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Differential Modulation of Adenylyl Cyclases I and II by Various G_β Subunits*

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The accepted dogma concerning the regulation of adenylyl cyclase (AC) activity by $G_{\beta\gamma}$ dimers states that the various isoforms of AC respond differently to the presence of free $G_{\beta\gamma}$. It has been demonstrated that AC I activity is inhibited and AC II activity is stimulated by $G_{\beta\gamma}$ subunits. This result does not address the possible differences in modulation that may exist among the different $G_{\beta\gamma}$ heterodimers. Six isoforms of G_β and 12 isoforms of G_γ have been cloned to date. We have established a cell transfection system in which G_β and G_γ cDNAs were cotransfected with either AC isoform I or II and the activity of these isoforms was determined. We found that while AC I activity was inhibited by both $G_{\beta1/\gamma2}$ and $G_{\beta5/\gamma2}$ combinations, AC II responded differentially and was stimulated by $G_{\beta1/\gamma2}$ and inhibited by $G_{\beta5/\gamma2}$. This finding demonstrates differential modulatory activity by different combinations of $G_{\beta\gamma}$ on the same AC isoform and demonstrates another level of complexity within the AC signaling system.

The heterotrimeric G-protein has been shown to be a central molecule that connects seven-transmembrane domain receptors to the many downstream effector molecules whose activities they regulate. The G-protein superfamily has been divided into many classes, originally based on the activity that the G_α subunit exerted on the effectors. For example, the G_α subunit that was found to activate adenylyl cyclase (AC)[1] was classified $G_{\alpha s}$ while the G_α subunit found to inhibit AC activity was called $G_{\alpha i}$ (reviewed in Refs. 1–4). In the last few years, the role of free $G_{\beta\gamma}$ subunits (disassociated from G_α upon receptor activation) in signal transduction has begun to be revealed (reviewed in Refs. 5–7). *In vitro* membrane reconstitution assays have clearly demonstrated that a number of AC isoforms are sensitive to $G_{\beta\gamma}$ subunits and that AC activity can either be stimulated or inhibited by $G_{\beta\gamma}$ subunits depending of the AC isoform in question. For example, the activity of AC type I is significantly inhibited while the activity of AC types II, IV, and presumably VII are activated by $G_{\beta\gamma}$ (6–13). This finding has led to the understanding of how it is possible that the classically known inhibitory receptors that are coupled to $G_{\alpha i/o}$ can actually stimulate AC activity in situations where AC isoforms that are activated by free $G_{\beta\gamma}$ subunits are present (11, 12, 14).

Six G_β and 12 G_γ isoforms have been cloned (reviewed in Refs. 4, 5). Most of the prior experiments investigating the role of free $G_{\beta\gamma}$ on AC activity were performed in cell-free systems with either baculovirus/Sf9 recombinant G_β and G_γ preparations (15, 16) or with purified brain $G_{\beta\gamma}$ preparations that consist of a mixture of various $G_{\beta\gamma}$ heterodimers (8, 9). There is little information about the possible variations between the effects of various G_β and G_γ subunits in the intact cell. This seems to be important since the various G_β and G_γ subunits do not necessarily have the same regulatory activities. Due to the recent cloning of various G_β and G_γ isoforms, it is now possible to study the activity of the various $G_{\beta\gamma}$ combinations. Indeed, it has recently been shown by us that activation of PLC-β_2 by $G_{\beta\gamma}$ is G_β isoform-independent ($G_{\beta1/\gamma2}$ being equally effective as $G_{\beta5/\gamma2}$) while MAPK/ERK and JNK/SAPK activation appeared to be G_β isoform-dependent and was much more efficiently activated with $G_{\beta1/\gamma2}$ than with $G_{\beta5/\gamma2}$ (17).

In this study, we have characterized the modulations of two AC isoforms by specific $G_{\beta\gamma}$ combinations. Utilizing cotransfection of G_β and G_γ together with AC isoforms I and II, we observed differential modulation of AC activities by specific combinations of $G_{\beta\gamma}$. We found that AC type I was inhibited by both $G_{\beta1/\gamma2}$ and $G_{\beta5/\gamma2}$. On the other hand, AC II activity was stimulated by one isoform of G_β ($\beta1$) while inhibited by another ($\beta5$).

EXPERIMENTAL PROCEDURES

Cell Cultures—COS-7 cells were obtained from ATCC (Bethesda, MD) and cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin.

Plasmids—cDNAs of AC I and II were used in the pXMD1 vector under control of the adenovirus-2 major late promoter (18). AC I cDNA was released from pSK-AC-I (19) using *Hin*dIII and *Xba*I and ligated after "fill-in" into the *Sma*I site of pXMDI. AC II cDNA was released from pSK-AC-II (20) by *Eco*RI and ligated to the *Eco*RI site of pXMDI. $G_{\beta1}$ and $G_{\beta5}$ cDNAs in pcDNAIII and $G_{\gamma2}$ in pcDM8 were described earlier (17, 21). $G_{\gamma2}$C68S in pcDM8 (a mutant that cannot undergo prenylation) was described earlier (21, 22). cDNA for G_α-transducin (αT) was provided by Dr. J. S. Gutkind. Constitutively active $G_{\alpha s}$ ($G_{\alpha s}$Q227L) was obtained from Dr. H. Bourne (23).

Transfection of COS Cells—COS-7 cells in 10-cm dishes were transfected with the indicated cDNAs by the DEAE-dextran chloroquine method (24). Vectors were added to complement the amount of cDNA in the transfection mixture to 6–7 μg. 24 h later, the cells were trypsinized and cultured for an additional 24 h in 24-well plates for AC activity

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[1] The abbreviations used are: AC, adenylyl cyclase; FS, forskolin; HRP, horseradish peroxidase; $G_{\alpha s}$Q227L, constitutively active $G_{\alpha s}$ subunit; IBMX, isobutylmethylxanthine; PBS, phosphate-buffered saline; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase.

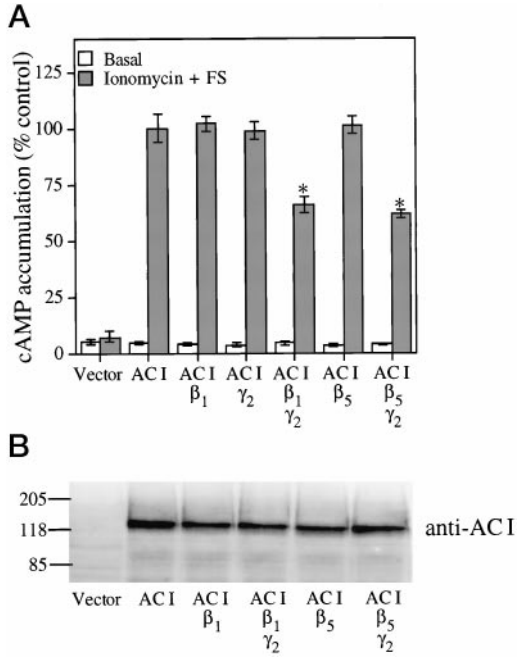

FIG. 1. **AC I activity is inhibited by $G_{\beta1/\gamma2}$ and $G_{\beta5/\gamma2}$.** *A*, effects of cotransfection of $G_{\beta/\gamma}$ combinations on the basal or stimulated (1 μM FS + 1 μM ionomycin) AC I activity. Transfections in 10-cm dishes contained 2 μg AC I, 2 μg $G_{\beta1}$, 2 μg $G_{\beta5}$, and 1 μg $G_{\gamma2}$ cDNAs, as indicated. cAMP accumulation is expressed as percent of control (AC I transfected alone) and is the mean ± S.E. of three experiments performed in triplicate. 100% cAMP accumulation is equivalent to 12,000–14,000 cpm (depending on the experiment). The significance of inhibition of AC I activity was determined by Student's *t* test (*, $p < 0.001$). *B*, aliquots of 15 μg of protein of COS cells transfected as above were analyzed by Western blotting using the anti-AC I antibody BBC-1. *Numbers* show positions of molecular weight markers.

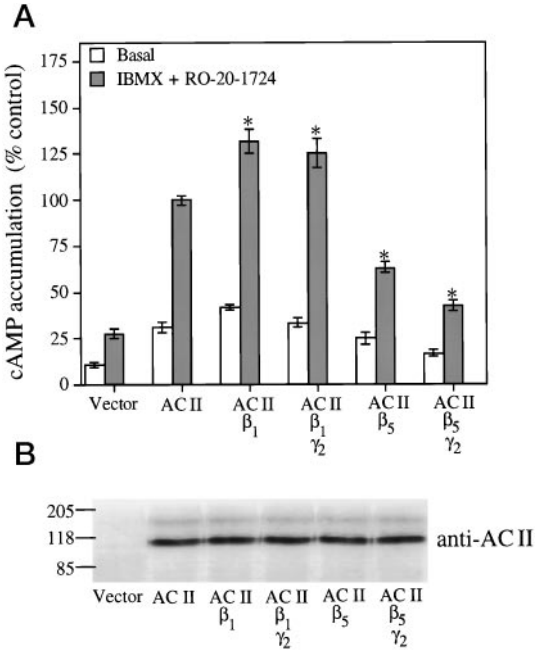

FIG. 2. **AC II activity is stimulated by $G_{\beta1}$ and $G_{\beta1/\gamma2}$ and is inhibited by $G_{\beta5}$ and $G_{\beta5/\gamma2}$.** *A*, cAMP was determined under basal conditions or after a 10-min exposure to IBMX and RO-20–1724 (0.5 mM each). Transfections contained 0.4 μg of $G_{\alpha s}$Q227L cDNA and, where indicated, 2 μg of AC II, 2 μg of $G_{\beta1}$, 2 μg of $G_{\beta5}$, and 1 μg of $G_{\gamma2}$ cDNAs. cAMP accumulation is expressed as percent of control (transfection with GαsQ227L and AC II) and is the mean ± S.E. of three experiments performed in triplicate. 100% cAMP accumulation is equivalent to 20,000–23,000 cpm (depending on the experiment). The significance of stimulation or inhibition of AC II activity was determined by Student's *t* test (*, $p < 0.001$). *B*, aliquots of 15 μg of protein of COS cells transfected as above were analyzed by Western blotting using the anti-AC II antibody BBC-4.

assay or in 10-cm dishes to check protein expression by Western blots. Transfection efficiency, as determined by staining for β-galactosidase (25) activity following transfection with the plasmid expressing the enzyme, was 60–80%.

Adenylyl Cyclase Assay—The assays were performed in triplicate as described (26). Cells cultured in 24-well plates were incubated for 3 h with 0.25 ml/well of fresh growth medium containing 5 μCi/ml [^{3}H]adenine. This medium was replaced with Dulbecco's modified Eagle's medium containing 20 mM Hepes (pH 7.4), 1 mg/ml bovine serum albumin, and the phosphodiesterase inhibitors isobutylmethylxanthine (IBMX) (0.5 mM) and RO-20–1724 (0.5 mM). Unless otherwise indicated, the AC stimulants forskolin (FS) or ionomycin (which increases intracellular Ca^{2+} and thus increases the activity of AC I) were immediately added at 1 μM concentrations, and the cells were incubated at 37 °C for 10 min. Reaction was terminated with 1 ml of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. Aliquots of 0.9 ml were neutralized and applied to a two-step column separation procedure (27). The [^{3}H]cAMP was eluted into scintillation vials and counted.

SDS-Polyacrylamide Gel Electrophoresis (PAGE) and Western Blots—Cells were washed with cold phosphate-buffered saline (PBS), scraped in PBS, and spun down at 5000 rpm (4 °C for 5 min), and cell pellets were mixed with 100 μl of Laemmli sample buffer (28), sonicated, and frozen. Prior to application on the gel, dithiothreitol (0.1 M final) was added, and the samples were incubated for 2 h at 37 °C. Proteins were separated on polyacrylamide gels (8% for AC and 12% for G_β) and transferred to nitrocellulose. Nitrocellulose was blocked in PBS containing 5% fat-free milk and 0.5% Tween-20 for 1 h followed by 1.5 h of incubation with either BBC-1 monoclonal antibody (against AC I), BBC-4 monoclonal antibody (against AC II) (29, 30), RA polyclonal antibody (against $G_{\beta1}$), or SGS polyclonal antibody (against $G_{\beta5}$) (17). Blots were washed 3 times with PBS containing 0.3% Tween-20 and secondary antibodies (horseradish peroxidase (HRP)-coupled rat anti-mouse or HRP-coupled goat anti-rabbit, Jackson Immunoresearch Laboratories, Inc.) diluted 1:20,000 in 5% fat-free milk plus 0.5% Tween-20 and incubated with the blot for 1 h, and the blot was extensively washed (>2 h) with PBS containing 0.3% Tween-20. Peroxidase activity was observed by the ECL chemiluminescence technique (Amersham Corp.).

RESULTS

AC I Activity Is Inhibited by $G_{\beta1/\gamma2}$ and $G_{\beta5/\gamma2}$—We have performed cotransfection experiments of AC I together with $G_{\beta1}$ or $G_{\beta5}$ and $G_{\gamma2}$. Activation of AC I is known to be dependent on the presence of Ca^{2+} ions (8). In the experiment shown in Fig. 1, we have assayed the activity of this isozyme in the presence of the Ca^{2+} ionophore ionomycin (1 μM) together with FS (1 μM). This combination was shown to synergistically stimulate AC I activity (11). The endogenous AC activity present in COS cells was not significantly affected by ionomycin and thus contains very little Ca^{2+}-stimulated AC isozymes. As shown in Fig. 1*A*, AC I activity was significantly inhibited upon cotransfection with either $G_{\beta1}$ or $G_{\beta5}$ together with $G_{\gamma2}$. The individual $G_{\beta1}$, $G_{\beta5}$, and $G_{\gamma2}$ subunits had no inhibitory activity on their own. Western blots of AC I protein (see Fig. 1*B*) reveals that the levels of AC I were not affected by the cotransfection of the other cDNAs. Our results thus indicate that the inhibitory effect mediated by $G_{\beta\gamma}$ subunits, originally found in membrane assays, can also be observed in a whole cell system and that both $G_{\beta1}$ and $G_{\beta5}$ are effective in the inhibition of AC I, provided that $G_{\gamma2}$ is present.

AC II Activity Is Stimulated by $G_{\beta1}$ and $G_{\beta1/\gamma2}$ but Is Inhibited by $G_{\beta5/\gamma2}$—The AC II subtype is known to be stimulated by free $G_{\beta\gamma}$ (8). We have performed cotransfection of AC II with constitutively active $G_{\alpha s}$ ($G_{\alpha s}$Q227L) and, where indicated, with $G_{\beta1}$ or $G_{\beta5}$ and with $G_{\gamma2}$. The amounts of cAMP in these cells were determined 2 days after transfection. In addition, we have treated the cells for 10 min with a mixture of the phosphodiesterase inhibitors, IBMX and RO-20–1724, and assayed the amounts of cAMP following this incubation. From the results shown in Fig. 2, it is clear that transfection with AC II

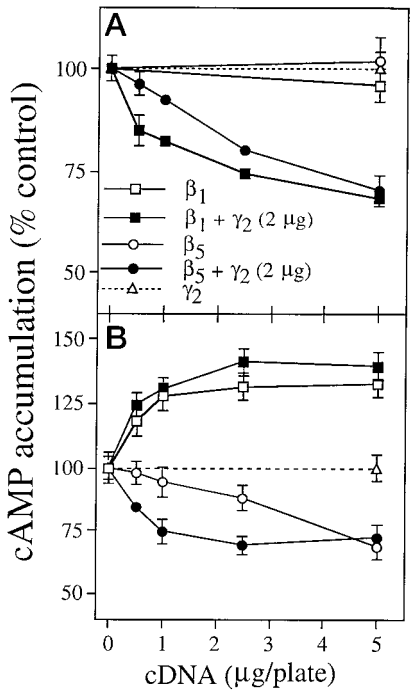

FIG. 3. **Dose dependence of the modulation of AC I and AC II by $G_{\beta/\gamma}$ combinations.** *A*, effect of increasing amounts of G_β cDNA on AC I activity. COS cells in 10-cm plates were transfected with 2 μg of AC I cDNA and, where indicated, with $G_{\beta1}$, $G_{\beta5}$, and 2 μg of $G_{\gamma2}$ cDNA. cAMP accumulation was measured 10 min after the addition of 1 μM FS and 1 μM ionomycin (see Fig. 1). *B*, the effect of increasing amounts of G_β cDNA on AC II activity. COS cells were transfected with 0.4 μg of $G_{\alpha s}$Q227L and 2 μg of AC II cDNA and, where indicated, with $G_{\beta1}$, $G_{\beta5}$, and 2 μg of $G_{\gamma2}$ cDNA. cAMP accumulation was measured 10 min after the addition of IBMX and RO-20–1724. Data are expressed as percent of controls (AC I transfected alone or AC II transfected together with $G_{\alpha s}$Q227L) and are the mean ± S.E. of three experiments performed in triplicate. 100% cAMP accumulation is equivalent to 10,000–12,000 cpm for AC I and 18,000–21,000 cpm for AC II (depending on the experiment).

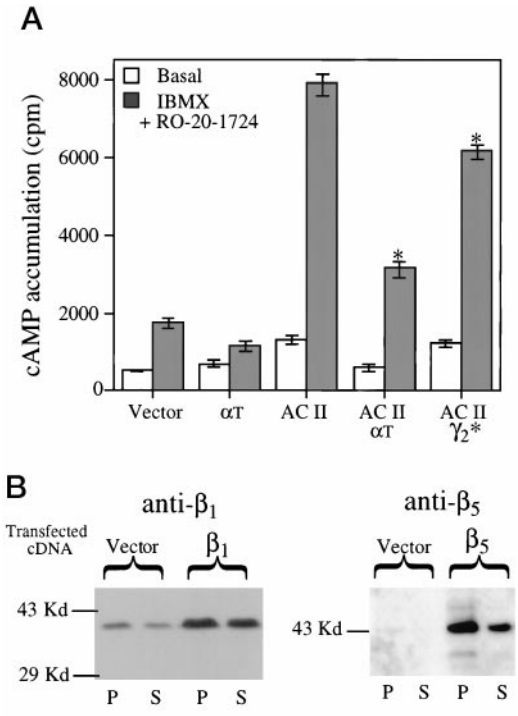

FIG. 4. **Endogenous $G_{\beta\gamma}$ of COS 7 cells is stimulatory for AC II.** *A*, effect of cotransfection with αT or $G_{\gamma2}$C68S on basal and $G_{\alpha s}$Q227L-stimulated AC II activity. Transfections contained 0.4 μg of $G_{\alpha s}$Q227L and, where indicated, 2 μg of AC II, 2 μg of $G_{\gamma2}$C68S (γ*), or 2 μg of αT cDNAs. cAMP accumulation (in cpm) is from one representative experiment out of three which gave similar results. The significance of inhibition of AC II activity was determined by Student's *t* test (*, $p < 0.001$). *B*, expression of $G_{\beta1}$ and $G_{\beta5}$ in transfected COS cells. Membranes were prepared from untransfected cells as well as from cells transfected with 2 μg of $G_{\beta1}$ or $G_{\beta5}$ cDNAs and solubilized with 1% cholate. Aliquots of 5 μg of protein of the cholate particulate (*P*) and soluble (*S*) fractions were separated by SDS-polyacrylamide gel electrophoresis (12% polyacrylamide), and the G_β subunits were detected by the RA polyclonal antibody (against $G_{\beta1}$) or SGS polyclonal antibody (against $G_{\beta5}$) (17). The procedure for the preparation of membranes and cholate solubilization was described previously (17).

increased the amounts of cAMP present in the cells under both conditions. As expected, transfection with $G_{\beta1}$ caused a further increase in cAMP accumulation. This is due to AC II activation since it was not observed when $G_{\beta1}$ was transfected to COS cells without the addition of AC II plasmid (data not shown) or when it was transfected with AC I (Fig. 1). The increase of AC II activity mediated by $G_{\beta1}$ was not significantly affected by the addition of $G_{\gamma2}$, which by itself had no effect. Interestingly, in contrast to $G_{\beta1}$, $G_{\beta5}$ caused a siginificant inihibition of AC II activity, and the addition of $G_{\gamma2}$ increased this inhibition. These results show that in contrast to AC I, AC II is differentially affected by $G_{\beta1}$ and $G_{\beta5}$, thus demonstrating a level of specificity within the G_β family members in regards to their ability to affect AC II activity. Western blot analysis of the AC II protein levels under the various transfection conditions revealed that AC II expression was not affected by the cotransfection with G_β and G_γ subunits.

G_β Subunits Modulate AC I and AC II in a Dose-dependent Manner—The experiments shown in Figs. 1 and 2 were performed with relatively large amounts of G_β and G_γ cDNAs. They were, therefore, repeated with $G_{\beta1}$ and $G_{\beta5}$ cDNAs at various concentrations. Fig. 3*A* demonstrates that the inhibition of AC I reaches maximal values when concentrations of cDNAs of $G_{\beta1}$ or $G_{\beta5}$ are above 2.5 μg. The efficiencies of inhibition by transfected $G_{\beta1}$ and $G_{\beta5}$ (in the presence of $G_{\gamma2}$) were similar. Half-maximal effect was observed with ~1 μg of $G_{\beta1}$ and 2 μg of $G_{\beta5}$ cDNAs. The inhibition observed for AC I activity was dependent on the presence of $G_{\gamma2}$ (2 μg/plate) for both $G_{\beta1}$ and $G_{\beta5}$.

As shown in Fig. 3*B*, the level of stimulation of AC II by $G_{\beta1}$ is dependent on the amount of $G_{\beta1}$ cDNA used, reaching a maximum above ~1 μg of transfected cDNA. The addition of 2 μg $G_{\gamma2}$ cDNA to the transfection mixture increased the effect observed with $G_{\beta1}$ cDNA alone by only a marginal value of approximately 10%. Conversely, transfection of $G_{\beta5}$ cDNA caused a marked inhibition of AC II activity, and this inhibition was greatly enhanced by the presence of transfected $G_{\gamma2}$. Maximal inhibition (~30%) was observed with 5 μg of $G_{\beta5}$ cDNA/10-cm culture dish, while in the presence of $G_{\gamma2}$, maximal inhibition (of the same level of 30%) was already observed with ~1 μg of $G_{\beta5}$ cDNA.

Endogenous $G_{\beta\gamma}$ in COS Has a Role in AC II Stimulation—Since we have demonstrated that the $G_{\beta5/\gamma2}$ combination can be inhibitory to AC II activity, it became of interest to characterize the role of endogenous $G_{\beta\gamma}$ in COS-7 cells with respect to AC II modulation. To investigate this question, we utilized a number of molecular tools that were shown to interfere with $G_{\beta\gamma}$ activity. A mutant form of $G_{\gamma2}$ that lacks the prenylation site ($G_{\gamma2}$C68S) and which therefore cannot anchor to the membrane has been shown to redirect G_β subunits into the cellular cytosol (21, 22). Additionally, wild-type G_α subunits such as αT combines with $G_{\beta\gamma}$ and interferes with $G_{\beta\gamma}$-mediated signaling (11, 14). Fig. 4*A* demonstrates the effect of cotransfection of the above mentioned proteins on AC II activity in COS-7 cells. A

marked inhibition of AC II activity was observed in cells cotransfected with αT. $G_{\gamma 2}$C68S also produced a significant inhibition of AC II activity although the efficacy of inhibition compared with αT was noticeably less. The cotransfection of αT and $G_{\gamma 2}$C68S with AC II did not have any effect on the expression of AC II (data not shown). These results suggest that $G_{\beta\gamma}$ in COS cells has a role in the stimulation of AC II activity. Fig. 4*B* demonstrates that COS cells do indeed express endogenous $G_{\beta 1}$, but appear to be devoid of $G_{\beta 5}$. Transfection of COS cells with $G_{\beta 1}$ or $G_{\beta 5}$ cDNAs led to a significant increase in the levels of $G_{\beta 1}$ and $G_{\beta 5}$ protein in the cell membranes.

DISCUSSION

There are many reports that show involvement of the $G_{\beta\gamma}$ dimer complex in the regulation of various effectors including PLC-β2, MAPK/ERK, JNK/SAPK, phosphatidylinositol 3-kinase, and several AC isozymes (5, 8, 14, 17, 31, 32). Most of these experiments were performed either in whole cells using $G_{\beta\gamma}$ scavengers (*i.e.* molecules that strongly interact with $G_{\beta\gamma}$ and block its ability to modulate effectors) or with cell membranes. In the latter case, the $G_{\beta\gamma}$ used for most of the experiments has been with either baculovirus/Sf9 recombinant G_β and G_γ preparations or with a mixture of a large repertoire of $G_{\beta\gamma}$ heterodimers, such as in $G_{\beta\gamma}$ preparations purified from bovine brain. Therefore, there has been little information regarding the possible differences in activities between the various G_β and G_γ subunits composing the $G_{\beta\gamma}$ dimers in intact cells. Following the cloning of specific G_β and G_γ isoforms, it has become possible to dissect the individual G_β and G_γ combinations that affect AC activity. In a previous study, using transfection of intact cells with $G_{\beta 1}$ or $G_{\beta 5}$ cDNAs, we showed that $G_{\beta 1}$ and $G_{\beta 5}$ differ in their ability to activate MAPK/ERK and JNK/SAPK but not in their capacity to activate PLC-β2 (17). Here, we have used the same approach to investigate the role of $G_{\beta 1}$ and $G_{\beta 5}$ in the modulation of two types of AC isozymes: AC I, previously shown to be inhibited by $G_{\beta\gamma}$ heterodimers, and AC II, previously reported to be stimulated by $G_{\beta\gamma}$ (8, 15, 16).

Our results demonstrate that indeed, as reported using *in vitro* reconstitution assays, $G_{\beta\gamma}$ inhibits AC I activity. We found that $G_{\beta 1}$ and $G_{\beta 5}$ do not markedly differ in their capacity to inhibit AC I and that in both cases, the inhibition was dependent on the cotransfection of a G_γ subunit. Our observations with AC II showed that while $G_{\beta 1/\gamma 2}$ yielded stimulation of this isoform, in agreement with previously reported activities of $G_{\beta\gamma}$ (7, 8, 11, 13, 14). $G_{\beta 5/\gamma 2}$ caused a marked inhibition of AC II activity, demonstrating selective effects of G_β subunits on the activity of this AC isoform. Interestingly, transfected $G_{\beta 1}$ was by itself sufficient in stimulating AC II activity. This is probably due to its capacity for recruiting endogenous G_γ present in the cell. $G_{\beta 5}$ also had some effect on its own, although its effect on the inhibition of AC II was significantly enhanced by cotransfection of $G_{\gamma 2}$. As previously proposed (17, 22), these results suggest a difference in the capacity of $G_{\beta 1}$ and $G_{\beta 5}$ to interact with G_γ subunits.

It is known that of the six cloned G_β isoforms, $G_{\beta 5}$ shares only a 53% homology compared with the other G_β isoforms (5). The unique sequence of $G_{\beta 5}$ may be the source of the different effects on AC II activity that we have observed. It should be noted that this specific activity could have been missed in prior studies in which $G_{\beta\gamma}$ was tested in *in vitro* membrane reconstitution assays due to the possibility that the concentration of $G_{\beta 5}$ in the pool of $G_{\beta\gamma}$ (usually purified from bovine brain) may have been too small. The activity of $G_{\beta 5}$ may have been masked by other G_β subunits present in the preparation, and therefore, the levels of $G_{\beta 5}$ were not high enough to observe any inhibitory effects on AC II activity.

In summary, $G_{\beta 1}$ and $G_{\beta 5}$ represent two distinct forms of the G_β subunit, as based on their sequence, expression pattern, and ability to affect downstream signaling proteins (17, 33). Our results show that they also differ in their capacity to affect the activity of AC type II. Future studies should allow complete characterization of the various $G_{\beta\gamma}$ combinations influencing the activity of each of the AC isozymes and elucidate the finer regulation of $G_{\beta\gamma}$ signaling within the AC system.

REFERENCES

1. Birnbaumer, L. (1992) *Cell* **71,** 1069–1072
2. Helper, J. R., and Gilman, A. G. (1992) *Trends Biochem. Sci.* **17,** 383–387
3. Guderman, T., Kalkbrenner, F., and Schultz, G. (1996) *Annu. Rev. Pharmacol. Toxicol.* **36,** 429–459
4. Simon, M., Strathmann, M., and Gautam, N. (1991) *Science* **252,** 802–808
5. Clapham, D. E., and Neer, E. J. (1997) *Annu. Rev. Pharmacol. Toxicol.* **37,** 167–203
6. Clapham, D. E., and Neer, E. J. (1993) *Nature* **365,** 403–406
7. Sunahara, R. K., Dessauer, C. W., and Gilman, A. G. (1996) *Annu. Rev. Pharmacol. Toxicol.* **36,** 461–480
8. Taussig, R., Quarmby, L. M., and Gilman, A. G. (1993) *J. Biol. Chem.* **268,** 9–12
9. Tang, W.-J., Krupinski, J., and Gilman, A. G. (1991) *J. Biol. Chem.* **266,** 8595–8603
10. Choi, E.-J., Xia, Z., Villacres, E. C., and Storm, D. R. (1993) *Curr. Opin. Cell Biol.* **5,** 269–273
11. Avidor-Reiss, T., Nevo, I., Saya, D., Bayewitch, M., and Vogel, Z. (1997) *J. Biol. Chem.* **272,** 5040–5047
12. Yoshimura, M., Ikeda, H., and Tabakoff, B. (1996) *Mol. Pharmacol.* **50,** 43–51
13. Chen, J., DeVivo, M., Dingus, J., Harry, A., Li, J., Sui, J., Carty, D. J., Blank, J. L., Exton, J. H., Stoffel, R. H., Inglese, J., Lefkowitz, R. J., Logothetis, D. E., Hildebrandt, J. D., and Iyengar, R. (1995) *Science* **268,** 1166–1169
14. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992) *Nature* **356,** 159–161
15. Iñiguez-Lluhi, J., Simon, M., Robishaw, J., and Gilman, A. (1992) *J. Biol. Chem.* **267,** 23409–23417
16. Ueda, N., Iñiguez-Lluhi, J., Lee, E., Smrcka, A., Robishaw, J., and Gilman, A. (1994) *J. Biol. Chem.* **269,** 4388–4395
17. Zhang, S., Coso, O., Lee, C., Gutkind, J., and Simonds, W. (1996) *J. Biol. Chem.* **271,** 33575–33579
18. Kluxen, F.-W., and Lübbert, H. (1993) *Anal. Biochem.* **208,** 352–356
19. Krupinski, J., Coussen, F., Bakalyar, H. A., Tang, W.-J., Feinstein, P. G., Orth, K., Slaughter, C., Reed, R. R., and Gilman, A. G. (1989) *Science* **244,** 1558–1564
20. Feinstein, P. G., Schrader, K. A., Bakalyar, H. A., Tang, W. J., Krupinski, J., Gilman, A. G., and Reed, R. R. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10173–10177
21. Garritsen, A., Gallan, P. V., and Simonds, W. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 7706–7710
22. Garritsen, A., and Simonds, W. (1994) *J. Biol. Chem.* **269,** 24418–24423
23. Zachary, I., Masters, S. B., and Bourne, H. R. (1990) *Biochem. Biophys. Res. Commun.* **168,** 1184–1193
24. Keown, W. A., Campbell, C. R., and Kucherlapati, R. S. (1990) *Methods Enzymol.* **185,** 527–537
25. Lim, K., and Chae, C. B. (1989) *BioTechniques* **7,** 576–579
26. Bayewitch, M., Rhee, M., Avidor-Reiss, T., Breuer, A., Mechoulam, R., and Vogel, Z. (1996) *J. Biol. Chem.* **271,** 9902–9905
27. Salomon, Y. (1991) *Methods Enzymol.* **195,** 22–28
28. Laemmli, U. K. (1970) *Nature* **227,** 680–685
29. Pfeuffer, E., Mollner, S., and Pfeuffer, T. (1991) *Methods Enzymol.* **195,** 83–91
30. Droste, M., Mollner, S., and Pfeuffer, T. (1996) *FEBS Lett.* **391,** 209–214
31. Lopez-Ilasaca, M., Crespo, P., Giuseppe Pellici, P., Silvio Gutkind, J., and Wetzker, R. (1997) *Nature* **275,** 394–397
32. Crespo, P., Xu, N., Simonds, W. F., and Gutkind, J. S. (1994) *Nature* **369,** 418–420
33. Watson, A., Katz, A., and Simon, M. (1994) *J. Biol. Chem.* **269,** 22150–22156