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# Association of Solubilized Angiotensin II Receptors with Phospholipase C-α in Murine Neuroblastoma NIE-115 Cells

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## SUMMARY

The peptide angiotensin II (AngII) has been reported to stimulate phosphoinositide-specific phospholipase C (PLC) activity in the murine neuroblastoma cell line N1E-115. In the present study, polyclonal antibodies raised against a PLC isoenzyme, PLC-α, reacted with a 60-kDa protein present in both membrane and cytosolic fractions of differentiated N1E-115 cells. In order to examine the possible association of PLC-α with cell surface AngII receptors (AngII-Rs), membranes from differentiated N1E-115 cells were solubilized, using the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). CHAPS (1%) solubilized AngII-Rs, from N1E-115 cells, that maintained their high affinity for agonists. Gel filtration analysis of the solubilized membranes revealed that the majority of the specific binding of $^{125}$I-AngII eluted as a large protein complex with a molecular mass of 380 kDa and that agonist binding was partially reduced by guanosine-5′-*O*-(3-thio)triphosphate (GTPγS), within this complex. CHAPS also effectively solubilized immunoreactive PLC-α, from N1E-115 cell membranes, that was similarly present within the 380-kDa AngII-binding complex. Anti-PLC-α antisera immunoprecipitated approximately 16% of the total phosphatidylinositol-4,5-bisphosphate-specific PLC activity in the 1% CHAPS extract and 40% of cytosolic PLC activity. Moreover, a 60-kDa $^{35}$S-Trans S-labeled protein, comigrating with immunoreactive PLC-α, was immunoprecipitated from the 1% CHAPS extract by the antisera. In addition, anti-PLC-α antisera immunoprecipitated approximately 20% of solubilized AngII-Rs prebound with $^{125}$I-AngII but failed to precipitate receptors prebound with the antagonist $^{125}$I-Sarc$^1$,Ile$^8$-AngII. The anti-PLC-α antisera also immunoprecipitated AngII-Rs when intact membranes were labeled with $^{125}$I-AngII before solubilization in 1% CHAPS, suggesting that the AngII-R interaction with PLC-α was not the result of detergent-promoted protein-protein interaction. On the other hand, monoclonal antibodies against another PLC isozyme, PLC-γ, did not precipitate AngII-Rs in solubilized N1E-115 membranes. Finally, the formation of the immunoprecipitated AngII-R-PLC-α complex was disrupted by the nonhydrolyzable guanine nucleotide analog GTPγS, suggesting that the interaction between AngII-Rs and PLC-α is likely to involve a heterotrimeric guanine nucleotide-binding protein in neuron-like cells.

The physiological, endocrine, and behavioral actions of AngII are mediated by specific receptors located in peripheral tissues and the brain (1). In the majority of cells expressing AngII-Rs, agonist occupancy stimulates the hydrolysis of $PIP_2$, yielding the two second messengers inositol 1,4,5-trisphosphate and diacylglycerol (2–9). Inositol 1,4,5-trisphosphate mobilizes sequestered stores of calcium (10, 11), whereas diacylglycerol stimulates protein kinase C (12). The ubiquity of this membrane transduction pathway suggests that it plays an important role in the cellular actions of AngII.

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Agonist stimulation of $PIP_2$ hydrolysis is presumed to be mediated by a phosphoinositide-specific PLC. At least nine PLC isozymes have been demonstrated to date (13–15). Although the mechanism by which receptor occupancy stimulates PLC activity remains unclear, the physical association of cell surface receptors with specific PLCs has been demonstrated in two previous studies. For instance, antisera directed against the bovine brain PLC isozyme, PLC-γ, have been reported to co-precipitate PLC-γ and the epidermal growth factor receptor from A431 cells (16). Additionally, the apparent association of rat liver vasopressin $V_1$ receptors with another PLC isozyme, PLC-α (17, 18), also has been reported (19).

Recently, we have demonstrated that the murine neuro-

**ABBREVIATIONS:** AngII, angiotensin II; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; PLC, phospholipase C; $PIP_2$, phosphatidylinositol-4,5-bisphosphate; AngII-R, angiotensin II receptor; DMEM, Dulbecco's modified Eagle's medium; PEI, polyethylenimine; 1,10-PA, 1,10-*o*-phenanthroline; SARILE, Sarc$^1$,Ile$^8$-angiotensin II; FCS, fetal calf serum; SDS, sodium dodecyl sulfate; G protein, guanine nucleotide-binding protein; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PAGE, polyacrylamide gel electrophoresis; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; GDPβS, guanosine-5′-*O*-(2-thio)diphosphate; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; bis-Tris, bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane.

blastoma cell line N1E-115 possesses AngII-Rs, the density of which was dramatically up-regulated by differentiation (20, 21). These AngII-Rs were regulated by guanine nucleotides and coupled to inositol trisphosphate-mediated increases in cytosolic free calcium through a pertussis toxin-insensitive pathway (6). In other studies, we demonstrated that AngII-Rs could be solubilized from N1E-115 membranes using the zwitterionic detergent CHAPS (22). The solubilized receptors retained the pharmacological properties characteristic of the receptors within intact membranes. Moreover, affinity purification of the solubilized N1E-115 membranes demonstrated that the binding of $^{125}$I-AngII was to a single 66-kDa protein. On the other hand, in the present study, gel filtration analysis of the solubilized membranes revealed that the majority of the binding activity eluted as a large complex of approximately 380 kDa, suggesting that AngII-Rs are likely to be associated with other proteins in the nonpurified CHAPS extract.

In the present report, we have continued the investigation of the association of cell surface AngII-Rs with other membrane proteins, by examining the interaction of AngII-Rs with PLC-$\alpha$ in differentiated N1E-115 cells. The results of the present study indicate that 1% CHAPS solubilized both AngII-Rs and PLC activity from differentiated N1E-115 cell membranes. Western blot analysis, utilizing anti-PLC-$\alpha$ polyclonal antisera, identified an immunoreactive protein with an apparent molecular mass of 60 kDa, in the 1% CHAPS extract. Furthermore, the anti-PLC-$\alpha$ antisera co-precipitated PLC activity and AngII-Rs that had been prelabeled with the agonist $^{125}$I-AngII, but not those labeled with the antagonist $^{125}$I-SARILE. On the other hand, monoclonal antibodies against PLC-$\gamma$, another major PLC isozyme present in N1E-115 cells (23), did not precipitate AngII-Rs. Collectively, these results suggest that membranous AngII-Rs may be coupled to PLC-$\alpha$ in a neuron-like cell line.

## Experimental Procedures

### Materials

Monoiodinated $^{125}$I-AngII and $^{125}$I-SARILE, as well as $^{3}$H-labeled inositol lipids and [$^{35}$S]methionine, were obtained from NEN/DuPont (Boston, MA). Unlabeled AngII and related peptides, HEPES, glycerol, aprotinin, 1,10-PA, Coomassie Brillant Blue, PEI, prestained molecular weight standards for SDS-PAGE, and Sephacryl S-300 resin were from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA) and were of the highest obtainable grade.

### Solubilization and Radioligand Binding Techniques

**N1E-115 cell culture.** N1E-115 cells were maintained and differentiated as previously described (6, 20–22). Cells were grown for 3 days in T75 or T150 plastic plates, in DMEM with high glucose, supplemented with 10% FCS, 2.5 $\mu$g/ml fungizone, 50 units/ml penicillin, and 50 $\mu$g/ml streptomycin, at 37° in a humidified 5% $CO_2$/95% $O_2$ atmosphere. Cell passages 20–30 were used throughout this study. N1E-115 cells were induced to differentiate by the replacement of normal growth medium with medium containing 0.5% FCS and 1.5% dimethylsulfoxide. Cells were subsequently maintained for 3 or 4 days before harvesting. Differentiated N1E-115 cells were used throughout this study.

**Membrane preparation and solubilization.** Medium was removed from culture dishes, and cells were rinsed three times in ice-cold 20 mM Tris·HCl, pH 7.4, 150 mM NaCl. Cells were then incubated for 10–15 min at 4° in 20 mM Tris·HCl, pH 7.4, removed with a rubber policeman, and homogenized with a Dounce homogenizer. The homogenate was centrifuged at 1000 × $g$ for 5 min. Membranes were collected by centrifugation of the resultant supernatant at 48,000 × $g$ for 30 min. The membrane pellet was resuspended in 50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 5 mM $MgCl_2$ (buffer A), and recentrifuged for 30 min at 48,000 × $g$. These washes were incorporated to eliminate any trace of soluble AngII-binding activity associated with the cytosol of N1E-115 and other cells (24). The membranes were resuspended in 25 mM sodium phosphate, 5 mM EGTA, 25% glycerol, 500 mM KCl (buffer B), and protein concentration was determined by the method of Bradford (25). The membranes were again collected by centrifugation at 48,000 × $g$ for 30 min and were resuspended to a final protein concentration of 6–8 mg/ml, in 1% CHAPS in buffer B supplemented with 0.3 trypsin inhibitory units/ml aprotinin, 100 $\mu$g/ml 1,10-PA, and 0.2% heat-inactivated BSA, unless otherwise specified. The 1% CHAPS homogenate was incubated for 1 hr at 4° on an orbital shaker and then centrifuged at 105,000 × $g$ for 90 min at 4°. The resulting supernatant was then removed, and the residual pellet was either resuspended, to measure unsolubilized protein and ligand binding, or discarded. Material was either used immediately or stored at −70°.

**$^{125}$I-AngII Labeling of N1E-115 cell membranes.** N1E-115 cell membranes were harvested as described. Membranes were adjusted to a protein concentration of 1 mg/ml and labeled with 0.25 nM $^{125}$I-AngII, in buffer A supplemented with 0.2% heat-inactivated BSA, aprotinin, and 1,10-PA, in the presence or absence of 1 $\mu$M AngII, for 60 min at 25°. Unbound ligand was removed by centrifugation at 20,000 × $g$ for 15 min. The pellet was resuspended at a protein concentration of 8 mg/ml, in 1% CHAPS in buffer B supplemented with aprotinin, 1,10-PA, and 0.2% heat-inactivated BSA, and was incubated for 60 min at 4°. The labeled solubilized material was centrifuged at 105,000 × $g$ for 90 min, and the supernatant was removed for binding assays and immunoprecipitation experiments.

**Receptor binding assay.** Radioligand binding assays were performed essentially as described (20–22). One hundred microliters of solubilized material were added to 150 $\mu$l of buffer A, with 0.2% heat-inactivated BSA, aprotinin, and 1,10-PA, containing $^{125}$I-AngII or $^{125}$I-SARILE (specific activity, 2200 Ci/mmol; NEN/DuPont) and various concentrations of unlabeled peptides, as required. Nonspecific binding was defined in the presence of 1 $\mu$M AngII. The reaction was terminated after a 60-min incubation at 25°, by dilution and rinsing of the assay tubes three times in 5 mM Tris·HCl, pH 7.4, 150 mM NaCl (buffer C), followed by filtration through 0.1% PEI-presoaked glass fiber filters, using a Skatron cell harvester. Dried filters were counted for 1 min in a LKB $\gamma$ scintillation counter, at 80.5% efficiency. In all experiments, binding data were analyzed by LIGAND (26).

**Gel filtration analysis of solubilized AngII-Rs.** Sephacryl S-300 resin (Sigma Chemical Co.) was poured into a 1- × 40-cm column and equilibrated at 4° with a buffer containing 50 mM HEPES (pH 7.4), 5 mM EDTA, 5 mM EGTA, 200 mM NaCl, 0.1% CHAPS, and 10% glycerol. Freshly solubilized AngII-Rs (approximately 2–2.5 mg of protein) were applied to the column, which was run at 0.2 ml/min at 4°. Fractions (0.5 ml) were collected in a LKB fraction collector and monitored at 280 nm. In binding assays, each fraction was split into two equal volumes, to measure total and nonspecific binding. Each tube received $^{125}$I-AngII (approximately 0.6 nM final concentration), 0.3 trypsin inhibitor units/ml aprotinin, and 100 $\mu$g/ml 1,10-PA. In selected experiments, GTP$\gamma$S (100 $\mu$M final concentration) was added to the receptor binding assay. In all cases, nonspecific binding was determined with 10 $\mu$M unlabeled SARILE and represented <10% of total bound radioactivity. Binding assays were carried out for 60 min at 22° and were terminated on a GF/B glass fiber filter that had been previously soaked in 0.3% PEI.

### Immunoprecipitation Techniques

**Anti-PLC-$\alpha$ polyclonal antisera.** PLC-$\alpha$ was purified from guinea pig uteri according to the method of Bennett and Crooke (18). Female New Zealand white rabbits were injected subcutaneously with 50 $\mu$g of purified PLC-$\alpha$ in Freund's complete adjuvant. Rabbits were boosted intramuscularly 3 weeks later with 25 $\mu$g of PLC-$\alpha$ in Freund's incom-

plete adjuvant. Blood was taken every 3 weeks. Serum fractions were subjected to 50% ammonium sulfate fractionation, to obtain an immunoglobulin fraction. When required, IgG was purified from preimmune and immune sera on Protein A columns.

**Protein separation and Western blotting.** Fifty or 100 μg of protein were separated by SDS-PAGE and electrophorectically transferred to nitrocellulose membranes in 25 mM Tris, pH 8.3, 192 mM glycine, 20% methanol. Nonspecific binding to the nitrocellulose was blocked with 3% normal goat serum in Tris-buffered saline (20 mM Tris·HCl, pH 7.4, 150 mM NaCl). The nitrocellulose was then incubated in diluted preimmune or anti-PLC-α immune serum. Unbound antibody was removed by three washes in Tris-buffered saline supplemented with 0.05% Tween-20. Immunoreaction products were detected using a 1/7500 dilution of alkaline phosphate-conjugated goat anti-rabbit IgG (Promega) and the chromogenic substrates nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Protein separation by isoelectric focusing/SDS-PAGE was performed according to the method of O'Farrell (27), with modifications as described by Woolkalis and Manning (28). Proteins were then transferred to nitrocellulose and immunoreactive products were detected as described above.

**PLC assay.** PLC activity was measured by the formation of water-soluble products from [*inositol*-2-$^3$H]$PIP_2$, essentially as described (18, 29). Briefly, phosphoinositide substrate was dried under a stream of $N_2$ and sonicated in 5 mg/ml deoxycholate. Enzymatic activity was measured over 10 min at 37°, in 50 mM bis-Tris, pH 7.0, 50 mM KCl, 1 mM $CaCl_2$, 2.4 mM deoxycholate, 20 μM substrate (15,000–25,000 cpm), in a final volume of 50 μl. Reactions were terminated by the addition of 250 μl of chloroform/methanol/concentrated HCl (50:50:0.3) and 75 μl of 1 N HCl with 5 mM EGTA. Samples were then centrifuged, and 150 μl of the upper phase were counted in liquid scintillant.

**$^{35}$S-Trans S label metabolic Labeling.** N1E-115 cells were washed twice in methionine-free DMEM supplemented with 0.5% FCS. Cells were then incubated for 20 hr in methionine-free DMEM containing 50 μCi/ml $^{35}$S-Trans S label and 0.5% FCS, at 37°, in a humidified 5% $CO_2$/95% $O_2$ environment. Plates were washed three times in $Ca^{2+}$/$Mg^{2+}$-free phosphate-buffered saline, and membranes were harvested as usual. Labeled membranes were solubilized in 1% CHAPS as described above, unless otherwise specified. The detergent extract was precleared by incubation with 30 μl of Protein A-Sepharose that had been pretreated with a nonimmune rabbit immunoglobulin fraction. The obtained nonimmune precleared supernatant was incubated overnight with preimmune or anti-PLC-α immune serum at 4°, on an orbital shaker. Immunoreactive products were pelleted by centrifugation for 3 min at 12,000 × *g*. The obtained pellet was washed three times in 50 mM $NaPO_4$, pH 8, 150 mM NaCl, 0.5% CHAPS, and subsequently boiled in Laemmeli buffer (30) for 3 min. Samples were separated on SDS-polyacrylamide gels, and the gels were dried and autoradiographed, with Kodak X-OMAT film, for 3–7 days.

**Immunoprecipitation of PLC activity.** Purified guinea pig uterus PLC-α (0.5 μg), N1E-115 cytosolic proteins (35 μg), or 1% CHAPS-extracted membrane proteins (175 μg) were incubated for 30 min, at 37°, with increasing concentrations of anti-PLC-α antiserum in 25 mM bis-Tris, pH 7.0, 100 mM KCl, 0.5 mM EGTA, 1 mg/ml BSA, containing aprotinin and 1,10-PA, in a final volume of 100 μl. One hundred microliters of 50% Protein A-Sepharose were then added, and the mixture was incubated for 30 min at 37°. Immunoreactive products were collected by centrifugation at 12,000 × *g* for 3 min. Enzymatic activity remaining in the supernatant was determined by the PLC assay described above. The removal of PLC-α immunoactivity by the antisera was also examined in these experiments, by immunoblotting of the remaining supernatant after exposure to increasing concentrations of PLC-α antiserum and addition of Protein A-Sepharose.

**Immunoprecipitation of AngII binding by anti-PLC-α antisera.** N1E-115 membrane proteins solubilized in CHAPS were labeled with $^{125}$I-AngII or $^{125}$I-SARILE, in buffer supplemented with 0.2% heat-inactivated BSA, aprotinin, and 1,10-PA, as described above. Nonspecific binding was defined in the presence of 1 μM AngII. The labeled extract was then precleared with nonimmune rabbit immunoglobulin-Protein A-Sepharose and was incubated with appropriate amounts of preimmune serum or anti-PLC-α immune serum overnight at 4°, on an orbital shaker. One hundred microliters of 20% Protein A-Sepharose were then added, and the mixture was incubated for 2 hr at 4°. Immune complexes were collected by centrifugation at 12,000 × *g* for 3 min. Pellets were washed three times in 50 mM sodium phosphate, pH 8, 150 mM NaCl, 0.5% CHAPS. Wash buffer was removed with a syringe, and the $^{125}$I-AngII or $^{125}$I-SARILE content of the obtained pellet was determined by a LKB γ counter.

## Results

**Solubilization and gel filtration analysis of AngII-Rs from differentiated N1E-115 cells.** Previous studies have demonstrated that murine neuroblastoma N1E-115 cells possess a high density of membranous AngII-Rs (20, 21) that can be solubilized by the zwitterionic detergent CHAPS (22). In a series of preliminary studies, we confirmed these earlier observations, particularly the efficacy of 1% CHAPS in solubilizing N1E-115 AngII-Rs. For instance, we found that 40–55% of specific $^{125}$I-AngII or $^{125}$I-SARILE binding and approximately 50% of total protein were solubilized when membrane proteins (6–8 mg/ml) were treated with 1% CHAPS (1 hr at 4°), as has been reported previously (22). Moreover, the rank order of potency of $^{125}$I-SARILE binding exhibited in intact membranes, [Sarc$^1$]AngII ≥ SARILE > AngII > angiotensin III ≫ angiotensin I, was identical in the solubilized material (data not shown). Of particular importance was the demonstration that the displacement of $^{125}$I-SARILE binding with AngII was biphasic, consisting of both high affinity ($K_{D_1}$ = 3.63 ± 0.21 nM, $B_{max_1}$ = 108 ± 16 fmol/mg of protein) and low affinity ($K_{D_2}$ = 34.03 ± 4.6 nM, $B_{max_2}$ = 170 ± 23 fmol/mg of protein; four experiments) components in the solubilized membranes, as we reported previously with intact membranes (20, 21). Finally, gel filtration analysis of solubilized N1E-115 membranes indicated that the specific binding of $^{125}$I-AngII eluted from the Sephacryl S-300 column in a major peak of 380 ± 20 kDa and a less prominent peak of 67 ± 3 kDa (Fig. 1). GTPγS decreased $^{125}$I-AngII binding by approximately 23% in the 380-kDa complex, with a corresponding increase in the binding activity present within the smaller peak (i.e., 67 kDa), consistent with the suggestion that some population of AngII-Rs remain associated with G proteins. These results are in general agreement with our previous findings (22), except that the current use of the Sephacryl S-300 column facilitated the detection of the 380-kDa complex but could not resolve a previously reported 120-kDa binding site that was probably lost in the leading edge of the 67-kDa peak.

**Solubilization of PLC-α.** The subcellular distribution of PLC-α in differentiated N1E-115 cells was examined by Western blot analysis, utilizing anti-PLC-α polyclonal antibodies. The anti-PLC-α antisera specifically reacted with a 60-kDa protein present in both cytosolic (Fig. 2C, *lane 1*) and membrane fractions (Fig. 2C, *lane 2*) of differentiated N1E-115 cells; this protein comigrated with purified guinea pig uterine PLC-α. Moreover, membrane-associated PLC-α was solubilized by 1% CHAPS with a high degree of efficiency (Fig. 2D, *lane S*), insofar as the residual CHAPS-insoluble protein contained very little immunologically detectable PLC-α (Fig. 2D, *lane P*). Interestingly, the solubilized PLC-α immunoactivity was present in the same 380-kDa protein complex that contained the

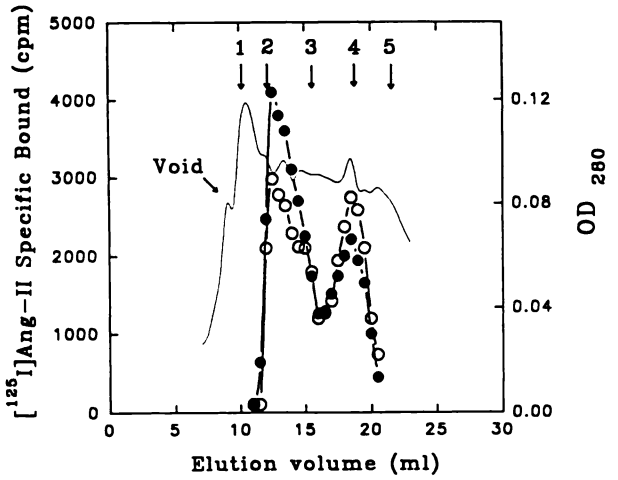


**Fig. 1.** Gel filtration analysis of solubilized AngII-Rs. Sephacryl S-300 size exclusion chromatography of CHAPS-solubilized N1E-115 cell membranes. Aliquots of solubilized Ang-II-Rs (2–2.5 mg) were applied to the Sephacryl S-300 column (1 × 40 cm), which was run at a flow rate of 2 ml/min, at 4°. Two major specific binding peaks eluted from the column, with approximate molecular masses of 380 ± 20 kDa (mean ± standard deviation; three experiments) and 67 ± 3 kDa (mean ± standard deviation, three experiments). ●, Binding activity in the eluted fractions measured in the absence of GTPγS; ○, data obtained from assays performed in the presence of GTPγS (100 μM). Molecular weight standards depicted in the graph represent thyroglobulin (669,000) (*1*), apoferritin (443,000) (*2*), β-amylase (200,000) (*3*), BSA (66,000) (*4*), and carbonic anhydrase (29,000) (*5*).

majority of AngII-binding activity (Fig. 2E, *lane I*). Preimmune immunoglobulins failed to react with proteins contained in either cytosolic or membrane fractions (Fig. 2, B, *lanes 1* and *2*, and E, *lane N*). Finally, the ability of 1% CHAPS to solubilize PLCs simultaneously was also assessed by measuring PLC enzymatic activity. When 20 μM $PIP_2$ was used as substrate, 1% CHAPS extracted 135% of total membranous PLC activity, resulting in a 2.9-fold enrichment of its specific activity (Table 1).

**Immunoprecipitation of PLC-α.** In order to demonstrate that the anti-PLC-α antibodies were directed against a PLC, the extent of immunoprecipitation of PLC activity from purified guinea pig PLC-α, N1E-115 cytosolic proteins, and 1% CHAPS-extracted membranes was determined. Maximally, 99% of purified PLC-α, 48% of cytosolic protein, and 16% of CHAPS-extracted membranous PLC activity were specifically immunoprecipitated by anti-PLC-α antisera (Fig. 3). As expected, the diminution of PLC activity in solubilized N1E-115 membranes after exposure to increasing concentrations of PLC-α antisera was associated with the quantitative precipitation of immunoreactive PLC-α (Fig. 3B). It is likely that the remaining PLC activity was attributable to the activity of other PLC isoforms. In this regard, Western blot analysis has indicated that N1E-115 cells also contain PLC-γ (23).

In other experiments, N1E-115 cells were metabolically labeled with $^{35}$S-Trans S label for 20 hr, to demonstrate the specific interaction of the polyclonal antisera with a 60-kDa protein. A 60-kDa $^{35}$S-labeled protein, comigrating with PLC-α, was precipitated by immune antiserum but not by preimmune antiserum (Fig. 4). The ability of the antisera to identify PLC-α specifically was further confirmed by two-dimensional gel electrophoresis. Western blot analysis revealed the presence of one immunoreactive 60-kDa protein, with an apparent pI of 6.6 (Fig. 5). The inability of the anti-PLC-α antisera to cross-react with purified PLC-δ, PLC-γ, or PLC-β further confirmed the specificity of the antisera for the PLC-α isoform.[1] Collectively, these data demonstrate that the anti-PLC-α antisera specifically recognize one 60-kDa protein and immunoprecipitate PLC activity, which is not attributable to three other PLC isoforms, in the 1% CHAPS extract of N1E-115 cell membranes.

**Immunoprecipitation of AngII binding by anti-PLC-α antibodies.** In addition to the precipitation of PLC-α immunoreactivity and PLC activity from solubilized N1E-115 membranes, the PLC-α antisera also co-precipitated AngII-Rs labeled with $^{125}$I-AngII. Maximal recovery of $^{125}$I-AngII in the immune precipitate was achieved with 100 μg of antiserum (Fig. 6), and this corresponded to the removal of approximately 20% of the $^{125}$I-AngII-binding activity present in solubilized membranes (data not shown). More specifically, 100 μg of anti-PLC-α antiserum precipitated 13.7 ± 0.8 fmol/mg of protein of specifically bound $^{125}$I-AngII (0.25 nM), compared with the precipitation of <0.2 ± 0.1 fmol/mg of protein by preimmune serum. Interestingly, the calculated percentage of AngII-binding activity that was precipitated by PLC-α was nearly equal to the proportion of agonist binding reduced by GTPγS (see Fig. 1).

As previously noted, N1E-115 cells also contain PLC-γ (23). To determine whether AngII-Rs were coupled to PLC-γ, as well as PLC-α, solubilized N1E-115 membranes were incubated with $^{125}$I-AngII and precleared with nonimmune mouse immunoglobulin-Protein A-Sepharose. The labeled extracts were

[1] S. G. Rhee, personal communication.

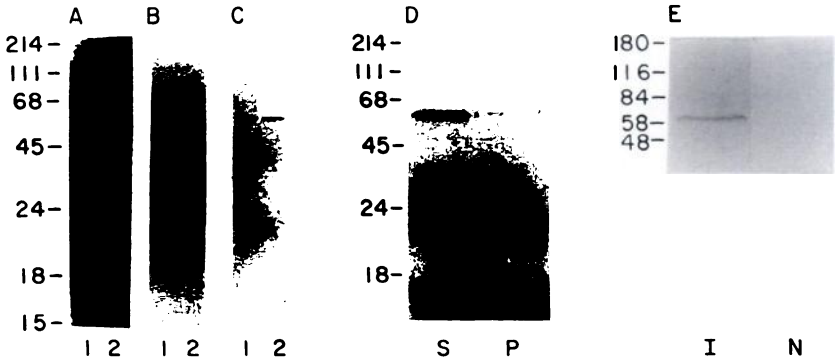


**Fig. 2.** Distribution of PLC-α in the differentiated N1E-115 cell preparation. A–C, 25 μg of cytosolic (*lanes 1*) or 25 μg of membrane (*lanes 2*) proteins were separated by electrophoresis on an 11% SDS-polyacrylamide gel and were transferred to nitrocellulose. Nitrocellulose was stained with Amido black (A) or probed with a 1/500 dilution of preimmune serum (B) or a 1/500 dilution of anti-PLC-α immune serum (C). Alternatively, 50 μg of 1% CHAPS-extracted membrane (D, *lane S*) or residual detergent pellet (D, *lane P*) proteins were separated, transferred to nitrocellulose, and probed with a 1/500 dilution of anti-PLC-α antiserum. E, 90 μg of the 380-kDa solubilized protein complex were treated similarly and probed with a 1/500 dilution of anti-PLC-α antiserum (*lane I*) or nonimmune serum (*lane N*). Immunoreactive proteins were detected with alkaline phosphatase-conjugated goat anti-rabbit IgG.

TABLE 1

**Subcellular distribution of PLC activity in N1E-115 cells**

N1E-115 cell fractions were assayed for PLC activity by measuring the formation of water-soluble inositol polyphosphates from 20 μM [$^3$H]$PIP_2$. Enzymatic activity was measured at 37° for 10 min.

| | Protein | Specific activity | Total activity |
|---|---|---|---|
| | *mg* | *nmol/min/mg of protein* | *nmol/min* |
| 48,000 × *g* membranes | 8.84 | 2.35 | 20.76 |
| 48,000 × *g* cytosol | 110.69 | 6.40 | 708.08 |
| 1% CHAPS extract | 4.06 | 6.88 | 27.95 |

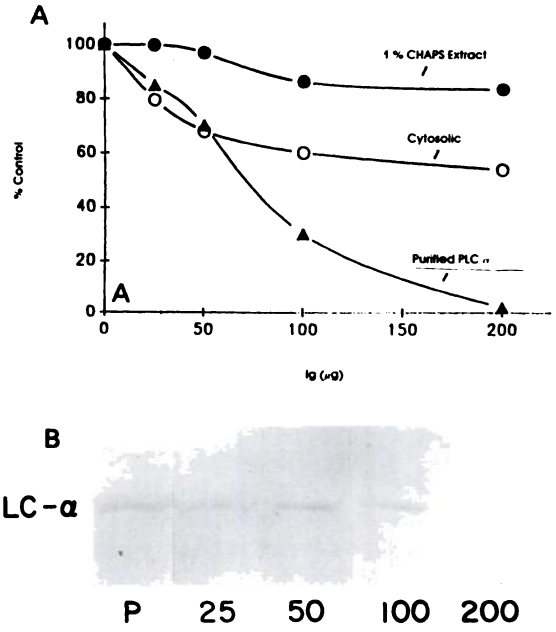


**Fig. 3.** Immunoprecipitation of N1E-115 cell PLC activity and PLC-α immunoreactivity by anti-PLC-α antisera. A, Purified guinea pig uterine PLC-α (0.5 μg), N1E-115 cell cytosol (35 μg), or 1% CHAPS extract (175 μg) was incubated with increasing amounts of anti-PLC-α antiserum. Immunoreactive products were precipitated by the addition of 100 μl of 50% Protein A-Sepharose, and the PLC activity remaining in the supernatant was quantitated by assaying enzymatic activity in the presence of 20 μM $PIP_2$. Nonspecific precipitation of PLC activity (3–17% of total activity) was defined in the presence of 200 μg of preimmune IgG. Reported values are the averaged results of three separate experiments. B, The supernatant remaining after immunoprecipitation with increasing amounts of PLC-α antiserum (25–200 μg) was separated by SDS-PAGE, transferred to nitrocellulose, and probed with a 1/500 dilution of anti-PLC-α immune serum.

subsequently incubated with either mouse IgG or anti-PLC-γ monoclonal antibodies. In a parallel series, the identical solubilized membranes were incubated with the polyclonal PLC-α antisera. In marked contrast to the efficacy of the PLC-α antisera (Fig. 7, *left*), the anti-PLC-γ antibodies failed to precipitate AngII-Rs (Fig. 7, *right*).

**Characterization of AngII-R coupling to PLC-α.** In order to examine the effect of solubilization on the coupling of AngII-Rs to PLC-α, intact membranes were radiolabeled with 0.25 nM $^{125}$I-AngII, and unbound ligand was removed by centrifugation. The labeled membranes were subsequently solubilized with 1% CHAPS, and the detergent extract was then incubated with antiserum. As described for the previous experiments, the anti-PLC-α antisera immunoprecipitated specifically bound $^{125}$I-AngII, whereas preimmune serum precipitated substantially less binding activity (Table 2). These data suggest

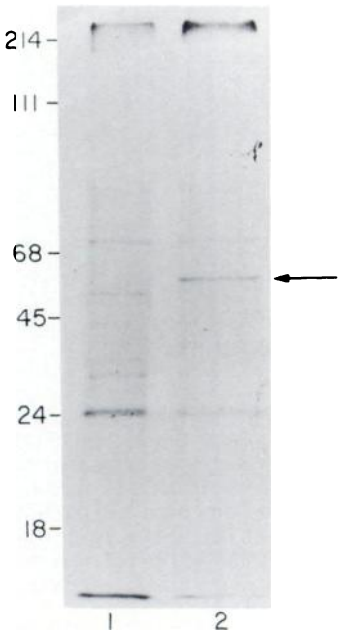


**Fig. 4.** Immunoprecipitation of a $^{35}$S-Trans S-labeled 60-kDa protein by anti-PLC-α antisera. Differentiated N1E-115 cells were metabolically labeled with 50 μCi/ml $^{35}$S-Trans S label for 20 hr. Cells were then washed, harvested, and homogenized, and a membrane pellet was obtained by centrifugation at 48,000 × *g* for 30 min. Membrane proteins were solubilized in 1% CHAPS under nondenaturing conditions, as described in Experimental Procedures. Thirty micrograms of the extracted proteins were then incubated with 50 μg of either preimmune serum (*lane 1*) or anti-PLC-α antiserum (*lane 2*). Immunoprecipitated proteins were collected after incubation with 20% Protein A-Sepharose and were separated by electrophoresis on an 11% SDS-polyacrylamide gel. Autoradiographs of the dried gels were developed after 3 days of exposure at −70°. The arrow indicates the immune-specific 60-kDa protein.

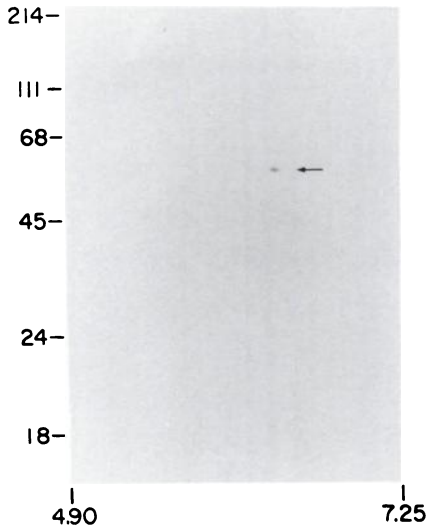


**Fig. 5.** Specific interaction of anti-PLC-α antisera with 1% CHAPS-extracted PLC-α. Two hundred-fifty micrograms of 1% CHAPS-extracted N1E-115 cell membranes were separated by two-dimensional gel electrophoresis. Proteins were transferred to nitrocellulose and probed with a 1/500 dilution of anti-PLC-α antiserum. Immunoreactive products were detected with alkaline phosphatase-conjugated goat anti-rabbit IgG, as described in Experimental Procedures, and are indicated by an *arrow*. A 1/500 dilution of preimmune serum failed to identify an immunoreactive product (data not shown).

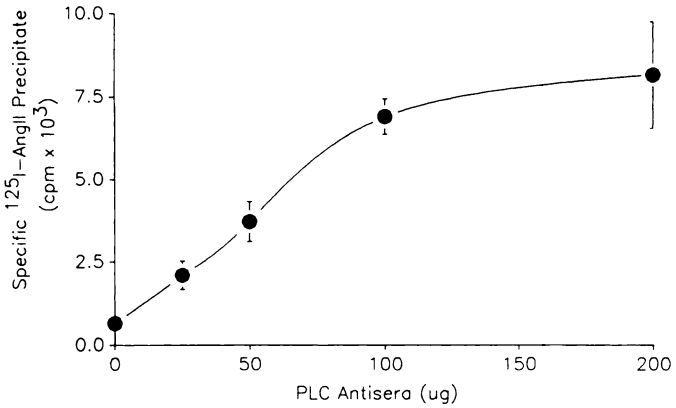


**Fig. 6.** Immunoprecipitation of $^{125}$I-AngII binding by anti-PLC-$\alpha$ antisera. The 1% CHAPS extract of differentiated N1E-115 cells was labeled with $^{125}$I-AngII, precleared with nonimmune rabbit immunoglobulin, and incubated with increasing amounts of anti-PLC-$\alpha$ antiserum. Immunoreactive products were collected by incubation with Protein A-Sepharose, followed by centrifugation at 12,000 × *g* for 3 min. The immunoprecipitate was washed three times, and $^{125}$I-AngII bound in the pellet was quantitated by $\gamma$ counting. The data represent the means and standard errors from five separate experiments.

that the association of the AngII-R and PLC-$\alpha$ detected by anti-PLC-$\alpha$ antisera occurs in intact N1E-115 cell membranes and is not merely an artifact of solubilization.

The coupling of cell surface receptors to membrane-associated PLC-$\alpha$ is presumed to be mediated by agonist-promoted interactions with G proteins. In order to determine whether agonist occupancy was similarly required for detection of an AngII-R-PLC-$\alpha$ complex, solubilized membranes were radiolabeled with 0.25 nM $^{125}$I-AngII or $^{125}$I-SARILE and then incubated with anti-PLC-$\alpha$ antiserum. In confirmation of our earlier findings, when solubilized N1E-115 membranes were labeled with an agonist, AngII-Rs were immunoprecipitated by anti-PLC-$\alpha$ antiserum (Fig. 8, *left*). However, when the same membranes were exposed to the antagonist $^{125}$I-SARILE, no specific binding was precipitated by the antiserum (Fig. 8, *right*). Moreover, immunoprecipitation of an AngII-R-PLC-$\alpha$ complex by the anti-PLC-$\alpha$ antiserum was disrupted by GTP$\gamma$S (Fig. 9). In contrast, GDP$\beta$S did not significantly affect the coupling of AngII-Rs with PLC-$\alpha$. Collectively, these results suggest that AngII-Rs may be coupled to PLC-$\alpha$ through a G protein and that agonist occupancy of AngII-Rs may be required for stabilization of this complex in differentiated N1E-115 cells.

## Discussion

The presence of AngII-Rs in the brain is now well established (31–34). However, detailed biochemical analysis of these proteins has proceeded slowly, because of their restricted anatomical localization. For this reason, we have recently characterized the properties of membranous AngII-Rs expressed on murine neuroblastoma N1E-115 cells as a model for the actions of AngII in the brain (6, 20–22). Cultured N1E-115 cells possess AngII-Rs that are pharmacologically indistinguishable from those in the brain (20), and their density is substantially increased when the neuronal phenotype of these cells is fully expressed during *in vitro* differentiation (21). Moreover, the binding of AngII-related agonists, but not antagonists, in these cells was partially regulated by guanine nucleotides, suggesting that some subpopulation of AngII-Rs were coupled to G proteins (20, 21). In this regard, we demonstrated that AngII stimulated the formation of inositol phosphates and the attendant mobilization of intracellular $Ca^{2+}$ through a pertussis toxin-insensitive pathway in N1E-115 cells (6). Because the ability of agonists such as AngII to stimulate $PIP_2$ hydrolysis is mediated by one or more of the PLCs that have been described (13–15), we have investigated the possible interaction of AngII-Rs with two prominent PLC isozymes present in solubilized membranes of differentiated N1E-115 cells, PLC-$\alpha$ and PLC-$\gamma$ (23).

In order for the analysis of AngII-R and PLC interactions to proceed, it was first necessary to solubilize these membrane proteins. Previously, we demonstrated that the zwitterionic detergent CHAPS solubilizes AngII-Rs from N1E-115 cells (22), and we have confirmed these earlier results in the present

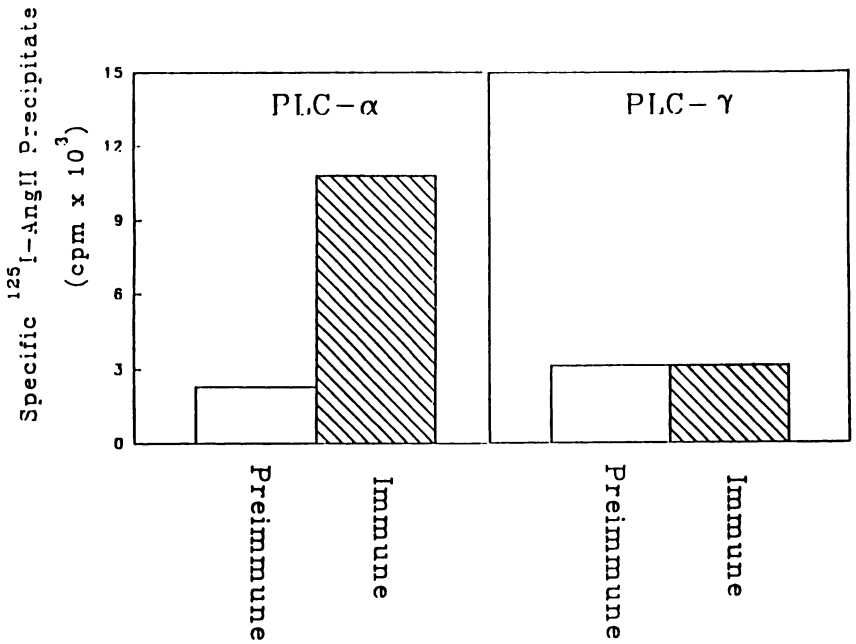


**Fig. 7.** Failure of anti-PLC-$\gamma$ antisera to immunoprecipitate AngII-Rs. Membranes from differentiated N1E-115 cells were labeled with $^{125}$I-AngII before solubilization in 1% CHAPS, precleared with nonimmune rabbit IgG (*left*) or nonimmune mouse IgG (*right*), and then incubated overnight with either anti-PLC-$\alpha$ antiserum (100 $\mu$g) (*left*), anti-PLC-$\gamma$ antiserum (20 $\mu$g/100 $\mu$l) (*right*), or the appropriate preimmune control. Immunoreactive products were collected by incubation with Protein A-Sepharose, followed by centrifugation at 12,000 × *g* for 3 min. The immunoprecipitates were washed three times, and the $^{125}$I-AngII bound in the pellet was quantified by $\gamma$ counting. Each *point* represents an average of four determinations, and the data displayed are representative of three separate experiments.

TABLE 2

**Immunoprecipitation of $^{125}$I-AngII binding by anti-PLC-α antisera**

Intact N1E-115 membranes were labeled with $^{125}$I-AngII and solubilized with 1% CHAPS. The extracted labeled proteins were precleared with nonimmune IgG and subsequently incubated with preimmune serum or immune anti-PLC-α antiserum. Immune complexes were collected by Protein A-Sepharose, and $^{125}$I-AngII binding was γ counted. Nonspecific binding was defined in the presence of 1 μM unlabeled AngII.

| | $^{125}$I-ANGII bound | |
|---|---|---|
| | Expt. 1 | Expt. 2 |
| | *cpm* | |
| Preimmune | | |
| Total | 787 ± 65 | 1434 ± 53 |
| Nonspecific | 561 ± 58 | 1372 ± 89 |
| Specific | 226 | 62 |
| Immune | | |
| Total | 2097 ± 54 | 2186 ± 100 |
| Nonspecific | 551 ± 36 | 926 ± 56 |
| Specific | 1546 | 1260 |

report. These solubilized receptors retain their expected specificity for AngII-related peptides, and the competition exhibited by AngII for $^{125}$I-SARILE-labeled sites demonstrated the presence of high and low affinity binding components in the solubilized extract. Moreover, gel filtration analysis of the solubilized membranes revealed that the majority of the AngII-binding activity eluted as a large complex of approximately 380 kDa on the Sephacryl S-300 column and that a small but significant portion (23%) of the activity remained responsive to guanine nucleotides. On the other hand, in our earlier studies affinity chromatography of solubilized membranes, followed by covalent cross-linking, suggested that purified AngII-Rs were 66 kDa (22). Collectively, these results suggest that AngII-Rs are likely to maintain some association with other proteins after solubilization in CHAPS.

Because AngII mobilizes intracellular $Ca^{2+}$ in N1E-115 cells (6), AngII-Rs must be functionally associated with the class of phosphoinositide-specific PLCs that mediate $PIP_2$ hydrolysis. In the present study, we demonstrated that polyclonal antisera raised against one particular form of PLC, PLC-α, identified an immunoreactive protein with an apparent molecular mass of 60 kDa, in cytosolic and membrane fractions of differentiated N1E-115 cells. Western blot analysis also indicated that 1% CHAPS effectively solubilized PLC-α as part of the 380-kDa AngII-binding complex present in N1E-115 membranes, and enzymatic analysis demonstrated the presence of PLC activity in the 1% CHAPS extract. More significantly, 40% of the cytosolic and 16% of the 1% CHAPS-extracted membrane PLC activity were removed by immunoprecipitation with the anti-PLC-α antisera. Furthermore, a 60-kDa protein was immunoprecipitated by the anti-PLC-α antisera from the 1% CHAPS extract of N1E-115 cell membranes metabolically labeled with $^{35}$S-Trans S label. Collectively, these data demonstrate that the anti-PLC-α antisera interact with a 60-kDa protein that retains PLC activity and that is located in the cytosol, membranes, and 1% CHAPS extract of differentiated N1E-115 cells.

In order to confirm further the specificity of the anti-PLC-α antisera in N1E-115 cells, CHAPS-solubilized membrane proteins were subjected to two-dimensional gel electrophoresis and transferred to nitrocellulose. Western blot analysis indicated that the anti-PLC- antisera detected a unique 60-kDa protein with an apparent pI of 6.6. These results are in contrast to other studies in which similar PLC-α antisera were used in both neuronal and non-neuronal tissues. For instance, in rat liver the 60-kDa protein detected by the PLC-α antisera is apparently present in two forms, with different isoelectric points.[2] Moreover, Mobbs *et al.* (35, 36) have reported that PLC-α antisera immunoreact with an estrogen-induced 70-kDa protein (HIP-70), in rat hypothalamus, that has the same amino-terminal sequence as PLC-α purified from guinea pig uterus (18). This protein also exists as two species, with different isoelectric points on two-dimensional gels. On the other hand, the guinea pig PLC-α antisera detect only a single species in both WB[2] and RBL-1 cells (18). At present, it seems likely that the multiple forms of PLC-α result from cell-specific post-translational modifications, such as phosphorylation, of a common precursor, although the functional significance of these

[2] P. He, S. K. Joseph, S. M. Fisher, R. A. Johanson, and J. R. Williamson. Rat liver phospholipase C-α: localization to the lumen of the endoplasmic reticulum and secretion in response to $Ca^{2+}$ mobilization. Submitted for publication.

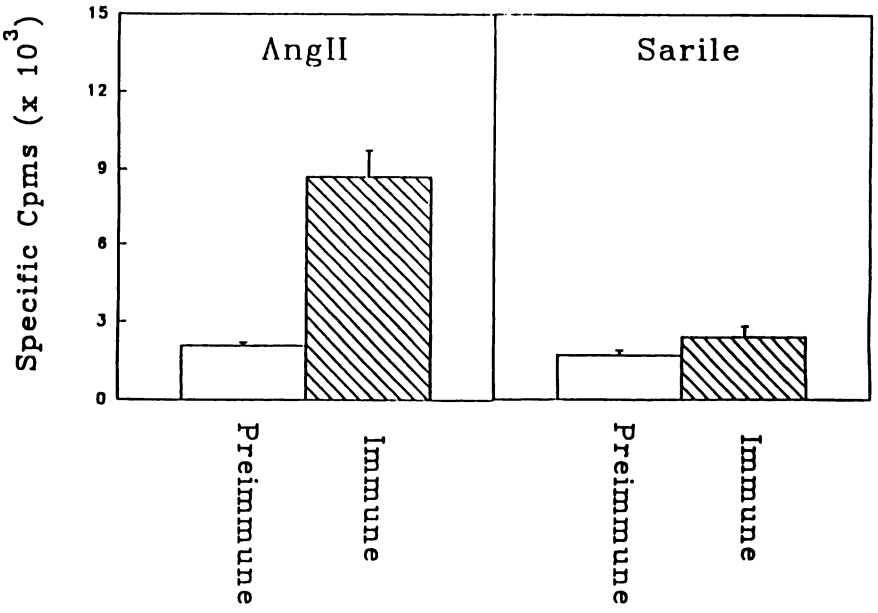


**Fig. 8.** Failure of anti-PLC-α antisera to immunoprecipate AngII-Rs labeled with $^{125}$I-SARILE. The 1% CHAPS extract from differentiated N1E-115 membranes was labeled with either $^{125}$I-AngII (*left*) or the antagonist $^{125}$I-SARILE (*right*) and was precleared with nonimmune IgG. The extract was then incubated with 100 μg of preimmune or immune anti-PLC-α antiserum. Immunoreactive products were collected by incubation with Protein A-Sepharose, followed by centrifugation at 12,000 × *g* for 3 min. The immunoprecipitates were washed three times, and the $^{125}$I-AngII bound in the pellet was quantified by γ counting. The data represent the means and standard errors from three separate experiments.

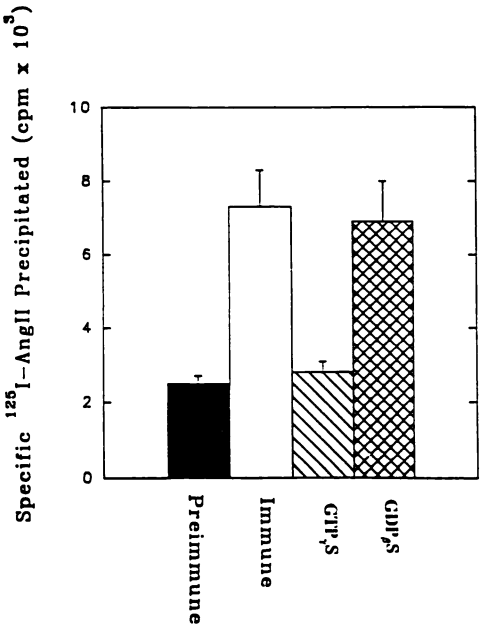


**Fig. 9.** Effect of guanine nucleotides on the immunoprecipitation of AngII-Rs by anti-PLC-$\alpha$ antisera. The 1% CHAPS extract of differentiated N1E-115 cells was labeled with $^{125}$I-AngII. Extracts were treated with 100 $\mu$M GTP$\gamma$S or GDP$\beta$S, as indicated. Samples were then precleared with nonimmune rabbit IgG and then incubated with preimmune serum or immune anti-PLC-$\alpha$ antiserum (100 $\mu$g). Immunoreactive products were collected by incubation with Protein A-Sepharose, followed by centrifugation at 12,000 × *g* for 3 min. The immunoprecipitates were washed three times, and the $^{125}$I-AngII bound in the pellet was quantified by $\gamma$ counting. The data represent the means and standard errors from three separate experiments.

forms remains unclear. Nonetheless, the ability of the antisera to immunoreact with a single protein, combined with their ability to immunoprecipitate PLC activity, demonstrates that these antisera specifically interact with the protein that is designated PLC-$\alpha$, in solubilized membranes from N1E-115 cells.

The mechanism by which receptor occupancy stimulates phosphoinositide-specific PLC activity is unclear. However, an apparent association of vasopressin $V_1$ receptors and PLC-$\alpha$ has recently been described in rat liver membranes (19). These studies demonstrated that high affinity $V_1$ receptors could be solubilized with the detergent lysophosphatidylcholine without prior exposure to an agonist. Immunoblotting of the solubilized fractions with anti-PLC-$\alpha$ antisera revealed that this protein had also been solubilized by the detergent. Moreover, specific vasopressin-binding activity and PLC-$\alpha$ immunoreactivity coeluted with the binding of $^{35}$S-GTP on a DEAE-Sepharose column and, most significantly, the anti-PLC-$\alpha$ antiserum immunoprecipitated $V_1$ receptors solubilized from rat liver, but not $V_2$ receptors from hog kidney. Collectively, these results suggest that there is a physical association between $V_1$ receptors, G proteins, and PLC-$\alpha$ in solubilized rat liver membranes and that prior agonist occupancy of the receptor is not required for its formation.

In the present report, we have demonstrated what appears to be a similar association between AngII-Rs and PLC-$\alpha$ in neuroblastoma N1E-115 cells, insofar as $^{125}$I-AngII-prelabeled receptors were immunoprecipitated by anti-PLC-$\alpha$ antisera in solubilized N1E-115 membranes. Approximately 20% of the total solubilized AngII-Rs were specifically immunoprecipitated by the antisera. Concomitantly, 16% of the total 1% CHAPS extract PLC activity was precipitated. These results indicate that the anti-PLC-$\alpha$ antisera simultaneously immunoprecipitate functional PLC-$\alpha$ and AngII-Rs, both of which appear to be part of a large 380-kDa protein complex. Moreover, successful immunoprecipitation of AngII-Rs by PLC-$\alpha$ antisera apparently required that the receptor be labeled with an agonist, because solubilized receptors labeled with the antagonist $^{125}$I-3 SARILE did not form an immunoreactive complex with PLC-$\alpha$. Because the gel filtration analysis of the solubilized membranes revealed that the majority of AngII-binding activity eluted as a large protein complex, even without prior receptor occupancy, these results suggest that an agonist may be required to stabilize an already existing physical association between AngII-Rs and PLC-$\alpha$ in solubilized N1E-115 membranes. Finally, the apparent association of AngII-Rs with PLC-$\alpha$ could also be demonstrated when intact N1E-115 cell membranes were labeled with $^{125}$I-AngII and subsequently solubilized with CHAPS. These data demonstrate that the AngII-R forms a specific complex with PLC-$\alpha$, which is not the result of nonspecific protein-protein interactions resulting from detergent exposure and micelle formation.

In addition to evidence implicating PLC-$\alpha$ in the activation of membrane-associated receptors for peptides such as vasopressin and angiotensin, it has recently been suggested that another PLC isozyme, PLC-$\gamma$, may also associate with other types of cell surface receptors. For instance, Margolis *et al.* (16) demonstrated that antisera directed against the PLC isoform PLC-$\gamma$ coimmunoprecipitated PLC-$\gamma$ and the epidermal growth factor receptor in A431 cells. It now appears that PLC-$\gamma$ is importantly involved in the action of several growth factors that possess intrinsic tyrosine kinase activity (16, 37). Immunoblotting techniques revealed that membranous PLC-$\gamma$ is present in differentiated N1E-115 cells (23); however, monoclonal antibodies raised against PLC-$\gamma$ did not precipitate AngII-Rs in solubilized fractions. This apparent lack of any association between AngII-Rs and PLC-$\gamma$ is interesting, because AngII is known to be mitogenic in vascular smooth muscle cells (38), although it is not known whether this is true in neuroblastoma cells. Nonetheless, it would appear that AngII-Rs are not associated with all of the PLC isozymes present in N1E-115 cells.

The involvement of G proteins in agonist stimulation of phosphoinositide hydrolysis is now well established (39, 40). Moreover, in solubilized hepatic membranes it has been demonstrated that vasopressin binding to $V_1$ receptors, GTP$\gamma$S binding, and PLC activity comigrated with PLC-$\alpha$ immunoreactivity after ion exchange chromatography and gel filtration analysis (19). In the present report, several observations support, but do not prove, the suggestion that the apparent association of AngII-Rs with PLC-$\alpha$ involves a heterotrimeric G protein. For instance, AngII agonist binding in solubilized membranes consisted of both high and low affinity components, and this biphasic binding is known to result from G protein interactions within intact N1E-115 cells (20). In addition, the majority of AngII-binding activity was present as a large molecular mass complex (380 kDa) in solubilized N1E-115 membranes, and a portion of this binding was responsive to guanine nucleotides. Interestingly, immunoreactive PLC-$\alpha$ was also present in this complex, although no reliable effect of GTP$\gamma$S on its chromatographic profile was observed. Most significantly, the formation of an immunoprecipitatable AngII-R-

PLC-α complex was prevented by the nonhydrolyzable GTP analog GTPγS, but not by GDPβS. However, it remains to be demonstrated that those AngII-Rs associated with PLC-α are the same population of AngII-Rs that remain responsive to GTPγS in the larger protein complex. Nonetheless, these results, collectively, suggest that stable coupling of a subpopulation of AngII-Rs with PLC-α may require the interposition of a G protein in solubilized N1E-115 membranes. Although the identity of this putative G protein is not known, in preliminary studies we have used antisera recognizing the α subunits of various G proteins to demonstrate the presence of $G_s$, $G_{i\alpha 1\text{-}3}$, $G_o$, and $G_Q$ in the solubilized N1E-115 extract. The presence of $G_Q$ is particularly noteworthy, because other investigators have suggested that its α subunit regulates the activity of one PLC, PLC-$\beta_1$ (41, 42). Thus, the potential role of $G_Q$ in the coupling of AngII-Rs to phosphoinositide-specific PLC in N1E-115 cells is worthy of study, although it is important to emphasize that these neuroblastoma cells apparently lack PLC-$\beta_1$ (23).

Despite the apparent physical association of AngII-Rs, G proteins, and PLC-α, AngII was incapable of stimulating PLC activity in N1E-115 membranes solubilized with 1% CHAPS. The lack of a demonstrable functional AngII-R-PLC interaction may be attributed to a number of factors. For instance, several studies have demonstrated that the activity of PLC isozymes is dependent on the phospholipid composition of cellular fractions (15). It seems likely, then, that a functional AngII-R-PLC complex may be dependent on a specific phospholipid environment more precisely duplicating the plasma membrane. Additionally, alteration of the membranous environment in which the interaction between AngII-Rs and PLC-α normally occurs may also impair regulatory events necessary for the expression of agonist-mediated PLC activity. In this regard, it has been demonstrated that PLC isozymes, including PLC-α, are phosphorylated by the phorbol ester phorbol 12-myristate 13-acetate *in vitro* (18) but that enzyme activity is not altered by such phosphorylation. In contrast, these same treatments are well known to modify agonist and guanine nucleotide stimulation of phosphoinositide hydrolysis in cultured cells (29, 43). These results suggest that the phosphorylation of PLC may alter the interaction of PLC with other modulatory proteins. To explore this possibility further, studies are currently being pursued to examine the effects of phosphorylation upon the formation and functionality of the AngII-R-PLC-α complex in solubilized N1E-115 membranes.

In summary, membrane-associated AngII-Rs and PLC-α were effectively solubilized by the synthetic detergent CHAPS in murine neuroblastoma N1E-115 cells. Antisera raised against PLC-α immunoprecipitated a complex of solubilized AngII-Rs and PLC-α from these membrane fractions. Moreover, the stability of this complex appeared dependent on agonist occupancy of the receptors, and the complex was likely to include a heterotrimeric G protein. Although these results emphasize a possible role for PLC-α in the membrane transduction process for some population of AngII-Rs that are coupled to $PIP_2$ hydrolysis, we cannot exclude the possibility that other interactions between these proteins may also occur. In this regard, the subcellular distribution of both AngII-binding proteins (24) and PLC-α (18)[2] is not confined to the plasma membrane, and it has been suggested that both AngII-Rs (44) and PLC-α[2] may undergo cellular translocation from multiple sites, including membranes, cytosol, nucleus, and other intracellular organelles. Thus, it is possible that actions of AngII other than those occurring at the cell surface, such as autocrine or nuclear effects, may involve an interaction between a subpopulation of AngII-Rs and PLC-α that need not reside exclusively in the plasma membrane. Regardless of the cellular distribution and molecular nature of their interaction, our results strongly suggest that PLC-α may mediate some of the actions of AngII within the nervous system.

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