A Possible Role for Caveolin as a Signaling Organizer in Olfactory Sensory Membranes*

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Fast kinetics and sensitivity of olfactory signaling raise the question of whether the participating proteins may be associated in supramolecular transduction complexes. We found evidence that caveolin proteins could play an important role in organizing signaling elements in olfactory sensory neurons. Western blot analysis indicated that caveolins are highly enriched in olfactory sensory membranes, where they co-localize in detergent-insoluble complexes with key components of the signaling pathways. Furthermore, the results of immunoprecipitation experiments suggest that G proteins and effector enzyme form preassembled subcellular complexes with caveolins. Since anti-caveolin antibodies and synthetic peptides derived from the scaffolding domains of caveolin-1 and caveolin-2 effectively attenuated second messenger responses in sensory cilia preparations in a characteristic manner, the data led to the suggestion that caveolins could mediate the assembly of signaling complexes within specialized membrane microdomains of olfactory sensory neurons.

In mammals, odorants bind to heptahelical receptors on specialized sensory neurons, stimulating heterotrimeric G proteins associated with the inner face of the plasma membrane. Consequently, effector enzymes are activated, resulting in a fast and transient pulse of second messengers, which finally trigger the electric response of the cell (1–5). The chemosensory signal transduction process is highly sensitive (6, 7) and extremely rapid (8). These findings fuel the hypothesis that the participating molecules may be organized in preassembled signaling complexes to minimize diffusion routes and to control cross-talk between different signaling pathways (9). Specialized membrane microdomains and scaffolding proteins have been proposed as structural organizers for such multimolecular signaling assemblies in various cell types (10–12).

There is accumulating evidence that caveolin proteins may be involved in organizing signal transduction pathways at the plasma membrane (13–17). Caveolins are 21–24-kDa membrane proteins, originally identified in trans-Golgi network-derived exocytotic vesicles (18) and flask-shaped membrane invaginations called caveolae (19). Caveolins are inserted into the lipid bilayer by an internal hydrophobic hairpin domain, which was illustratively described as the caveolin “greasy elbow configuration” (20). Caveolins strongly bind cholesterol (21) and reportedly form high molecular weight oligomers (22, 23). Three caveolin subtypes have been identified, with caveolin-1 (24) and caveolin-2 (25) being ubiquitously expressed in many cell types, whereas caveolin-3 was found to be muscle-specific (26, 27). It has been proposed that caveolin-1 and caveolin-2 could function cooperatively in the formation and stabilization of large lipid microdomains (28).

Simons and Ikonen propose a “raft hypothesis” for the lateral organization of lipid bilayers (12). Accordingly, assemblies of sphingolipids and cholesterol create biochemically distinct microdomains (rafts) in a glycerophospholipid environment. Such rafts may recruit and exclude specific sets of membrane proteins based on their physicochemical properties and may therefore be viewed as platforms to concentrate signaling molecules within the lipid bilayer. The properties of caveolin could enable this protein to sequester raft lipids, eventually forming and organizing large stabilized raft domains in the plasma membrane (28, 29). In addition, caveolins reportedly interact with multiple components of G protein-mediated signaling pathways, including receptors, G proteins, and effector enzymes (14, 30–33). Accordingly, caveolin-mediated clustering and dispersion of raft domains could contribute to regulating and shaping G protein-mediated signaling.

In this study we addressed the question of whether caveolins may play such a role in olfactory sensory neurons. We examined their subcellular distribution in the main olfactory epithelium (MOE) and vomeronasal organ (VNO) of the rat and evaluated their possible function in the odor-induced second messenger signaling.

EXPERIMENTAL PROCEDURES

Materials

Male and female adult Harlan Sprague-Dawley rats were purchased from Charles River (Sulzfeld, Germany). The odorants citralvala (3,7-dimethyl-2,6-octadienitrile), hedione (3-oxo-2-pentylcyclopentanecarboxylic acid methyl ester), eugenol (2-methoxy-4-(2-propenyl)phenol), lilial (para-butyl-a-methylhydrocinnamic aldehyde), and lyral (4-(4-hydroxy-4-methylpentyl)-3-cyclhexene-1-carboxylic acid) were provided by DROM (Baierbrunn, Germany). Isovaleric acid (3-methylbutanoic acid), triethylamine, GTPγS, and goat anti-rabbit IgG-conjugated horseradish peroxidase were purchased from Sigma. Forskolin and 3-isobutyl-1-methylxanthine were supplied by Calbiochem. Antibodies against Gaα1, Gαo, Gαq, adenylyl cyclase type III, phospholipase C β2 (PLCβ2), caveolin-1, caveolin-3, as well as agarose-conjugated caveolin-1 antibodies were provided by Santa Cruz Biotechnology (Santa Cruz, CA). Caveolin-2-specific antibodies were purchased from Affiniti (Manhead, UK).

1 The abbreviations used are: MOE, main olfactory epithelium; VNO, vomeronasal organ; GTPγS, guanosine 5′-O-(3-thiotriphosphate); PLCβ2, phospholipase C β2; IP3, inositol 1,4,5-trisphosphate; Mes, 4-morpholineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; TBST, Tris-buffered saline-Tween; DIG, detergent-insoluble glycolipid-enriched membrane domains.

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Peptides

Peptides corresponding to the scaffolding domains of caveolin-1 (amino acid positions 82–101, DGIWKASPTTFTVTKYWYR) and caveolin-2 (amino acid positions 54–73, DVKWICSHALFESKTVKYR) were synthesized by Dr. H. Kalbacher (University of Tubingen, Germany) and purified by high pressure liquid chromatography. The purity was confirmed by mass spectrometric analysis. A control peptide located N-terminal from the scaffolding domain of caveolin-1 (amino acid positions 53–81, RDPKHNLNDVFKIDFEDVIAEPEGTHSF) was purchased from Interactiva (Ulm, Germany).

Methods

Isolation of Sensory Cilia from Main Olfactory Epithelia—Olfactory cilia were prepared using the calcium shock method according to Anholt (34). Briefly, after a short wash of the olfactory epithelia in ice-cold Ringer solution (120 mM NaCl, 5 mM KCl, 1.6 mM K$_2$HPO$_4$, 25 mM NaHCO$_3$, 7.5 mM glucose, pH 7.4), the tissue was subjected to Ringer solution supplemented with 10 mM calcium chloride and gently stirred for 5 min at 4 °C. Detached cilia were isolated by three sequential centrifugation steps for 5 min at 7,700 × g. The supernatants were collected, and the resulting pellets were resuspended in Ringer solution including 10 mM calcium as described above. The cilia preparation was collected by a final centrifugation step of the pooled supernatants for 15 min at 27,000 × g. The resulting pellet containing the cilia was resuspended in hypotonic TME buffer (10 mM Tris, 3 mM MgCl$_2$, 2 mM EGTA, pH 7.4) and stored at −70 °C.

Isolation of Sensory Microvilli Fragments from Vomeronasal Organs—Microvilli fractions from rat VNO were prepared as described previously (35). Briefly, VNOs were removed from fertile female rats, washed twice in Ringer solution, and stored frozen in liquid nitrogen until further use. VNOs from 30–60 animals were thawed on ice, minced, and subsequently stirred for 10 min at 4 °C in Ringer solution containing 10 mM calcium chloride. After removing the debris by centrifugation (10 min, 3,000 × g), the supernatant was collected while the pellet was resuspended in Ringer solution containing 10 mM calcium and processed as described above. The pooled supernatants were centrifuged for 10 min at 45,000 × g, and the resulting pellet containing the microvilli membrane fragments was resuspended in hypotonic TME buffer and stored in aliquots at −70 °C. Protein concentrations were measured by the Bradford method (36).

Isolation of Triton X-100-insoluble Membrane Domains—Detergent-insoluble membrane fractions were purified as described previously (37). Briefly, freshly dissected rat olfactory epithelium from the nasal septum and the ethmoid turbinate area from VNOs were collected and minced in TME buffer. An aliquot of 1 ml of protein solution (4 μg/ml) was diluted with lysis buffer (25 mM Mes, 150 mM NaCl, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin, leupeptin, and pepstatin, pH 6.5), adjusted to a final concentration of 1% (w/v) Triton X-100 or, alternatively, 60 mM octyl glycoside, and solubilized for 10 min on ice. Subsequently, samples were mixed with an equal volume of 80% sucrose in lysis buffer, placed on the bottom of a 12-ml centrifuge tube, and overlaid with a discontinuous sucrose gradient (6 ml of 30% sucrose, 4 ml of 5% sucrose, both prepared in lysis buffer). Samples were centrifuged at 200,000 × g for 16 h. Fractions of 1 ml were collected from the top (fraction 1–12), whereas the pellet of the gradient was resuspended in 1 ml of lysis buffer (fraction 13). Aliquots of each fraction were stored at −70 °C and subjected to SDS-PAGE.

Immunoprecipitation—Aliquots of detergent-insoluble membrane fractions of olfactory epithelium or isolated olfactory cilia isolated by equilibrium sucrose density centrifugation were lysed for 10 min on ice with immunoprecipitation buffer (50 mM Tris, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each of aprotinin, leupeptin, pepstatin, pH 7.4) containing 60 mM octyl glycolide. The lysate was centrifuged at 12,000 × g for 15 min, and the supernatant was incubated overnight at 4 °C with an agarose-conjugated anti-caveolin-1 antibody; after three washes, bound protein was eluted with an aliquot of 5 × sample buffer (625 mM Tris/HCl, pH 6.8, 50% glycerol, 5% SDS, 7.5 mM dithiothreitol, 0.05% bromphenol blue), boiled for 2 min, and subsequently subjected to SDS-PAGE and immunoblotting.

SDS-PAGE and Western Blot Analysis—Aliquots of sucrose gradient fractions or protein samples from sensory epithelia (38) were mixed with 5% sample buffer, boiled for 2 min, and subjected to SDS-polyacrylamide gel electrophoresis using the Laemmli buffer system (39). The separated proteins were transferred onto nitrocellulose membranes using a semidyblotting system (Amersham Pharmacia Biotech). The blot was stained with Ponceau S, dried, and stored at 4 °C until further use. For Western blot analysis, nonspecific binding sites were blocked with 5% nonfat milk powder (Naturaffor, Dietmannsried, Germany) in 10 mM Tris/HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween 20 (TBST). Subsequently, the blots were incubated overnight at 4 °C with specific antibodies diluted in TBST containing 3% nonfat milk powder. After three washes with TBST, a horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000 dilution in TBST with 3% milk powder) was applied. After three washes with TBST, the ECL system was used to visualize bound antibodies.

Stimulation Experiments and Second Messenger Determination—Stimulation experiments were performed for 2 min at 37 °C in a shaking water bath as described previously (40). Briefly, test substances (e.g., odorants, forskolin, or GTP·S) were diluted in reaction buffer (200 mM NaCl, 10 mM EGTA, 50 mM Mops, 2.5 mM MgCl$_2$, 1 mM dithiothreitol, 0.05% sodium cholate, 1 mM ATP, 4 μM GTP, 12 mM free calcium calculated and adjusted as described (41), pH 7.4). To prevent degradation of cAMP and IP$_3$, stimulation was performed in the presence of either 1 mM 3-isobutyl-1-methylxanthine or 20 mM LiCl. The reaction was started by mixing 200 μl of prewarmed reaction buffer with 30 μl of isolated olfactory cilia. After stopping the reaction by the addition of 100 μl of ice-cold perchloric acid (7%), quenched samples were stored on ice for 20 min, followed by determination of the concentrations of cAMP or IP$_3$, according to Steiner et al. (42) and Palmer et al. (43), respectively. The applied concentrations of the different modulators in the results
section represent concentrations during pretreatment of the microvilli preparations or ligand concentrations in the reaction buffer.

RESULTS

To analyze the expression of caveolins in rat olfactory neurons, preparations of MOE and VNO were probed with subtype-specific anti-caveolin antisera on Western blots. For this purpose, total MOE and VNO samples were prepared by homogenizing freshly dissected tissue in TME buffer. Sensory cilia and microvilli fragments were separated from cells by the addition of calcium chloride and purified by multiple centrifugation procedures.

**FIG. 2.** Subcellular fractionation of olfactory sensory tissue from the MOE. Tissue from the MOE was homogenized in either lysis buffer with 1% Triton X-100 (A–C) or 60 mM octyl glycoside (D) and fractionated by discontinuous sucrose gradient centrifugation. Equal aliquots of each fraction were subjected to SDS-PAGE, and the gels were stained with Coomassie Blue (A). Fractions 1–8 represent the 5–30% sucrose layers, fractions 9–12 represent the 40% sucrose layer, and fraction 13 represents the insoluble pellet. Equivalent gels were blotted on nitrocellulose membranes, where immunoreactivity of caveolin-1 (B) and caveolin-2 (C) was visualized with subtype-specific antibodies (1:1,000 dilution). The bulk of caveolin immunoreactivity was found in fractions 3 and 4, which exclude more than 90% of total cellular protein as determined by densitometric analysis of the Coomassie gels. In contrast, solubilization with octyl glycoside shifted caveolin-1 immunoreactivities to the low density fractions 10–13 (D).

**FIG. 3.** Subcellular fractionation of olfactory sensory tissue from the VNO. Low density Triton X-100-insoluble fractions were obtained from sensory tissue of the VNO using a sucrose density gradient centrifugation procedure. Equal aliquots were collected from the top of the gradient and subjected to SDS-PAGE. Upon solubilization at 4 °C with Triton X-100, endogenous caveolin-1 (A) and caveolin-2 (B), visualized with subtype-specific antibodies (1:1,000), are restricted to the low density fractions 3 and 4, whereas upon treatment with octyl glycoside, caveolin-1 is localized to the high density fractions 10–13 (C).
gation steps. Equal amounts of protein from each preparation were subjected to SDS-PAGE and blotted onto nitrocellulose membranes. The membranes were subsequently probed with caveolin-1- and caveolin-2-specific antibodies, followed by peroxidase-conjugated secondary antibodies and chemiluminescence detection.

As shown in Fig. 1, both antibodies recognized a band of about 25 kDa in all preparations, consistent with the predicted molecular masses of caveolin-1 and -2 of about 22,000 (30). Comparing the relative band intensities, we found both caveolin subtypes enriched in cilia and microvilli fractions of MOE and VNO, respectively. Centrifugation at high speed of cilia and microvilli lead to a further enrichment of immunoreactivity of both caveolin subtypes in the membrane pellet (data not shown). Antibodies directed to the muscle-specific subtype caveolin-3 did not label a polypeptide at the predicted size of this caveolin subtype (data not shown).

Caveolins have been found to segregate into detergent-insoluble glycolipid-enriched membrane domains (DIGs) (31). To test whether this may also be the case for caveolins expressed in olfactory tissues, we isolated low density membrane fractions from MOE and VNO using sucrose gradient ultracentrifugation. In the past, association with detergent-insoluble cytoskeleton proteins was made responsible for Triton X-100 insolubility of G protein a-subunits in Triton X-100-insoluble fractions of the MOE. Triton X-100-insoluble membrane fractions were purified by sucrose density gradient centrifugation, and equal aliquots of each of 13 fractions were subjected to SDS-PAGE. Subsequently, Ga, and Go, were detected by Western blot analysis with Ga subtype-specific antibodies (1:1,000 dilution). Upon solubilization with Triton X-100, the bulk of Ga (A) and Go (B) co-migrated with caveolin-enriched low density fractions, whereas in octyl glycoside extracts, Ga was found in high density fractions (C).

The data indicate that caveolin-1 and -2 are expressed in both rat olfactory organs, MOE and VNO. Furthermore, enrichment of both caveolin subtypes in sensory membranes points to a possible colocalization with components of olfactory signal transduction cascades in the appropriate subcellular compartments. Caveolins have been found to segregate into detergent-insoluble glycolipid-enriched membrane domains (DIGs) (31). To test whether this may also be the case for caveolins expressed in olfactory tissues, we isolated low density membrane fractions from MOE and VNO using sucrose gradient ultracentrifugation. In the past, association with detergent-insoluble cytoskeleton proteins was made responsible for Triton X-100 insolubility of G protein a-subunits in Triton X-100-insoluble fractions of the MOE. Triton X-100-insoluble membrane fractions were purified by sucrose density gradient centrifugation, and equal aliquots of each of 13 fractions were subjected to SDS-PAGE. Subsequently, Ga, and Go, were detected by Western blot analysis with Ga subtype-specific antibodies (1:1,000 dilution). Upon solubilization with Triton X-100, the bulk of Ga (A) and Go (B) co-migrated with caveolin-enriched low density fractions, whereas in octyl glycoside extracts, Ga was found in high density fractions (C).
insolubility at 4 °C. The detection of multiple glycosylphosphatidylinositol-anchored proteins in DIGs, however, established Triton X-100 insolubility as the basis for isolating lipid rafts and associated proteins (44).

Olfactory epithelia from MOE and VNO were homogenized in Triton X-100-containing lysis buffer at 4 °C, and after centrifugation, equal volumes of each of the 13 fractions were subjected to SDS-PAGE. Proteins were visualized by Coomassie Blue staining (Fig. 2A), revealing a highly skewed protein distribution along the gradient. As determined by protein quantification for each fraction (data not shown), more than 90% of the total protein was found in high density fractions representing Triton X-100-soluble components (lanes 9–13 in Fig. 2A). Only 10% of the total protein localized to the low density fractions (lanes 1–8). In contrast, when the fractions were subjected to Western blot analysis as described above, most of the caveolin-1 and -2 immunoreactivity was found concentrated in low density fractions 3–5 of the MOE (Fig. 2, B and C) and the VNO (Fig. 3, A and B). Replacing Triton X-100 by 60 mM octyl glycoside completely abolished this pattern, leaving all caveolin-1 immunoreactivity from MOE (Fig. 2D) and from VNO (Fig. 3C) in the high density fractions 10–13. Similar results were obtained for caveolin-2 (data not shown). These results demonstrate that both caveolin subtypes show a strong tendency to associate with DIGs isolated from olfactory epithelia as described for other tissues (45).

To test whether key components of the olfactory G protein-coupled signal transduction cascades are enriched within DIG fractions from rat olfactory tissues along with caveolins, we monitored the distribution of two major olfactory Go subtypes from MOE, Go and Go, (46) in sucrose density gradients. Western blot analysis with subtype-specific anti-Go antibodies detected some immunoreactivity for Go, (Fig. 4A) and Go, (Fig. 4B) in Triton X-100-soluble high density fractions 7–12. The most of these proteins, however, were concentrated in Triton X-100-insoluble low density fractions 4–6. Replacing Triton X-100 by 60 mM octyl glycoside in the lysis buffer released the immunoreactivity for both proteins (data shown for Go, only) into the soluble high density fractions (Fig. 4C).

Similar results were obtained when preparations from VNO were used (Fig. 5). Go and Go, subtypes have been suggested to mediate chemosensory signal transduction in this tissue (47–49). Again, the majority of both G protein α-subunits co-migrated with the Triton X-100-insoluble low density fractions. Comparable experiments with an antibody against a conserved domain of all five known rat Gβ subtypes revealed a similar distribution (data not shown).

To test whether further downstream signaling molecules of the olfactory transduction pathways co-migrate in the low density fractions along with caveolins and G proteins, the distributions of two key effector enzymes, adenylyl cyclase III and PLCβ (50, 51), were monitored in sucrose density gradients. Again, homogenates from MOE were prepared as described above and subjected to sucrose gradient centrifugation. After SDS-PAGE separation of each fraction, the proteins were blotted onto nitrocellulose membranes and probed with antibodies specific to rat adenylyl cyclase III (Fig. 6A) and PLCβ (Fig. 6B) proteins. We found significant immunoreactivity for both enzymes in Triton X-100-insoluble low density fractions 4–6, suggesting their co-localization with caveolins and G proteins in common lipid compartments of sensory membranes.

Several studies indicate that in vivo, caveolin 1 and caveolin 2 form a hetero-oligomeric complex (for review, see Ref. 16). To explore whether caveolin-1 may interact with caveolin-2, co-immunoprecipitation experiments were performed using a caveolin-1-specific antibody conjugated to agarose; therefore, Triton X-100-insoluble low density membrane fractions iso-
lated by sucrose density centrifugation were solubilized with octyl glycoside and subsequently used in immunoprecipitation experiments; to assess the specificity of the procedure, protein samples were incubated either with non-immune rabbit IgG (Fig. 7, panel A, Cav1) or alternatively, antibodies used in Western blotting were preincubated with the specific peptide (Fig. 7, panel B, Cav1). As demonstrated in Fig. 7, caveolin-1 was effectively precipitated by its respective antibody (panel A, Cav1) compared with the labeling of the caveolin-1 antibody observed in isolated olfactory cilia (panel A, Ci), whereas no immunostaining was detectable in control samples (panel A, IgG and Cav1P). When caveolin-1 precipitates were probed with caveolin-2-specific antibodies, an intense immunoreactivity for caveolin-2 was observed (Fig. 7, panel B, Cav2), indicating that hetero-oligomeric complexes of caveolin-1 and caveolin-2 exist in the olfactory sensory epithelium.

Caveolin forms higher ordered complexes with a variety of signal transduction molecules (for review: see Ref. 16); to determine if caveolin-1 is associated with olfactory G protein α-subunits, no staining for both G protein-subtypes was detected in control samples, whereas in the caveolin precipitates, a significant amount of Gαo (panel C) and Gαi (panel D) was detected. To explore whether effector enzymes involved in olfactory signaling are also associated with caveolin, immunoprecipitates of caveolin-1 antibodies were also assessed for adenylyl cyclase and PLCβ2 immunoreactivity; Fig. 7 shows that adenylyl cyclase (panel E) and PLCβ2 (panel F) are both co-eluted with caveolin-1, indicating that G protein α-subunits as well as effector enzymes of the olfactory signaling pathways form complexes with caveolin-1.

To examine the functional significance of interactions between caveolins and other olfactory signaling molecules, we performed two different experimental approaches. Using antibodies specific to caveolin-1 (Fig. 8A) and caveolin-2 (Fig. 8B), we attempted to sterically protect caveolin proteins from homophilic interactions. In addition, we sought to competitively replace caveolins from potential interaction partners with synthetic peptides corresponding to the amino acid sequence of caveolin-1 (Fig. 9A) and caveolin-2 (Fig. 9B) scaffold-
Samples of cilia preparations were preincubated with different dilutions of antibodies specific to caveolin-1 or caveolin-2 and subsequently stimulated with a mixture of odorants inducing cAMP responses (citralva, hedione, eugenol; 10 μM each) (40) or odorant components eliciting IP₃ formation (lilial, lyril, ethylvanillin; 10 μM each) (54). Alternatively, downstream signaling elements were activated directly with GTPγS for G proteins and forskolin for adenyl cyclase. The reaction was stopped by the addition of perchloric acid, and subsequently stimulated with a mixture of odorants inducing cAMP responses (citralva, hedione, eugenol; 10 μM each) (40). Preincubation of cilia with synthetic peptides representing the scaffolding domain showed no effect on either cAMP (Fig. 9C) or IP₃ (not shown) signaling. As observed for the antibody experiments, the reduction of odorant-induced cAMP production was most pronounced. Both peptides evoked gradually smaller effects when the second messenger response was induced by GTPγS (30–40% of control values) or forskolin (45–65% of control values).

**DISCUSSION**

The results of this study suggest that caveolins may play an important role in olfactory signaling by assembling elements of the transduction machinery in microdomains of the chemosensory membranes. Immunoreactivity for caveolin-1 and caveolin-2 was found to be enriched in membrane protein fractions from cilia and microvilli preparations, the specialized chemosensory compartments of olfactory epithelia and vomeronasal organs, respectively. Furthermore, caveolin-1 and caveolin-2 co-migrated in low buoyant density fractions along with several key components of the olfactory signal transduction cascades, including G proteins, adenyl cyclase III, and phospholipase C. This indicates a possible co-localization of these molecules in situ in detergent-insoluble membrane domains, such as rafts or caveolae. Co-immunoprecipitation experiments further support the idea of a possible molecular association of caveolins with key olfactory signaling molecules. Most importantly, antibodies to both caveolins as well as synthetic peptides representing the scaffolding domain effectively suppressed the odor-induced second messenger formation.

It has been suggested that caveolins may act as scaffolding proteins to organize preassembled signaling complexes at the plasma membrane (30, 16), but models for molecular mechanisms underlying this function remain incomplete. Simons and
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co-workers (29, 55) recently proposed that caveolin-1 might stabilize lipid raft microdomains. Small and highly dynamic lipid domains that are rich in cholesterol and sphingolipids are thought to form spontaneously in biological membranes based on the differential miscibility of lipids (12, 29, 56–58). Caveolins may stabilize these domains, since they bind cholesterol (21) and oligomerize to form large assemblies of up to 600 kDa (23). Cooperatively, caveolin-1 and caveolin-2 may therefore induce the formation of large lipid domain clusters, regulating their size by intermolecular cross-linking. This possibility is particularly attractive because the oligomerizing property of caveolin-2 may be modified by phosphorylation through protein kinases (55), thus offering a potential cue for physiological regulation.

In this context, our observations are consistent with the notion that in chemosensory membranes caveolins may contribute to assemble the olfactory signal transduction machinery in microdomains, thereby shaping and regulating the odor-induced second messenger responses. Antibodies to both caveolins effectively attenuated the odor-induced second messenger formation (Fig. 8). This inhibitory effect could result from steric or competitive hindrance of a direct interaction between caveolins and other signaling molecules. The same may hold true for the attenuating effect of synthetic peptides, representing the caveolin scaffolding domains (Fig. 9). The observation that scaffolding domains in fact interact with G proteins (52) and other signaling molecules (53, 59–63) is supportive to this idea. However, it was found that putative interaction partners bind differentially to each of the caveolins (53). Furthermore, scaffolding peptides from the two caveolins elicited different effects on G protein function and adenylyl cyclase III activity (25, 64). In the view of these findings it is somewhat surprising that peptides representing the scaffolding domains of caveolin-1 and caveolin-2 equally affected the olfactory signal transduction cascades.

A solution for this controversy may be provided by a slightly different consideration. The scaffolding domain peptides, irrespective of whether they represent caveolin-1 or caveolin-2, as well as antibodies to both proteins could disturb the homo- and heterophilic interactions between caveolin proteins themselves, a view that is consistent with the role of the scaffolding domain in caveolin oligomerization (20, 23, 65). This could trigger the dispersion of large stable lipid domain clusters into small, dynamic microdomains, releasing the different signaling molecules into a much larger diffusion territory and, consequently, reducing the transduction efficiency. This notion is consistent with the finding that all used antibodies and peptides attenuate second messenger formation equally effectively and that scaffolding domain-derived peptides only partially affect co-precipitation of olfactory G proteins and effecter enzymes (data not shown). Furthermore, it would provide a possible explanation for the observation that the inhibitory effect was more pronounced when second messenger formation was induced via odorant receptors as compared with responses induced by direct activation of G proteins and adenylyl cyclase III. It seems an intriguing question, whether caveolin-mediated coalescence and dispersion of lipid domains could play a role in regulating the olfactory signal transduction processes or G protein-coupled signaling cascades in general.

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