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G_q and G_{11} are concurrently activated by bombesin and vasopressin in Swiss 3T3 cells

Stefan Offermanns*, Eva Heiler, Karsten Spicher, Günter Schultz

Institut für Pharmakologie, Freie Universität Berlin, Thielallee 69/73, 14195 Berlin, Germany

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Abstract

The α-subunits of the widely expressed G-proteins G_q and G_{11} indistinguishably activate β-isoforms of phospholipase C. In this report we have tested whether differences exist in the activation of both G-proteins via phospholipase C-linked receptors. We found that bombesin and vasopressin, with very similar potencies and time dependencies, induce the activation of both G_q and G_{11} in Swiss 3T3 cells, suggesting that these G-proteins, at least in part, serve interchangable functions.

Key words: G-protein; Phospholipase C; Bombesin; Vasopressin

1. Introduction

A wide variety of hormones, neurotransmitters and growth factors acting on G-protein coupled receptors lead to the activation of phospholipase C (PLC), resulting in the generation of inositol 1,4,5 trisphosphate and diacylglycerol [1,2]. In the majority of cells, stimulation of PLC via these receptors involves G-proteins of the G_q family, the α-subunits of which, upon receptor activation, regulate the activity of β-isoforms of PLC [3]. Among the G-proteins of the G_q family, G_q and G_{11} are nearly ubiquitously expressed, and their α-subunits exhibit 88% sequence identity [4–6]. Both α_q and α_{11} share indistinguishable properties with regard to stimulation of various PLC-β isoforms [7–9]. In the present study we tested whether differences exist between G_q and G_{11} with regard to their activation by different receptors. As a model system, we chose Swiss 3T3 cells which express bombesin and vasopressin receptors linked to the activation of PLC [10–12].

2. Materials and methods

2.1. Materials

Bombesin and vasopressin were from Sigma (Deisenhofen, Germany); sources of other substances have been described [13,14].

2.2. Cell culture and membrane preparation

Swiss 3T3 cells were grown in Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum, and membranes were prepared by nitrogen cavitation.

2.3. Photolabeling of membrane proteins

[α-^{32}P]GTP azidoanilide was synthesized and purified as described [13]. Cell membranes (100–200 μg of protein per assay tube) were incubated at 30°C in a buffer containing 0.1 mM EDTA, 5 mM $MgCl_2$, 30 mM NaCl, 1 mM benzamidine and 50 mM HEPES-NaOH (pH 7.4). After 3 min of preincubation in the absence and presence of receptor agonist, samples were incubated for another 10 min with 10–20 nM [α-^{32}P]GTP azidoanilide (130 kBq per tube). The final assay volume was 60 μl. After stopping the reaction by cooling the samples on ice, samples were centrifuged at 4°C for 5 min at 12,000 × *g*, and pellets were resuspended in 60 μl of the above buffer supplemented with 2 mM glutathione. Suspended membranes were then irradiated for 10 s at 4°C with a 254 nm UV lamp (Vilber Lourmat, Torcy, France).

2.4. Immunoprecipitation

For immunoprecipitation, photolabeled membranes were pelleted and solubilized in 40 μl of 2% (w/v) sodium dodecyl sulfate (SDS) at room temperature. Thereafter, 120 μl of precipitation buffer (1% (w/v) Nonidet P-40, 1% (w/v) desoxycholate, 0.5% (w/v) SDS, 150 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 10 mg/ml aprotinin and 10 mM Tris-HCl, pH 7.4) was added. Solubilized membranes were centrifuged at 4°C for 10 min at 12,000 × *g* to remove insoluble material, and 10 ml of antisera were added to the supernatants. Following incubation of samples at 4°C for 2 h at constant rotation, 60 μl of 10% (w/v) Protein A–Sepharose beads were added, and samples were incubated overnight. Sepharose beads were pelleted (12,000 × *g*, 3 min) and washed twice with 1 ml of washing buffer A (600 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.5% (w/v) SDS, 1% (w/v) Nonidet P-40) and twice with washing buffer B (300 mM NaCl, 10 mM EDTA, 100 mM Tris-HCl, pH 7.4). Preparation of samples for SDS-PAGE was performed as described [14].

2.5. SDS-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting and antisera

SDS-PAGE of photolabeled proteins was performed on 13% (w/v) acrylamide gels as described by Blank et al. [15]. Photolabeled membrane proteins were visualized by autoradiography of the dried gels with Kodak X-OMAT AR-5 films. Blotting of membrane proteins separated by SDS-PAGE, processing of immunoblots and detection of immunoreactive proteins by a chemiluminescence procedure (Amersham, Braunschweig, Germany) have been described [16]. Peptide antisera against G-protein α-subunits were obtained after injection of synthetic peptides corresponding to specific regions of α-subunits coupled to keyhole limpet hemocyanine into rabbits. Table 1 shows the sequences of peptides against which the antisera specific for α_s (AS 348), $\alpha_{q/11}$ (AS 368), α_{11} (AS 255) and the $\alpha_{i\ common}$ antiserum (AS 266) were

*Corresponding author. *Present address:* Division of Biology 147-75, California Institute of Technology, Pasadena, CA 91125, USA. Fax: (1) (818) 796-7066.

Abbreviations: G-protein, regulatory heterotrimeric guanine nucleotide-binding protein; PLC, phospholipase C; G_x and α_x, nomenclature to describe a particular G-protein and its α-subunit.

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raised. Specificity of antisera was controlled by testing their selectivity for α-subunits expressed in *E. coli* (K. Spicher, unpublished data).

2.6. Miscellaneous and reproducibility

Protein was determined using the BCA protein assay system (Pierce, Rockford, IL, USA). The experiments shown are representative for at least two independently performed experiments using different membrane preparations.

3. Results and discussion

We first identified the G-protein α-subunits expressed in Swiss 3T3 cells (Fig. 1). Immunoblotting of Swiss 3T3 cell membranes with an antiserum raised against the C-terminus of α_q and α_{11} (Table 1) showed the expression of two proteins of 43 and 41.5 kDa. The 43 kDa protein was also recognized by an antiserum directed against α_{11}. Thus, the 41.5 kDa protein represents the G_q α-subunit whereas the 43 kDa protein corresponds to the G_{11} α-subunit. Using the same SDS-gel system, Berstein et al. [17] found identical migration properties for α_i and α_{11}. In addition, an antibody specific for the α-subunits of the G_i-type G-proteins, the $\alpha_{i\ common}$ antiserum, detected a protein of about 40 kDa, and an antiserum raised against a specific sequence common to all α_s subtypes recognized a protein of about 45 kDa.

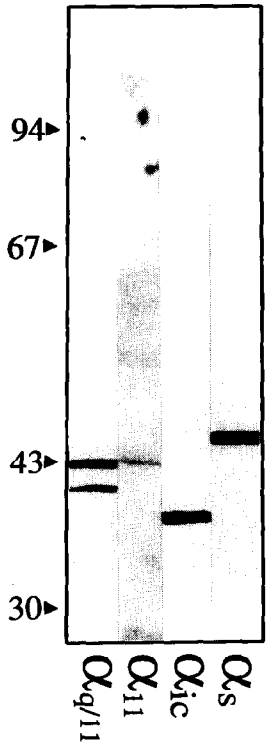

Fig. 1. G-protein α-subunits in membranes of Swiss 3T3 cells. Membrane proteins were separated by SDS-PAGE and blotted onto nitrocellulose filters. The filters were cut into strips which were incubated with different antibodies. α_q/11, $\alpha_{q/11}$ antiserum (AS 368); α_{11}, α_{11} antiserum (AS 255); α_{ic}, $\alpha_{i\ common}$ antiserum (AS 266); α_s, α_s antiserum (AS 348). Bound antibodies were visualized by a chemiluminescence procedure as described. Shown are autoluminograms of nitrocellulose strips. Numbers on the left indicate molecular masses of marker proteins.

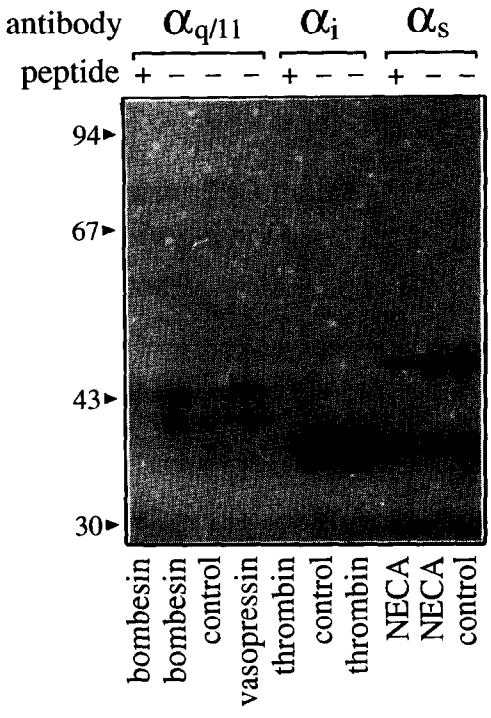

Fig. 2. Stimulation of photolabeling of G-protein α-subunits in membranes from Swiss 3T3 cells. Membranes (200 μg of protein per tube) were incubated in the absence (control) or presence of the indicated receptor agonists for 10 min and were photolabeled with [α-^{32}P]GTP azidoanilide. Membranes were solubilized and incubated with the $\alpha_{q/11}$, α_i or α_s antisera. Immunoprecipitation was performed as described. Precipitated proteins were subjected to SDS-PAGE. Shown is an autoradiogram of an SDS gel with the molecular masses of marker proteins on the left. To verify the specificity of the immunoprecipitating antisera, antisera were in some cases (peptide +) preincubated with the peptide (5 μg/ml) against which they were raised. $\alpha_{q/11}$, $\alpha_{q/11}$ antiserum (AS 368); α_i, α_i common antiserum (AS 266); α_s, α_s antiserum (AS 348). Receptor agonists were applied in the following concentrations: bombesin, 1 μM; vasopressin, 1 μM; thrombin, 1 U/ml; NECA (5′-*N*-ethylcarboxamidoadenosine), 1 μM.

To test the interaction of known PLC-linked receptors with G_q and G_{11} in Swiss 3T3 cells, membranes were photolabeled with [α-^{32}P]GTP azidoanilide in the absence and presence of bombesin or vasopressin. Thereafter, membranes were solubilized, α_q and α_{11} were immunoprecipitated with the $\alpha_{q/11}$ antiserum, and the immunoprecipitates were subjected to SDS-PAGE (Fig. 2). Both agonists led to the incorporation of [α-^{32}P]GTP azidoanilide into both the 41.5 kDa G_q and the 43 kDa G_{11} α-subunits. Immunoprecipitation could be blocked by preincubation of the anti-peptide antiserum with the corresponding peptide. The $\alpha_{i\ common}$ and the α_s antisera precipitated photolabeled 40 and 45 kDa proteins, respectively. These 40 kDa G_i and 45 kDa G_s α-subunits showed a slightly increased incorporation of [α-^{32}P]GTP azidoanilide in response to thrombin and the adenosine A_2 receptor agonist 5′-*N*-ethylcarboxamidoadenosine, respectively.

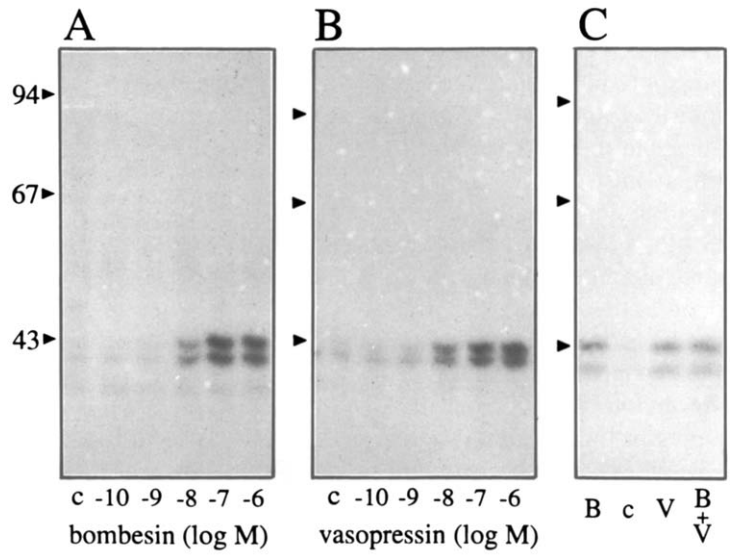

Fig. 3. Concentration dependence of bombesin- and vasopressin-induced photolabeling of G_q and G_{11} α-subunits in Swiss 3T3 cells. Membranes (150 μg/tube) were incubated for 10 min with bombesin (panel A) or vasopressin (panel B) at increasing concentrations as indicated and photolabeled with [α-^{32}P]GTP azidoanilide. Solubilized membranes were immunoprecipitated with the $\alpha_{q/11}$ antiserum (AS 368) as described, and precipitated proteins were subjected to SDS-PAGE. (Panel C) Membranes were incubated with 1 μM of bombesin (B), 1 μM of vasopressin (V) or both agonists at 1 μM together (B + V), and photolabeling was performed as in Panel A/B. c, control (without agonists). Shown are autoradiograms of SDS gels. Arrows on the left indicate the position of marker proteins.

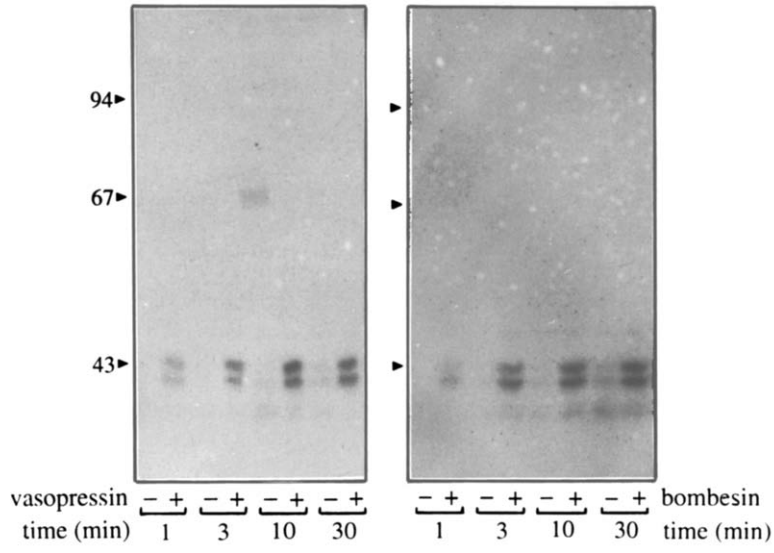

Fig. 4. Time-course of vasopressin and bombesin-induced photolabeling of G_q and G_{11} α subunits in Swiss 3T3 cells. Membranes (150 μg/tube) in the absence (−) or presence of vasopressin (1 μM) and bombesin (1 μM) for the indicated time intervals with [α-^{32}P]GTP azidoanilide. Photolabeled membranes were solubilized, and membrane proteins were precipitated with the $\alpha_{q/11}$ antiserum. Precipitated proteins were subjected to SDS-PAGE. Shown are autoradiograms of SDS gels with the molecular masses of marker proteins on the left.

Table 1
Peptide antisera used for immunoprecipitation of G-protein α-subunits

Antiserum	Peptide sequence	G-protein α-subunit	
		Recognized	Amino acid
$\alpha_{q/11}$ (AS 368)	(C) L Q L N L K E Y N L V	$\alpha_{q/11}$	349–359[a]
α_{11} (AS 255)	(C) V T T F E H Q Y V N A I K	α_{11}	121–133[a]
α_{ic} (AS 266)	(C) N L R E D G E K A A R E V	$\alpha_{i1/2/3}$	22–34[b]
α_s (AS 348)	(C) R M H L R Q Y E L L	α_s	385–394[b]

(C) indicates that an N-terminal cysteine was added to the original peptide sequence in order to facilitate coupling to keyhole limpet hemocyanine. [a][4]. [b][18].

To confirm the activation of G_q and G_{11} via the bombesin and vasopressin receptors and to compare the potency of bombesin and vasopressin to induce interaction of their respective receptors with G_q and G_{11}, we performed photolabeling experiments in the presence of increasing concentrations of both agonists (Fig. 3). Bombesin and vasopressin concentration-dependently stimulated photolabeling of the 43 kDa G_{11} and of the 41.5 kDa G_q α-subunits immunoprecipitated by the $\alpha_{q/11}$ antiserum (Fig. 3). Activation of both G_q and G_{11} via bombesin and vasopressin receptors could be observed at an agonist concentration of 10 nM and reached a maximum at about 1 μM. No obvious difference in the potency of bombesin or vasopressin to activate G_q and G_{11} could be observed. Fig. 3C shows that the amount of radioactivity incorporated into precipitated α_q and α_{11} was very similar regardless of whether maximally effective concentrations of bombesin and vasopressin alone and in combination were applied, suggesting that both activated receptors 'talked' to the same pool of G_q and G_{11}.

In a further series of experiments, we tested whether differences exist with regard to the time–course of bombesin and vasopressin induced activation of G_q and G_{11}. Therefore, membranes were incubated for increasing time periods with [α-^{32}P]GTP azidoanilide in the absence or presence of agonists (Fig. 4). Bombesin and vasopressin induced photolabeling of α_q and α_{11} could be observed after 1 min and reached a maximum after about 10 min. Basal photolabeling remained low during the whole time–course. No difference between the time–course of G_q and G_{11} activation by each of the studied receptor agonists could be observed.

These data demonstrate that neither G_q nor G_{11} is preferentially activated via bombesin or vasopressin receptors in Swiss 3T3 cells. Activation of G_q and G_{11} through each receptor, on the contrary, exhibited a remarkable parallelism with regard to the agonist concentration dependence and the time dependence. Earlier studies have shown that activation of PLC by bombesin and vasopressin in intact Swiss 3T3 cells is similarly superimposable in terms of agonist concentration–response behaviour and time–course. In view of the indistinguishable properties of G_q and G_{11} regarding the activation of β-isoforms of PLC [7–9], both G-proteins may interchangably couple receptors to PLC. Thus, G_q and G_{11} obviously represent an example of at least partial functional redundancy among some G-protein subtypes.

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References

[1] Meldrum, E., Parker, P.J. and Carozzi, A. (1991) Biochim. Biophys. Acta 1092, 49–71.
[2] Rhee, S.G. and Choi, K.D. (1992) J. Biol. Chem. 267, 12393–12396.
[3] Sternweis, P.C. and Smrcka, A.V. (1992) Trends Biochem. Sci. 17, 502–506.
[4] Strathmann, M. and Simon, M.I. (1990) Proc. Natl. Acad. Sci. USA 87, 9113–9117.
[5] Gutowski, S., Smrcka, A.V., Nowak, L., Wu, D., Simon, M. and Sternweis, P.C. (1991) J. Biol. Chem. 266, 20519–20524.
[6] Mitchell, F.M., Mullaney, I., Godfrey, P.P., Arkinstall, S.J., Wakelam, M.J.O. and Milligan, G. (1991) FEBS Lett. 287, 171–174.
[7] Taylor, S.J. and Exton, J.H. (1991) FEBS Lett. 286, 214–216.
[8] Wu D., Lee, C.H., Rhee, S.G. and Simon, M.I. (1992) J. Biol. Chem. 267, 1811–1817.
[9] Hepler, J.R., Kozasa, T., Smrcka, A.V., Simon, M.I., Rhee, S.G., Sternweis, P.C. and Gilman, A.G. (1993) J. Biol. Chem. 268, 14367–14375.
[10] Rozengurt, E. (1986) Science 234, 161–166.
[11] Battey, J.F., Way, J.M., Corjay, M.H., Shapira, H., Kusano, K., Harkins, R., Wu, J.M., Slattery, T., Mann, E. and Feldman, R.I. (1991) Proc. Natl. Acad. Sci. USA 88, 395–399.
[12] Lopez-Rivas, A., Mendoza, S.A., Nonberg, E., Sinnett-Smith, J. and Rozengurt, E. (1987) Proc. Natl. Acad. Sci. USA 84, 5768–5772.
[13] Offermanns, S., Schultz, G. and Rosenthal, W. (1991) Methods Enzymol. 195, 286–301.
[14] Laugwitz, K.-L., Spicher, K., Schultz, G. and Offermanns, S. (1994) Methods Enzymol. 237, 283–294.
[15] Blank, J.L., Ross, A.H. and Exton, J.H. (1991) J. Biol. Chem. 266, 18206–18216.
[16] Offermanns, S., Seifert, R., Metzger, J., Jung, G., Lieberknecht, A., Schmidt, U. and Schultz, G. (1992) Biochem. J. 282, 551–557.
[17] Berstein, G., Blank, J.L., Smrcka, A.V., Higashijima, T., Sternweis, P.C., Exton, J.H. and Ross, E.M. (1992) J. Biol. Chem. 267, 8081–8088.
[18] Jones, D.T. and Reed, R.R. (1987) J. Biol. Chem. 262, 14241–14249.