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Close association of the α subunits of G_q and G_{11} G proteins with actin filaments in WRK_1 cells: Relation to G protein-mediated phospholipase C activation

(cytoskeleton)

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ABSTRACT **A selective polyclonal antibody directed toward the C-terminal decapeptide common to the α subunits of G_q and G_{11} G proteins ($G_{\alpha q}/G_{\alpha 11}$) was prepared and used to investigate the subcellular distribution of these proteins in WRK_1 cells, a rat mammary tumor cell line. In immunoblots, the antibody recognized purified $G_{\alpha q}$ and $G_{\alpha 11}$ proteins and labeled only two bands corresponding to these α subunits. Functional studies indicated that this antibody inhibited vasopressin- and guanosine 5′-[α-thio]triphosphate-sensitive phospholipase C activities. Immunofluorescence experiments done with this antibody revealed a filamentous labeling corresponding to intracytoplasmic and perimembranous actin-like filament structures. Colocalization of $G_{\alpha q}/G_{\alpha 11}$ and F-actin filaments (F-actin) was demonstrated by double-labeling experiments with anti-$G_{\alpha q}/G_{\alpha 11}$ and anti-actin antibodies. Immunoblot analysis of membrane, cytoskeletal, and F-actin-rich fractions confirmed the close association of $G_{\alpha q}/G_{\alpha 11}$ with actin. Large amounts of $G_{\alpha q}/G_{\alpha 11}$ were recovered in the desmin- and tubulin-free F-actin-rich fraction obtained by a double depolymerization–repolymerization cycle. Disorganization of F-actin filaments with cytochalasin D preserved $G_{\alpha q}/G_{\alpha 11}$ and F-actin colocalization but partially inhibited vasopressin- and fluoroaluminate-sensitive phospholipase C activity, suggesting that actin-associated $G_{\alpha q}/G_{\alpha 11}$ proteins play a role in signal transduction.**

Guanine nucleotide-binding proteins (G proteins) constitute a large family of heterotrimeric proteins that relay information from cell-surface receptors to effectors. Although the mechanisms of receptor-mediated G protein activation and coupling to effectors have been extensively examined (for reviews, see refs. 1–3), little information is available on the structural and topological organization of receptors, G proteins, and effectors as they exist in their native environment.

Data derived from different experimental approaches indicate that G proteins in the membrane are associated within large polymeric structures from which they can be released during the process of guanine nucleotide- or receptor-mediated activation (refs. 4 and 5 and the references therein). There is also experimental evidence suggesting that proteins involved in signal-transduction pathways are compartmentalized in distinct membrane domains and that they segregate upon hormonal stimulation. Moreover, the cytoskeleton is supposed to play an important role in these mechanisms. Thus, (*i*) drugs that disrupt cytoskeletal structures, such as cytochalasins or colchicine, were shown to alter the generation of second messengers in a variety of cell types like neutrophils, platelets, lymphocytes, myometrial cells, and frog adrenocortical cells (6–13). (*ii*) There is evidence for a compartmentation of the α subunit of the G protein involved in adenylyl cyclase inhibition ($G_{\alpha i}$) in the coupling of muscarinic, opiate, and α_2-adrenergic receptors to their effectors in NG108-15 cells probably mediated by the cytoskeleton (14). (*iii*) Lateral cosegregation of receptors and $G_{\alpha i}$ during capping in lymphocytes has been shown to depend upon cytoskeletal reorganization (15). (*iv*) Several proteins involved in signal transduction have been found associated with cytoskeletal structures: $G_{\alpha i}$ and β subunits of G proteins (G_β) in human neutrophils and in S49 lymphoma cells, respectively (16, 17), phospholipase C in several cell lines (18, 19), and protein kinase C in REF52 cells (20). (*v*) Conversely, reorganization of the cytoskeleton, which is an early event in the response of neutrophils to fMet-Leu-Phe (21–23) and in activation of T-helper lymphocytes (10), depends upon $G_{\alpha i}$ proteins.

Little is known about the subcellular localization of $G_{\alpha q}/G_{\alpha 11}$ G proteins that couple hormonal receptors to phospholipase C β_1 and β_2 (24, 25). In the present study, we have used a selective polyclonal antibody against $G_{\alpha q}/G_{\alpha 11}$ subunits, and we demonstrate by an approach combining confocal microscopy and cytoskeletal purification technique that the bulk of $G_{\alpha q}/G_{\alpha 11}$ closely associates with actin filaments (F-actin) in WRK_1 cells.

MATERIALS AND METHODS

Sources of Antibodies. The decapeptide LQLNLKEYNLV corresponding to the $G_{\alpha q}$ and $G_{\alpha 11}$ common C terminal was synthesized and used to generate antibodies in New Zealand White rabbits. Antibodies were purified by affinity chromatography on antigenic decapeptide-coupled Affi-Gel column, as described (26), and stored at −20°C at 1.5 mg of protein per ml.

Antibodies directed against G_β from calf brain were prepared, purified, and characterized as described (26). Purified $G_{\alpha q}$ and $G_{\alpha 11}$ were provided by J. H. Exton (Howard Hughes Medical Institute, Vanderbilt University, Nashville). Monoclonal antibodies directed against actin and desmin were purchased from Amersham and Sigma, respectively. Peroxidase-conjugated and fluorescent secondary antibodies were from Pierce and Nordic Immunological Laboratories, respectively.

Cell Culture. WRK_1 cells, a rat mammary tumor cell line expressing high amounts of vasopressin receptor coupled to

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Abbreviations: F-actin, actin filaments; G_α, α subunit of G protein; G_β, β subunit of G protein; GTP[γS], guanosine 5′-[γ-thio]triphosphate.

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phospholipase C, were cultured, as described (27). When specified, confluent WRK_1 cells were incubated for 90 min at 37°C with cytochalasin D, colchicine, or vehicle.

Fractionation of WRK_1 Cells. Crude membranes and cytoskeletal preparations were obtained as previously described (18, 28).

Polymerized actin and actin-binding protein-rich fractions were prepared as described (18). Briefly, crude cytoskeletal preparations derived from $\approx 40 \times 10^6$ cells were incubated 60 min at 4°C in 10 ml of a medium containing 0.6 M KI, 100 mM Hepes (pH 6.5), 100 mM KCl, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 1 μM bestatin, 1 μM pepstatin, and amastatin at 2 μg/ml) and centrifuged (40,000 $\times$ *g* for 20 min at 4°C). Soluble depolymerized material in the supernatant was repolymerized by dialysis four times for 1 hr at 4°C against 1 liter of a buffer containing 10 mM Hepes (pH 6.8), 1 mM EGTA, 2 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 5 mM benzamidine. The polymerized actin was recovered by centrifugation (12,000 $\times$ *g* at 4°C for 5 min) and submitted to a second depolymerization–repolymerization cycle.

SDS/PAGE and Immunoblotting. Proteins were resolved by SDS/PAGE (13% acrylamide), as described by Blank *et al.* (29), and transferred to nitrocellulose. An enhanced chemiluminescence kit from Amersham was used for antibody detection.

Immunocytochemistry. Cells were fixed for 1 min with 3% (vol/vol) formaldehyde in a medium containing 80 mM Pipes (pH 6.5), 5 mM EDTA, and 2 mM $MgCl_2$, and fixed for an additional 8 min with 3% (vol/vol) formaldehyde in 100 mM sodium borate, pH 11. Cells were then incubated 30 min in phosphate-buffered saline (PBS)/0.1% (wt/vol) sodium borohydride. Subsequently cells were permeabilized by incubation in PBS/0.2% Triton X-100, incubated overnight at 4°C with the antibody to be tested, washed, and further incubated for 60 min at 37°C with a secondary antibody. After washings, cells were postfixed for 20 min with 3% formaldehyde/PBS and incubated in the presence of 50 mM NH_4Cl for 10 min. The coverslips were mounted in 1,4-diazabicyclo[2.2.2]octane (100 mg/ml)/PBS/50% (vol/vol) glycerol.

Images were obtained by using a confocal krypton/argon mixed gas laser scanning microscope (MRC 600 Bio-Rad). Excitation was at 488 nm and 568 nm, respectively, for fluorescein and rhodamine. Fluorescent images taken in an equatorial plane (defined as the midpoint between the top and the bottom of the cell) were digitalized, and pseudocolor images were generated by using an arbitrary color code for light intensity: blue < green < red < yellow < white.

Phospholipase C Assays. Inositol phosphate accumulation in intact cells was measured as described (30). For phospholipase C assays on acellular preparations, *myo*-[^{3}H]inositol-labeled membranes (20–50 μg of protein per tube) were used as described (31).

RESULTS

The purified polyclonal antibody raised against the thyroglobulin-coupled carboxyl-terminal decapeptide of $G_{\alpha q}/G_{\alpha 11}$ exhibited the expected specificity because (*i*) as evaluated by ELISA tests, the antibody reacted with high affinity with the $G_{\alpha q}/G_{\alpha 11}$ terminal decapeptide and exhibited <2% cross-reactivity with the corresponding decapeptides from $G_{\alpha s}$, $G_{\alpha i}$ family, $G_{\alpha o}$, $G_{\alpha t2}$, $G_{\alpha z}$, $G_{\alpha 16}$, $G_{\alpha 12}$, and $G_{\alpha 13}$, or with pure actin (data not shown). (*ii*) Immunoblotting of SDS/PAGE-resolved proteins from rat liver and WRK_1 cell plasma membranes revealed the presence of only two bands of 43 kDa and 42 kDa that comigrated with pure $G_{\alpha q}$ and $G_{\alpha 11}$ (Fig. 1). These signals were undetectable when the antibody was preincubated for 4 hr with an excess of the $G_{\alpha q}/G_{\alpha 11}$ terminal decapeptide (data not shown). (*iii*) Preincubation of WRK_1 cell plasma

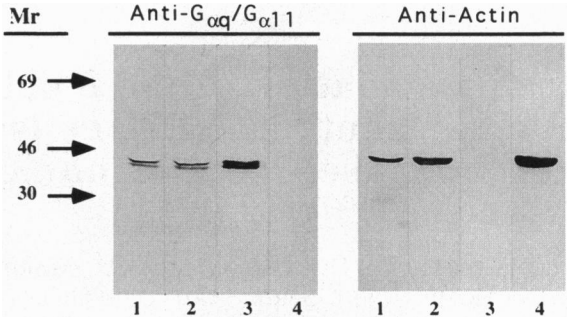

FIG. 1. Immunoblot analysis of WRK_1 cell and rat liver membranes using anti-$G_{\alpha q}/G_{\alpha 11}$ and anti-actin antibodies. Membrane proteins (50 μg) from WRK_1 cells (lane 1) and rat liver (lane 2) were resolved by SDS/PAGE and subsequently immunoblotted by using anti-$G_{\alpha q}/G_{\alpha 11}$ (*Left*) or anti-actin antibodies (*Right*). Lane 3 shows the results with a mixture of purified $G_{\alpha q}$ and $G_{\alpha 11}$ (50 ng of each); lane 4 shows the results with pure actin (100 ng) Mr, position of molecular weight standards.

membranes for 4 hr at 4°C in the presence of anti-$G_{\alpha q}/G_{\alpha 11}$ antibody produced a dose-dependent inhibition of vasopressin-sensitive phospholipase C activity (maximal inhibition, 66 ± 7%, $n = 4$) with no change in basal activity. This inhibition disappeared when the antibody was saturated overnight with an excess of its antigenic peptide (Fig. 2*A*). (*iv*) The anti-$G_{\alpha q}/G_{\alpha 11}$ antibody also inhibited guanosine 5′-[α-thio]triphosphate (GTP[γS])-induced phospholipase C activation but did not affect the calcium-dependent activity (Fig. 2*B*). (*v*) The anti-$G_{\alpha q}/G_{\alpha 11}$ antibody could also specifically immunoprecipitate $G_{\alpha q}$ and $G_{\alpha 11}$ proteins (data not shown).

On the basis of these observations, we concluded that the anti-$G_{\alpha q}/G_{\alpha 11}$ antibody is suitable for investigating the intracellular distribution of $G_{\alpha q}/G_{\alpha 11}$.

In WRK_1 cells, immunoreactivity revealed with the anti-$G_{\alpha q}/G_{\alpha 11}$ antibody was found associated with the plasma membrane and intracellular structures resembling actin filaments (Fig. 3*A*). Control experiments done with anti-$G_{\alpha q}/G_{\alpha 11}$ antibody saturated with its antigenic decapeptide or with the secondary antibody added alone did not reveal specific labelings (Fig. 3 *C–D*). Such data demonstrated the specificity of the immunocytochemical reaction (see below). Double-labeling

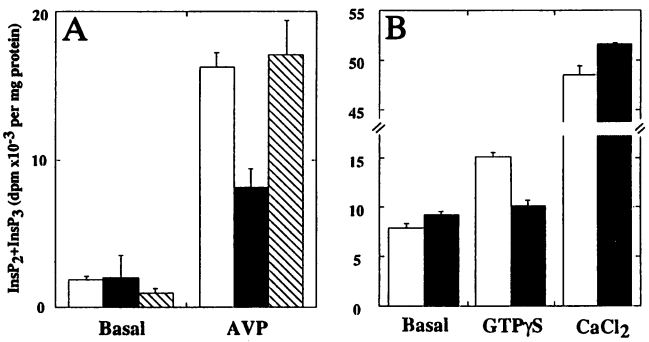

FIG. 2. Effect of anti-$G_{\alpha q}/G_{\alpha 11}$ antibody on basal and stimulated phospholipase C activity from WRK_1 cell membranes. Membranes (60 μg of protein) from *myo*-[^{3}H]inositol-prelabeled WRK_1 cells were incubated 4 hr at 4°C in the presence of 150 μg of protein of preimmune rabbit IgG (open bars), 150 μg of protein of anti-$G_{\alpha q}/G_{\alpha 11}$ antibody (black bars), or 150 μg of protein of anti-$G_{\alpha q}/G_{\alpha 11}$ antibody saturated with 1000-fold molar excess of $G_{\alpha q}/G_{\alpha 11}$ terminal decapeptide (hatched bars). Phospholipase C activity was assayed for 6 min, and inositol bis- ($InsP_2$) and tris ($InsP_3$) phosphate that accumulated were separated by filtration through Dowex 1 × 8 columns and measured. (*A*) Assay with or without 100 nM [Arg^8]vasopressin plus 100 μM GTP. (*B*) Assay under basal conditions with 100 μM GTP[γS] or with 100 μM free calcium. Results are the means ± SEMs of triplicate determinations derived from three distinct experiments.

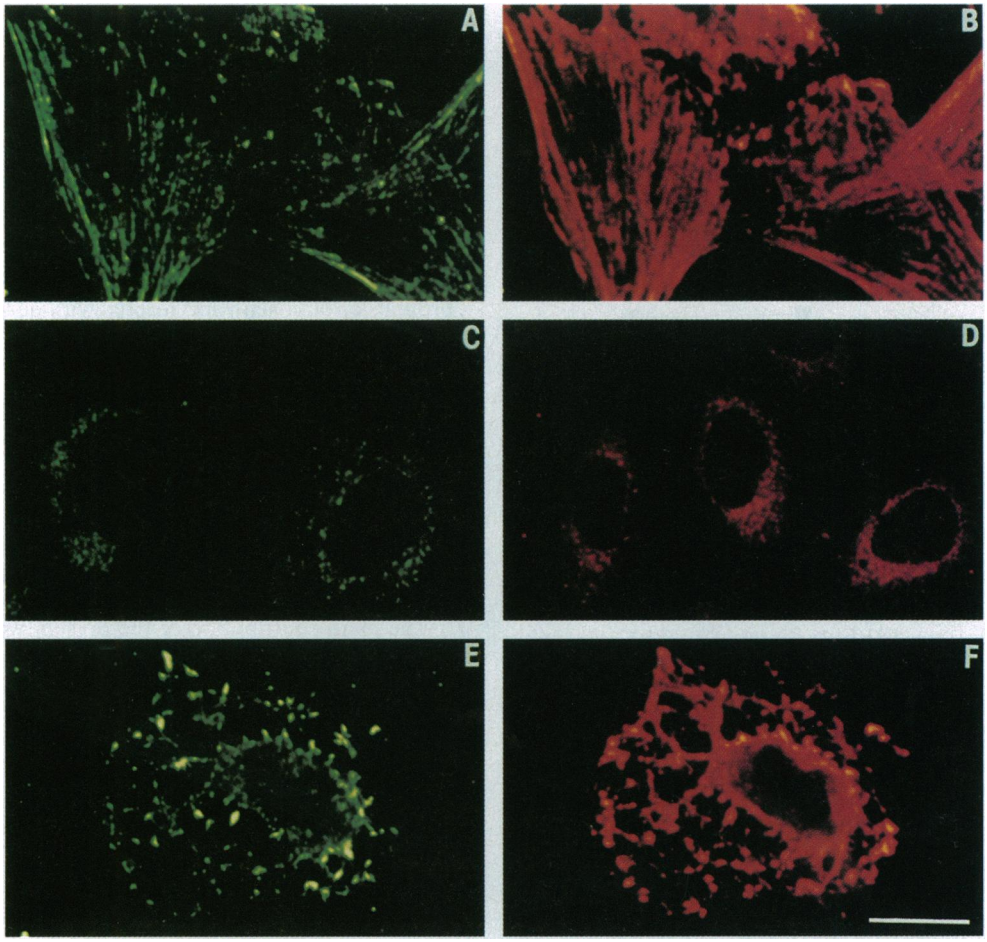

FIG. 3. Influence of cytochalasin D treatment on the $G_{\alpha q}/G_{\alpha 11}$ and actin localization in WRK_1 cells. WRK_1 cells were incubated 90 min at 37°C with 2 μM of cytochalasin D (*E, F*) or with vehicle (*A–D*). Cells were then incubated with (*A, B, E,* and *F*) or without (*C* and *D*) a mixture of rabbit anti-$G_{\alpha q}/G_{\alpha 11}$ (dilution 1/50) and mouse anti-actin (dilution 1/1000) antibodies. After washings, all preparations were incubated with a mixture of fluorescein isothiocyanate conjugated anti-rabbit IgG (1/40 dilution) and rhodamine-conjugated anti-mouse IgG (1/40 dilution). Fluorescein isothiocyanate (*A, C,* and *E*) and rhodamine (*B, D,* and *F*) labelings were analyzed as described on the same cell preparation. (Bar = 25 μm.)

experiments with anti-$G_{\alpha q}/G_{\alpha 11}$ and anti-actin antibodies were done to substantiate a colocalization of $G_{\alpha q}/G_{\alpha 11}$ and F-actin. As already demonstrated by ELISA tests and further illustrated in Fig. 1, pure actin could not be labeled with anti-$G_{\alpha q}/G_{\alpha 11}$ antibody and pure $G_{\alpha q}/G_{\alpha 11}$ could not be labeled with the monoclonal anti-actin antibody. The double-labeling experiments showed almost identical distribution patterns of the immunoreactive materials detected by anti-$G_{\alpha q}/G_{\alpha 11}$ and anti-actin antibodies (Fig. 3 *A–B*). In both cases the labeling appeared punctate. In a situation where F-actin filaments were disorganized (1 μM cytochalasin D), the distribution of anti-$G_{\alpha q}/G_{\alpha 11}$ and anti-actin immunoreactive materials was markedly altered, but their colocalization was preserved (Fig. 3 *E–F*). This effect reverted after 30 min in a medium without cytochalasin D (data not shown). Incubation with 1 μM colchicine, which leads to microtubular disorganization, had no detectable effect on the distribution pattern of the immunoreactive material (data not shown).

The close association of $G_{\alpha q}/G_{\alpha 11}$ with actin filaments was confirmed by using F-actin-rich fractions prepared from cytoskeletons of WRK_1 cells. Anti-$G_{\alpha q}/G_{\alpha 11}$ immunoreactive material was present in cytoskeletal fractions (Fig. 4, lane 1). As expected, the F-actin-rich fraction prepared by a two-step polymerization–depolymerization procedure did not contain detectable amounts of desmin, a constituent of intermediate filaments (Fig. 4). By contrast, anti-$G_{\alpha q}/G_{\alpha 11}$ immunoreactive material could be easily detected in this F-actin-rich repolymerized fraction. Immunoblotting experiments done with antibodies raised against G_β subunit showed the presence of immunoreactive material in the cytoskeletal and membrane fractions but not in the F-actin-rich fraction.

Alteration of WRK_1 cytoskeleton by cytochalasin D (Fig. 3 *E* and *F*) (*i*) did not permeabilize the cells because their intracellular calcium concentration was not affected (intracellular $[Ca^{2+}]$ = 89 ± 5 and 92 ± 7 nM for the control or

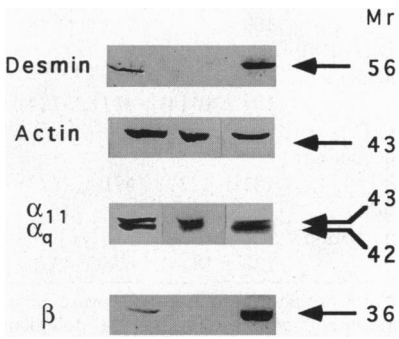

FIG. 4. Immunoblot analysis of WRK_1 cell subcellular fractions using anti-$G_{\alpha q}/G_{\alpha 11}$, anti-$G_\beta$, anti-desmin, and anti-actin antibodies. Cytoskeletal (lane 1), F-actin-rich (lane 2), and crude membrane (lane 3) fractions (50 μg of protein each) were prepared from WRK_1 cells, as indicated, resolved by SDS/PAGE, and subsequently immunoblotted using antisera to $G_{\alpha q}/G_{\alpha 11}$, G_β, desmin, and actin. Results are representative of four different experiments. Mr, position of molecular weight standards.

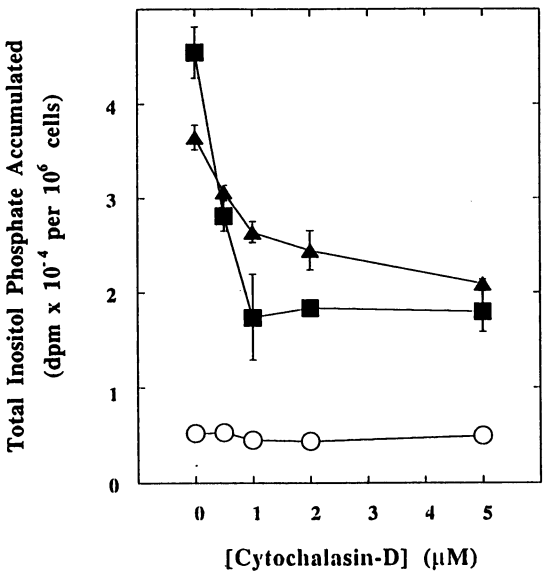

FIG. 5. Effect of cytochalasin D treatment on WRK_1 cells inositol phospholipid turnover. *myo*-[^{3}H]Inositol-prelabeled WRK_1 cells were incubated 90 min before the experiment with increased amounts of cytochalasin D or vehicle. Cells were then further incubated, as described in Table 1, without effector (○), with 100 nM [Arg^8]-vasopressin (■), or with 10 μM aluminium chloride/30 mM sodium fluoride (▲). Total inositol phosphates that accumulated were measured and expressed as dpm per 10^6 cells. Results indicate the means ± SEMs of triplicate determinations from four different experiments.

cytochalasin D-treated cells, respectively), (*ii*) did not modify basal or forskolin-stimulated levels of cAMP, and only weakly reduced (13 ± 6%) cAMP production induced by aluminium fluoride (data not shown), (*iii*) did not affect the basal level of inositol polyphosphate accumulation (Fig. 5), and (*iv*) inhibited both the vasopressin- and the aluminium fluoride-stimulated inositol phosphate production. The inhibition was dose-dependent (IC_{50} = 0.5 μM), partial (maximal inhibition of 40%), and reversible (Table 1). Colchicine treatment, which drastically disrupted the microtubule network in WRK_1 cells (data not shown), did not affect either the intracellular distribution of $G_{\alpha q}/G_{\alpha 11}$ or the vasopressin- and aluminium fluoride-stimulated inositol phosphate accumulation (Table 1).

Table 1. Effect of cytochalasin D and colchicine treatments on inositol phospholipid turnover in WRK_1 cells

Treatment	Total inositol phosphates accumulated, % of control		
	Basal	AVP	NaF
None	100	712 ± 58 (4)	713 ± 60 (4)
Cytochalasin D (1 μM) during preincubation and assay	104 ± 10 (4)	427 ± 57 (4)	471 ± 50 (4)
Cytochalasin D (1 μM) during preincubation only	101 ± 4 (3)	691 ± 21 (3)	699 ± 36 (3)
Colchicine (1 μM) during preincubation and assay	120 ± 18 (4)	676 ± 43 (3)	692 (2)

myo-[^{3}H]Inositol-prelabeled WRK_1 cells were incubated 90 min before the experiments with cytochalasin D, colchicine, or vehicle. Cells were then equilibrated for 15 min at 37°C in a PBS/lithium medium and further incubated 30 min in the same medium without effectors (basal conditions), with 0.2 μM [Arg^8]vasopressin (AVP), or with 30 mM sodium fluoride/10 μM aluminium chloride. Labeled inositol phosphates that accumulated during the incubation period were determined. Results, expressed as percentage of total inositol phosphates that accumulated in control cells, represent the means ± SEMs of the number of distinct experiments shown in parentheses (100% = 6700 ± 350 dpm/10^6 cells).

DISCUSSION

The polyclonal antibody raised against the carboxyl-terminal decapeptide common to $G_{\alpha q}$ and $G_{\alpha 11}$ and purified by a two-step affinity-chromatography procedure satisfied the main criterion used to assess the selectivity of antibodies raised against synthetic epitopes. The antibody exhibited <2% cross-reactivity with the corresponding epitopes from other G proteins or pure actin and reacted with purified $G_{\alpha q}$ and $G_{\alpha 11}$. It did not react with other proteins on SDS/PAGE-resolved proteins from WRK_1 cells and rat liver membranes and at least partially inhibited endogenous G proteins involved in phospholipase C activation. Finally, the signals revealed by the antibody could be abolished by incubation with an excess of the synthetic decapeptide common to $G_{\alpha q}$ and $G_{\alpha 11}$. As expected, this antibody did not discriminate between these two proteins. Therefore, no conclusions about their respective role and localization can be drawn from the present study. In the following discussion, the immunoreactive material detected by our purified antibody will be designated as $G_{\alpha q}/G_{\alpha 11}$.

The main contribution of the present work is to provide a series of experimental arguments favoring the conclusion that in WRK_1 cells, $G_{\alpha q}/G_{\alpha 11}$ is closely associated with actin filaments. These arguments are as follows: (*i*) The demonstration of a colocalization of immunoreactive materials revealed by anti-actin and anti-$G_{\alpha q}/G_{\alpha 11}$ antibodies in experimental conditions where the absence of cross-reactivity could be established, (*ii*) the association of $G_{\alpha q}/G_{\alpha 11}$ with an F-actin-rich fraction obtained after two depolymerization–repolymerization cycles, (*iii*) the persistence of $G_{\alpha q}/G_{\alpha 11}$ and actin labelings on actin-filament network after its disorganization by cytochalasin D treatment, and (*iv*) the absence of interaction between $G_{\alpha q}/G_{\alpha 11}$ and globular actin [$G_{\alpha q}/G_{\alpha 11}$ labeling was not altered when cytosolic proteins like globular actin were washed out before fixation with formaldehyde (data not shown)]. It is not possible, however, to assess whether $G_{\alpha q}/G_{\alpha 11}$ found in the plasma membrane fraction corresponds to the G protein associated directly with the lipid bilayer or with actin filaments that are closely associated with the plasma membrane. Indeed, we were unable to prepare an actin-free membrane fraction, whatever the plasma membrane treatments used (incubation with DNase I or with cytochalasin D, mechanical stirring with glass beads).

The association of $G_{\alpha q}/G_{\alpha 11}$ with the cytoskeleton seems exclusively due to its association with actin filaments because there is no evidence of a contribution of microtubules and intermediate filaments in this association. Indeed, the intracellular distribution of $G_{\alpha q}/G_{\alpha 11}$ was unaffected by a colchicine treatment, and F-actin-rich fraction does not contain any detectable amounts of desmin (Fig. 4). In contrast, other G protein subunits, like $G_{\alpha s}$, seem to be associated with tubulin rather than with actin filaments. Thus, colchicine treatment inhibits both cAMP accumulation and $G_{\alpha s}$ GTPase activity in rat myometrium and in rat anterior pituitary lobe, respectively (12, 32), and stimulates adenyl cyclase activity in S49 lymphoma cells (11).

Similar distribution patterns of $G_{\alpha q}/G_{\alpha 11}$ were observed in human adrenal glomerulosa (a secretory cell type), in human myometrium (a muscle cell type), and in neurons from cortex (data not shown), suggesting that this distribution is a general phenomenon. Conversely, Vaziri and Downes (19) reported that $G_{\alpha q}/G_{\alpha 11}$ was readily detectable in plasma membranes and in ghosts from turkey erythrocytes but was absent from cytoskeleton preparations. Similarly Wilson *et al.* (33) reported that in semi-thin sections of rat pituitary, anti-$G_{\alpha q}/G_{\alpha 11}$ antibodies predominantly labeled the plasma membrane of most cells and only weakly stained the Golgi region. Yet, these data

do not exclude the possibility of an association of $G_{\alpha q}/G_{\alpha 11}$ with membrane-bound F-actin because (*i*) the organization of the turkey erythrocyte cytoskeleton is different from that found in mammalian cells (34) and (*ii*) cultured cells contain intracellular stress fibers unlike cells *in situ* (this study and refs. 19 and 29).

Preliminary immunocytochemical experiments using specific antibodies directed against $G_{\alpha o1}$ and $G_{\alpha i3}$ indicate that the labeling patterns obtained with these antibodies clearly differ from those obtained with $G_{\alpha q}/G_{\alpha 11}$: no labeling of structures resembling F-actin filament can be observed (data not shown). Concerning other G protein subunits, immunoblotting experiments indicate that in contrast to $G_{\alpha q}/G_{\alpha 11}$, the G_{β} subunit was less abundant in crude cytoskeleton fraction than in plasma membranes and not detectable in F-actin-rich fractions (Fig. 4). Such data do not, however, signify that G_{β} (and probably G_{γ}) is absent from the F-actin filaments because $G_{\beta\gamma}$ subunits might have been solubilized by Triton X-100 during cytoskeleton preparation.

The observed close association of $G_{\alpha q}/G_{\alpha 11}$ with F-actin raised the question of its functional role. The actin filament directly in contact with the plasma membrane (peripheral pool) may represent the active pool of $G_{\alpha q}/G_{\alpha 11}$ susceptible to interact with hormonal receptor and to activate phospholipase C. The $G_{\alpha q}/G_{\alpha 11}$ found associated with internal F-actin may constitute an intracellular pool of spare G proteins available for sustained or repeated hormonal stimulation. This hypothesis implies that $G_{\alpha q}/G_{\alpha 11}$ may cycle from one pool to the other upon hormonal stimulation. Several experimental observations fit this model: (*i*) the rapid depalmitoylation of $G_{\alpha q}/G_{\alpha 11}$, as well as the reorganization of F-actin network generally observed after hormonal stimulation, favored the hypothesis of $G_{\alpha q}/G_{\alpha 11}$ translocation (35, 36). (*ii*) The partial inhibition of vasopressin-sensitive phospholipase C activity observed upon cytochalasin D treatment may be due to an interference of its dynamic renewal in the absence of organized actin filaments. The model also provides some basis for the proposal by Rodbell and colleagues (4, 5) of an organization of G protein large-polymeric structures and fits with the disaggregation theory of signal transduction applied to multimeric G proteins, as recently developed by Jahangeer and Rodbell (5).

Aside from this model, the close association between actin and $G_{\alpha q}/G_{\alpha 11}$ might suggest a structural role for $G_{\alpha q}/G_{\alpha 11}$ that could stabilize the interaction between the actin filament and the plasma membrane due to their hydrophobic palmitate tails (37) and coupling with seven transmembrane receptors. This association may also favor the dynamic renewal of actin filaments due to the GTPase activity of $G_{\alpha q}/G_{\alpha 11}$ G proteins as recently observed for small G proteins such as Rho and Rac in mast cells (36). This association may also be involved in the disaggregation of the F-actin network seen upon hormonal stimulation of phospholipase C, as observed in many cellular systems, disaggregation favoring exocytosis, and thus secretion processes (for review, see ref. 38). Finally, the presence of $G_{\alpha q}/G_{\alpha 11}$ on actin filaments would be consistent with a role of these proteins in vesicular transport, as has already been suggested (33).

In conclusion, the tight association of $G_{\alpha q}/G_{\alpha 11}$ with F-actin demonstrated here by biochemical and immunochemical approaches gives further insight into the role of the cytoskeleton in the dynamics of hormonal receptor coupling with G proteins. Moreover, such a localization raises the question of another function for $G_{\alpha q}/G_{\alpha 11}$.

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