Distinct Roles for Ga2, Ga3, and Gβγ in Modulation of Forskolin- or Gs-mediated cAMP Accumulation and Calcium Mobilization by Dopamine D2S Receptors*

Mohammad H. Ghahremani‡‡‡, Peihua Cheng‡, Paola M. C. Lembo¶, and Paul R. Albert¶

From the ‡Department of Pharmacology and Therapeutics, McGill University, Montreal H3G 1Y6, Canada and the ¶Neuroscience Research Institute, University of Ottawa, Ottawa K1H 8M5, Canada

Previous studies have shown that a single G protein-coupled receptor can regulate different effector systems by signaling through multiple subtypes of heterotrimeric G proteins. In LD2S fibroblast cells, the dopamine D2S receptor couples to pertussis toxin (PTX)-sensitive Gi/Go proteins to inhibit forskolin-or prostaglandin E1-stimulated cAMP production and to stimulate calcium mobilization. To analyze the role of distinct Gi/o protein subtypes, LD2S cells were stably transfected with a series of PTX-insensitive Gi/o protein Cys→Ser point mutants and assayed for D2S receptor signaling after PTX treatment. The level of expression of the transfected Ga mutant subunits was similar to the endogenous level of the most abundant Gi/o proteins (Ga1, Ga2, Ga3). D2S receptor-mediated inhibition of forskolin-stimulated cAMP production was retained only in clones expressing mutant Ga2. In contrast, the D2S receptor utilized Ga3 to inhibit PGE1-induced (Gα-coupled) enhancement of cAMP production. Following stable or transient transfection, no single or pair set of mutant Gi/o subtypes rescued the D2S-mediated calcium response following PTX pretreatment. On the other hand, in LD2S cells stably transfected with GRK-CT, a receptor kinase fragment that specifically antagonizes Gβγ subunit activity, D2S receptor-mediated calcium mobilization was blocked. The observed specificity of Ga2 and Ga3 for different states of adenyl cyclase activation suggests a higher level of specificity for interaction of Ga subunits with forskolin-versus Gi/o activated states of adenyl cyclase than has been previously appreciated.

A wide variety of physiological functions and pathological conditions are regulated by hormones and neurotransmitters which transduce intracellular signals by coupling to heterotrimeric guanine nucleotide-binding proteins (G proteins). Upon receptor activation, G proteins dissociate into Ga and Gβγ subunits which in turn regulate the activity of effector molecules (1–3). The family of Ga subunits is divided into structural and functional homologues, for example, Ga proteins couple positively to AC to increase intracellular production of cAMP; Ga proteins couple negatively to AC and are inactivated by PTX; and Ga proteins couple to PLC-β subtypes to increase [Ca2+]i, and are insensitive to PTX. The Gβγ subunits of G proteins couple to a variety of cell-specific effectors including AC types II and IV, PLC-β2 and PLC-β3, inwardly rectifying potassium channels, and N-type calcium channels (4, 5). In addition, G protein-coupled receptors appear to utilize particular combinations of subunits to initiate specific types of responses (6).

The dopamine D2S receptor couples to PTX-sensitive G proteins (Gαi/o) to initiate multiple signaling pathways (7, 8). In cells of neuroendocrine origin the D2S couples to “inhibitory” pathways, including inhibition of adenyl cyclase, activation of potassium channels to hyperpolarize the cell membrane, and inhibition of L-type calcium channels (9–12), which in concert mediate inhibition of hormone secretion and gene transcription, and inhibition of cell proliferation (13–18). By contrast, when expressed in cells of mesenchymal lineage (e.g. Ltk–fibroblast or Chinese hamster ovary cells), the same receptor mediates stimulation of phospholipase C activity to induce calcium mobilization, and activation of the mitogen-activated protein kinase cascade leading to enhanced gene transcription and cell proliferation (8, 14, 17, 19–23). These findings suggest that the same receptor mediates different cellular responses depending on the repertoire of cell-specific effectors that are expressed. To address the pathways that underlie cell-specific signaling we have studied the G protein specificity of D2S receptor coupling, based on the hypothesis that different G protein subunits mediate receptor coupling to inhibitory versus stimulatory signaling events.

PTX acts to uncouple Gi/o proteins by ADP-ribosylating these subunits at a conserved carboxyl-terminal domain cysteine (Cys) residue (24). By mutating the conserved Cys residue in Ga1, Ga2, Ga3, and Ga to a ribosylation-resistant serine (Ser) residue we have generated a series of PTX-insensitive mutants of Ga protein (G-PTX). Because the Cys residue is a structurally conservative change, the mutant G proteins remain functional following PTX pretreatment (25–28). We have assessed the contribution of individual or specific combinations of Ga protein subunits to D2S-mediated signaling. The D2S receptor utilizes distinct single Ga subunits to inhibit cAMP accumulation depending on the method of AC activation. In contrast, calcium mobilization induced by the D2S receptor is not reconstituted with single or combinations of Ga subunits.
but is blocked by inhibiting Gβγ function. These results indicate a strong dependence on Gα subtype for D2S-mediated inhibition of AC that is not observed for stimulation of calcium mobilization.

**EXPERIMENTAL PROCEDURES**

**Materials**—Apolimorphine, dopamine, EGTA, forskolin, PGE_2, isobutylmethylxanthine, and PTX were from Sigma. Fura-2-AM was purchased from Molecular Probes (Eugene, OR) and hygromycin B from Gibco. HEPES and all other chemicals were from Sigma. Media, sera, and Geneticin (G418) were obtained from Life Technologies, Inc. Plasmids pY3 and pCMV-LaCl II were obtained from the American Type Culture Collection (Manassas, VA). Endonucleases and DNA polymerase were purchased from New England Biolabs (Mississauga, Ontario, Canada). Taq polymerase has been purchased from Pharmacia Biotech Inc. (Baie d‘Urfe, QB). The cDNAs encoding wild-type rat Goα, Goγ, and Goγ were generously provided by Dr. Randall Reed, Johns Hopkins University, Baltimore, MD. The Goα antibody was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA) and anti-Goα-1,2 and anti-Goγ were obtained from Calbiochem (San Diego, CA). Anti-RGS-His antibody was purchased from Qiagen (San Clarita, CA).

**Expression of Ltk**—cells stably transfected with rat dopamine D2S receptor (LD2S) (13) were maintained in minimum Eagle’s medium (MEM) with 10% FBS in a humidified atmosphere of 5% CO_2, 95% air at 37 °C. The cells were routinely passaged using 0.05% trypsin, 0.02% EDTA in HBBS. For PTX treatment, the cells were treated with 50 ng/ml PTX for 16 h prior to experimentation.

**Site-directed Mutagenesis**—Site-directed mutagenesis was performed using the Altered Sites ITM system (Promega). PTX-insensitive Goα mutants were generated using rat cDNAs (29) encoding Goα1, Goα2, and Goγ3 subunits. The cyteline 351 codon (352 for Goα2) i.e. TGT, was mutated to TCT in order to encode serine using the following oligonucleotides: Goα-PTX, TCCGGGGCTCTGGCTTGTA; Goγ1-PTX, AACCTAAAGACTCTGGTC; Goγ2-PTX, ATCAACTGGAAGGACTCTGG; and Goγ3-PTX, AAGGAACTGGGCCCTTACTA. The mutation was confirmed by endonuclease restriction analysis and Sanger dideoxynucleotide chain termination using modified T7 DNA polymerase (Pharmacia Biotech Inc.).

**His-GRK-CT Construct**—The OK-GRK2 cDNA (30) was partially digested with BstEI and EcoRV endonucleases and the 1506-bp fragment encoding the C-terminal domain was subcloned from the EcoRV site of pcDNA3 (Invitrogen) mammalian expression vector in the cyteline pattern promoter. His-GRK-CT-His-GRK-CT construct—The OK-GRK2 cDNA (30) was partially digested with BstEI and EcoRV endonucleases and the 1506-bp fragment encoding the C-terminal domain was subcloned from the EcoRV site of pcDNA3 (Invitrogen) mammalian expression vector in the cyteline pattern promoter. His-GRK-CT-His-GRK-CT construct was confirmed by DNA sequencing.

**Transfected**—LD2S cells plated at 50% confluence were co-transfected with 30 μg each of the mutant Goα subunits (Goα-PTX, G1-PTX, G2-PTX, and G3-PTX) and 2 μg of pY3 using calcium phosphate co-precipitation method (32) and cultured in MEM, 10% FBS containing 400 μg/ml hygromycin-B for 2–3 weeks. Antibiotic-resistant clones of each transfection were picked (24 clones/transfection) and tested for the expression of corresponding Goα proteins using Northern blot analysis.

**Stable Transfection**—LD2S cells plated at 50% confluence were co-transfected with 30 μg each of the mutant Goα subunits (G1-PTX, G1-PTX, G2-PTX, and G3-PTX) and 2 μg of pY3 using calcium phosphate co-precipitation method (32) and cultured in MEM, 10% FBS containing 400 μg/ml hygromycin-B for 2–3 weeks. Antibiotic-resistant clones of each transfection were picked (24 clones/transfection) and tested for the expression of corresponding Goα proteins using Northern blot analysis.

**CaMP Measurement**—Equivalent numbers of cells were plated in 6-well plates and grown to 70–80% confluence. After rinsing with HBBS buffer (118 mM NaCl, 4.6 mM KCl, 1.0 mM CaCl_2, 10 mM glucose, and 20 mM Hepes, pH 7.2) the cells were incubated with or without experimental compounds in 1 ml/well of HBBS + 100 μM isobutylmethylxanthine at 37 °C. After 20 min the media were recovered and stored at −20 °C. Samples were analyzed by specific radiomunoassay to detect cAMP (34). Percent inhibition was calculated using the following formula: % inhibition = 100 − [(100/EM)/(S/Cl)] × 100, where D = cAMP in dopamine-treated cells; C = control or nontreated cells (basal cAMP); S = stimulated cAMP in forskolin- or PGE_2-treated cells.

**Measurement of [Ca^{2+}]_{i}**—Cells were grown to 80% confluence, harvested with trypsin/EDTA, resuspended in 1 ml of HBBS with 2 μM Fura-2 AM and incubated at 37 °C for 45 min with shaking (100 rpm). The cells were washed twice with HBBS, resuspended in 2 ml of HBBS, and subjected to fluorometric measurement. The fluorescence ratio of emission at 510 nm to 440 nm (F510/F440) was monitored in a Perkin-Elmer LS-50 spectrofluorometer at an excitation wavelength of 340/380 nm and an emission wavelength of 510 nm. Calibration was done using 0.1 μM Triton-X 100 and 20 mM Tris base to determine R_{min} and 10 mM EGTA (pH > 8) to obtain R_{max} (34) and the fluorescence ratio was converted to [Ca^{2+}]_{i} (34), based on a K_{D} of 227 nM for the Fura 2-calcium complex. Experimental compounds were added directly to cuvette from 100-fold concentrated solutions at the times indicated in the figure.
Distinct Roles for $\text{G}_\alpha2$, $\text{G}_\alpha3$, and $\text{G}_\beta\gamma$

RESULTS

Expression of Mutant $\text{G}_\alpha/o$ Subtypes in LD2S Cells—In order to investigate the importance of individual $\text{G}_\alpha$ subtypes in dopamine-mediated responses, PTX-insensitive mutants of $\text{G}_\alpha/o$ were generated and stably transfected into LD2S cells (Ltk$^-$ cells stably transfected with the rat D2S receptor cDNA). Transfected clones expressing the highest levels of individual mutant $\text{G}_\alpha/o$ RNA were identified by Northern blot analysis (data not shown) and were named $\text{RG}_i$, $\text{RG}_1$, $\text{RG}_2$, and $\text{RG}_3$ for clones expressing $\text{G}_\alpha/i$, $\text{G}_\alpha/1$, $\text{G}_\alpha/2$, and $\text{G}_\alpha/3$, respectively. Cell extracts from clones of interest were subjected to Western blot analysis to assess the protein level the overexpression of $\text{G}_\alpha$ proteins (Fig. 1). Wild-type LD2S cells expressed all four $\text{G}_\alpha/o$ subunits, although $\text{G}_\alpha/o$ and $\text{G}_\alpha/3$ appeared to be the most abundant based on densitometric analysis. Comparison of $\text{G}_\alpha/o$ and $\text{G}_\alpha/3$ expression in each transfectant to LD2S (wild type) indicates that transfectant cell lines expressed approximately 2-fold more than the corresponding endogenous $\text{G}_a$ subunit. This indicates that approximately equal amounts of mutant and wild-type protein were produced in the transfected cell lines.

$\text{G}_\alpha2$-PTX Mediates D2S-induced Inhibition of Forskolin-stimulated cAMP Accumulation—In LD2S cells, dopamine did not alter the basal cAMP production (21). Upon addition of forskolin (10 $\mu$M), cAMP levels were increased by 4.5-fold compared with basal (2.22 ± 0.17 versus 0.50 ± 0.04 pmol/ml) (Fig. 2A). Dopamine (10 $\mu$M) inhibited forskolin-stimulated cAMP accumulation in these cells by 84.5 ± 12.2% (n = 5), an action that was mimicked by apomorphine (1 $\mu$M, not shown) and was largely reversed by pretreatment with PTX (Fig. 2A), indicating the involvement of $\text{G}_\alpha/o$ proteins. PGE$_1$ has been shown to induce a concentration-dependent increase in cAMP production indicating the presence of endogenous $\text{G}_\alpha$-coupled PGE$_1$ receptors (30). In LD2S cells, PGE$_1$ (1 $\mu$M) increased cAMP accumulation by 7–8-fold basal cAMP (1.65 ± 0.25 versus 0.196 ± 0.002 pmol/ml) (Fig. 2B). The greater effect of PGE$_1$ may be related to the specific isoforms of adenylyl cyclase present in LD2S cells. Activation of D2S receptors with apomorphine (1 $\mu$M) inhibited PGE$_1$-induced cAMP production by 66.3 ± 7.3% (Fig. 2B) and this action of apomorphine was largely reversed after PTX treatment, implicating $\text{G}_\alpha/o$ proteins.

The PTX sensitivity of dopamine-mediated inhibition of forskolin-induced cAMP production was examined in wild-type LD2S and stable clones expressing the mutant $\text{G}_\alpha/o$ subunits. Dopamine inhibited forskolin-stimulated cAMP accumulation in all clones expressing mutant $\text{G}_\alpha/o$ proteins, as observed in LD2S cells (wild type). However, PTX blocked dopamine action in all clones except for those clones which express the mutant $\text{G}_2$ subunits. In multiple experiments, the percent inhibition by dopamine of forskolin-stimulated cAMP accumulation was unaltered by PTX in only $\text{RG}_2$-3 and $\text{RG}_2$-4 clones, whereas in other clones a significant attenuation of dopamine action by PTX was observed (Fig. 3). These results indicate that the PTX-insensitive mutant of $\text{G}_2$ is functional and that $\text{G}_2$ is the only subunit required for D2S-mediated inhibition of forskolin-induced cAMP production in LD2S cells.
Ga3-PTX Mediates D2S Inhibition of PGE1-stimulated cAMP Production—The ability of D2S receptor activation to inhibit Gs-coupled stimulation of cAMP accumulation was tested in the LD2S clones expressing PTX-insensitive Ga3i1 mutants. In these clones PGE1 (1 μM) induced a 7–8-fold increase in basal cAMP and apomorphine inhibited PGE1-stimulated cAMP production by 60–70%, comparable to wild-type LD2S cells. Upon pretreatment with PTX, apomorphine-mediated inhibition was completely reversed in RGi3-2, RG3-1–5, and RG3-1–10, expressing G1-PTX, RG2-3 and RG2-4, expressing G2-PTX; RG3-2, expressing G3-PTX.

Adenylyl Cyclase Expression in LD2S Cells—To further investigate the role of AC expression in LD2S cells, we performed semi-quantitative reverse transcriptase-PCR to determine the relative expression of AC subtypes I–VI, since their regulation has been well characterized compared with other subtypes (VII–X) (21, 35–37). The PCR was performed with different primers specific for each AC subtype.
concentrations of cDNA (0.1, 0.5, and 1.0 \( \mu \)g/reaction) and repeated at least twice for each concentration. Each PCR reaction amplified a single, specific product with the predicted size for each AC subtype, and the sequence was confirmed by sequencing the subcloned fragment. The specificity of the primers used was not altered with change in cDNA concentration, but the intensity of the product increased with concentration (Table I). Mouse brain RNA was used as positive control and was found to express all the subtypes of AC (data not shown). In LD2S cells, RNA for AC I and VI was expressed more abundantly than AC III, AC IV, and AC II (ACI > ACVI > ACIII > ACIV > ACII) and AC V RNA was not detected in these cells (Table I). These results indicate that LD2S cells express AC subtypes at different levels, which may direct the specificity of signaling through AC in these cells.

**G_{i/o}** Protein Subtypes Involved in Calcium Mobilization in LD2S Cells—In LD2S cells, the D2S receptor couples to PI turnover to induce mobilization of calcium from ionomycin-sensitive intracellular stores (8). In LD2S cells dopamine induced a 2–2.5-fold increase in \( [\text{Ca}^{2+}]_{i} \) (Fig. 5) which was blocked by the D2 receptor antagonist spiperone and was not observed in D2 receptor-negative Ltk— cells (data not shown), indicating that this effect is mediated by the D2S receptor. The increase in \( [\text{Ca}^{2+}]_{i} \), induced by dopamine was completely inhibited by PTX pretreatment, suggesting mediation via G_{i/o} proteins.

Each of the clones expressing mutant G proteins responded to dopamine with a 2–2.5-fold increase in \( [\text{Ca}^{2+}]_{i} \) (Fig. 6). Following pretreatment with PTX, none of the mutant G protein transfectants exhibited a D2S-mediated calcium response (Fig. 6). In order to test whether more than one G protein could rescue the calcium response, Ltk— cells were transfected with different pairs of the four G-PTX mutant constructs along with D2S receptor and assayed for \( [\text{Ca}^{2+}]_{i} \), by 1.6-fold (Fig. 6), similar to that in LD2S cells (33). This indicates that the Ltk— cells express sufficient levels of transiently transfected cDNAs to mediate a full functional response. However, after PTX treatment, none of the mutant combinations rescued the dopamine response (Fig. 6). In these experiments, the increase of \( [\text{Ca}^{2+}]_{i} \), induced by 100 \( \mu \)M ATP served as a positive control for cellular responsiveness and was unchanged following PTX pretreatment, since the ATP response is mediated through a G_{q} coupled P2-purinergic receptor. Similarly, in LD2S cells transiently transfected with pairs of G-PTX plasmids, PTX pretreatment blocked completely the dopamine-mediated calcium responses for all combinations (data not shown). The dopamine response was also tested in another series of transfections in which a double dose (60 \( \mu \)g) of single G-PTX plasmid was transiently transfected in LD2S cells. As for the stable LD2S clones (see above), the single G-PTX did not mediate the D2S calcium response in these

**TABLE I**

Expression of different adenylyl cyclase subtypes in LD2S cells

| Adenylyl cyclase | cDNA concentration (\( \mu \)g/reaction) |
|-----------------|----------------------------------------|
|                 | 0.1 | 0.5 | 1.0 |
| ACI             | +   | +   | +   |
| ACII            | –   | –   | ±   |
| ACIII           | +   | ±   | +   |
| ACIV            | –   | ±   | ±   |
| ACV             | –   | –   | ±   |
| ACVI            | +   | +   | +   |

a The reverse transcriptase-PCR was performed for ACI-VI with 0.1, 0.5, and 1.0 \( \mu \)g of cDNA as indicated. The data have been obtained from at least two independent experiments for each condition. Specific primers for each subtype of AC amplified only a single product with the predicted size corresponding to the subtype they have been targeted to. Positive (+) and negative (−) signs indicate the presence and the absence of the specific product on ethidium bromide stained gels, respectively, and “±” represents a weakly detectable product. As a positive control, all primer pairs yielded same size products from mouse brain RNA (not shown).

**Fig. 5.** PTX blocks D2S-induced calcium mobilization in LD2S cells expressing PTX-insensitive single G_{i/o}-PTX mutants. Ltk— cells expressing D2S receptor (LD2S, A) and LD2S cells expressing G_{i2}-PTX (RG-1, B), G_{i1}-PTX (RG1–10, C), G_{i2}-PTX (RG2–4, D), and G_{i3}-PTX (RG3–2, E) mutant G proteins were treated without (solid line) or with (dash line) PTX (50 ng/ml, 16 h) and changes in intracellular \( [\text{Ca}^{2+}]_{i} \) in response to dopamine (10 \( \mu \)M) or ATP (10 \( \mu \)M) were measured.
transfections (data not shown). The protein level of the Ga1
and Ga2 was increased by more than 2-fold in Ltk- cells
transiently transfected by both of these proteins (Fig. 6,
inset). Based on these results, none of the Ga/o subunits, alone or in
combination, mediated calcium mobilization induced by do-
pamine in LD2S cells.

Gbγ Subunits Mediate D2S-induced Calcium Mobilization in
LD2S Cells—In order to investigate the role of Gbγ subunit of
Ga/o proteins in D2S-mediated increase in [Ca2+]i, LD2S cells
were stably transfected with the His6-tagged carboxyl-terminal
of GRK-2 (GRK-CT), which contains a pleckstrin homology
domain that is known to bind and inactivate free Gbγ subunits
(38). The relative level of His-GRK-CT protein in clones ex-
pressing His-GRK-CT was determined by Western blot using
an antibody against the His epitope (Fig. 7, inset). As shown in
Fig. 7, the dopamine-induced increase in [Ca2+]i was reduced
by 80% compared with LD2S cells in the clone expressing
His-GRK-CT (RD-21). In contrast, dopamine mediated inhibi-
tion of forskolin- and PGE1-stimulated cAMP accumulation
was not significantly different between LD2S and RD-21 cells
(data not shown). In another clone expressing lower levels
(20%) of His-GRK-CT, the increase in [Ca2+]i induced by dopamine was reduced by only 30% (data not shown).
These results suggest that D2S-mediated stimulation of [Ca2+]i
is mediated by Gbγ subunits and is more dependent on Gbγ
subunits than particular Ga subunits.

DISCUSSION
State-dependent Modulation of Adenylyl Cyclase via Distinct
Ga Proteins—The dopamine-D2S receptor is coupled to inhibition
of adenylyl cyclase in a wide variety of cell types (13, 14,
39–41). Indeed, inhibition of adenylyl cyclase by receptors that
couple to Ga/o appears to be a ubiquitous pathway (2, 5, 6). In
intact cells, adenylyl cyclase can exist in at least three states:
basal, forskolin-stimulated, or Gαo-stimulated (36). In Ltk-
cells, the D2S receptor inhibits cAMP production stimulated by
either forskolin or PGE1, but does not inhibit the basal level of
cAMP level (Fig. 2, (21)). By contrast, in pituitary cells the D2S receptor inhibits all three states, i.e. basal, forskolin-stimulated, and vasoactive intestinal peptide-stimulated cAMP accumulation (13, 14, 39). The basal levels of cAMP are at least 5-fold lower in Ltk- fibroblast cells as compared with GH4C1 pituitary cells (34), and perhaps it is already at a minimum level. Furthermore, our results show that Ltk- cells have an undetectable level of ACV, and ACII and IV are weakly expressed (Table I). This could explain why D2S receptor activation induced no change in basal cAMP production in LD2S cells. For example, the dopamine-D3 receptor appears to couple exclusively to ACV (42).

To address the G protein specificity of D2S receptor signaling, LD2S cells were transfected stably with individual PTX-insensitive mutants of Gαs subtypes and treated with PTX to inactivate endogenous Gαo proteins. When stimulated by forskolin, inhibition of cAMP accumulation by D2S receptor activation is mediated exclusively through the Gαo2 subtype (Fig. 3). This agrees with findings in pituitary cells. Using antibodies to Gαo subunits, Izenwasser and Cote (43) have reported that inhibition of cAMP accumulation by D2 receptors in pituitary tumor cells utilizes Gα1 and/or Gα2, since their antibody detects both subtypes equally. Furthermore, using PTX-insensitive mutants, Senogles (39) has shown that in GH4C1 cells expressing D2S receptor, D2S inhibition of forskolin-induced adenyl cyclase is routed through Gαo1. Our results indicate that in Ltk- fibroblast cells, the D2S receptor couples preferentially to the Gαo2 subtype to inhibit activation of adenyl cyclase by forskolin.

On the other hand, inhibition of PGE1-stimulated cAMP accumulation, a Gαo2-coupled AC pathway, by D2S receptor activation was mediated through Gα3 in the LD2S cells, and not by Gα2 as for forskolin (Fig. 4). This indicates that D2S receptor utilizes distinct Gαo proteins to mediate inhibition of AC depending on the pathway of activation of AC. This is consistent with previous findings in GH4C1 pituitary cells, in which antisense depletion of Gα2 only marginally reduced D2S-mediated inhibition of cAMP accumulation (44). However, when ACII was stimulated by calmodulin or forskolin, Gα1 mediated inhibition of ACI. This indicates that the extent of inhibition of a particular AC subtype (ACI) by Gα1 is dependent on the activation pathway. Following D2S receptor activation, Gα2 may also selectively inhibit the forskolin-activated state in AC subtypes that predominate in Ltk- cells, whereas Gα3 preferentially inhibits the Gα3-activated state. Distinct conformational changes in AC upon interaction with forskolin or Gα3 could explain the selective inhibition of Gα1 subtypes. The crystal structure of the catalytic domain of ACII reveals that forskolin binds to two symmetrical sites to prevent hydration and enhance dimerization of the C1-C2 domains (45). The binding site for Gα1o has not been determined, but it may bind to the a2a3 region of C1α, which is close to the catalytic domain, on the opposite site of the Gα3 site (46). In this case, Gα1o protein could alter the preferable alignment by blocking the "counterclockwise" rotation of C1 (47). In the forskolin-bound conformation, AC may preferentially recognize specific Gα1o protein subtypes distinct from those recognized by the Gα3-bound conformation of AC. Further structural studies may reveal the molecular basis for state-dependent G protein selectivity in inhibition of AC.

Gβγ and Calcium Mobilization—In LD2S cells transfected with single or pairs of PTX-insensitive Gβγ proteins cDNAs to yield a greater than 2-fold protein expression, dopamine failed to induce calcium mobilization after PTX treatment, suggesting that Gα subunits plays a minor or secondary role in this pathway. On the other hand, inhibition of Gβγ signaling in LD2S cells expressing GRK-CT correlated with an inhibition of
D2S-induced calcium mobilization, indicating that this process is mediated through Gβγ subunits rather than the Go subunit. This result is consistent with the fact that Gβγ subunits of Gi/o proteins can activate PLC-β2 and PLC-β3 (5). Recent results indicate that the D2S receptor increases calcium mobilization in LD2S cells via activation of PLC-β2 (33), implicating Gβγ-signaling in the calcium mobilization pathway. The lack of activity of individual Go subunits to mediate calcium mobilization was partly unexpected. In NG108-15 neuroblastoma cells, PTX-insensitive Gz signaling was partly unexpected. In NG108-15 neuroblastoma cells, PTX-insensitive Go, did mediate coupling to inhibition of calcium channel activation (25), a Gβγ-mediated response (5). It has been estimated that a 10-fold higher amount of Gβγ is required to activate PLC-β2 in vitro than is required for Go-mediated activation of AC (48). It may be that multiple Gα subtypes, rather than a single subtype, must be activated to release sufficient Gβγ subunits to induce calcium mobilization in LD2S cells.

Conclusion—The dopamine D2S receptor couples to Gα2 to inhibit forskolin-induced cAMP production. On the other hand, when AC is activated by a Gs-coupled receptor (PGE1 receptor), PLC-β2 and PLC-β3 are activated in LD2S cells, indicating that this process is mediated through Gi/o subunits rather than the Gα3 subunit. Therefore, the dopamine D2S receptor utilizes different Gα protein subunits to regulate a diversity of effector functions within the cell. Moreover, this study shows that the PTX-insensitive Gi/o mutants provide useful tools for the dissection of G protein coupling to receptors.

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