The Sweet Smell of Success: Conclusive Evidence that Cyclic AMP Hydrolysis Does Not Trigger Fast Adaptation in Olfactory Receptor Cells

Anita L. Zimmerman
Department of Molecular Pharmacology and Physiology, Brown University Medical School, Providence, RI 02912

We are often taught that it is almost impossible to publish negative results. Yet, in this issue, Boccaccio, Lagostena, Hagen, and Menini (2006) have assembled a very impressive array of negative results that answer a critical question in olfactory transduction. In an elegant set of experiments, these authors have ruled out a suspected mechanism of fast olfactory adaptation and have, by default, provided further evidence for another mechanism in the process.

A characteristic feature of sensory transduction is adaptation to the stimulus. When we sit in chairs, we quickly cease to notice their existence; when we enter a bright room, we squint only briefly, as our photoreceptors quickly adapt. Our sense of smell also adapts rapidly to the sustained presence of an odor: a flower smells most intense when first put to the nose. The primary mechanism of olfactory transduction has largely been worked out over the last couple of decades. However, mechanisms of odorant adaptation, especially as they relate to the roles of Ca$^{2+}$, are still being pieced together (Schild and Restrepo, 1998; Menini, 1999; Zufall and Leinders-Zufall, 2000; Firestein, 2001; Matthews and Reisert, 2003; Menini et al., 2004). The difficulty resides in the complex nature of the adaptive response. As one might expect, brief exposures to an odorant produce successively smaller responses (fast adaptation). However, prolonged odorant exposure adds an additional level of complexity, with slow oscillatory responses whose amplitude gradually declines during odorant exposure (Matthews and Reisert, 2003).

In olfactory transduction, an odorant molecule binds to a membrane protein on the cilia of olfactory sensory neurons. Odorant binding to the odor receptor protein activates a GTP-binding protein, which in turn stimulates adenylyl cyclase III to increase the production of cAMP. cAMP then activates cyclic nucleotide-gated (CNG) ion channels, and the ensuing influx of Na$^{+}$ and Ca$^{2+}$ depolarizes the cell. Next, another ion channel gets into the act: the increase in cytoplasmic [Ca$^{2+}$] stimulates Ca$^{2+}$-activated Cl$^{-}$ channels, which catalyze the net efflux of Cl$^{-}$ from the cell because of an unusually high intracellular Cl$^{-}$ concentration in these cells. Thus, the olfactory cell’s depolarizing response to odorants is generated by both cation influx and Cl$^{-}$ efflux. Ultimately, the depolarizing receptor potential initiates action potentials that carry the signal to the brain.

Ca$^{2+}$-activated Cl$^{-}$ channels are not the only Ca$^{2+}$-dependent proteins in the olfactory transduction cascade. In association with calmodulin or another Ca$^{2+}$-binding protein, Ca$^{2+}$ has three other important actions: (1) it stimulates a phosphodiesterase (PDE1C2) to hydrolyze cAMP; (2) it stimulates Ca$^{2+}$-calmodulin–dependent protein kinase II to phosphorylate, and thereby inhibit, adenylyl cyclase III; and (3) it inhibits the opening of olfactory CNG channels. Each of these actions of intracellular Ca$^{2+}$ would tend to reduce the odorant response, but which mechanism(s) does the olfactory sensory neuron use in fast adaptation?

Previous work suggests that the reduction in odorant response during fast adaptation is a Ca$^{2+}$-dependent process that does not involve a change in cAMP synthesis (Kurahashi and Menini, 1997), and most likely results from Ca$^{2+}$-dependent inhibition of the CNG channels. A large amount of accumulated evidence has revealed strong inhibition of olfactory CNG channels by Ca$^{2+}$, in combination with calmodulin and/or another endogenous Ca$^{2+}$-binding protein (Kramer and Siegelbaum, 1992; Chen and Yau, 1994; Balasubramanian et al., 1996; Kleene, 1999; Bradley et al., 2001; Trudeau and Zagotta, 2003; Bradley et al., 2004), and this inhibition appears to involve more than one subunit type within the channel (Bradley et al., 2001). Furthermore, work with normal (Kurahashi and Menini, 1997) and transgenic (Munger et al., 2001) olfactory sensory neurons implicates CNG channel inhibition in fast olfactory adaptation. However, because the synthesis and hydrolysis of cAMP are also Ca$^{2+}$ dependent, it is important to also examine their potential role in adaptation. Kurahashi and Menini (1997) have shown that odorant stimulation and release of caged cAMP produce the same Ca$^{2+}$-dependent adaptation in olfactory cells, indicating that fast adaptation occurs downstream of the cyclase.

Correspondence to Anita L. Zimmerman:
anita_zimmerman@brown.edu

Abbreviation used in this paper: CNG, cyclic nucleotide-gated.
Here, Boccaccio et al. (see p. 171 of this issue) set out to test whether the third Ca\(^{2+}\)-dependent process (cAMP hydrolysis) might participate in fast adaptation. They also asked whether fast adaptation only involved CNG channels, or whether Ca\(^{2+}\)-activated Cl\(^{-}\) channels were also important. To address these questions, they performed a set of difficult, rigorous experiments using whole-cell voltage clamp of isolated mouse olfactory sensory neurons, with flash photolysis of caged compounds (cAMP, 8-Br-cAMP, and Ca\(^{2+}\)).

Boccaccio et al. (2006) reasoned that if fast adaptation requires cAMP hydrolysis, then it should be quantitatively different in the presence of the hydrolysereistant nucleotide, 8-Br-cAMP, as compared with normal cAMP. They measured whole-cell currents in response to repetitive photorelease of cAMP or 8-Br-cAMP. With each cyclic nucleotide, the second flash gave a smaller current response, consistent with fast adaptation triggered by Ca\(^{2+}\) entry through CNG channels that were opened by cyclic nucleotide produced with the first flash. Furthermore, the fractional reduction in the responses was the same with both cAMP and 8-Br-cAMP, suggesting equivalent adaptation with and without hydrolysis. Removal of extracellular Ca\(^{2+}\) eliminated the adaptation in both cases, indicating a common Ca\(^{2+}\) requirement, as seen with odorant-induced adaptation. To further ensure that hydrolysis was not a factor, they performed control experiments that included the phosphodiesterase inhibitor, IBMX. These experiments confirmed the lack of involvement of cAMP hydrolysis in fast adaptation.

Having settled this issue, Boccaccio et al. examined the potential involvement of Ca\(^{2+}\)-activated Cl\(^{-}\) channels. These channels are clearly involved in primary olfactory transduction, but are they also involved in adaptation? It is conceivable that Ca\(^{2+}\) entering through the CNG channels could not only activate the Cl\(^{-}\) channels but also desensitize them, leading to a component of fast adaptation. Boccaccio et al. eliminated this possibility in two ways. First, they used ion substitution to substitute the reversal potential of Cl\(^{-}\) at their holding potential of −50 mV to eliminate a contribution of Cl\(^{-}\) current to the whole-cell current measured during adaptation. This manipulation did not affect measured adaptation, suggesting that Ca\(^{2+}\)-activated Cl\(^{-}\) channels are not involved in the process. Second, they investigated whether the Ca\(^{2+}\)-activated Cl\(^{-}\) channels could themselves adapt (i.e., desensitize) to Ca\(^{2+}\). For this experiment, they released caged Ca\(^{2+}\) intracellularly in the absence of cAMP or 8-Br-cAMP. Since the CNG channels were closed in the absence of agonist, they did not contribute to the measured whole-cell current and did not interfere with measurements of Ca\(^{2+}\)-induced effects on the Cl\(^{-}\) channels. Repetitive flashes of the caged Ca\(^{2+}\) gave equivalent responses, indicating that the Ca\(^{2+}\)-activated Cl\(^{-}\) channels do not desensitize to Ca\(^{2+}\), and therefore cannot contribute to fast olfactory adaptation. These results are consistent with earlier work in which Ca\(^{2+}\)-activated Cl\(^{-}\) channels were found to be insensitive to Ca\(^{2+}\)-calmodulin (Kleeke, 1999; Reisert et al., 2003).

The work of Boccaccio et al. takes us a large step closer to a complete understanding of adaptation in olfactory sensory neurons, though some questions remain. It is now clear that the main event in fast adaptation is inhibition of the ciliary CNG channels by Ca\(^{2+}\) in combination with calmodulin or a similar Ca\(^{2+}\)-binding protein. The inhibition by Ca\(^{2+}\) is terminated when the Ca\(^{2+}\) is extruded by Na\(^{+}\)/Ca\(^{2+}\) exchange, and the influx declines with the fall in CNG channel open probability. Interestingly, however, although the onset of CNG channel inhibition by Ca\(^{2+}\)-calmodulin is very fast, recovery is very slow; the inhibition is sustained for several seconds after removal of Ca\(^{2+}\)-calmodulin (Bradley et al., 2001, 2004, 2005; Munger et al., 2001). This slow recovery of channel open probability is not yet understood in molecular terms, but its duration is consistent with the measured time course of fast adaptation in the olfactory neurons. Furthermore, whereas changes in cAMP synthesis and hydrolysis are clearly not involved in fast adaptation, their regulation by Ca\(^{2+}\)-calmodulin is very fast, requiring that hydrolysis was not a factor, a mechanism to be studied in more detail. It will be important to define the mechanistic basis for these slower processes. Finally, as these mechanisms unfold, it becomes interesting to see how the mechanisms of adaptation in olfactory sensory neurons will relate to adaptation and feedback mechanisms in other neural systems.

**References**


