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$G\alpha_{15}$ and $G\alpha_{16}$ Couple a Wide Variety of Receptors to Phospholipase C*

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The murine G-protein α-subunit $G\alpha_{15}$ and its human counterpart $G\alpha_{16}$ are expressed in a subset of hematopoietic cells, and they have been shown to regulate β-isoforms of inositide-specific phospholipase C. We studied the ability of a variety of receptors to interact with $G\alpha_{15}$ and $G\alpha_{16}$ by cotransfecting receptors and G-protein α-subunits in COS-7 cells. Activation of β_2 adrenergic and muscarinic M_2 receptors in cells expressing the receptors alone or together with $G\alpha_q$, $G\alpha_{11}$, or $G\alpha_{14}$ led to a very small stimulation of endogenous phospholipase C. However, when the receptors were coexpressed with $G\alpha_{15}$ and $G\alpha_{16}$, addition of appropriate ligands caused a severalfold increase in inositol phosphate production which was time- and dose-dependent. A similar activation of phospholipase C was observed when several other receptors which were previously shown to couple to members of the G_i and G_s family were coexpressed with $G\alpha_{15/16}$. In addition, stimulation of inositol phosphate formation via receptors naturally coupled to phospholipase C was enhanced by cotransfection of $G\alpha_{15}$ and $G\alpha_{16}$. These data demonstrate that $G\alpha_{15}$ and $G\alpha_{16}$ are unique in that they can be activated by a wide variety of G-protein-coupled receptors. The ability of $G\alpha_{15}$ and $G\alpha_{16}$ to bypass the selectivity of receptor G-protein interaction can be a useful tool to understand the mechanism of receptor-induced G-protein activation. In addition, the promiscuous behavior of $G\alpha_{15}$ and $G\alpha_{16}$ toward receptors may be helpful in finding ligands corresponding to orphan receptors whose signaling properties are unknown.

A wide variety of hormones and neurotransmitters regulate cellular functions by binding to transmembranous receptors which couple to and activate heterotrimeric guanine nucleotide binding proteins (G-proteins).[1] Receptor-activated G-proteins then regulate different cellular effectors, such as specific enzymes and ion channels (1–4). Sixteen mammalian genes encoding G-protein α-subunits, which define the individual G-proteins, have been identified, and they have been grouped into four families, $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$, according to sequence homologies (5). Many of the factors which determine the specificity of G-protein-mediated signal transduction are still unknown. Nonetheless, the selective coupling of an activated receptor to a distinct pattern of G-proteins is regarded as an important requirement to achieve accurate signal transduction. For example, receptors which upon activation lead to stimulation of adenylyl cyclase primarily couple to G_s, whereas the receptor-mediated pertussis toxin-insensitive activation of phospholipase C is due to the coupling of receptors to members of the G_q family (6–8).

The G_q family consists of five members whose α-subunits show different expression patterns. Whereas $G\alpha_q$ and $G\alpha_{11}$, which are 88% identical, seem to be almost ubiquitously expressed and are primarily responsible for coupling receptors in a pertussis toxin-insensitive manner to phospholipase C β-isoforms (7–9), the expression of $G\alpha_{14}$, which is 81% identical with $G\alpha_q$, is more restricted (10). The human $G\alpha_{16}$ and its murine counterpart $G\alpha_{15}$ are only expressed in a subset of hematopoietic cells (10, 11). $G\alpha_{15}$ and $G\alpha_{16}$, which are 85% identical, have been placed into the G_q family since their sequences show the highest similarity toward $G\alpha_q$ (57%). All five members of the $G\alpha_q$ family share functional properties, *i.e.* they can regulate the β-isoforms of phospholipase C (12–14). Purified $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{16}$ indistinguishably activate different isoforms of phospholipase C-β in a reconstituted system (15, 16). Recent data, however, demonstrate that receptors for interleukin 8 and C5a interact selectively with G_{16} but not with G_q and G_{11} (17–19), demonstrating that there are differences among members of the G_q family with regard to receptor interaction. In the present study, we show that a wide variety of structurally and functionally different receptors couple to G_{15} and G_{16} but not to other members of the G_q family, indicating that G_{15} and G_{16} are unique, *i.e.* they possess the ability to nonselectively couple a large variety of receptors to phospholipase C.

EXPERIMENTAL PROCEDURES

Materials—Carbachol, isoproterenol, dopamine, 8-hydroxy-2-(di-*n*-propylamino)tetralin, thrombin, serotonin, [Arg^8]vasopressin, [D-Ala^2, *N*-MePhe^4,Gly^5-ol]enkephalin, 11α,9α-epoxymethano-PGH_2 (U46619), and *N*-formyl-Met-Leu-Phe were from Sigma. CGS-21680 was from RBI (Natick, MA) and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) was from Life Technologies, Inc.

Transient Transfection and Labeling of COS-7 Cells—COS-7 cells were cultured as described (13). For transfection experiments, cells were seeded in 24-well plates at a density of 4×10^4 cells per well and grown overnight. Cells were then washed with phosphate-buffered saline, and 0.4 μg of DNA mixed with 2 μl of lipofectamine (Life Technologies, Inc.) in 0.25 ml of Opti-MEM was added to each well. In cotransfection experiments with two different plasmids, 0.2 μg of each plasmid was added. In control experiments, the total amount of DNA was maintained constant by adding DNA from a vector encoding β-galactosidase. After 5 h at 37 °C, 0.25 ml of DMEM containing 20% (v/v) fetal bovine serum was added to each well. About 24 h after transfection, cells were labeled for 20–24 h with 120 pmol of *myo*-[2-^{3}H]inositol (758.5 GBq/mmol; Du Pont NEN) per well as described (13).

Determination of Inositol Phosphate Levels—Labeled cells were washed with phosphate-buffered saline and then incubated for 10 min at 37 °C with 0.25 ml of inositol-free DMEM containing 10 mM LiCl.

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[1] The abbreviations used are: G-protein, heterotrimeric guanine nucleotide-binding protein; DMEM, Dulbecco's modified Eagle's medium; fMLP, *N*-formyl-methionine-leucine-phenylalanine; C5a, active cleavage product of the complement factor 5; 5-HT, 5-hydroxytryptamine (serotonin); U46619, 11α,9α-epoxymethano-PGH_2.

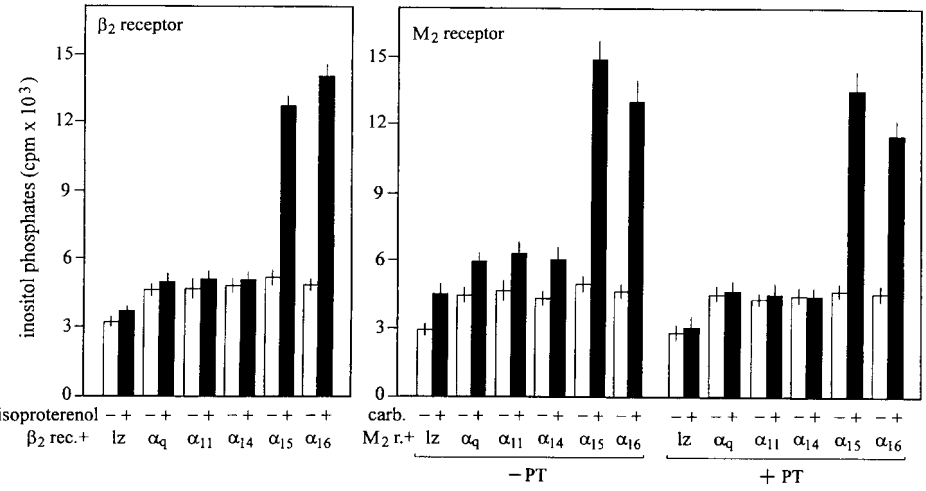

FIG. 1. **Accumulation of inositol phosphates in COS-7 cells that coexpress the β_2 adrenergic or the M_2 muscarinic receptor and Gα subunits.** COS-7 cells were cotransfected with cDNAs encoding the the β_2 adrenergic (*left panel*) or the M_2 muscarinic receptor (*right panel*) and cDNAs encoding β-galactosidase (*lz*) or α-subunits of the $G\alpha_q$ family, $G\alpha_q$ (α_q), $G\alpha_{11}$ (α_{11}), $G\alpha_{14}$ (α_{14}), $G\alpha_{15}$ (α_{15}), and $G\alpha_{16}$ (α_{16}) as described under "Experimental Procedures." After 48 h, [^{3}H]inositol-labeled cells were incubated in the absence (−; *open bars*) or presence (+) of 10 μM isoproterenol (*closed bars*, *left panel*) and 10 μM carbachol (*carb.*; *closed bars*, *right panel*) for 20 min, and levels of inositol phosphates were determined as described. Cells which were cotransfected with the M_2 muscarinic receptor cDNA and Gα subunit cDNAs were processed as described (−*PT*) or were pretreated with 100 ng/ml pertussis toxin (+*PT*) for 18 h prior to incubation with ligand. Shown are mean values of triplicates ± S.D.

Thereafter, medium was aspirated, and the indicated agents were added in DMEM containing 10 mM LiCl. Inositol phosphate formation was stopped after 20 min by removing the medium and adding 0.2 ml of 10 mM ice-cold formic acid. After keeping the samples on ice for 20 min, 0.45 ml of 10 mM NH_4OH was added, and the whole sample was loaded onto a column containing 0.75 ml of anion exchange resin (AG 1-X8; Bio-Rad) equilibrated with 5 mM borax and 60 mM sodium formate. Total inositol phosphates were then separated and measured as described (20). If not stated otherwise, measurements were done in triplicate representing three independently transfected wells.

Determination of Cellular cAMP Levels—For determination of cAMP levels, cells were grown and transfected in 24-well plates as described. 48 h after transfection, cells were preincubated for 15 min with DMEM containing 300 μM 3-isobutyl-1-methylxanthine and 20 μM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20–1724). Thereafter, medium was replaced by DMEM containing both phosphodiesterase inhibitors and the indicated concentrations of ligands. At the end of this treatment (20- min incubation time), the reaction was stopped by aspiration of the medium and addition of 150 μl of ice-cold 10% (w/v) trichloroacetic acid. Samples were kept for 10 min on ice, and 90 μl of 1 M Tris (pH 9.8) was added to neutralize the sample. cAMP was determined by the competitive binding assay (21, 22). Briefly, 100 μl of the sample were incubated for 2 h with 2 pmol of [8-^{3}H]cAMP (925 Gbq/mmol; Amersham) and 62.5 μg of cAMP-dependent protein kinase purified from porcine heart (Sigma) in a final volume of 200 μl at 4 °C. Thereafter, 100 μl of 4% (w/v) charcoal in 5 mM EDTA and 50 mM Tris-HCl (pH 7.5) was added, and samples were immediately centrifuged for 2 min at 12,000 × *g*. To determine the amount of [8-^{3}H]cAMP bound to the binding protein, supernatants were counted in a liquid scintillation counter. Each experiment was calibrated by running a set of cAMP standards along with the unknown test sample. For the standard samples, the log of total counts/min bound was plotted *versus* the log of total cAMP per sample (labeled plus unlabeled), and the amount of cAMP in the test sample was calculated from the resulting standard curve (21). Assays were done in triplicate representing three independently transfected wells.

COS Cell Expression Vectors—cDNAs corresponding to G-protein α-subunits $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$ were carried by the cytomegalovirus vector pCIS (12, 13, 17, 19, 20). A β-galactosidase construct inserted into pCIS was used as a transfection control. cDNAs encoding the human muscarinic M_2, the human β_2 adrenergic, the murine 5-$HT_{1C/2C}$, and the human thrombin receptors were in the vector pCIS. The human dopamine D_{1A} construct was in pCMV5 (23), the rat μ opioid receptor and the human adenosine A_{2A} receptor encoding cDNAs were in pRc/CMV (Invitrogen), the human vasopressin V_2 receptor and the human fMLP receptor cDNAs were in the vector pcDNAI/Amp (Invitrogen), the human 5-HT_{1A} receptor construct was in pSVL (Pharmacia Biotech Inc.), the cDNA encoding the human vasopressin V_{1A} receptor was carried by the pcD3 vector (24), and the human thromboxane A_2 receptor cDNA was in pCDM8 (Invitrogen).

RESULTS

In order to study the interaction of different receptors and G-proteins of the G_q family, cDNA clones encoding receptors and G-protein α-subunits were transiently cotransfected into COS-7 cells, and inositol phosphate production in response to receptor ligands was measured. First, we tested the β_2 adrenergic and the M_2 muscarinic receptor, which have been shown to couple primarily to G_s and $G_{i/o}$, respectively (25–28), to determine if they can mediate ligand-dependent inositol phosphate production in COS-7 cells (Fig. 1). When both receptors were expressed alone, there was a slight increase in the inositol phosphate production in response to the respective ligands. In the case of the muscarinic M_2 receptor, this increase could be blocked by pretreatment of cells with pertussis toxin and is presumably mediated by βγ-subunits of G_i (29). Cotransfection of G-protein α-subunits $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{14}$, all of which have been shown to be expressed at high levels and to mediate receptor-dependent phospholipase C activation in COS cells (12, 13, 17, 19, 20), slightly increased the basal inositol phosphate production, but had no effect on the ligand-dependent increase in inositol phosphate formation. In contrast, ligand-dependent inositol phosphate production was severalfold enhanced when both receptors were cotransfected with $G\alpha_{15}$ and $G\alpha_{16}$ (Fig. 1).

Fig. 2 shows that the β_2 adrenergic or the M_2 muscarinic receptor-mediated increase in inositol phosphate production in cells cotransfected with $G\alpha_{15}$ or $G\alpha_{16}$ was linear with time for at least 30 min. Isoproterenol- and carbachol-induced inositol phosphate formation in cells transiently expressing $G\alpha_{15}$ or $G\alpha_{16}$ and the corresponding receptors was concentration-dependent (Fig. 3). Half-maximal and maximal effects of carbachol were observed at concentrations of 0.1 and 1–10 μM, whereas isoproterenol-stimulated inositol phosphate production was half-maximal and maximal at concentrations of 0.3 and 10 μM, respectively. We then tested the ability of isoproterenol and carbachol to stimulate inositol phosphate formation in the presence of $G\alpha_{15}$ and $G\alpha_{16}$ in order to test if the ligand concentration dependence was in the same range as the dosage required to regulate the natural effector target of their

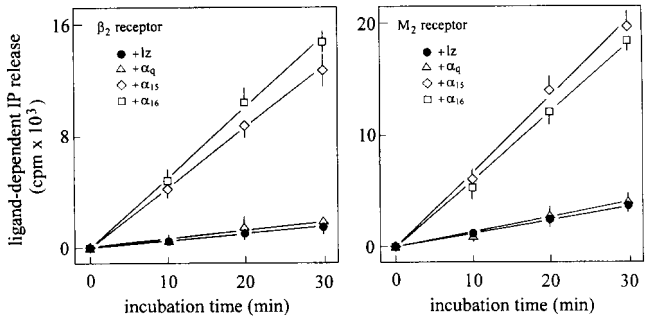

FIG. 2. **Time course of the β_2 adrenergic and M_2 muscarinic receptor-mediated inositol phosphate formation in COS-7 cells.** COS-7 cells were cotransfected with cDNAs encoding the β_2 adrenergic (*left panel*) or the M_2 muscarinic receptor (*right panel*) and cDNAs encoding β-galactosidase (●, lz) or $G\alpha_q$ (Δ, α_q), $G\alpha_{15}$ (◇, α_{15}), and $G\alpha_{16}$ (□, α_{16}) as described under "Experimental Procedures." Cells were incubated in the absence or presence of 10 μM isoproterenol (*left panel*) and 10 μM carbachol (*right panel*) for the indicated time periods (*abscissa*), and released inositol phosphates were measured as described. Shown is the ligand-dependent inositol phosphate release, and data points represent mean values of triplicates ± S.D.

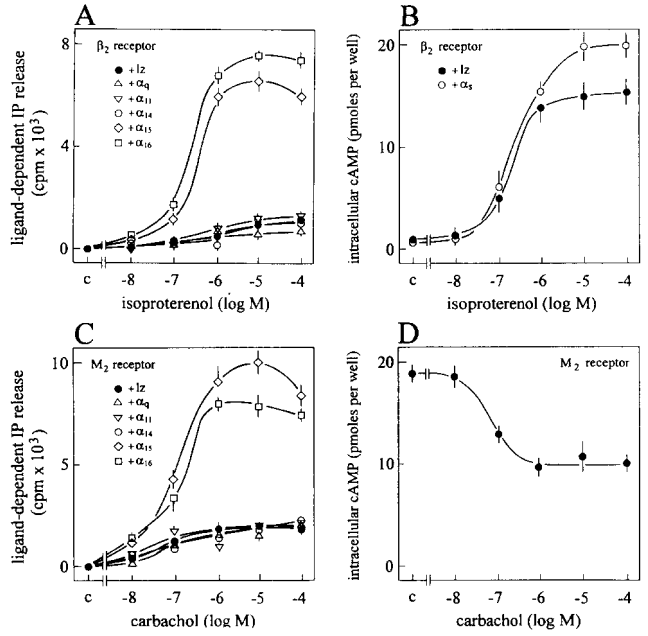

FIG. 3. **Accumulation of inositol phosphates and cAMP in COS-7 cells transfected with the β_2 adrenergic or M_2 muscarinic receptor cDNAs.** *A* and *C*, COS-7 cells were cotransfected with cDNAs encoding the β_2 adrenergic (*A*) or the M_2 muscarinic receptor (*C*) and cDNAs encoding β-galactosidase (●, lz) or α-subunits of the $G\alpha_q$ family, $G\alpha_q$ (Δ, α_q), $G\alpha_{11}$ (▽, α_{11}), $G\alpha_{14}$ (○, α_{14}), $G\alpha_{15}$ (◇, α_{15}), and $G\alpha_{16}$ (□, α_{16}) as described under "Experimental Procedures." Cells were then incubated with the indicated concentrations of isoproterenol (*A*) and carbachol (*C*), and the ligand-dependent inositol phosphate formation was determined as described. *B*, COS-7 cells were cotransfected with cDNAs encoding the β_2 adrenergic receptor and β-galactosidase (●, lz) or $G\alpha_s$ (○, α_s). Cells were incubated at increasing concentrations of isoproterenol for 20 min, and cellular cAMP content was determined as described under "Experimental Procedures." *D*, COS-7 cells were cotransfected with cDNAs encoding the the β_2 adrenergic and M_2 muscarinic receptor (0.2 μg of each per well). Transfected cells were incubated with 2 μM isoproterenol and the indicated concentrations of carbachol (*abscissa*) for 20 min, and the carbachol-induced decrease in cellular cAMP content was measured as described. Basal cAMP levels (in the absence of any ligand) were 1.2 ± 0.2 pmol per well. Values are mean values ± S.D.

respective receptors. Therefore, in a set of parallel experiments we measured the effect of increasing concentrations of both ligands on the cellular cAMP content in COS-7 cells transfected with the β_2 or the M_2 receptor cDNAs. Isoproterenol induced an increase in the intracellular cAMP content in cells expressing the β_2 receptor, and the extent of this effect was slightly increased in cells coexpressing $G\alpha_s$. In order to study the M_2 receptor-mediated decrease in intracellular cAMP, cells were cotransfected with the muscarinic M_2 receptor and the β_2 adrenergic receptor. When cAMP levels were increased through the stimulation of the β_2 receptor, a carbachol-dependent decrease of the cAMP content could be observed. In both cases, effects mediated by the β_2 and the M_2 receptor were dose-dependent and occurred with a very similar dose-response relationship as the $G\alpha_{15}/G\alpha_{16}$-dependent stimulation of phospholipase C via both receptors (Fig. 3). This shows that the activated β_2 and the M_2 receptor regulated the intracellular cAMP content in COS-7 cells with a very similar efficacy as they induced inositol phosphate formation when cotransfected with $G\alpha_{15}$ and $G\alpha_{16}$ into COS-7 cells.

To further determine the spectrum of receptors able to activate G_{15} and G_{16}, we cotransfected COS-7 cells with cDNAs of a variety of different receptors alone or together with $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, or $G\alpha_{16}$. We then measured the effect of increasing agonist concentrations on inositol phosphate formation. Fig. 4 shows the results obtained with three receptors which are naturally coupled to the stimulation of adenylyl cyclase, the vasopressin V_2, the dopamine D_{1A}, and the adenosine A_{2A} receptor (23, 30–32). When these receptors were expressed alone, a small dose-dependent increase in the inositol phosphate formation could be observed. In cells coexpressing the receptors and $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{14}$, the ligand-dependent inositol phosphate production was the same as in cells transfected with the receptors alone. However, when the vasopressin V_2, the dopamine D_{1A}, and the adenosine A_{2A} receptors were coexpressed with $G\alpha_{15}$ or $G\alpha_{16}$, the ligand-dependent inositol phosphate formation increased severalfold.

We then tested the ability of $G\alpha_{15}$ and $G\alpha_{16}$ to couple receptors, which activate G_i and G_o proteins, to the production of inositol phosphates (Fig. 5). The μ opioid receptor, the 5-HT_{1A} receptor, and the fMLP receptor, which have been shown to activate G-proteins of the G_i family (33–35), also mediated a small increase in the formation of inositol phosphates when expressed alone. No significant increase in the ligand-dependent inositol phosphate formation was observed when the receptors were coexpressed with $G\alpha_q$ or $G\alpha_{11}$. Coexpression of the fMLP or the 5-HT_{1A} receptor but not the μ opioid receptor and $G\alpha_{14}$ slightly increased the ligand-dependent inositol phosphate production. Ligand-induced release of inositol phosphates was again markedly increased when the receptors were coexpressed with $G\alpha_{15}$ and $G\alpha_{16}$, indicating that the receptors can couple to $G\alpha_{15}$ and $G\alpha_{16}$, but not to $G\alpha_q$ or $G\alpha_{11}$.

To prove that receptors which activate phospholipase C in a pertussis toxin-insensitive manner by coupling to $G\alpha_{q/11}$ can also activate $G\alpha_{15}$ and $G\alpha_{16}$, we expressed the thrombin, the thromboxane A_2, the vasopressin V_{1A}, and the 5-$HT_{1C/2C}$ receptors (24, 36–38) together with $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$ in COS-7 cells (Fig. 6). Since COS cells express $G\alpha_q$ and $G\alpha_{11}$ (13), the effects of cotransfected $G\alpha_q$ family members on the inositol phosphate formation mediated by phospholipase C-linked receptors is much weaker, as the receptors can interact with endogenous $G\alpha_q$ and $G\alpha_{11}$. Nevertheless, the tested phospholipase C-coupled receptors mediated the stimulation of inositol phosphate production, and the ligand-dependent portion could be significantly increased when the receptors were coexpressed with $G\alpha_q$ family members, including $G\alpha_{15}$ and $G\alpha_{16}$.

Thus, different receptors which couple to G_s, G_i, and G_q family members can be functionally linked to endogenous phospholipase C when coexpressed with $G\alpha_{15}$ and $G\alpha_{16}$ in COS cells, indicating that they all activate $G\alpha_{15}$ and $G\alpha_{16}$.

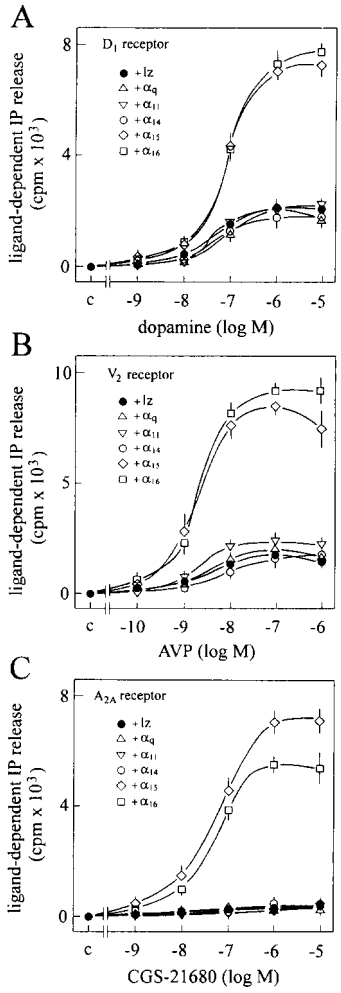

FIG. 4. **Formation of inositol phosphates in COS-7 cells coexpressing vasopressin V_2, dopamine D_1, or adenosine A_{2A} receptors and G-protein α-subunits.** COS-7 cells were cotransfected with cDNAs encoding the dopamine D_1 receptor (*A*), the vasopressin V_2 (*B*), or the adenosine A_{2A} receptor (*C*) and cDNAs encoding β-galactosidase (●, lz) or α-subunits of the $G\alpha_q$ family, $G\alpha_q$ (Δ, α_q), $G\alpha_{11}$ (▽, α_{11}), $G\alpha_{14}$ (○, α_{14}), $G\alpha_{15}$ (◇, α_{15}) and $G\alpha_{16}$ (□, α_{16}) as described under "Experimental Procedures." Cells were then incubated with the indicated concentrations of dopamine (*A*), [Arg⁸]vasopressin (*AVP*; *B*), and CGS-21680 (*C*), and the ligand-dependent inositol phosphate formation was determined as described. Shown are mean values of triplicates ± S.D.

DISCUSSION

In this paper, we demonstrate that $G\alpha_{15}$ and $G\alpha_{16}$ are capable of coupling a variety of receptors to the stimulation of inositol phosphate formation when coexpressed with the receptor in COS-7 cells. Receptors which are not linked to a pertussis toxin-insensitive regulation of phospholipase C under physiological conditions mediated little increase in inositol phosphate formation when expressed alone. This increase was not altered by coexpression of $G\alpha_q$ and $G\alpha_{11}$. In some cases, coexpression of $G\alpha_{14}$ led to a slight increase of the basal ligand-dependent inositol phosphate formation (Fig. 5). All receptors tested gained the ability to mediate a severalfold increase in the inositol phosphate production when they were coexpressed with $G\alpha_{15}$ and $G\alpha_{16}$, indicating that they functionally interacted with $G\alpha_{15}$ and $G\alpha_{16}$. In addition, the inositol phosphate formation mediated by several receptors which are physiologically linked to phospholipase C in a pertussis toxin-insensitive manner, could be increased by coexpression of $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15}$, and $G\alpha_{16}$. Thus, these receptors were able to act through all the transfected Gα subunits.

The receptors shown to activate $G\alpha_{15}$ and $G\alpha_{16}$ represent a wide spectrum of structurally and functionally different ligand binding proteins, demonstrating that $G\alpha_{15}$ and $G\alpha_{16}$ can be activated by a wide variety of receptors which serve very different functions under physiological conditions. This appears to be a unique feature of $G\alpha_{15/16}$; other G-proteins usually are selectively activated by a defined spectrum of receptors. A certain degree of specificity in the receptor G-protein interaction is regarded as a prerequisite for proper signal transduction. Thus, a G-protein which can nonselectively link functionally different receptors in a given cell to the same effector would be thought to produce inappropriate signaling and block specific intracellular information processing pathways. However, $G\alpha_{15}$ and $G\alpha_{16}$ exhibit very restricted expression patterns. Expression has only been shown in a subset of hematopoietic cells, and especially at premature stages in different cell lineages (11, 39). Therefore, coupling of most of the tested receptors to $G\alpha_{15}$ and $G\alpha_{16}$ in the COS cell system is presumably without direct physiological significance, since many of these receptors and $G\alpha_{15}$ and $G\alpha_{16}$ may not be coexpressed *in vivo*.

The chemokine receptors for C5a and interleukin 8 have been shown to activate $G\alpha_{16}$, but not $G\alpha_q$ and $G\alpha_{11}$ (17–19), and they are known to be expressed in mature cells of the immune system, especially in leukocytes. Therefore, they have been implicated in the physiological regulation of $G\alpha_{15/16}$ activity. Whereas chemokine receptors for interleukin 8, C5a, and fMLP can undoubtedly couple to $G\alpha_{15}$ and $G\alpha_{16}$ (17–19, Fig. 5), our current work indicates that this ability is not restricted to this receptor class, but rather due to the unique properties of $G\alpha_{15/16}$. In addition, expression of chemokine receptors and $G\alpha_{15/16}$ seems to be regulated in a reciprocal manner during leukocyte development. For example, in undifferentiated HL-60 cells which express high levels of $G\alpha_{16}$, chemokine receptors are absent or at low levels, whereas chemokine receptor expression increases and the expression of $G\alpha_{16}$ dramatically decreases during differentiation of HL-60 cells (11, 40–42). The effects of chemokine receptors in myeloid cells are mainly pertussis toxin-sensitive (43–45), indicating that they are mediated by G_i-type G-proteins. Recently it has been shown that the fMLP receptor primarily functions through pertussis toxin-sensitive G-proteins even when stably expressed in undifferentiated HL-60 cells where $G\alpha_{16}$ is present (46). Chemokine receptors probably interact with $G\alpha_{15}$ and $G\alpha_{16}$ under some conditions *in vivo*; it is, however, tempting to speculate that the main receptors physiologically coupled to $G\alpha_{15}$ and $G\alpha_{16}$ are still to be identified. They might be involved in the regulation of growth and differentiation of hematopoietic cells, and $G\alpha_{15/16}$ may allow the coupling of diverse receptors to the stimulation of phospholipase C.

Since a variety of receptors can activate $G\alpha_{15}$ and $G\alpha_{16}$ while being unable to activate $G\alpha_q$ and $G\alpha_{11}$, the question arises of which structural determinants of $G\alpha_{15}$ and $G\alpha_{16}$ are responsible for their ability to become activated by many different receptors. The carboxyl-terminal 55 amino acids of $G\alpha_{15}$ and $G\alpha_{16}$ are most divergent from $G\alpha_q$, $G\alpha_{11}$, and $G\alpha_{14}$ (9–11). This region includes the very carboxyl terminus of the α-subunit which has been shown to affect receptor specificity (47, 48). Interestingly, $G\alpha_{15}$ and $G\alpha_{16}$ possess a unique insert of several amino acids including different charged residues between helix α4 and helix α5 which are just adjacent to a region homologous to residues 311–329 of transducin (10, 11, 49). This region of transducin has also been implicated in the interaction with receptor (50). However, the situation appears to be more complicated as shown by a recent study in which different chimeras between $G\alpha_{11}$ and $G\alpha_{16}$ were examined for their ability to

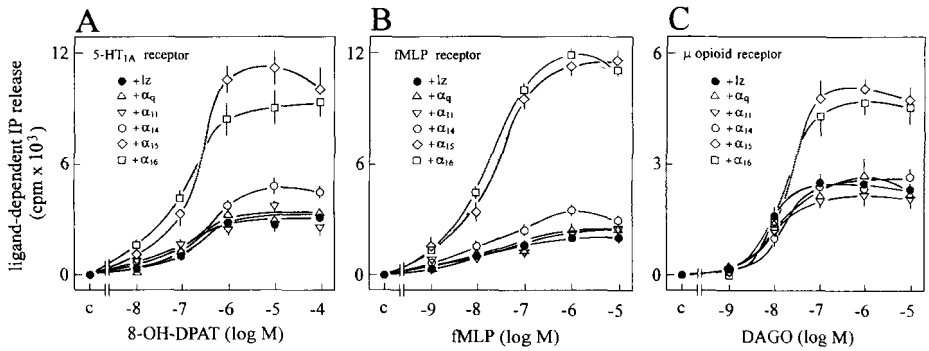

FIG. 5. **Accumulation of inositol phosphates in COS-7 cells cotransfected with cDNAs encoding the 5-HT_{1A}, the fMLP or the μ opioid receptor, and G-protein α-subunits.** COS-7 cells were cotransfected with cDNAs encoding the 5-HT_{1A} (*A*), the fMLP (*B*), or the μ opioid receptor (*C*) and cDNAs encoding β-galactosidase (●, lz) or α-subunits of the $G\alpha_q$ family, $G\alpha_q$ (Δ, α_q), $G\alpha_{11}$ (▽, α_{11}), $G\alpha_{14}$ (○, α_{14}), $G\alpha_{15}$ (◇, α_{15}), and $G\alpha_{16}$ (□, α_{16}) as described under "Experimental Procedures." Cells were then incubated with increasing concentrations of 8-hydroxy-2-(di-*n*-propylamino)tetralin (*A*), fMLP (*B*), and [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin (*C*), and the ligand-dependent inositol phosphate formation was determined as described. Shown are mean values of triplicates ± S.D.

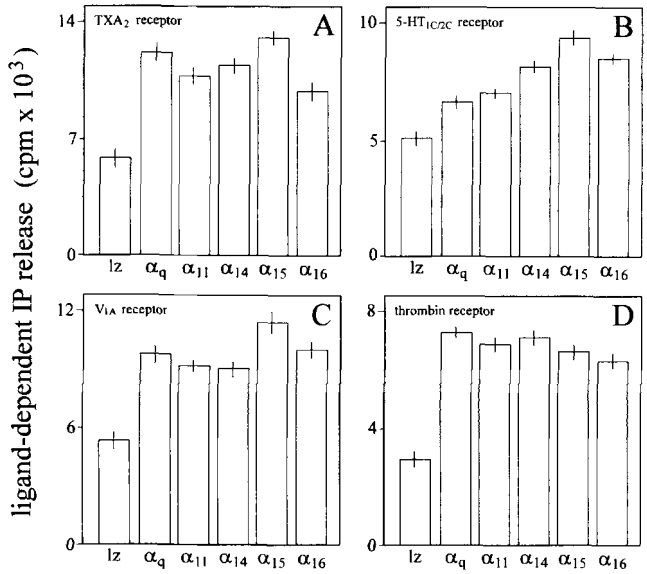

FIG. 6. **Formation of inositol phosphates in COS-7 cells coexpressing thromboxane A_2, 5-$HT_{1C/2C}$, vasopressin V_{1A} or thrombin receptors, and G-protein α-subunits.** COS-7 cells were cotransfected with cDNAs encoding the thromboxane A_2 receptor (*A*), the 5-$HT_{1C/2C}$ (*B*), the vasopressin V_{1A} receptor (*C*), or the thrombin receptor (*D*) and cDNAs encoding β-galactosidase (*lz*) or α-subunits of the $G\alpha_q$ family, $G\alpha_q$ (α_q), $G\alpha_{11}$ (α_{11}), $G\alpha_{14}$ (α_{14}), $G\alpha_{15}$ (α_{15}), and $G\alpha_{16}$ (α_{16}) as described under "Experimental Procedures." Cells were then incubated in the absence or presence of 3 μM U46619 (*A*), 10 μM serotonin (*B*), 1 μM [Arg^8]vasopressin (*C*), or 3 units/ml thrombin (*D*), and the ligand-dependent inositol phosphate formation was determined as described. Shown are mean values of triplicates ± S.D.

interact with the C5a receptor, which couples to $G\alpha_{16}$ but not to $G\alpha_{11}$ (51). This study suggests that the carboxyl-terminal 133 amino acids of $G\alpha_{16}$ do not alone account for its ability to interact with the C5a receptor, and that multiple regions of $G\alpha_{16}$ are responsible for the functional difference between $G\alpha_{11}$ and $G\alpha_{16}$, including a segment which comprises residues 220–240 of $G\alpha_{16}$. Thus, there are obviously many domains of $G\alpha_{15}$ and $G\alpha_{16}$ which directly or indirectly may affect the structure of G-protein and in this way may modulate the specificity of receptor G-protein interaction.

The fact that $G\alpha_{15}$ and $G\alpha_{16}$ interact with a wide variety of receptors can be useful for understanding the molecular details of the receptor G-protein interaction, once more structural data pertaining to receptors and different G-protein α-subunits are available. In addition, $G\alpha_{15/16}$ promiscuity may facilitate the examination of orphan receptors whose ligands and signal transduction properties are unknown. Cotransfection of orphan receptors and $G\alpha_{15}$ or $G\alpha_{16}$ into COS cells and subsequent determination of phospholipase C activity can be a way to search for ligands independent of the physiological signaling properties of the receptor.

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