NO was achieved by applying the NO scavenger, 2-phenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl (PTIO), by the DOC method. Relative response was 0.46 ± 0.09 ($n = 8$) with 10 mM PTIO. This clearly indicates that NO is involved as an activating factor in the transduction cascade of the sugar receptor cells. PTIO hardly affected the responses to salt or water.

Next we examined the effect of increasing NO in the sugar receptor. NO was continuously released from an NO donor, 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC 7), dissolved in the solution inside the tip recording electrode. When we applied freshly prepared 8 mM NOC 7 solution (in 20 mM MOPS, pH 6.6) to a sensillum, two kinds of impulses with different amplitudes appeared. Following small impulses (0.56 ± 0.01 mV, $n = 20$), large impulses (0.84 ± 0.01 mV, $n = 20$) started to appear with ~15 s of latency. By comparison with standard impulses derived from sugar, salt, water and 'fifth' receptor cells according to Ozaki *et al.* (2003), the small impulses were attributed to those derived from the water receptor cells whereas the large impulses were attributed to those from sugar receptor cells. It is very likely that NO induced the impulses from sugar receptor cells. The long latency for the impulses from sugar receptor cells might be the time necessary for NO to penetrate the membrane and to be concentrated until at a sufficient level to activate sGC in the sugar receptor cells.

Using the NOS inhibitor, N^{G}-nitro-L-arginine methyl ester (L-NAME, 250 μM), we examined if NOS is involved in the transduction pathway in the sugar receptor cells. This reagent specifically decreased the response to 100 mM sucrose giving relative response of 0.66 ($n = 7$). However, the same amount of the inactive enantiomer of the L-NAME, N^{G}-nitro-D-arginine methyl ester (D-NAME), had no effect, giving average relative response of 0.94 ($n = 7$). These results indicate that the NOS is involved in the neuronal activation of the sugar receptor cells in response to sucrose.

CNG conductance on the dendritic plasma membrane

Following Murakami and Kijima (2000), labella were dissected from pupae aged 12–24 h before the emergence. The sensilla were cut at their middle of the largest type and incubated in the fly Ringer solution. In 20 min, the sensory processes grew out from the cut end of the sensilla. When Ca^{2+} in the extracellular solution was reduced, tips of the processes swelled into small spheres. Using patch electrodes

containing Ca^{2+}-free solution, we obtained inside-out patch membranes from such swollen parts. We recorded the membrane current under the ramp voltage from –70 to 70 mV to monitor the *I–V* relationship of the membrane conductance. In a small number of membrane patches, we observed the conductance increase in response to 1 mM cGMP applied to the intracellular side of the patch membrane. Although these data are preliminary, they indicate the presence of CNG conductance on the plasma membrane.

Ca^{2+} shifts induced by taste stimuli in the isolated receptor cell

We developed the cell culture protocol for the taste receptor cells (Murata *et al.*, 2003). Twenty labella of pupae aged 4–5 days after the pupation were disrupted by the incubation with 0.4 mg/ml papain in nominally Ca^{2+}-free solution for 40 min, then shaken with a mixer at 1500 r.p.m. for 2 min. Obtained cell suspension was plated and cultured in modified Leibovitz's L-15 medium at 29°C. We found bipolar cells survived for 2–6 days in the culture, some of which extended long processes. By monitoring the intracellular Ca^{2+} in these bipolar cells with the Ca^{2+}-sensitive fluorescent dye, fluo-3, we found that some cells were responsive specifically to sucrose, NaCl or LiCl. The response to sucrose was dependent on the Ca^{2+} in the extracellular solution. Therefore the taste responses are likely to occur through the opening of cation channels that allow the influx of Ca^{2+} into the cells.

Discussion

Our tip recording experiments demonstrated that NO works for the impulse-generation in the sugar receptors as well as that NO is produced by the stimulation with sugar, which suggests that it is produced and functions for the impulse-generation in the same sugar receptor cell. This result raises the possibility that NO activates sGC to produce cGMP that can function as a second messenger. This possibility is supported by our preliminary observation that the dendritic membranes of the receptor cells contain the conductance gated by cGMP (CNG conductance). Thus it is very likely that the sugar receptor cells contain the NO-cGMP cascade as a part of the signal transduction pathways.

It is noteworthy that the IP$_3$ gated channels (Ozaki and Amakawa, 1992; Koganezawa and Shimada, 2002) or the ionotropic receptors (Murakami and Kijima, 2000) could contribute to the NOS activation by allowing Ca^{2+} entry into the sugar receptor cells. In fact, we have preliminarily observed the transient increase of Ca^{2+} in the excited receptor cells. That observation might reflect the preceding process of the NOS activation. However, CNG channels or voltage gated channels could also be a route for the Ca^{2+}. The relationships between those mechanisms and the NO–cGMP cascade are to be studied. The study may lead to elucidation of the overall transduction mechanism in the sugar receptor cells.

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