Pertussis Toxin-insensitive Activation of the Heterotrimeric G-proteins G\textsubscript{i}/G\textsubscript{o} by the NG108-15 G-protein Activator*

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A ligand-independent activator of heterotrimeric brain G-protein was partially purified from detergent-solubilized extracts of the neuroblastoma-glioma cell hybrid NG108-15. The G-protein activator (NG108-15 G-protein activator (NG-GPA)) increased \([\text{[35S]}\text{GTP}\gamma\text{S}]\) binding to purified brain G-protein in a magnesium-dependent manner and promoted GDP dissociation from G\textsubscript{o},. The NG-GPA also increased GTP\gamma\text{S} binding to purified, recombinant G\textsubscript{i1}, G\textsubscript{o}, and G\textsubscript{ar}, but minimally altered nucleotide binding to purified transducin. The NG-GPA increased GTP\gamma\text{S} binding to membrane-bound G-proteins and inhibited basal, forskolin- and hormone-stimulated adenylyl cyclase activity in DDT\textsubscript{1}-MF-2 cell membranes. In contrast to G-protein-coupled receptor-mediated activation of heterotrimeric G-proteins in DDT\textsubscript{1}-MF-2 cell membrane preparations, the action of the NG-GPA was not altered by treatment of the cells with pertussis toxin. ADP-ribosylation of purified brain G-protein also failed to alter the increase in GTP\gamma\text{S} binding elicited by the NG-GPA. Thus, the NG-GPA acts in a manner distinct from that of a G-protein coupled receptor and other recently described receptor-independent activators of G-protein signaling. These data indicate the presence of unexpected regulatory domains on G\textsubscript{i}/G\textsubscript{o} proteins and suggest the existence of pertussis toxin-insensitive modes of signal input to G\textsubscript{i}/G\textsubscript{o} signaling systems.

The basal level of activated G-proteins, the magnitude of agonist-induced activation of G-protein, and the coupling specificity between G-protein coupled receptors (GPCR) and G-proteins varies with cell type. These cell-type specific differences in G-protein activation are often independent of the type and/or amount of G-protein expressed by the cell, indicating the existence of additional non-receptor proteins that regulate the activation state of heterotrimeric G-proteins by influencing nucleotide exchange, nucleotide hydrolysis, and/or subunit interactions. Such proteins include the NG108-15 G-protein activator (NG-GPA) (1), Activators of G-protein signaling 1–3 (2, 3), LGN (4, 5), calnuc (6, 7), the Drosophila AGS3/LGN ortholog PINS (8–10), phosphatidylethanolamine-binding protein (11), GAP-43 (12), RapL (13–15), polycystins (16, 17), phosducin or phosducin-like2 (18), G-26 (19, 20) and the family of proteins known as regulators of G-protein signaling. Most such regulatory proteins identified to date preferentially interact with the G-proteins G\textsubscript{i1}, G\textsubscript{o}, and G\textsubscript{ar} and exert varying effects on nucleotide exchange, nucleotide hydrolysis, and subunit interactions.

An expression cloning system developed to identify the NG-GPA led to the isolation of AGS1 to AGS3, which interact with different conformations or subunits of G-proteins and activate the signaling system by distinct mechanisms (2, 3). The NG-GPA, which increased GTP\gamma\text{S} binding to G\textsubscript{i2}, G\textsubscript{3}, and G\textsubscript{o,i}, is clearly distinct from AGS2 and AGS3. In contrast to the activation of G-proteins by a GPCR, AGS1, and other accessory proteins, the effect of the NG-GPA on GTP\gamma\text{S} binding to brain G-protein and membrane bound G-proteins was not altered by ADP-ribosylation of G-proteins with pertussis toxin. These data indicate that the NG-GPA likely interacts with unexpected regulatory domains on G\textsubscript{i} subunits and suggest the existence of pertussis toxin-insensitive modes of signal input to G\textsubscript{i}/G\textsubscript{o} signaling systems.

EXPERIMENTAL PROCEDURES

Materials—[\textsuperscript{32}P]NAD (30 Ci/mmole) and [\textsuperscript{32}P]GTP (800 Ci/mmole) were purchased from PerkinElmer Life Sciences. G-proteins were purified from bovine brain as described and consisted of ~63% G\textsubscript{ar}, 4% G\textsubscript{o}, 16% G\textsubscript{i1}, 16% G\textsubscript{i2}, 1%, G\textsubscript{o,1–3} were expressed in Sf9 cells and kindly provided by Dr. Stephen Graber. All other materials were obtained as described previously (1, 22). The partially purified preparations of the NG-GPA were generated essentially as described previously (1) following large scale tissue culture preparation at the Finnish Biotechnology Institute in Turku, Finland. Five independent preparations were generated from the one large scale culture of cells with some variability in the relative degree of enrichment. Harvested cells were lysed, and membranes were solubilized with 1% Triton X-100 (2:1) in 50 mM Tris-HCl, pH 7.4, 5 mM MgCl\textsubscript{2}, 0.6 mM EDTA. Detergent extracts were then fractionated by DEAE ion exchange, ultrafiltration, gel filtration, and lectin affinity chromatography (1).

Nucleotide Binding Assays—Guanine nucleotide binding to purified brain G-protein or cell membranes was determined essentially as described previously (1, 22) with the exception that the solution phase assays in the present study used 1.25 mM G-protein and assay volume was 50 μl. In each experiment, parallel samples containing NG-GPA without G-protein were processed to determine the background binding of \([\text{[32P]}\text{GTP}]/\text{S}\) to any guanine nucleotide-binding proteins co-fractionating with the NG-GPA. This level of background binding to NG-GPA...
RESULTS AND DISCUSSION

A large scale preparation of cells obtained from the Finnish Biotechnology Institute (yield ~826 mg of membrane protein) was solubilized and fractionated exactly as described previously (1) to generate partially purified NG-GPA. The NG-GPA increased nucleotide binding to G-protein over a time course of 150 min compared with the nucleotide binding obtained with G-protein alone (Fig. 1A). The action of the NG-GPA required the divalent cation magnesium (Fig. 1B) and was dependent upon the concentration of NG-GPA (Fig. 1C). Assays involved preincubation of the NG-GPA with G-protein for 1 h at 4 °C (1). The action of the NG-GPA did not require preincubation, although a lag phase in the augmented GTP- S binding was observed when all assay components were mixed at time 0 (Fig. 1D). Radiiodination of the peak activity fraction obtained from size exclusion chromatography revealed six major and six minor species following SDS-PAGE and autoradiography.  

In addition to activating bovine brain G-protein, the NG-GPA also increased GTP- S binding to recombinant Gαi2, and Gαi3 (Fig. 2A). The augmented GTP- S binding observed with the NG-GPA involved accelerated dissociation of GDP from Gαi subunit (Fig. 2B). The NG-GPA had little effect on nucleotide binding to purified transducin, Gβγ. The NG-GPA peak activity fractions under standard incubation conditions described above was approximately equal to that of [35S]GTP- S binding to brain G-protein alone.

**GDP Dissociation Assay—**Gαo (20 μM) was incubated in the presence or absence (vehicle) of the NG-GPA in buffer containing 10 μg/ml bovine serum albumin, 2.3 μl of [35S]GTP, 1 μM GTP, 50 mM Tris-HCl, pH 7.6, 5 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 10 mM MgCl2 in a total volume of 200 μl with 0.005% Thesit at 30 °C for 20 min. Samples were then cooled to 24 °C and dissociation initiated by addition of 1 mM GDP. 10-μl aliquots of the 200-μl samples were processed by vacuum filtration just prior to addition of GDP and at increasing times following the addition of GDP.

Miscellaneous Procedures—Brain G-protein was treated with pertussis toxin as previously described (23). The effectiveness of ADP-ribosylation of brain G, by pertussis toxin was determined by “back ADP-ribosylation” of an aliquot of the treated material using [32P]NAD.

Adenylyl cyclase activity assays were conducted in DDT1-MF2 cells stably transfected with the α2-AD arachidonic receptor (21).

**RESULTS AND DISCUSSION**

A large scale preparation of cells obtained from the Finnish Biotechnology Institute (yield ~826 mg of membrane protein) was solubilized and fractionated exactly as described previously (1) to generate partially purified NG-GPA. The NG-GPA increased nucleotide binding to G-protein over a time course of 150 min compared with the nucleotide binding obtained with G-protein alone (Fig. 1A). The action of the NG-GPA required the divalent cation magnesium (Fig. 1B) and was dependent upon the concentration of NG-GPA (Fig. 1C). Assays involved preincubation of the NG-GPA with G-protein for 1 h at 4 °C (1). The action of the NG-GPA did not require preincubation, although a lag phase in the augmented GTP- S binding was observed when all assay components were mixed at time 0
with a regulatory domain(s) on G_{i/G_o} proteins distinct from the regions that are key for G-protein regulation by a GPCR and other proteins that influence the activation state of G_{i/G_o} proteins. Of particular note is that these data indicate that modification of G_{i/G_o} by pertussis toxin may not prevent the activation of these G-proteins within the cell by non-receptor proteins.

The NG-GPA and related accessory proteins provide unexpected mechanisms for regulation of the G-protein activation cycle. In addition to the obvious interest related to GPCR signaling, such mechanisms may also provide alternative binding partners for G-protein subunits that allow G-proteins to serve functions within the cell unrelated to a GPCR. As such, these proteins and the concepts advanced with their discovery provide unexpected avenues for therapeutics and understanding disease mechanisms.

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REFERENCES
1. Sato, M., Ribas, C., Hildebrandt, J. D., and Lanier, S. M. (1996) J. Biol. Chem. 271, 30002–30006.
2. Cismowski, M., Takekawa, A., Ma, C., Lizzano, J. S., Xie, X., Faenkrantz, H., Lanier, S. M., and Duzic, E. (1996) Nat. Biotechnol. 17, 478–833.
3. Takekawa, A., Cismowski, M. J., Ribas, C., Chung, P., Hazard, S. J., Duzic, E., and Lanier, S. M. (1999) J. Biol. Chem. 274, 33202–33205.
4. Mochizuki, N., Cho, G., Wen, B., and Insel, P. A. (1996) Gene (Amst.) 181, 39–43.
5. Blumberg, J. B., Chandler, I. L., and Lanier, S. M. (2002) J. Biol. Chem., 277, 5897–5903.
6. Mochizuki, N., Hibi, M., Kanai, Y., and Insel, P. A. (1995) FEBs Lett. 373, 155–158.
7. Weiss, T. S., Chamberlain, C. E., Takeda, T., Lin, P., Hahn, K. M., and Farquhar, M. G. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 14961–14966.
8. Yu, F. W., Morin, X., Cai, Y., Yang, X. H., and Chia, W. (2000) Cell 100, 399–409.
9. Belluzie, Y., Radovic, A., Woods D. F., Hough C. D., Parmentier M. L., and O’Kane C. J. (2001) Cell 106, 355–366.
10. Schaefter, M., Protzemerki, M., Donner, D., Forbe, M., and Knoblach, J. J. A. (2001) Cell 107, 185–194.
11. Krasiliov, T., Koch, T., Kahl, E., and Holll, V. (2001) J. Biol. Chem. 276, 39772–39778.
12. Strittmatter, S. M., Valenzuela, D., Sudo, Y., Linder, M. E., and Fishman, M. C. (1991) J. Biol. Chem. 266, 22465–22471.
13. Jordan, J. D., Carey, K. D., Stork, P. J., and Iyengar, R. (1999) J. Biol. Chem. 274, 21507–21510.
14. Meng, J., Glick, J. L., Polakis, P., and Casey, P. J. J. (1999) J. Biol. Chem. 274, 36663–36669.
15. Mochizuki, N., Ohha, Y., Goykawa, E., Kurata, T., Murakami, T., Ozaki, T., Kitabatake, A., Nagashima, K., and Matsuda, M. (1999) Nature 400, 891–894.
16. Delmas, P., Nomura, H., Li, X., Lakkis, M., Luo, Y., Segal, Y., Fernandez-Fernandez, J. M., Harris, P., Frischau A. M., Brown, D. A., and Zhou, J. (2002) J. Biol. Chem. 277, 11276–11283.
17. Parnell, S. C., Magenbein, B. S., Maser, R. L., Zien, C. A., Frischauf, A. M., and Calvet, J. P. (2002) J. Biol. Chem. 277, 15566–15572.
18. McLoughlin, J. N., Thoulin, C. D., Bray, S. M., Martin, M. M., Elton T. S., and Willardson B. M. (2002) J. Biol. Chem. 277, 34885–34895.
19. Luo, Y., and Denker, B. M. (1999) J. Biol. Chem. 274, 10685–10688.
20. Natchonin, M., Gasiom, K. G., and Artemyev, N. O. (2001) Biochemistry 40, 5322–5328.
21. Marjamaki, A., Sato, M., Boset-Alard, R., Yang, Q., Limon-boulez, I., Legrand, C., and Lanier, S. M. (1997) J. Biol. Chem. 272, 16466–16467.
22. Yang, Q., and Lanier, S. M. Mol. Pharmacol. 56, 651–656.
23. Carty, D. J. (1994) Methods Enzymol. 237, 63–70.
24. Peterson, Y. K., Bernard, M., Ma, H. C., Bernard, M., Hazard, S. J., Graber, S. G., and Lanier, S. M. (2000) J. Biol. Chem. 275, 33193–33196.
25. Bernard, M., Peterson, Y. K., Chung, P., Bernard, M., Jourdan, J., and Lanier, S. M. (2001) J. Biol. Chem. 276, 1585–1593.
26. Kipple, R. J., Kippley, M. E., Betts, L., Sondek, J., and Sidorenko, D. P. (2002) Nature 416, 875–881.
27. Cismowski, M. J., Ma, C., Ribas, C., Xie, X., Spruyt, M., Lizzano, J. S., Lanier, S. M., and Duzic, E. (2000) J. Biol. Chem. 275, 23421–23424.
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