

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

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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  $\text{Ca}^{2+}$ , 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 adenylate cyclase III to increase the production of cAMP. cAMP then activates cyclic nucleotide-gated (CNG) ion channels, and the ensuing influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  depolarizes the cell. Next, another ion channel gets into the act: the increase in cytoplasmic  $[\text{Ca}^{2+}]$  stimulates  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels, which catalyze the net efflux of  $\text{Cl}^-$  from the cell because of an unusu-

ally high intracellular  $\text{Cl}^-$  concentration in these cells. Thus, the olfactory cell's depolarizing response to odorants is generated by both cation influx and  $\text{Cl}^-$  efflux. Ultimately, the depolarizing receptor potential initiates action potentials that carry the signal to the brain.

$\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels are not the only  $\text{Ca}^{2+}$ -dependent proteins in the olfactory transduction cascade. In association with calmodulin or another  $\text{Ca}^{2+}$ -binding protein,  $\text{Ca}^{2+}$  has three other important actions: (1) it stimulates a phosphodiesterase (PDE1C2) to hydrolyze cAMP; (2) it stimulates  $\text{Ca}^{2+}$ -calmodulin-dependent protein kinase II to phosphorylate, and thereby inhibit, adenylate cyclase III; and (3) it inhibits the opening of olfactory CNG channels. Each of these actions of intracellular  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -dependent process that does not involve a change in cAMP synthesis (Kurahashi and Menini, 1997), and most likely results from  $\text{Ca}^{2+}$ -dependent inhibition of the CNG channels. A large amount of accumulated evidence has revealed strong inhibition of olfactory CNG channels by  $\text{Ca}^{2+}$ , in combination with calmodulin and/or another endogenous  $\text{Ca}^{2+}$ -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 implicate CNG channel inhibition in fast olfactory adaptation. However, because the synthesis and hydrolysis of cAMP are also  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -dependent adaptation in olfactory cells, indicating that fast adaptation occurs downstream of the cyclase.

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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  $\text{Ca}^{2+}$ -dependent process (cAMP hydrolysis) might participate in fast adaptation. They also asked whether fast adaptation only involved CNG channels, or whether  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  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  $\text{Ca}^{2+}$ ).

Boccaccio et al. (2006) reasoned that if fast adaptation requires cAMP hydrolysis, then it should be quantitatively different in the presence of the hydrolysis-resistant 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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  eliminated the adaptation in both cases, indicating a common  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels. These channels are clearly involved in primary olfactory transduction, but are they also involved in adaptation? It is conceivable that  $\text{Ca}^{2+}$  entering through the CNG channels could not only activate the  $\text{Cl}^-$  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 set the reversal potential of  $\text{Cl}^-$  at their holding potential of  $-50$  mV to eliminate a contribution of  $\text{Cl}^-$  current to the whole-cell current measured during adaptation. This manipulation did not affect measured adaptation, suggesting that  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels are not involved in the process. Second, they investigated whether the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels could themselves adapt (i.e., desensitize) to  $\text{Ca}^{2+}$ . For this experiment, they released caged  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ -induced effects on the  $\text{Cl}^-$  channels. Repetitive flashes of the caged  $\text{Ca}^{2+}$  gave equivalent responses, indicating that the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels do not desensitize to  $\text{Ca}^{2+}$ , and therefore

cannot contribute to fast olfactory adaptation. These results are consistent with earlier work in which  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels were found to be insensitive to  $\text{Ca}^{2+}$ -calmodulin (Kleene, 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  $\text{Ca}^{2+}$  in combination with calmodulin or a similar  $\text{Ca}^{2+}$ -binding protein. The inhibition by  $\text{Ca}^{2+}$  is terminated when the  $\text{Ca}^{2+}$  is extruded by  $\text{Na}^+/\text{Ca}^{2+}$  exchange, and the influx declines with the fall in CNG channel open probability. Interestingly, however, although the onset of CNG channel inhibition by  $\text{Ca}^{2+}$ -calmodulin is very fast, recovery is very slow; the inhibition is sustained for several seconds after removal of  $\text{Ca}^{2+}$ -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  $\text{Ca}^{2+}$  appears to contribute to slower processes (e.g., oscillations) that remain 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.

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