Amplification of Odor-Induced Ca\textsuperscript{2+} Transients by Store-Operated Ca\textsuperscript{2+} Release and Its Role in Olfactory Signal Transduction

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1Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland, Baltimore, Maryland 21201; and 2Section of Neurobiology and 3Department of Neurosurgery, Yale University, New Haven, Connecticut 06520

Zufall, Frank, Trese Leinders-Zufall, and Charles A. Greer. Amplification of odor-induced Ca\textsuperscript{2+} transients by store-operated Ca\textsuperscript{2+} release and its role in olfactory signal transduction. J. Neurophysiol. 83: 501–512, 2000. A critical role of Ca\textsuperscript{2+} in vertebrate olfactory receptor neurons (ORNs) is to couple odor-induced excitation to intracellular feedback pathways that are responsible for the regulation of the sensitivity of the sense of smell, but the role of intracellular Ca\textsuperscript{2+} stores in this process remains unclear. Using confocal Ca\textsuperscript{2+} imaging and perforated patch recording, we show that salamander ORNs contain a releasable pool of Ca\textsuperscript{2+} that can be discharged at rest by the SERCA inhibitor thapsigargin and the ryanodine receptor agonist caffeine. The Ca\textsuperscript{2+} entry in the dendrite and soma but not in the cilia, the site of odor transduction. We deplete the stores to show that odor stimulation causes store-dependent Ca\textsuperscript{2+} mobilization. This odor-induced Ca\textsuperscript{2+} release does not seem to be necessary for generation of an immediate electrophysiological response, nor does it contribute significantly to the Ca\textsuperscript{2+} transients in the olfactory cilia. Rather, it is important for amplifying the magnitude and duration of Ca\textsuperscript{2+} transients in the dendrite and soma and is thus necessary for the spread of an odor-induced Ca\textsuperscript{2+} wave from the cilia to the soma. We show that this amplification process depends on Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release. The results indicate that stimulation of ORNs with odorants can produce Ca\textsuperscript{2+} mobilization from intracellular stores without an immediate effect on the receptor potential. Odor-induced, store-dependent Ca\textsuperscript{2+} mobilization may be part of a feedback pathway by which information is transferred from the distal dendrite of an ORN to its soma.

INTRODUCTION

Olfactory receptor neurons (ORNs) are the chemoreceptive cells that convert odor molecules into electrical membrane signals, a process known as odor transduction. It is clear that two ubiquitous second messengers, cAMP and Ca\textsuperscript{2+}, play pivotal roles in odor transduction. cAMP is the primary second messenger produced in the olfactory cilia when odor molecules bind to olfactory receptors, thus causing the activation of a G-protein–coupled adenyl cyclase/cAMP second-messenger cascade leading to the opening of cAMP-gated cation channels (CNG channels) (for review, see Ache and Zhananazarov 1995; Reed 1992; Restrepo et al. 1996). The substantial Ca\textsuperscript{2+} permeability of the CNG channels (Dzeja et al. 1999; Frings et al. 1995) then causes a rapid Ca\textsuperscript{2+} increase in the lumen of the olfactory cilia (Leinders-Zufall et al. 1997, 1998). This Ca\textsuperscript{2+} signal controls both excitation and adaptation. By activating Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} channels conducting a depolarizing Cl\textsuperscript{−} current, it serves to increase the gain of transduction (Kleene 1993; Kleene and Gesteland 1991; Kurahashi and Yau 1993; Lowe and Gold 1993; Reuter et al. 1998). The Ca\textsuperscript{2+} rise also mediates adaptation by modulation of CNG channel activity (Kurahashi and Menini 1997; Liu et al. 1994) and Ca\textsuperscript{2+}/calmodulin kinase II–dependent attenuation of adenyl cyclase (Leinders-Zufall et al. 1999; Wei et al. 1998).

Because of the crucial role of Ca\textsuperscript{2+} in olfactory signal transduction, it is necessary to develop a detailed understanding of the mechanisms underlying Ca\textsuperscript{2+} regulation and homeostasis. Here, we investigate the contribution of intracellular Ca\textsuperscript{2+} stores. This study was motivated, in part, by our previous finding that brief focal odor stimulation of olfactory cilia produced a wavelike Ca\textsuperscript{2+} elevation beginning in the apical cilia and spreading through the dendrite toward the soma, generating a global Ca\textsuperscript{2+} rise in the entire ORN (Leinders-Zufall et al. 1997, 1998). Unlike the monotonic decay of the Ca\textsuperscript{2+} transients in the cilia, Ca\textsuperscript{2+} elevations in the dendrite and soma often had a more complex appearance and lasted up to 10 times longer than the ciliary signals, leading us to hypothesize that they may depend on secondary Ca\textsuperscript{2+} mobilization.

Two other compelling reasons make the study of Ca\textsuperscript{2+} stores in ORNs necessary. First, odorants sometimes also cause formation of InsP\textsubscript{3} (Boekhoff et al. 1990; Ronnett et al. 1993). It has been proposed that InsP\textsubscript{3} mediates a second transduction mechanism by opening InsP\textsubscript{3}-gated channels (Boekhoff et al. 1990; Schild et al. 1995), but this proposal is at odds with experiments suggesting that odor transduction is mediated solely by the cAMP pathway (Belluscio et al. 1998; Brunet et al. 1996). Intracellular Ca\textsuperscript{2+} stores could provide an alternative target for odor-induced InsP\textsubscript{3}. Second, imaging intracellular Ca\textsuperscript{2+} is increasingly used as a method to visualize odor-induced activity, construct sensitivity profiles of individual ORNs, and relate this information to the structure of odor receptor genes (Bozza and Kauer 1998; Malnic et al. 1999; Rawson et al. 1997; Sato et al. 1994; Tarelius et al. 1995; Touhara et al. 1999). If these data are to be interpreted correctly, more information on the precise mechanisms by which Ca\textsuperscript{2+} is generated in ORN compartments is required. As our results show, odor-induced mobilization of Ca\textsuperscript{2+} from intracellular stores needs to be taken into account.

METHODS

Preparation of isolated ORNs

Isolated ORNs were prepared following protocols described previously (Leinders-Zufall et al. 1997). Briefly, ORNs were acutely dis-
associated from the nasal epithelium of adult land-phase tiger salamanders without the use of enzymes. To avoid movement artifacts, suspended cells were placed in a laminar flow chamber on glass coverslips that had been previously coated with 0.01% poly-L-lysine and 0.1% laminin to immobilize the neurons and their normally motile cilia on the substrate. Only those cilia that did not change their position during the course of an experiment were included in the analysis. ORNs were continuously superfused with physiological Ringer solution containing (in mM) 115 NaCl, 2.5 KCl, 1.0 CaCl₂, 1.5 MgCl₂, 4.5 HEPES, and 4.5 Na-HEPES, pH 7.6, adjusted to 240 mOsm. To avoid a contribution to the measured Ca²⁺ signals from regenerative action potential discharges, 4 µM tetrodotoxin (TTX) was added to this solution in all Ca²⁺ imaging experiments. TTX was also present in the Ringer solution during the electrophysiological experiments with the exception of the receptor potential recordings of Fig. 7. All measurements were carried out at room temperature.

Calcium imaging

Imaging techniques were essentially as described earlier (Leinders-Zufall et al. 1997, 1998). ORNs (n = 57) were loaded with the Ca²⁺ indicator fluo-3 AM (18 µM; Molecular Probes, Eugene, OR). A laser scanning confocal system (Bio-RAD MRC-600, Hercules, CA) attached to an Olympus IMT-2 inverted microscope was employed to visualize Ca²⁺-mediated fluorescence changes. A 60×, 1.4 numeric aperture oil immersion objective (Nikon) was used; images were additionally magnified three to four times using the confocal’s electronic zoom setting. Time-series images were made by collecting 64 × 64 pixel fluorescence images at a rate ranging from 0.1 to 3 Hz depending on the experiment. For the higher spatial resolution required in some experiments, four individual frames (768 × 512 pixels/frame) were averaged together, using the Kalman filter function of the confocal system. Data are presented in arbitrary fluorescence units or as relative changes in fluorescence intensity normalized to baseline fluorescence (ΔF/F). For the quantification of Ca²⁺ signals, regions of interest were outlined and the average pixel values in these regions were measured. Usually, these regions corresponded to entire ORN compartments such as soma, dendrite, knob, and individual cilia, as schematized in Fig. 1A. We also tested whether equivalent results were obtained when smaller areas that showed some degree of inhomogeneity in fluorescence level were taken as representative region (see, for example, Fig. 1, B and G). In no case did the results obtained by these two approaches differ qualitatively, and none of the conclusions depend critically on the small quantitative differences seen (compare for example Fig. 1, F and G).

Electrophysiological recording and drug application

For all current-clamp and voltage-clamp recordings (n = 46), we employed the perforated patch-clamp technique with ampheproticin B (Leinders-Zufall et al. 1995). This approach ensures the least possible disturbance of the internal milieu of the neurons and avoids artificial Ca²⁺ buffering of the intracellular compartments. The patch pipettes were filled with the following solution (in mM): 17.7 KCl, 105.3 KOH, 82.3 methanesulfonic acid, 5.0 EGTA, and 10 HEPES, pH 7.5 (KOH), adjusted to 224 mOsm. In some experiments K⁺ was replaced by Cs⁺. Current and voltage recording, stimulation sequences, data acquisition, and on-line analysis were controlled by an EPC-9 patch-clamp amplifier in combination with Pulse software (HEKA Electronic, Lambrecht/Pfalz, Germany) and a Macintosh computer. Currents were filtered at 300 Hz (3 dB, 8-pole low-pass Bessel). The indifferent electrode consisted of a Ag-AgCl wire connected to the bath solution via an agar bridge. All data reported here have been corrected for junction potentials.

Thapsigargin (Research Biochemicals International, Natick, MA) was initially dissolved in ethanol to give a 2-mM stock solution. The agent was diluted to the final concentration immediately before use, sonicated, and applied by bath perfusion. Final concentrations of thapsigargin solutions contained 0.01% (vol/vol) ethanol. In control experiments (n = 4) we found that this concentration of ethanol alone had no effect on intracellular Ca²⁺ levels. Other stimuli such as the odorant cione (eucalyptol, 1.3,3-trimethyl-2-oxacyclo[2,2,2]-octane), the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), or the ryanodine receptor agonist caffeine were ejected from multibarrel glass pipettes that were placed within 5–10 µm from the cilia or the cell soma. Stimulus pipettes were located downstream from the cells to avoid prestimulation. Unless otherwise stated, all chemicals were obtained from Sigma (St. Louis, MO). Our basic paradigm to deplete intracellular Ca²⁺ stores was to add 200 nM thapsigargin to the bath solution for 5 min. This treatment resulted, over the course of 10–25 min after the initial application of the drug, in Ca²⁺ accumulation followed by restoration of the Ca²⁺ signal to prestimulation levels (see Figs. 1 and 3).

Data analysis

For off-line analysis, eight-bit confocal image files were transferred to a Macintosh G3 microcomputer and analyzed with customized NIH Image 1.61 software. Additional data analyses and calculations were performed using Igor Pro software (WaveMetrics, Lake Oswego, OR). Unless otherwise stated, data are expressed as means ± SD. Statistical tests were performed with SuperAnova 1.1 (Abacus Software, Berkeley, CA). Fisher’s LSD (least significant difference) test was used as a post hoc comparison of the ANOVA. Composite images were prepared using Adobe Photoshop 3.0 and printed on a Fujix Pictography 3000 color printer.

RESULTS

Spatial segregation of store-operated Ca²⁺ release in ORN compartments

To test for the existence of functional Ca²⁺ stores in ORNs, we applied thapsigargin, a potent and irreversible inhibitor of sarcoplasmic-endoplasmic reticulum Ca²⁺-ATPases (SERCAs) that mediate the uptake of cytosolic Ca²⁺ into endoplasmic reticulum stores (Pozzan et al. 1994; Thastrup et al. 1990; Thomas and Hanley 1994; Treiman et al. 1998). To visualize changes in the intracellular Ca²⁺ concentration, ORNs were loaded with the Ca²⁺ indicator dye fluo-3 AM, and confocal fluorescence imaging was done as described previously (Leinders-Zufall et al. 1997, 1998). Figure 1 illustrates the effect of bath-applied thapsigargin (200 nM) on Ca²⁺ fluorescence in the various cellular compartments of an ORN. At rest before stimulation (Fig. 1B), fluorescence intensity was generally rather low except for domains of higher fluorescence in discrete spots at the rim of the nucleus and at proximal and distal portions of the dendrite. The distribution of these fluorescence spots resembled the distribution of cellular organelles such as endoplasmic reticulum, mitochondria, and variagated and multivesicular bodies (Simmons et al. 1981). The soma contains a large elliptically shaped nucleus that allows only a thin rim of surrounding cytoplasm on the lateral and basal aspects of the soma. The light and dark areas within the soma reflect the nuclear “checkerboard” pattern of chromatin typical for mature salamander ORNs (Simmons et al. 1981). Consistent with our previous results, resting Ca²⁺ fluorescence within single cilia was below detection level in most ORNs. Thapsigargin induced a substantial long-lasting elevation in intracellular Ca²⁺ (Fig. 1C). This thapsigargin-evoked Ca²⁺ rise overlapped spatially with the distribution of endoplasmic reticulum, occurring in the knob, dendrite, and soma, but not in the olfactory cilia. This effect of
thapsigargin was highly robust and reproducible in virtually every ORN tested (soma: $\Delta F/F = 26.4 \pm 12.3\%$, mean $\pm$ SD; dendrite: $\Delta F/F = 19.9 \pm 8.9\%$; knob: $\Delta F/F = 9.0 \pm 6.8\%; n = 17$). The elevated $Ca^{2+}$ recovered to near baseline levels over the course of 10 min after superfusing the ORN with Ringer solution (Fig. 1D).

Given that thapsigargin acts as an irreversible SERCA inhibitor, a likely explanation for this observation is that the $Ca^{2+}$ stores were emptied at this point and $Ca^{2+}$ clearance took place. This notion was supported further in other experiments in which we applied thapsigargin for a prolonged time (200 nM for 30 min, not shown). Despite the continued presence of thapsigargin in the bath solution, the $Ca^{2+}$ elevation always recovered to baseline levels within 10–25 min after the initial application of thapsigargin ($n = 9$). Together with additional experiments shown in Fig. 3, this suggests that the recovery of the $Ca^{2+}$ signal as seen in Fig. 1D does not reflect reversibility of the thapsigargin-induced effect but instead is evidence for an almost complete depletion of the thapsigargin-sensitive $Ca^{2+}$ pools.

The thapsigargin-induced fluorescence changes were plotted as a function of time (Fig. 1, E–G). In Fig. 1, E and F, spatially averaged pixel values were obtained from entire ORN compartments as outlined in Fig. 1A. No measurable signal was detected in the cilia of this cell (Fig. 1E), or in cilia from any other ORN exposed to thapsigargin ($n = 29$). Within the soma, dendrite, and knob the thapsigargin-induced $Ca^{2+}$ rise occurred approximately simultaneously (Fig. 1F). The onset of this signal was initiated within 30 s and reached a maximum by $\sim 3$–4 min after drug application (Fig. 1F). Figure 1G shows the thapsigargin-induced $Ca^{2+}$ rise analyzed in four smaller regions within the soma compartment (the 4 areas are denoted by white lines shown in B).
dose of thapsigargin (400 nM), intracellular Ca$^{2+}$ arose much faster, but cell death occurred regularly during the course of these experiments, making the use of higher thapsigargin concentrations impracticable.

To strengthen further the evidence that store-operated Ca$^{2+}$ release is compartmentalized in ORNs, we utilized caffeine to induce Ca$^{2+}$ release. Caffeine is known as a stimulator of neuronal ryanodine receptors that act as intracellular Ca$^{2+}$ release channels (Garaschuk et al. 1997; McPherson et al. 1991; cf. Taylor and Broad 1998). When a pulse of caffeine (10 mM) was focally applied onto the soma, dendrite and cilia of an ORN, there was a transient rise in Ca$^{2+}$ in all cellular compartments except for the cilia ($n = 14$; Fig. 2, A–C). The Ca$^{2+}$ transients rose to a peak within 2 s and decayed back to baseline within 13–42 s after termination of the stimulus. They reached fluorescence changes of $\Delta F/F = 23.1 \pm 5.3\%$, $n = 5$ (analyzed in the dendritic compartment), which are in a similar range as those induced by thapsigargin. When the duration of the caffeine pulses was increased, there was no further increase in the measured Ca$^{2+}$ signals, indicating that the responses were close to their maximum (data not shown). Another known action of caffeine, inhibition of phosphodiesterase (PDE), was almost certainly not involved in the caffeine effect. PDE is known to be highly concentrated in the ORN cilia, and application of the PDE inhibitor IBMX leads to robust ciliary Ca$^{2+}$ elevations (Leinders-Zufall et al. 1997). The absence of such Ca$^{2+}$ increases after caffeine stimulation argues against a significant inhibition of olfactory PDE by the caffeine stimuli used here.

Together, the results of Figs. 1 and 2 indicate that salamander ORNs contain functional Ca$^{2+}$ stores in their knob, dendrite, and soma. The fact that Ca$^{2+}$ increases were readily induced by thapsigargin or caffeine before the activation of any Ca$^{2+}$ entry pathway provides evidence that even at rest the stores contain a releasable pool of Ca$^{2+}$. Because thapsigargin acts as an uptake inhibitor, the data suggest that Ca$^{2+}$ stores in ORNs continuously leak Ca$^{2+}$ and that this loss is counterbalanced by active uptake (sequestration) of Ca$^{2+}$ by the SERCA pumps into the stores. The fact that we have selectively increased Ca$^{2+}$ in the dendritic knob but not in the cilia by releasing it from Ca$^{2+}$ stores in the knob shows that this Ca$^{2+}$ remains in the knob and does not spread into the cilia. Ca$^{2+}$ release from thapsigargin-sensitive stores is therefore unlikely to be significantly involved in the primary odor transduction process or in its Ca$^{2+}$-dependent feedback regulation through modulation of enzymatic cascades present in the olfactory cilia.

**Thapsigargin discharges Ca$^{2+}$ from caffeine-sensitive stores**

To test that the source of the thapsigargin-induced Ca$^{2+}$ increase was indeed intracellular, we lowered the extracellular Ca$^{2+}$ concentration to 0.6 μM, which is sufficient to eliminate odor- and cyclic nucleotide–induced Ca$^{2+}$ responses in these cells (Leinders-Zufall et al. 1997, 1998). Under these conditions thapsigargin still elicited a substantial increase in Ca$^{2+}$ with no obvious difference in the spatial distribution of the signal compared with normal extracellular Ca$^{2+}$ concentrations (Fig. 3A). However, there was a strong modification in the temporal behavior of the thapsigargin-induced Ca$^{2+}$ rise in that the response became more transient, now recovering back to baseline within 3–4 min during the continuous presence of the drug (Fig. 3A; $n = 7$). This shift from a sustained to a more transient response following removal of extracellular Ca$^{2+}$ is consistent with previous results in a number of nonexcitable cell types and is sometimes interpreted as evidence for the existence of capacitative Ca$^{2+}$ entry (Putney 1986; cf. Thomas and Hanley 1994). In the “capacitative entry” model the initial Ca$^{2+}$ transient induced by thapsigargin is caused by release from intracellular stores, whereas the sustained Ca$^{2+}$ elevation reflects Ca$^{2+}$ entry caused by the activation of store-operated Ca$^{2+}$ entry channels located in the plasma membrane, but it is unclear whether such capacitative Ca$^{2+}$ entry occurs in neurons (see Garaschuk et al. 1997). The results of Fig. 3A may suggest that this is the case in ORNs, although more specific testing will be required for a final assessment of this possibility.

Figure 3B illustrates an experiment in which an ORN was stimulated by two successive thapsigargin applications (200 nM) that were interrupted by a 10-min washing period. Although the first stimulus induced a Ca$^{2+}$ rise that gradually recovered back to baseline over the course of ~12 min, the second application was unable to elicit any detectable Ca$^{2+}$ increase ($n = 3$). This provides further evidence that a one-time treatment with 200 nM thapsigargin was sufficient to discharge the thapsigargin-sensitive Ca$^{2+}$ pools to a large degree if not completely. Therefore this stimulus was utilized in all subsequent experiments for discharging Ca$^{2+}$ stores.

To investigate the relation between thapsigargin- and caffeine-depleted stores, an ORN was stimulated with a 5-s pulse of caffeine (10 mM) as described in Fig. 2, and the resulting Ca$^{2+}$ transients were analyzed in the knob (Fig. 3C) and dendrite (Fig. 3D). The experiment was done with low external Ca$^{2+}$ concentration (0.6 μM) to avoid release-activated Ca$^{2+}$ entry. After the caffeine test pulse, thapsigargin was applied, causing store depletion. A second caffeine stimulus was now unable to induce a Ca$^{2+}$ transient in the knob ($n = 4$; Fig. 3C). This indicates that caffeine and thapsigargin target the same set of Ca$^{2+}$ pools within the knob. In the dendrite, however, the
guished by the results of Fig. 3E. When the same protocol was repeated 6.6 min later, the initial large Ca\textsuperscript{2+} transient was fully recovered, indicating that the stores underwent spontaneous refilling. However, when this protocol was repeated after treatment of the cell with thapsigargin (200 nM), the initial large Ca\textsuperscript{2+} transient failed to recover, whereas there was very little effect on the smaller Ca\textsuperscript{2+} transients seen with repetitive caffeine stimulation (n = 5). These findings indicate that refilling of caffeine-sensitive stores in ORNs is mediated by SERCA pumps. The data demonstrate that thapsigargin and caffeine overlap, at least partially, in their actions, thus targeting the same set of Ca\textsuperscript{2+} stores. There may be a second type of caffeine-sensitive Ca\textsuperscript{2+} pool in these cells that is resistant or less sensitive to thapsigargin treatment (for comparison see Thomas and Hanley 1994), although a nonuniform internal thapsigargin concentration cannot be ruled out entirely.

$Ca^{2+}$ stores control the waveform of odor-induced $Ca^{2+}$ transients in the dendrite and soma but not in the cilia

What is the precise role of thapsigargin-sensitive Ca\textsuperscript{2+} stores in Ca\textsuperscript{2+} signaling of ORNs? The endoplasmic reticulum may serve 1) as an intracellular source for Ca\textsuperscript{2+} being involved in Ca\textsuperscript{2+} mobilization and Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release following cellular stimulation, or 2) it may mediate rapid sequestration and uptake to govern clearance of Ca\textsuperscript{2+} after an odor stimulus (Miller 1991; Simpson et al. 1995). To distinguish between these possibilities, we emptied Ca\textsuperscript{2+} stores with thapsigargin and examined the effect of this treatment on stimulus-induced Ca\textsuperscript{2+} responses in the ORN compartments.

The cilia of an ORN were stimulated with a brief pulse of the PDE inhibitor IBMX (300 μM for 1 s), and confocal images were acquired at the peak of the resulting Ca\textsuperscript{2+} response (Fig. 4, A–C). IBMX treatment is known to stimulate the odor-sensitive cAMP pathway present in the cilia of these cells causing CNG channel opening and Ca\textsuperscript{2+} entry (Firestein et al. 1991b; Leinders-Zufall et al. 1997). As reported previously, IBMX caused the generation of a characteristic Ca\textsuperscript{2+} wave spreading through the ORN with distinct spatiotemporal properties starting in the cilia and eventually leading to a global Ca\textsuperscript{2+} rise in the dendrite and soma (Fig. 4B). After recovery of this Ca\textsuperscript{2+} wave, Ca\textsuperscript{2+} stores were emptied with thapsigargin (200 nM for 5 min) until the resulting Ca\textsuperscript{2+} elevation recovered back to baseline as shown in Fig. 3B. Following this treatment, a second IBMX pulse of the same strength was applied that evoked a Ca\textsuperscript{2+} transient within the cilia very similar to that of the control measurement. But in contrast to the control situation, the propagation of the Ca\textsuperscript{2+} wave into the dendrite and soma was now strongly reduced. This can be seen in the confocal image of Fig. 4C in which the cilia exhibited enhanced Ca\textsuperscript{2+} fluorescence in response to IBMX but the fluorescence intensity in the dendrite and soma resembled more that of the unstimulated cell shown in Fig. 4A. Closely similar results were obtained in a total of six ORNs (see Table 1 for a more complete analysis of the data).

An equivalent effect was observed when the cells were stimulated with odorant (cineole, 1 s, 300 μM) instead of IBMX. Cineole triggered a similar Ca\textsuperscript{2+} wave as IBMX, starting in the cilia and then propagating toward the soma (Fig. 4D). A characteristic property of this Ca\textsuperscript{2+} wave is the pro-
longed Ca$^{2+}$ elevation in the dendrite and soma, which is distinct from the more transient Ca$^{2+}$ rises in the cilia and the olfactory knob. Consistent with the results of Fig. 4, A–C, Ca$^{2+}$ store depletion with thapsigargin caused a 2.5- to 11-fold decrease in the peak amplitude of the transients as well as a 2- to 4-fold shortening of the response duration in the dendrite and soma ($n = 4$; Fig. 4D). There was no detectable effect of store depletion on the ciliary odor-induced Ca$^{2+}$ transients ($n = 4$; Fig. 4D). A moderate degree of response shortening was seen in the olfactory knob ($n = 4$; Fig. 4D). Data from several independent experiments are summarized in Table 1. We conclude therefore that a substantial portion of the prolonged Ca$^{2+}$ responses in the dendrite and soma results from Ca$^{2+}$ release by thapsigargin-sensitive stores. This provides evidence that Ca$^{2+}$ released from thapsigargin-sensitive pools, at least under the conditions of odor stimulation employed here, amplifies and prolongs incoming Ca$^{2+}$ signals, thus boosting the magnitude and duration of odor-induced Ca$^{2+}$ transients in the ORN dendrite and soma giving rise to the propagation of a characteristic Ca$^{2+}$ wave from the cilia to the soma.

These data provide evidence against a significant contribution of Ca$^{2+}$ released from thapsigargin-sensitive stores to the ciliary Ca$^{2+}$ signals elicited by odor stimulation. Together with the results of Figs. 1 and 2, they thus confirm and extend the notion that there is no detectable back spread of Ca$^{2+}$ from the dendritic knob during the odor response and that each cilium can function as a Ca$^{2+}$ signaling unit that is relatively independent from Ca$^{2+}$ changes in other cilia and the dendritic knob.

**Depolarization-induced Ca$^{2+}$ transients depend on the filling state of thapsigargin-sensitive stores: evidence for Ca$^{2+}$-induced Ca$^{2+}$ release (CICR)**

Having provided evidence that odor stimuli can trigger Ca$^{2+}$ mobilization from thapsigargin-sensitive stores in the olfactory knob, dendrite, and soma, we next investigated whether Ca$^{2+}$ entering the ORN through voltage-operated Ca$^{2+}$ channels (VOCCs) contributes to this effect. To test for the presence of such CICR, we examined whether depolarization-induced Ca$^{2+}$ transients are affected by Ca$^{2+}$ store depletion (Fig. 5). If CICR contributes to the depolarization-induced Ca$^{2+}$ transients, then their magnitude should be diminished after store depletion.

When the soma of an ORN was stimulated with a 1-s
TABLE 1. Effect of store depletion on magnitude and time course of IBMX and odor-induced Ca$^{2+}$ responses in ORN compartments

<table>
<thead>
<tr>
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<th>IBMX</th>
<th>Cineole</th>
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<tr>
<td></td>
<td>$\Delta F/F$, %</td>
<td>Half recovery time, s</td>
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<tr>
<td>Cilia</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>14.3 ± 5.7</td>
<td>3.0 ± 1.0</td>
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<tr>
<td>Thapsigargin</td>
<td>14.1 ± 5.9</td>
<td>3.0 ± 1.0</td>
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<tr>
<td>Knob</td>
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</tr>
<tr>
<td>Control</td>
<td>25.9 ± 6.4</td>
<td>12.0 ± 8.7</td>
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<tr>
<td>Thapsigargin</td>
<td>14.3 ± 4.1</td>
<td>7.8 ± 7.9</td>
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<tr>
<td>Dendrite</td>
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<tr>
<td>Control</td>
<td>37.8 ± 9.5</td>
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<tr>
<td>Thapsigargin</td>
<td>9.1 ± 2.0</td>
<td>10.3 ± 6.1</td>
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<tr>
<td>Soma</td>
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<td>50.3 ± 6.8</td>
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<tr>
<td>Thapsigargin</td>
<td>8.5 ± 0.3</td>
<td>7.7 ± 5.5</td>
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Values are means ± SD. Number of neurons stimulated with IBMX is 6 and with cineole is 4. Ca$^{2+}$ responses were analyzed in the various intracellular compartments before and after store depletion by thapsigargin (200 nM) as shown in Fig. 6. Fluorescence changes ($\Delta F/F$) represent the peak of the responses. For each ORN, the Ca$^{2+}$ responses of 3–8 individual cilia were averaged. IBMX (300 μM) was applied for 1 s. The odor stimulus was a 1-s pulse of cineole (300 μM). Note that store depletion has virtually no effect on the ciliary Ca$^{2+}$ responses, whereas there is a strong reduction in response magnitude and duration in the other ORN compartments. IBMX, 3-isobutyl-1-methylxanthine; ORN, olfactory receptor neuron.

pulse of KCl (120 mM), a rapid Ca$^{2+}$ elevation was detected in the soma ($\Delta F/F = 44 ± 28%$; half recovery time = 21 ± 6 s; n = 9) and in other cellular compartments (Fig. 5A) but not in the cilia as shown previously (Leinders-Zufall et al. 1997). Following store depletion by thapsigargin (200 nM for 5 min), a second identical KCl stimulus elicited a Ca$^{2+}$ response that was strongly reduced both in peak amplitude and duration as compared with the control measurement ($\Delta F/F = 13 ± 14%$; half recovery time = 12.2 ± 7 s; n = 9; Fig. 5B). As with odor stimulation, the effect of store depletion on depolarization-induced Ca$^{2+}$ transients was somewhat variable causing peak amplitude decreases ranging from 2.5- to 12-fold (n = 9). A comparable decrease in depolarization-induced Ca$^{2+}$ transients was never seen in control experiments without thapsigargin treatment, indicating that the described effect was not caused by dye bleaching (n = 4). Thus a large fraction of the depolarization-induced Ca$^{2+}$ transients in the knob, dendrite, and soma of ORNs appears to depend on CICR.

Despite the fact that thapsigargin is widely accepted as a relatively specific inhibitor of SERCA pumps, previous work has shown that it sometimes can also act as an inhibitor of voltage-gated Ca$^{2+}$ channels, at least when used at micromolar concentrations (Rossier et al. 1993; Shmigol et al. 1995; Treiman et al. 1998). Although we did not utilize such high doses in the current study, we considered the possibility that the effect on depolarization-induced Ca$^{2+}$ transients as seen in Fig. 5B was mediated by thapsigargin-induced blockade of VOCCs. VOCCs were investigated in response to ramp depolarization under voltage clamp (holding potential, −60 mV) using the perforated patch technique (see METHODS for details). Voltage-gated Na$^+$ currents were inhibited by TTX (4 μM), and K$^+$ outward currents were blocked to a large degree with Cs$^+$, which was dialyzed from the patch pipette into the cytoplasm via the pores formed by amphotericin B. A typical recording example is depicted in Fig. 5C illustrating the resulting current-voltage (I-V) curve. Salmon ORNs exhibited relatively small currents through VOCCs with an activation threshold near −40 mV and a peak amplitude ≤60 pA (n = 6), consistent with previous results (Firestein and Werblin 1987). These currents were blocked completely by Cd$^{2+}$ (500 μM; Fig. 5C). Figure 5D illustrates that thapsigargin had no direct effect on VOCCs (n = 5). Thus thapsigargin-mediated inhibition of VOCCs is unlikely to account for the effects documented in Fig. 5B.

Taken together, the results of Figs. 4 and 5 indicate that an unexpectedly large fraction of the odor-induced Ca$^{2+}$ elevations in the knob, dendrite, and soma (but not in the cilia) of
salamander ORNs is mediated by Ca^{2+}-induced Ca^{2+} release from thapsigargin-sensitive stores. We show that CICR is present in ORNs and that its extent is sufficient to account for the store-operated amplification of odor-induced Ca^{2+} transients. The Ca^{2+} release cannot be explained by cAMP diffusing from the cilia to the soma: when we lowered the external Ca^{2+} concentration to 0.6 \mu M, which does not attenuate cAMP formation, there was no Ca^{2+} mobilization in any of the ORN compartments (Leinders-Zufall et al. 1997, 1998). We therefore conclude that the prolonged Ca^{2+} responses seen in the ORN dendrite and soma following a brief pulse of odorant result primarily from CICR.

**Depletion of Ca^{2+} stores by thapsigargin induces membrane hyperpolarization and activation of a K^{+} conductance**

To begin to assess the relation between store-operated Ca^{2+} release and the electrical properties of the cells, we examined whether Ca^{2+} depletion by thapsigargin leads to a change in membrane potential (Fig. 6). Thapsigargin sometimes depolarizes neurons through activation of cationic conductances (Knox et al. 1996). In salamander ORNs, however, thapsigargin (200 nM) induced a slow tonic hyperpolarization of \(-2.2 \pm 1.6\) mV (from a resting potential of \(-60\) mV, \(n = 6\)) lasting for \(7.2 \pm 1.3\) min. The reversal potential of this hyperpolarization was derived from a plot of the magnitude of the thapsigargin-induced voltage change as a function of the holding potential, yielding \(E_{\text{rev}} = -71\) mV (\(n = 16\); regression: \(P = 0.84\); data not shown). The fact that thapsigargin application was associated with membrane hyperpolarization but not with depolarization rules out that Ca^{2+} entry through VOCCs contributed to the thapsigargin-induced Ca^{2+} rises documented above.

Figure 6B illustrates that thapsigargin produced a decrease in input resistance (monitored through hyperpolarizing current pulses), from \(4.1 \pm 0.4\) G\(\Omega\) to \(3.3 \pm 0.6\) G\(\Omega\) (\(n = 6\)). The onset and recovery of this resistance change paralleled the general time course of the thapsigargin-induced hyperpolarization, suggesting that Ca^{2+} store depletion evoked the activation of specific ion channels.

To test this notion further, we measured the effect of thapsigargin in response to stimulation with a depolarizing voltage ramp on whole cell currents in voltage-clamped ORNs (holding potential, \(-60\) mV). In this case, unlike the experiment shown in Fig. 5, \(C\) and \(D\), K\(^{+}\) was not replaced with Cs\(^{+}\) in the intracellular pipette solution. Figure 6C illustrates that, under these ionic conditions, thapsigargin induced the activation of an outward current (thap, 5 min). Digital subtraction of the two \(I-V\) curves (thap, 5 min − control) revealed a conductance with outwardly rectifying properties and a characteristic N-shape showing a local minimum near \(+50\) mV, close to the expected reversal potential for Ca^{2+} flux through VOCCs (Fig. 6D; \(n = 9\)). Because this type of conductance is usually associated with the activation of Ca^{2+}-dependent K\(^{+}\) channels (Meech and Standen 1975; Thomas 1984), the main effect of thapsigargin-

![Figure 6](http://jn.physiology.org/)
induced Ca\(^{2+}\) store depletion on ORN membrane properties is a net hyperpolarization caused by the activation of putative Ca\(^{2+}\)-activated K\(^+\) channels. Such a conductance was never seen when the ORNs were dialyzed with Cs\(^+\) (see Fig. 5D). Ca\(^{2+}\)-dependent K\(^+\) channels have previously been shown to be present in the cell body, dendrite, and knob of ORNs (Maue and Dionne 1987). In eight of nine experiments, we also observed activation of a small inward current with an amplitude of \(\pm 10\) pA in response to thapsigargin (see arrow in Fig. 6D). This current component was somewhat reminiscent of calcium release-activated calcium currents (I\(_{\text{CRAC}}\)) that have been described in a number of nonexcitable cell types (cf. Parekh and Penner 1997) and could underlie the effects observed in Fig. 3A. Detailed investigation of this thapsigargin-evoked inward current, however, was beyond the scope of the current study.

**Lack of effect of Ca\(^{2+}\) store depletion on odor-induced receptor potential**

Thus far our results indicate that odor stimulation causes mobilization of Ca\(^{2+}\) from intracellular stores via CICR and that Ca\(^{2+}\), if discharged by thapsigargin, tends to hyperpolarize the cells through activation of a K\(^+\) conductance. This raises the question of whether odor-induced Ca\(^{2+}\) mobilization serves to shape the receptor potential thus modifying chemoelectrical signaling and odor processing in ORNs.

To test this idea, odor-induced receptor potentials were analyzed before and after store depletion with thapsigargin (Fig. 7). Surprisingly, however, we found that the waveform of the receptor potential was nearly independent of the filling state of Ca\(^{2+}\) stores. In the example shown in Fig. 7A, the cell had a resting potential of \(-60\) mV before thapsigargin application. A 1-s pulse of cineole (300 \(\mu\)M) induced a typical depolarizing receptor potential to \(-24\) mV lasting for \(-3\) s. During the rising phase, a transient burst of action potential discharges occurred, which was followed by a silent period. After repolarization there was a pronounced afterhyperpolarization (AHP) lasting for a few seconds. The cell was then treated with thapsigargin (200 nM for 22 min), and the same odor stimulus was applied. As shown in Fig. 7, A and B, there was neither a change in the resting potential nor a significant difference in the waveform of the odor-induced receptor potential. Closely similar results were obtained in a total of seven ORNs.

Overall, these results reveal that pharmacological disruption of Ca\(^{2+}\) store function has no immediate effect on the electrophysiological responses to odors, at least under the conditions of odor stimulation used here, indicating that the receptor potential and its underlying transduction currents are relatively independent of the filling state of Ca\(^{2+}\) stores. Thus, stimulation of ORNs with odorants can produce Ca\(^{2+}\) mobilization from intracellular stores without changing significantly the electrical properties of the ORNs. This situation is reminiscent of a class of synaptic responses involving Ca\(^{2+}\) release from intracellular stores in some dendritic spines (Takechi et al. 1998).

**Discussion**

A combination of confocal imaging and electrophysiological recording provided insight into the role of intracellular Ca\(^{2+}\) stores in odor transduction of ORNs. Several new findings emerge from this work. 1) ORNs contain a releasable pool of Ca\(^{2+}\) that can be discharged at rest applying the SERCA pump inhibitor thapsigargin or the ryanodine receptor agonist caffeine. 2) These Ca\(^{2+}\) stores are distributed differentially within the ORNs; they are present in the soma, dendrite, and knob, but not in the cilia, the site of primary odor transduction. 3) SERCA pumps mediate the refilling of caffeine-sensitive stores. 4) Ca\(^{2+}\) released from thapsigargin-sensitive pools serves to amplify odor-induced Ca\(^{2+}\) transients in the knob, dendrite, and soma, but not in the cilia; this effect underlies Ca\(^{2+}\) wave propagation from the cilia to the soma. 5) The amplification is primarily due to Ca\(^{2+}\)-induced Ca\(^{2+}\) release. 6) Thapsigargin-induced Ca\(^{2+}\) release tends to hyperpolarize the ORN membrane potential by stimulating a Ca\(^{2+}\)-activated K\(^+\) current. 7) Thapsigargin-sensitive stores do not seem to be necessary for generation of an immediate electrophysiological
odor response, at least under the experimental conditions described here. It seems likely that our results will also apply to mammals, although we have not yet tested whether thapsigargin-sensitive stores exist in mammalian ORNs. However, there are also differences in Ca\(^{2+}\) signaling between salamander and mammalian ORNs. For example, human ORNs frequently appear to respond with a decrease in Ca\(^{2+}\) to odorant stimulation (Rawson et al. 1997), whereas such responses were not observed in salamander (unpublished observations).

It should be noted that a small percentage of ORNs both in amphibians and rodents sometimes exhibit inhibitory odor responses (Duchamp-Viret et al. 1999; Morales et al. 1997). Such inhibitory responses were also seen infrequently in our experiments (unpublished observations). Because thapsigargin-induced Ca\(^{2+}\) release tended to hyperpolarize the ORNs, store-dependent Ca\(^{2+}\) release could potentially be involved in these inhibitory odor responses. However, the small percentage of ORNs that exhibited such responses prevented us from testing this hypothesis directly.

**Store-operated Ca\(^{2+}\) release amplifies odor-induced Ca\(^{2+}\) transients via CICR**

A key result presented in this communication is that thapsigargin-sensitive Ca\(^{2+}\) stores determine the waveform of odor-induced Ca\(^{2+}\) transients. Following store depletion, the magnitude and duration of odor-induced Ca\(^{2+}\) elevations was strongly reduced relative to the control response (2.5- to 11-fold). This effect occurred only in the dendrite and soma and to a lesser degree in the knob, but not in the cilia. This provides evidence that one function of Ca\(^{2+}\) stores is to serve as an intracellular source for Ca\(^{2+}\) involved in Ca\(^{2+}\) mobilization and release following an odor stimulus. We show that CICR is present in ORNs and that its extent is sufficient to explain the store-dependent amplification of odor-induced Ca\(^{2+}\) transients.

The fact that odor-induced Ca\(^{2+}\) responses in the soma and dendrite depend critically on the filling state of Ca\(^{2+}\) stores has two important implications for experiments in which Ca\(^{2+}\) imaging of the soma is utilized to construct odor sensitivity profiles of single ORNs (Bozza and Kauer 1998; Malnic et al. 1999; Rawson et al. 1997; Sato et al. 1994; Tareilus et al. 1995; Touhara et al. 1999). First of all, our results indicate that the odor-induced Ca\(^{2+}\) rise in the dendrite and cell body is causally related to the responses in the cilia and therefore can be used as an assay for imaging odor responsiveness. Thus Ca\(^{2+}\) imaging at the soma of mammalian ORNs, where it has not been possible so far to measure intracellular Ca\(^{2+}\) in the cilia, is a valid method. However, some caution must be exercised if the magnitude of the cell body responses is used for generating odor receptor sensitivity profiles, because the concentration dependence of these responses may not reflect with fidelity the concentration dependence and sensitivity of the responses in the cilia. From our experiments, it would appear that odor spectra based on somatic Ca\(^{2+}\) imaging need to be interpreted in the light of our evidence that they reflect, at least in part, the filling state of Ca\(^{2+}\) stores. This filling state may change over the course of an experiment in which an ORN is challenged with a large number of different odor stimuli. Also, Ca\(^{2+}\) release imposes a threshold on the measured responses such that their magnitudes become highly nonlinear with stimulus strength. Weak odor stimuli that may still be sufficient to produce ciliary Ca\(^{2+}\) transients may not be sufficient to induce Ca\(^{2+}\) release at the soma. In this case, one would underestimate the true sensitivity of an odor receptor for a given odorant by analyzing the Ca\(^{2+}\) responses in the cell body. Evidence for this notion has already been provided: weak activation of CNG channels produces Ca\(^{2+}\) elevations that are spatially restricted to the cilia and the knob, whereas strong CNG channel activation produces Ca\(^{2+}\) rises in all cellular compartments (Leinders-Zufall et al. 1997).

**Compartmentalization of Ca\(^{2+}\) release mechanisms**

We show that Ca\(^{2+}\) can be raised in the knob by discharging it from Ca\(^{2+}\) stores but that this Ca\(^{2+}\) remains in the knob and does not spread to the cilia thus providing evidence for the spatial segregation of store-operated Ca\(^{2+}\) release in divergent regions of an ORN. This effect is not due to a diffusion barrier between the olfactory knob and the cilia because the spread of other substances such as horseradish peroxidase (Kauer 1981) and cAMP/cGMP (Firestein et al. 1991a; Kurahashi 1990; Leinders-Zufall et al. 1995) is relatively unhindered. If cAMP is dialyzed from a patch pipette into the soma of an ORN, the resulting CNG channel activation in the cilia occurs after only a few hundred milliseconds.

The separation of distinct Ca\(^{2+}\) signaling systems in spatial domains of an ORN is well-suited for olfactory signaling. It has been shown that the dynamics of the ciliary Ca\(^{2+}\) transients determine the rate of odor adaptation (Leinders-Zufall et al. 1998). Partitioning of Ca\(^{2+}\) signaling in neuronal compartments ensures that the more sustained store-dependent Ca\(^{2+}\) signals in the knob, dendrite, and soma do not interfere with the signals in the cilia. This suggests that the Ca\(^{2+}\) signals in the dendrite/soma serve separate functions than the ciliary Ca\(^{2+}\) transients. In fact, our finding that store-dependent Ca\(^{2+}\) does not spread into the cilia provides evidence that it is not involved significantly in gain control and adaptation of ciliary enzymatic cascades.

**Ca\(^{2+}\) release provides a signal that can spread from the dendrite to the nucleus**

The filling state of Ca\(^{2+}\) stores had very little influence on the odor-induced receptor potential in these experiments, but more tests will be needed to investigate specifically whether this is also the case with different conditions of odor stimulation, e.g., using repeated stimuli of varying durations and intervals. Nonetheless, this leaves an open question: on the one hand odor stimuli generate a striking store-dependent Ca\(^{2+}\) wave propagating from the cilia to the soma; on the other this effect seems relatively unimportant for the electrical odor responses that are transmitted to the brain. Given this evidence, we suggest that store-operated Ca\(^{2+}\) release serves alternative yet unknown functions beyond odor transduction. This notion is consistent with a growing body of evidence in other cells indicating that propagating Ca\(^{2+}\) waves can provide a molecular signal by which information is transferred from distal parts of a neuron to its nucleus (cf. Berridge 1998). There is increasing evidence that somatic Ca\(^{2+}\), together with cAMP, has important roles in nuclear gene activation (Dolmetsch et al. 1997; Hardingham et al. 1997). It will be interesting to test
directly whether store-dependent Ca\textsuperscript{2+} release is part of a feedback pathway involved in neuronal gene transcription, specifically of the odor receptor genes.

Model of Ca\textsuperscript{2+} signaling in ORNs: Ca\textsuperscript{2+} regulation differs between the cilia and the dendrite/soma

In Fig. 8, we have summarized diagrammatically both the experimental results presented here and results taken from other published studies. Following odor recognition, Ca\textsuperscript{2+} signaling begins in the olfactory cilia. The primary pathway for Ca\textsuperscript{2+} entry into the cilia are CNG channels gated by odor-induced cAMP elevations (see INTRODUCTION). The CNG channels are highly compartmentalized and exist at high density in the cilia. Thus far, CNG channels represent the only known Ca\textsuperscript{2+} entry pathway into ORN cilia, although InsP\textsubscript{3}-gated cation channels have also been proposed to mediate this function (see INTRODUCTION). If the stimulus strength is sufficient, the ciliary Ca\textsuperscript{2+} transients are followed by a regenerative Ca\textsuperscript{2+} wave that spreads through the entire ORN, leading to a global Ca\textsuperscript{2+} elevation in the knob, dendrite, and soma. A likely starting signal for this Ca\textsuperscript{2+} wave is the passive electrotonic spread of membrane depolarization initiated by the transduction current in the cilia. Depolarization leads to Ca\textsuperscript{2+} entry through voltage-operated Ca\textsuperscript{2+} channels into the ORN dendrite and soma. This Ca\textsuperscript{2+} signal then triggers CICR from intracellular stores, most likely through activation of ryanodine receptors. A possible role of InsP\textsubscript{3} receptors in this process remains to be explored; the synthesis of a novel membrane-permeant caged InsP\textsubscript{3} ester (Li et al. 1998) opens up new experimental strategies. Refilling of the stores occurs through thapsigargin-sensitive SERCA pumps. Store release may also cause activation of a third Ca\textsuperscript{2+} entry pathway in the dendrite and soma via capacitative Ca\textsuperscript{2+} influx.

Because Ca\textsuperscript{2+} is critical for setting the gain and adaptation level of ORNs, the intracellular Ca\textsuperscript{2+} concentration must be tightly regulated. The diagram of Fig. 8 indicates some of the mechanisms by which Ca\textsuperscript{2+} can be extruded from ORNs. There is increasing evidence for the presence of a Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange mechanism in olfactory cilia (Noë et al. 1997; Reisert and Matthews 1998) and in the ORN dendrite (Jung et al. 1994). A Ca\textsuperscript{2+}-ATPase has also been isolated from ORNs (Lo et al. 1994). We hypothesize that Ca\textsuperscript{2+} extrusion can be independently controlled between cilia and dendrite/soma, given that stimulus-induced Ca\textsuperscript{2+} dynamics as well as Ca\textsuperscript{2+} entry and storage sites are fundamentally different between these cellular compartments. It will be interesting to test whether there is compartmentalization of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger and Ca\textsuperscript{2+}-ATPase between cilia and other ORN regions, in analogy to Ca\textsuperscript{2+} extrusion in photoreceptors (Krizaj and Copenhagen 1998).

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REFERENCES


