Characterization of a Phosphoinositide-mediated Odor Transduction Pathway Reveals Plasma Membrane Localization of an Inositol 1,4,5-Trisphosphate Receptor in Lobster Olfactory Receptor Neurons*

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The role of phosphoinositide signaling in olfactory transduction is still being resolved. Compelling functional evidence for the transduction of odor signals via phosphoinositide pathways in olfactory transduction comes from invertebrate olfactory systems, in particular lobster olfactory receptor neurons. We now provide molecular evidence for two components of the phosphoinositide signaling pathway in lobster olfactory receptor neurons, a G protein α subunit of the Gq family and an inositol 1,4,5-trisphosphate-gated channel or an inositol 1,4,5-trisphosphate (IP3) receptor. Both proteins localize to the site of olfactory transduction, the outer dendrite of the olfactory receptor neurons. Furthermore, the IP3 receptor localizes to membranes in the ciliary transduction compartment of these cells at both the light microscopic and electron microscopic levels. Given the absence of intracellular organelles in the sub-micron diameter olfactory cilia, this finding indicates that the IP3 receptor is associated with the plasma membrane and provides the first definitive evidence for plasma membrane localization of an IP3R in neurons. The association of the IP3 receptor with the plasma membrane may be a novel mechanism for regulating intracellular cations in restricted cellular compartments of neurons.

Ca2+ plays a central role in many physiological processes and regulates a plethora of ion channels, enzymes, and structural proteins. A pervasive mechanism for mobilizing Ca2+ is the direct gating of a receptor ion channel (IP3R) in the endoplasmic reticulum (ER) by the ubiquitous signaling molecule, inositol 1,4,5-trisphosphate (IP3), thereby permitting release of the ion from ER stores (1, 2). IP3-induced calcium release has been implicated in such diverse cellular processes as oogenesis (3), T-cell receptor signaling (4), and long term depression (5). The enzyme phospholipase C liberates IP3, along with diacylglycerol, from the membrane phospholipid phosphoinositide 4,5-bisphosphate. The phosphoinositide (PI) signaling pathway can be regulated by both intrinsic receptors and by ligand-activated seven transmembrane-domain external receptors that in turn activate both the α and βγ subunits of heterotrimeric G proteins.

The role of PI signaling is still being resolved in olfactory transduction (6–11). Compelling functional evidence for PI signaling in olfactory transduction comes from invertebrate olfactory systems, in particular spiny lobster olfactory receptor neurons (ORNs). Several lines of evidence support the hypothesis that PI signaling mediates excitation in spiny lobster ORNs. Intracellular dialysis of IP3 mimics the odor-induced inward current in cultured lobster ORNs (12). Odors elevate IP3 in biochemical preparations of the outer dendrites of lobster ORNs, the site of olfactory transduction in these cells (13). IP3 activates unitary currents in cell-free inside-out patches of outer dendritic membrane (14). Antisera against PI pathway-specific Goq/11 proteins block the excitatory odor response in these cells (15). Finally, PIs regulate the activity of a sodium-activated channel that has been implicated in amplifying the transduction current in lobster ORNs (16, 17).

Gq and phospholipase C proteins have been molecularly identified from the olfactory organ of clawed lobster (18, 19). In an effort to characterize further the molecular substrate for PI signaling in lobster ORNs, we isolated cDNAs from spiny lobster olfactory organ that encode a protein conserved with IP3Rs, in addition to the Goq/11 protein. Consistent with a role in olfactory transduction in this animal, both messages are expressed in neural tissue, including the olfactory organ, and both proteins are localized to ORN dendrites. Antisera directed against a unique region of the lobster IP3R localize the protein to the outer dendritic membrane of lobster ORNs. Furthermore, our studies avoid a major complication that has confounded attempts to localize unequivocally IP3Rs to the plasma membrane in neurons where ER and other membranous compartments are closely apposed to the plasma membrane; intracellular membranous compartments, including ER, are absent in the sub-micron diameter outer dendrites of lobster ORNs (20) (Fig. 1). Therefore, our findings provide the first definitive evidence for plasma membrane association of an IP3R. Association of the IP3R with the plasma membrane may be a novel

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1The abbreviations used are: IP3, IP3 receptor; IP3, inositol 1,4,5-trisphosphate; ER, endoplasmic reticulum; PI, phosphoinositide; ORNs, olfactory receptor neurons; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR; PBS, phosphate-buffered saline; RPA, ribonuclease protection assay; RACE, rapid amplification of cDNA ends; RPA, ribonuclease protection assay; MOPS, 4-morpholinepropanesulfonic acid; bp, base pair; kb, kilobase pairs.
mechanism for regulating intracellular ions within restricted cellular compartments of neurons.

EXPERIMENTAL PROCEDURES

RNA Extraction—Olfactory organs (lateral antennular filament, 100 per isolation) were harvested into a dry ice/ethanol bath from freshly caught specimens of the spiny lobster, Panulirus argus, and stored at −80 °C until used. The organs were homogenized by mortar and pestle and then by Polytron, and total RNA was extracted with guanidinium thiocyanate followed by centrifugation on a cesium chloride cushion.

Cloning of IP₃R—Degenerate oligonucleotides were designed against regions of conserved amino acid and nucleotide sequence corresponding to the final putative transmembrane region (TTCTCTCATGATCAT(CT/T)ATCAT/C/TGT, sense) and the carboxyl tail (TA(A/G)TGCCACAT-­GTGTCG(T)TC, antisense) of vertebrate and Drosophila IP₃Rs. Total olfactory organ RNA (10 μg) was reverse-transcribed with SuperScript II reverse transcriptase (Life Technologies, Inc.) using the antisense primer. The resulting cDNA served as template for polymerase chain reaction (PCR) amplification. The PCR cycling profile was as follows: 94 °C for 5 min, 60 °C for 2 min, and 72 °C for 3 min × 1 cycle; 94 °C for 1 min, 59 °C (−1 °C cycle) for 1 min, and 72 °C for 2 min × 9 cycles; 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min × 30 cycles. The resulting 203-bp product (78/79-1) was fully sequenced. The cDNA was extended into the 3′-untranslated region by 3′-rapid amplification of cDNA ends (RACE (21)). A unidirectional cDNA minilibrary was constructed in the bacteriophage agt22A vector system (Life Technologies, Inc.) from olfactory organ poly(A)+ RNA. cDNA ligated into the phage arms was reverse-transcribed from olfactory organ poly(A)+ RNA with the antisense degenerate primer. The library was screened by plaque hybridization at high stringency using clone 78/79-1 as probe. Several identical clones were isolated. Three more overlapping clones were also isolated from the library by PCR. 5′-RACE (Life Technologies, Inc. (21)) was used to isolate the 5′ end of the cDNA, extending the contig into the 5′-untranslated region. Sequencing of cDNAs was done by standard chain termination methods (22) or by the Taq DyeDeoxy Terminator and DyePrimer Cycle sequencing protocols (Applied Biosystems) at the University of Florida’s Interdisciplinary Center for Biotechnology DNA Sequencing Core Laboratory. cDNA sequences were translated, assembled, and analyzed with GeneRunner (Hastings Software, Inc.) and BioImage DNA Sequence Film Reader software (BioImage). To control for errors that may have resulted from the actions of the DNA polymerases, the coding region of the IP₃R cDNA was re-amplified in duplicate, independent RT-PCRs; the consensus sequence is reported.

Cloning of Gₛₐ₁₋₃—Degenerate oligonucleotide primers were designed against a common Ga sequence (amino acids KWHCFE, sense primer (AA/AG/TGGGAT/ATC/CA/TG/TA/G, antisense primer (ACTG/AC/TA/AG/GT/TG/CT/TC, antisense primer (TAC/AAG/AG/GT/TG/CT/TC/TC/TT, where 1 is inosine). Total RNA was reverse-transcribed, as described, using the antisense primer. The PCR cycling profile was as follows: 94 °C for 5 min, 50 °C for 2 min, and 72 °C for 1.5 min × 1 cycle; 94 °C for 30 s, 49 °C (−1 °C cycle) for 30 s, and 72 °C for 1.5 min × 9 cycles; 94 °C for 30 s, 40 °C for 30 s, and 72 °C for 1.5 min × 30 cycles. The PCR product was diluted 1:1000 and served as template for a second, identical PCR. The resulting 435-bp product was gel-purified, ligated into the plasmid pGem-T, and transformed into JM109 Escherichia coli for subcloning. After colonies were screened by PCR for inserts of appropriate size, individual clones were selected for sequencing by standard chain termination methods. One clone (RTG-4) was fully sequenced. The 3′ and 5′ ends of the cDNA were isolated by 3′- and 5′-RACE. Again, two independent clones, which spanned the entire contig from the 5′-to the 3′-untranslated regions, were amplified in duplicate RT-PCRs, sequenced, and a consensus sequence translated for analysis.

Ribonuclease Protection Assay (RPA) and Northern Blotting—The RPA was done according to the RPA II kit (Ambion) protocols. RNA samples (1.5 and 3 μg of olfactory organ poly(A)+ RNA and 5 and 10 μg of brain total RNA, along with yeast RNA controls) were hybridized in solution to a 32P-labeled antisense riboprobe transcribed from clone 78/79-1. The RNA samples were then subjected to RNase digestion (RNase A and T1) and separated on an 8% urea, 5% acrylamide gel. For the Northern, 100 μg of total RNA from olfactory organ and brain (IP₃R) or 150 μg from olfactory organ (Gₛ) were denatured with 15% glyoxal.
RESULTS

Isolation of an IP3R cDNA from Olfactory Organ—Degenerate oligonucleotides reflecting conserved amino acid residues of vertebrate and Drosophila IP3Rs were used in a reverse transcription-polymerase chain reaction (RT-PCR) against lobster olfactory organ total RNA. A 203-bp cDNA fragment similar to known IP3Rs was amplified. By using a combination of 3′-RACE, 5′-RACE, and screening of an olfactory organ-specific IP3R cDNA minilibrary, the entire coding region and partial 5′- and 3′-untranslated regions were isolated. The contiguous cDNA contains an 8349-bp open reading frame encoding a 2783-amino acid protein of 320,000 predicted molecular weight (Fig. 2). The initiating methionine is proposed at the first methionine in the open reading frame; a consensus translation initiation sequence (23) is present at this point. The protein contains several residues predicted to be subject to post-translational modification as follows: one consensus site for extraacellular N-glycosylation (Asn2315), three possible sites for phosphorylation by protein kinase A (Ser1001, Ser1051, and Ser2509) and one putative site for tyrosine phosphorylation (Tyr1832), as well as numerous putative sites for phosphorylation by caspin kinase II and protein kinase C. All sites require experimental confirmation of their relevance to the native protein. Unlike many vertebrate IP3Rs, there is no consensus sequence for ATP binding, consistent with the insensitivity of native lobster olfactory IP3Rs to ATP (12), although there is a related NADD/FAD consensus binding sequence (Gly2020, Gly2026) (24).

The deduced amino acid sequence for the full-length clone is similar to other IP3Rs and shows equivalent similarity to type 1 and type 2 IP3Rs but is less similar to type 3 IP3Rs (Fig. 3; Drosophila, 59% identity; rat (type 1), 57%; rat (type 2), 55%; and rat (type 3), 40%). Like other IP3Rs, it is clearly only distantly related to ryamidine receptors, with the greatest similarity seen in the channel domain. The lobster IP3R shows no similarity to the Drosophila plasma membrane-localized calcium-selective channel TRP (25) or related proteins. The entire lobster IP3R sequence seems to maintain the distribution of conserved and variable regions of IP3Rs (Fig. 3), except for one portion that is completely absent in the other IP3Rs identified to date, amino acids Thr1192-Leu1196 comprise a lysine-rich, hydrophilic stretch of the receptor (Fig. 3). Comparison of this stretch using a variety of search algorithms yielded no significant matches with any other sequences.

Isolation of a Gq cDNA from Olfactory Organ—A 435-bp cDNA product was amplified with RT-PCR and degenerate oligonucleotide primers; this product was subcloned, sequenced, and conceptually translated. A comparison of the deduced amino acid sequence with sequences in the GenBank database revealed that this product was similar to members of the Gq family of G proteins. This product was extended to the 5′-untranslated region by 5′-RACE and to the 3′-untranslated region by 3′-RACE.

The assembled full-length clone has an open reading frame of 1059 bp coding for 353 amino acids (Fig. 4). The predicted protein has a calculated molecular mass of 41.5 kDa. The deduced amino acid sequence for the full-length clone shows a high degree of identity to other known Gq proteins (Fig. 4; Drosophila, 84%; Limulus, 85%; and mouse, 82%) and is less similar to other Go types (e.g. Drosophila Gao, 51% (GenBank access number P16378)). The spiny lobster protein is 99% identical to the clawed lobster Gaq11 (GenBank access number P91950). The position of the initiating methionine was selected for several reasons. It is the first methionine in the open reading frame. There is a well conserved consensus initiation sequence (23). Initiating transcription at this position results in a protein of identical length to most Gq proteins (Fig. 4).

Sequence analysis of the lobster Gaq11 shows it to contain several motifs characteristic of other Gq proteins (Fig. 4 (26–28)) as follows: putative sites for palmitoylation, two N-termi-
nal cysteines (Cys³, Cys⁴); an absence of N-terminal myristoylation sites (although putative myristoylation sites do exist at several sites in the middle of the protein); a putative cholera toxin ADP-ribosylation site (Arg177) but no cysteine at the C-terminal subject to pertussis toxin ADP-ribosylation; and the G40TGES “GAG box” sequence that is present in the GTP-binding domain of other Gq proteins.

Expression in Neural Tissues—A ribonuclease protection assay (RPA) using a 203-bp lobster IP₃R probe highly conserved with other IP3Rs indicates that the lobster IP₃R is expressed in olfactory organ and brain, although at much higher levels in brain (Fig. 5A). RT-PCR from olfactory organ, brain, muscle, hepatopancreas, and antennal gland indicates that a sequence containing the unique hydrophilic region (Thr1159–Leu1186) as well as flanking sequence is expressed in olfactory organ, brain, and muscle but not in the other tissues (Fig. 5B). Northern blot analysis of brain RNA shows a single band, greater than 10 kb, when probed with the same conserved probe used for the RPA (Fig. 5C). A Northern blot of olfactory organ total RNA was also probed with a riboprobe transcribed from the Gₐq/11 clone RTG-4 (nucleotides 1049–1483 of the complete clone) at high stringency. A single band of 4.6 kb is seen (Fig. 6).

Localization of the IP₃R to the Plasma Membrane of Olfactory Receptor Neurons—A polyclonal antibody (antibody 23341) was generated against a synthetic peptide (Glu1170–Lys1185; Fig. 3) contained within the unique hydrophilic region and affinity purified against the antigenic peptide (several commercially available antibodies to IP₃Rs were unable to recognize the lobster protein, data not shown). The antisera recognize a single protein band much greater than 220 kDa on Western blots of dendritic membrane protein obtained by scraping the olfactory sensilla from the olfactory organ (Fig. 7). This band is not detectable in a membrane protein preparation of the organ minus the sensilla and is abolished by preabsorption with the antigenic peptide. The presence of the high molecular weight band in the sensilla-only lane indicates that this protein is enriched in, if not specific to, the sensilla.

The antisera (antibody 23341) were also immunoreactive with the cut tips of the sensilla, and this immunolabeling could be abolished by preabsorption of the antisera with the antigenic peptide (data not shown). These results were corroborated by immunohistochemistry followed by electron microscopy (Fig. 8, A and B). Grünert and Ache (20) previously showed that only the plasma membrane and microtubules of the outer den-
Dendritic segments are found in the tips of the olfactory sensilla. The immunogold labeling of putative IP<sub>3</sub>R protein observed in the present study appears to be associated with both of these ultrastructural features (Fig. 8A). Evidence for association with the plasma membrane, in particular, derives from the observation that a major portion of the gold label in most sections tends to be distributed at the perimeter of the outer dendritic segments. Polyclonal antisera C-19 (Santa Cruz Technology) that recognize Ga<sub>q/11</sub> localize the antigen to the cut tips of dendritic cilia in situ but do not label the tissue upon

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**Fig. 3. Schematic of the lobster IP<sub>3</sub>R (GenBank<sup>TM</sup> accession number AAC61691).** The three domains of IP<sub>3</sub>Rs (ligand binding, modulatory/transducing, and channel) are indicated. Also shown are consensus sites for PKA-dependent phosphorylation (three), tyrosine kinase-dependent phosphorylation (one) and N-glycosylation (one), as well as the six proposed transmembrane regions. The unique hydrophilic sequence (Thr<sup>1158</sup>–Leu<sup>1186</sup>) is expanded, with the antigenic peptide in **bold**. Percent amino acid identities, as compared with the lobster IP<sub>3</sub>R, are shown for the Drosophila IP<sub>3</sub>R (GenBank<sup>TM</sup> accession number A44360) and the rat type 1 (A38579), type 2 (S17796), and type 3 (A46719) IP<sub>3</sub>Rs, in blocks of 125 amino acids.

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**Fig. 4. Amino acid sequence of the spiny lobster Ga<sub>q/11</sub>.** Amino acid identities, as compared with the lobster Ga<sub>q/11</sub>, are shown for the Drosophila Ga<sub>q/11</sub> (GenBank<sup>TM</sup> accession number P23625), Limulus Ga<sub>q/11</sub> (AAB48510), and the mouse Ga<sub>q</sub> (P21279) proteins. The spiny lobster Ga<sub>q/11</sub> contains several motifs indicative of the Ga family, including N-terminal cysteines (*) that may be sites for palmitoylation, a putative cholerin toxin ADP-riboseylation site (●), and a GAG box (double underline).
preabsorption with the antigenic peptide (data not shown). These results are consistent with Western blot data reported earlier (15).

DISCUSSION

We have shown that the spiny lobster olfactory organ expresses two genes that are critical for PI signaling, a Goq,11 subunit and an IP₃R. We confirmed that the encoded proteins are in the appropriate cells and the appropriate cellular compartment within those cells to play a role in olfactory transduc-

preabsorption with the antigenic peptide (data not shown). These results are consistent with Western blot data reported earlier (15).

FIG. 5. The lobster IP₃R is expressed in olfactory organ and brain. A, ribonuclease protection assay with olfactory organ and brain RNAs. Lane 1, 1.5 µg of olfactory organ poly(A)⁺ RNA; lane 2, 3 µg of olfactory organ poly(A)⁺ RNA; lane 3, 5 µg of brain total RNA; lane 4, 10 µg of brain total RNA; lane 5, yeast RNA control. Closed arrow, protected band at 203 bp. This band does not appear in the yeast control. A second, smaller band (open arrow) is nonspecific, as it also appears in the control. B, RT-PCR screening of olfactory organ (lane 1), brain (lane 2), muscle (lane 3), hepatopancreas (lane 4), and antennal gland (lane 5) cDNAs show a product of approximately 744 bp in olfactory organ, brain, and muscle but not the nonexcitable tissues. No template (lane 6) and template minus reverse transcriptase controls (data not shown) result in no product. C, Northern blot of 100 µg of total RNA from olfactory organ (lane 1) and brain (lane 2). A faint single band greater than 10 kb is seen in brain (arrow), but no signal is seen in olfactory organ.

FIG. 6. The lobster Gq is expressed in olfactory organ. Northern blot of 150 µg of total RNA from olfactory organ. A single band is seen at 4.2 kb (arrow).

Fig. 7. The lobster IP₃R protein is enriched in the olfactory sensilla. Western blot of membrane protein from the olfactory sensilla (lanes 1 and 3) and the olfactory organ minus the sensilla (lane 2 and 4). Lanes 1 and 2 were incubated with a 1:500 dilution of the primary antisera 23341, and lanes 3 and 4 were incubated with antisera preabsorbed with the antigenic peptide. A single band greater than 220 kDa is observed in the sensilla (arrow) but not in the remaining organ. Preabsorption abolishes the immunoreactivity. Nonspecific immunoreactivity seen at the origin of the gel in both the sensilla lanes is likely due to the retention of cuticular pigment at the stacker/separator gel interface.

The Goq,11 subunit we cloned has the sequence features characteristic of the Go family (26–28). These include a putative cholera toxin ADP-ribosylation site, N-terminal cysteines that are putative substrates for palmitylation, and a “GAG box” sequence in the GTP-binding domain. The high degree of sequence identity between the spiny lobster Goq,11 and homo-

FIG. 7. The lobster IP₃R protein is enriched in the olfactory sensilla. Western blot of membrane protein from the olfactory sensilla (lanes 1 and 3) and the olfactory organ minus the sensilla (lane 2 and 4). Lanes 1 and 2 were incubated with a 1:500 dilution of the primary antisera 23341, and lanes 3 and 4 were incubated with antisera preabsorbed with the antigenic peptide. A single band greater than 220 kDa is observed in the sensilla (arrow) but not in the remaining organ. Preabsorption abolishes the immunoreactivity. Nonspecific immunoreactivity seen at the origin of the gel in both the sensilla lanes is likely due to the retention of cuticular pigment at the stacker/separator gel interface.
logues found in other species is expected in this conserved family of G protein α subunits. It is also consistent with the finding that antisera against mammalian Gα11 proteins block the excitatory odor response in these cells (15).

The primary structure of the cloned IP₃R reveals several putative functional motifs that are consistent with IP₃Rs characterized electrophysiologically in cultured lobster ORNs. The cloned IP₃R does not contain a complete ATP-binding motif, although it does contain a related NAD/FAD-binding motif (Fig. 2). Unlike mammalian IP₃Rs, ATP does not modulate lobster IP₃Rs (12). ATP insensitivity may be a feature of lobster calcium-release channels, as lobster ryanodine receptors are similarly insensitive to ATP (29). The cloned IP₃R also contains putative sites for phosphorylation by protein kinase A and by protein kinase C. The single-channel open probabilities of lobster olfactory IP₃Rs are sensitive to phosphorylation by both of these kinases.²

Lobster ORNs appear to express two functionally different types of IP₃Rs based on their conductance and kinetic properties (12, 14). It is unclear whether these two IP₃Rs, differentiated on the basis of electrophysiological properties, represent the products of different genes, alternative splicing of transcripts from the same gene, different states of posttranslational modification, or are heteromultimers with different subunit stoichiometry. Screening of olfactory organ cDNA libraries, olfactory organ and brain RNA, and genomic DNA yielded only the single cDNA reported in this study, and Northern blot analysis of brain RNA showed only a single band (Fig. 5C), but the existence of one or more additional isoforms cannot be excluded. Interestingly, at least two alternative transcripts of a single IP₃R gene have been observed in Drosophila (30).

Ultrastructural evidence presented here and in an earlier study (20) shows that the outer dendritic compartment lacks intracellular membranous organelles. As the same fixation methods are sufficient to preserve mitochondria, ER, and related structures in the inner dendrite (20), we assume that these structures would have been preserved in the outer dendrite if present. Immunogold label associated with the microtubules of the outer dendritic segments (Fig. 8A) may reflect transport of the IP₃R protein from the Golgi complex to the plasma membrane via these cytoskeletal elements (31–33). In the absence of possible contamination from ER and other intracellular membranes, our results indicate that the lobster olfactory IP₃R is associated with the plasma membrane in this cellular compartment. The high homology of the lobster olfactory IP₃R with mammalian IP₃Rs argues that it is an integral membrane protein. Our data do not allow us to conclude that the receptor is integral in the plasma membrane per se, since we cannot exclude the possibility of submembranous cisternae or caveolae, which have been shown to contain IP₃R-like proteins in non-neuronal cells (34). It is unknown whether the lobster IP₃R contains a signal sequence that directs the receptor to the plasma membrane. An intriguing possibility is that the unique stretch of amino acids (Thr¹¹⁵⁹–Leu¹¹⁸⁶) might constitute such a signal, but whether these amino acids target this protein to the plasma membrane, or rather are involved in some other function perhaps specific to lobster IP₃Rs, remains to be determined.

Although IP₃Rs typically are not associated with the plasma membrane in neurons, recent evidence localizes type 1 IP₃Rs to the plasma membrane of the outer segments of mammalian retinal cone cells (35). Earlier, IP₃Rs were implicated in the plasma membrane of vertebrate olfactory cilia (36) and presynaptic nerve terminals (37). Recruiting an IP₃R to the plasma membrane may be related to the anatomy of the neuronal compartment. In Drosophila photoreceptors, for example, depletion of internal IP₃-sensitive Ca²⁺ stores leads to the activation of TRP channels on the nearby plasma membrane (38). In lobster olfactory receptors, the extremely thin (0.1 μm) outer dendrite (20) presumably would impede diffusion of either IP₃ or Ca²⁺ between the site of olfactory transduction and the ER at the distal end of the inner dendrite (39). Thus, IP₃Rs associated with the plasma membrane could regulate the levels of cations in spatially constrained cellular compartments, as do intracellular IP₃Rs in larger cellular compartments.

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² D. A. Fadool and B. W. Ache, unpublished observations.
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