Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4062–4066, May 1993
Biochemistry

G_o mediates the coupling of the μ opioid receptor to adenylyl cyclase in cloned neural cells and brain

(G protein subtypes/μ and δ opioids/opioid tolerance/SH-SY5Y cells/rat brain membranes)

BRUCE D. CARTER AND FEDOR MEDZIHRADSKY*

Departments of Biological Chemistry and Pharmacology, The University of Michigan Medical School, Ann Arbor, MI 48109-0606

Communicated by J. L. Oncley, December 21, 1992 (received for review May 26, 1992)

ABSTRACT **In membranes from SH-SY5Y human neuroblastoma cells differentiated with retinoic acid, the μ-selective agonist Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol (DAMGO) inhibited cAMP formation with an IC_{50} of 26 nM. Two separate antibodies raised against distinct regions of the $G_{o\alpha}$ sequence attenuated the effect of DAMGO by 50–60%, whereas antibodies to $G_{i\alpha1,2}$ or $G_{i\alpha3}$ reduced the μ-opioid signal insignificantly or to a lesser extent. In contrast, inhibition of adenylyl cyclase by the δ-opioid agonist Tyr-D-Pen-Gly-Phe-D-Pen-OH (DPDPE; Pen = penicillamine) was very sensitive to the $G_{i\alpha1,2}$ antibody. In membranes from rat brain striatum, coupling of the μ opioid receptor to adenylyl cyclase was also maximally blocked by antibodies to $G_{o\alpha}$. After long-term treatment of the cells with DAMGO, the content of $G_{o\alpha}$ was reduced by 26%, whereas the levels of $G_{i\alpha1,2}$, $G_{i\alpha3}$, and $G_{s\alpha}$ were unaltered. Addition of G_o, purified from bovine brain, to membranes from pertussis toxin-treated SH-SY5Y cells restored the inhibition of adenylyl cyclase by DAMGO to 70% of that in toxin-untreated cells. To comparably restore the effect of DPDPE, much higher concentrations of G_o were required. By demonstrating mediation of cAMP-dependent signal transduction by G_o, these results describe (*i*) an additional role for this G protein present at a high concentration in brain, (*ii*) preferential, although not exclusive, interaction of μ and δ opioid receptors with different G protein subtypes in coupling to adenylyl cyclase, and (*iii*) reduced levels of G_o following chronic opioid treatment of SH-SY5Y cells with μ opioids.**

The coupling of many receptors, including the opioid receptors, to their effector is mediated by guanine nucleotide-binding proteins (G proteins) (1, 2). After the initial characterization of G_s and G_i based on their ability to stimulate or inhibit adenylyl cyclase, respectively, another inhibitory G protein, referred to as G_o, was described (3). The diversity of G proteins has continued to expand with the discovery of many subtypes of the α, β, and γ subunits that make up these heterotrimers. To date, three and four subtypes of $G_{i\alpha}$ (4) and $G_{o\alpha}$ (5), respectively, have been characterized and shown to exhibit considerable promiscuity in their interaction with receptors: a given receptor may interact with one of several G proteins, and one type of G protein can couple to different effectors. Thus, the α_2-adrenergic receptor couples to both $G_{i\alpha2}$ and $G_{i\alpha3}$ (6), whereas $G_{i\alpha2}$ transduces signals to both adenylyl cyclase (7) and K^+ channels (8). Although G_o interacts with phospholipase C (9) and Ca^{2+} (10) and K^+ (11) channels, no direct evidence has yet been presented for the involvement of this G-protein subtype in the transduction of the cAMP signal. On the other hand, G_o is present at a particularly high concentration in brain, where multiple receptor types couple to adenylyl cyclase (12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Several lines of evidence indicate that opioid receptors interact with different G proteins: in reconstitution studies both G_i and G_o restored the characteristics of the μ receptor (13, 14), whereas only G_i subtypes were effective in restoring the κ receptor (15). Purification of opioid receptor from rat brain yielded complexes containing either G_i or G_o (16) and, in the NG108-15 neuroblastoma × glioma hybrid cells, the δ receptor interacted with multiple subtypes of G proteins, including G_{i2}, G_o (17, 18), and G_{i3} (18). Although these studies demonstrate the capability of opioid receptors to couple to various G proteins, they do not identify those interactions that transduce the signal to a given effector.

Opioid receptors were shown to couple to the effectors adenylyl cyclase (19) and K^+ and Ca^{2+} channels (20). In the NG108-15 hybrid cells, the inhibition of adenylyl cyclase by δ opioids was mediated by G_{i2} (21), whereas the coupling of the δ opioid receptor to Ca^{2+} channels in these cells occurred through G_o (10). In SH-SY5Y neuroblastoma cells, μ agonists inhibited the calcium current in a naloxone-sensitive manner (22). However, the results of these and other studies recently reviewed (12) did not disclose a G protein that transduces the signal from the μ opioid receptor to adenylyl cyclase.

We have previously described the functional coupling of μ, δ, and κ opioid receptors to G proteins in brain membranes (23, 24) and have subsequently characterized the μ opioid receptor–G protein complex formed upon receptor occupancy by agonists (25). Recently, we have quantified the functional responses of G protein and adenylyl cyclase in normal (26) and opioid-tolerant (27) SH-SY5Y neural cells. In the present study, we identify G-protein subtypes that modulate the coupling of μ and δ opioid receptors to adenylyl cyclase. The results provide direct evidence for an additional function of G_o in cAMP-dependent signal transduction. A preliminary account of these findings has been presented (28).

MATERIALS AND METHODS

Materials. The anti-G protein antibodies AS/7, EC/2, RM/1, and GO/1 raised against the C terminus of $G_{i\alpha1,2}$, $G_{i\alpha3}$, $G_{\alpha s}$, and $G_{\alpha o}$, respectively, and GC/2 directed at the N-terminal region of $G_{\alpha o}$ were purchased from New England Nuclear. The μ-selective opioids D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP; Pen = penicillamine) (29) and Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol (DAMGO) were from Peninsula Laboratories and Sigma, respectively, whereas the δ opioids Tyr-D-Pen-Gly-Phe-D-Pen-OH (DPDPE) and ICI

Abbreviations: G protein, guanine nucleotide-binding protein; DAMGO, Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol; DPDPE, Tyr-D-Pen-Gly-Phe-D-Pen-OH; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2; G_s, G_i, and G_o, stimulatory, inhibitory, and other (inhibitory) G proteins; $G_{s\alpha}$, $G_{i\alpha}$, and $G_{o\alpha}$, α subunits of G_s, G_i, and G_o; Pen, penicillamine.

*To whom reprint requests should be addressed at the Department of Biological Chemistry.

174,864 (30) were obtained through the Narcotic Drug and Opioid Basic Research Center at the University of Michigan. The cAMP assay kit was purchased from Diagnostic Products (Los Angeles). Human neuroblastoma SH-SY5Y cells were kindly provided by June L. Biedler (Memorial Sloan–Kettering Cancer Center, New York). The two preparations of G_o, purified from bovine brain, were generous gifts from M. E. Linder and A. G. Gilman (University of Texas Southwestern Medical Center; preparation 1) and A. E. Remmers and R. R. Neubig (University of Michigan; preparation 2).

Cell Culture. The SH-SY5Y cells (passage 1–20) were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum in tissue culture flasks under 10% CO_2. For all experiments, cells differentiated with 10 μM retinoic acid were used (31). Opioid tolerance was induced by addition of 10 μM DAMGO to the medium on day 6 or 7 of differentiation. The cells were collected 24 hr later (27).

Membrane Isolation. The surface-growing cells were rinsed with phosphate-buffered saline and lifted by incubation with a Ca^{2+}- and Mg^{2+}-free modified Puck's solution for 10 min, and the membranes were isolated as described (26). The striatal region from rat brain was dissected at 4°C. The tissue was homogenized and further treated as described for the SH-SY5Y cells (26). The membranes from both sources were used immediately after isolation.

Adenylyl Cyclase Assay. As described (26), freshly isolated membranes (7.5 μg of protein per tube) were incubated in the presence or absence of opioid in a buffer medium (pH 7.4), containing ATP and its regenerating system, for 15 min at 37°C. The reaction was terminated by the addition of HCl, and the concentration of cAMP was determined by using a radioligand binding assay from Diagnostic Products.

Use of G Protein-Specific Antisera. The titers of the individual antisera were determined by correlating various dilutions with binding of the antibodies under conditions for the adenylyl cyclase assay. After incubating membranes with various dilutions of the antisera, the unbound antibody was removed by centrifugation at 16,000 × g for 5 min. The pellet was washed twice in phosphate buffer containing 100 mM NaCl and 1% bovine serum albumin, resuspended in the same buffer containing ^{125}I-labeled anti-rabbit antiserum, and incubated 45 min at 25°C. The membranes were then pelleted and washed twice as described above. The final pellet was dissolved in 10% SDS, and the radioactivity was determined by liquid scintillation counting. The antisera dilutions required for half-maximal binding were 1:1020 for AS/7, 1:2380 for GC/2, and 1:1500 for EC/2. Based on these titers, a 1:80 dilution was selected to effectively block all immunoreactive sites. A similar concentration of these antisera was used to prevent the coupling of other receptors to G protein (7, 21, 32).

Western Blotting. SH-SY5Y membranes prepared in Tris·HCl (pH 7.4) were subjected to SDS/10% PAGE (25–80 μg of protein per lane). After transfer to Immobilon (Millipore), the proteins were probed with a 1:1000 dilution of either the G protein-specific or nonimmune antisera described above using 3% nonfat dried milk as a blocking agent. Immunoreactivity was visualized using ^{125}I-labeled secondary antibodies and autoradiography. Quantitation was achieved by excising the radiolabeled bands and scintillation counting. The antibodies were used at protein concentrations that were within their linear range of effectiveness ($r^2 > 0.98$, based on three different concentrations). Protein concentration was determined according to the procedure of Lowry *et al.* (33).

Reconstitution with Purified G_o. The procedure employed was based on that described by Probiner *et al.* (34), who demonstrated the insertion of >80% of the added G protein into membranes of rat hepatocytes. After the exposure of SH-SY5Y cells to pertussis toxin at 100 ng/ml for 24–48 hr, membranes were prepared as described above, incubated for 20 min at 30°C with oligomeric G_o, and used as such. The adenylyl cyclase assay was implemented as described above. The two preparations of G_o were isolated from bovine brain as described (3, 35) and were 95% pure (preparation 1; M. E. Linder, personal communication) or had a G_o/G_i ratio of 10:1 (preparation 2; A. E. Remmers, personal communication). Since these preparations of G_o were solubilized in detergents, appropriate controls were carried out to assess possible effects of these agents on the observed phenomena.

Data Analysis. The dose–response curves of adenylyl cyclase assays were determined by the computer program GRAPHPAD operated in its nonlinear regression mode. The results of replicate experiments were analyzed simultaneously. The values for IC_{50} and maximum inhibition (I_{MAX}) were determined by fitting the data to an equation (on a logarithmic scale) that describes many dose–response functions: $Y = A + (B - A)/[1 + (10^X/10^C)^D]$, where Y is the percent inhibition, X is the logarithm of the ligand concentration, A is the minimum (zero) and B is the maximum of the curve (I_{MAX}), C is the IC_{50}, and D is the slope factor.

RESULTS

DAMGO inhibited cAMP formation in membranes from differentiated SH-SY5Y cells with an IC_{50} of 25.8 nM and an I_{MAX} of 47.2%. Exposure of the cells to 10 μM DAMGO for 24 hr increased the IC_{50} 4.5-fold (Fig. 1). Pertussis toxin treatment of the cells (100 ng/ml in the medium for 24 hr) prior to membrane preparation strongly attenuated the effects of DAMGO, thus implicating the involvement of G_i and/or G_o (Fig. 2). To determine which G proteins were mediating the inhibition, the membranes were initially incubated with nonimmune serum or with specific antibodies raised against different subtypes of G proteins (Fig. 3). Although the preincubation slightly attenuated both basal activity and maximum inhibition of adenylyl cyclase, the extent of enzyme inhibition by opioids was always determined relative to basal activity under identical conditions. While nonimmune serum did not significantly change the effect of DAMGO on adenylyl cyclase activity, the antibody to $G_{o\alpha}$, GC/2, attenuated enzyme inhibition by 50%, decreasing the maximum from 33.1% obtained in the presence of nonimmune serum to 16.5% (this antibody also reduced the inhibition of adenylyl cyclase by the opioid alkaloid agonist levorphanol by a similar extent). On the other hand, the

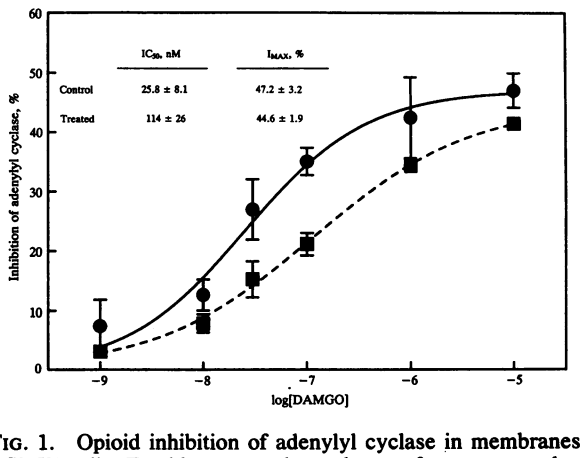

FIG. 1. Opioid inhibition of adenylyl cyclase in membranes of SH-SY5Y cells. Freshly prepared membranes from untreated cells (●) and cells exposed to 10 μM DAMGO for 24 hr (■) were incubated with various concentrations of DAMGO. Subsequently, the cAMP concentration was determined as described in *Materials and Methods*. The inhibition of adenylyl cyclase was related to the enzyme activity in membranes from untreated cells [29.7 ± 2.1 pmol cAMP per min per mg of protein (mean ± SEM, n = 14)]. The values depicted are the means and SEM obtained in four experiments.

antibody to $G_{i\alpha 1,2}$, AS/7, decreased the DAMGO signal by only 11%. The EC/2 antibody, specific for $G_{i\alpha 3}$, also attenuated the response of adenylyl cyclase, but the attenuation was less than that of GC/2 on $G_{o\alpha}$. Even the combined, additive effects of the two G_i-specific antibodies (AS/7 plus EC/2) were limited to a 38% reduction of the DAMGO signal (data not shown). The appropriate concentrations of the antisera were based on their titers, determined with saturation binding of the respective antibodies (Fig. 3, *Upper Inset*). To substantiate the involvement of G_o in mediating the inhibition of cAMP formation, another antibody to $G_{o\alpha}$, specific for a different sequence of the protein, was employed. The GO/1 antibody, which recognizes the C terminus, was equally effective as GC/2: it decreased opioid inhibition of adenylyl cyclase by 58% (n = 2) (data not shown). It has previously been ascertained that, under the conditions applied, the δ-selective antagonist ICI 174,864 does not alter the effect of DAMGO on adenylyl cyclase in SH-SY5Y cells (26, 27).

Signal transduction by δ opioids in SH-SY5Y cells was examined by incubating the membranes with DPDPE in the presence of the μ receptor antagonist CTOP at 1 μM, a concentration shown to prevent the interaction of 10 μM DPDPE with the μ receptor (Fig. 3, *Lower Inset*). Under these conditions, the $G_{i\alpha 1,2}$ antibody AS/7 was the most effective in inhibiting δ opioid signaling: it reduced the maximal DPDPE inhibition of adenylyl cyclase by 68.5%, followed by the $G_{o\alpha}$ and $G_{i\alpha 3}$ antibodies with 46.9% and 29.9% attenuation, respectively. In brain membranes (Fig. 4), maximal inhibition of cAMP formation by DAMGO, determined in the presence of ICI 174,864 to prevent interaction of the μ peptide with δ receptors, was 18.9%. This μ-selective signal decreased by 65% after incubation with the antibody to $G_{o\alpha}$, GC/2. In contrast, the antiserum to $G_{i\alpha 1,2}$ had no

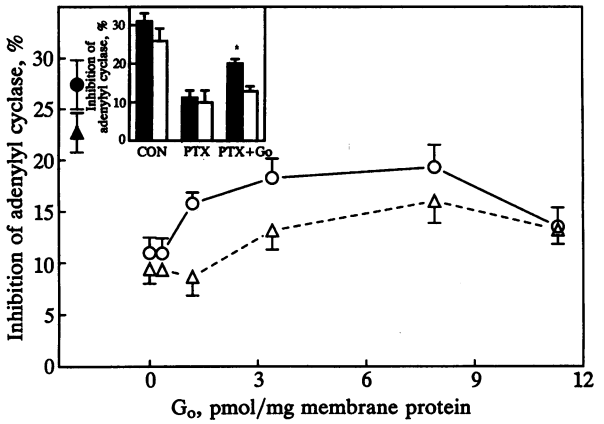

FIG. 2. Reconstitution of opioid inhibition of adenylyl cyclase using purified G_o. After treatment of SH-SY5Y cells with (open symbols) or without (closed symbols) pertussis toxin at 100 ng/ml for 24–48 hr, cell membranes were prepared and incubated with various concentrations of purified (bovine brain) heterotrimeric G_o present in 0.005% Lubrol (preparation 1). Subsequently, the inhibition of adenylyl cyclase by 1 μM DAMGO (○, ●) or 1 μM DPDPE in the presence of 1 μM CTOP (△, ▲) was determined. Shown are mean values (±SEM) from four to six experiments. (*Inset*) Inhibition of adenylyl cyclase by 1 μM DAMGO (solid bars) or 1 μM DPDPE plus 1 μM CTOP (open bars) in membranes from control cells (CON) or cells treated with pertussis toxin (PTX) at 100 ng/ml before and after reconstitution with purified G_o solubilized in 0.005% Lubrol and 0.004% cholate (preparation 2). Depicted is the maximum recovery of opioid signal, achieved with 8 pmol of G_o per mg of membrane protein. The mean values (±SEM) from four experiments are shown. The star indicates a significant difference ($P < 0.05$) in enzyme inhibition compared to that in membranes of toxin-treated cells with no G_o added. The control experiments were carried out in the presence of detergent.

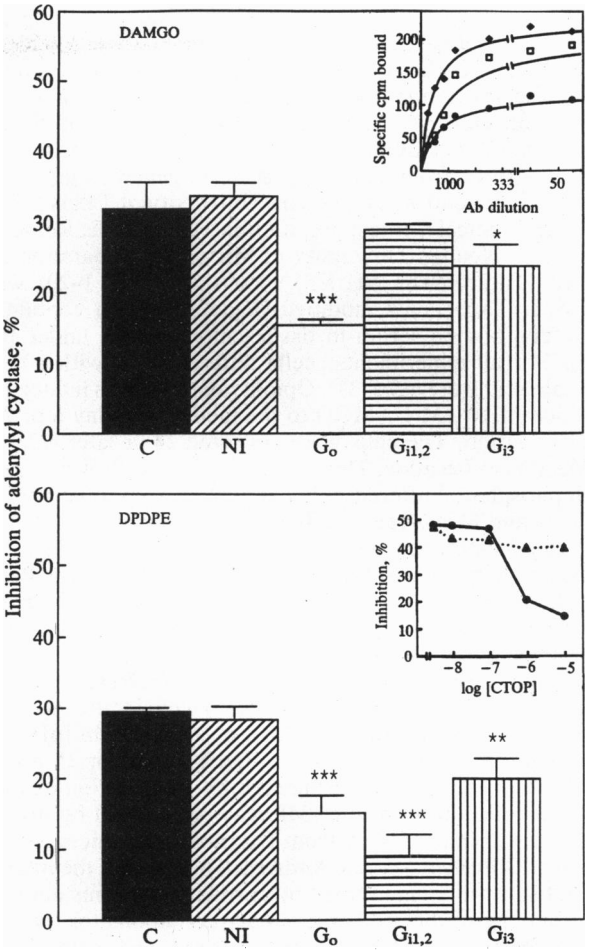

FIG. 3. Opioid inhibition of adenylyl cyclase in membranes of SH-SY5Y cells in the presence of G-protein antibodies. The membranes were preincubated with buffer (C), nonimmune serum (NI), or antisera (at their maximally effective concentration) raised against the α subunits of either G_o (GC/2), $G_{i1,2}$ (AS/7), or G_{i3} (EC/2). Subsequently, the membranes were incubated with 1 μM DAMGO (*Upper*) or 1 μM DPDPE in the presence of 1 μM CTOP (*Lower*), followed by the determination of cAMP. The inhibition of adenylyl cyclase was related to the enzyme activity in membranes from cells treated with nonimmune serum [21.0 ± 2.17 pmol cAMP per min per mg of protein (mean ± SEM, n = 12)]. The basal activity in the membranes preincubated with nonimmune serum was not significantly different from that in the untreated (control) membranes. Shown are the mean values and SEM obtained in three to six experiments. The stars indicate the significance of the mean values compared to that obtained with nonimmune serum: ★★★, $P < 0.01$; ★★, $P < 0.05$; ★, $P < 0.08$ (based on an unpaired, two-tailed t test). (*Upper Inset*) Saturation binding (specific cpm bound vs. antibody dilution) of AS/7 (□), GC/2 (◆), and EC/2 (●) to determine their titers. Presented are mean values obtained in three experiments. (*Lower Inset*) Inhibition of adenylyl cyclase in membranes of untreated SH-SY5Y cells by 200 nM DAMGO (●) or 1 μM DPDPE (▲) in the presence of varying concentrations of CTOP. Presented are data from a representative experiment replicated twice. Ab, antibody.

significant effect, and the antiserum specific for $G_{i\alpha 3}$ inhibited with marginal significance.

In membranes from SH-SY5Y cells exposed to DAMGO for 24 hr, which were shown to have desensitized receptor–effector coupling (Fig. 1), the quantity of $G_{o\alpha}$ was decreased by 25.8% relative to membranes from cells cultured in opioid-free medium (Fig. 5). None of the other tested G proteins showed a consistent pattern of change in opioid tolerance relative to control: the levels of $G_{i\alpha 1,2}$, $G_{i\alpha 3}$, or $G_{s\alpha}$ in treated membranes differed by <8% from control (Fig. 5). It should be noted that for the stimulatory G protein subtype

$G_{s\alpha}$, two forms (42 kDa and 52 kDa) were reported (1). In the present study, the smaller species was difficult to identify and was, therefore, not quantified. Nonimmune serum showed no reactivity under the experimental conditions applied.

In membranes from pertussis toxin-treated cells, μ opioid receptor signaling was reconstituted with two separate preparations of G_o from bovine brain (Fig. 2). While preparation 1, at 1 pmol of membrane protein per mg, restored 29% of the inhibition of adenylyl cyclase by DAMGO, higher concentrations of G_o were required to initiate recovery of the DPDPE effect. Maximal recovery (50%) was obtained with 8 pmol of G_o. Higher concentrations progressively inhibited basal adenylyl cyclase activity (data not shown). Preparation 2 of G_o yielded similar results for the μ response, restoring it to 45% of that in the toxin-untreated cells. In addition, in these experiments the recovery of the DPDPE effect was statistically not significant (Fig. 2 *Inset*).

DISCUSSION

While the involvement of G_i subtypes in mediating the inhibition of adenylyl cyclase by several receptors, including dopamine D_2 (36), angiotensin II (34), and α_2-adrenergic (7) receptors, was ascertained, no direct evidence for a role of G_o in this signaling pathway has yet been presented. In contrast to $G_{i\alpha}$, the activated α subunit of G_o failed to inhibit adenylyl cyclase (37, 38). The "G_o-like" protein that inhibited adenylyl cyclase in S49 cyc$^-$ membranes to a lesser degree then several $G_{i\alpha}$ subtypes was different from the G_o that is abundantly present in brain (39). Although, in NG108-15 neuroblastoma × glioma hybrids, α subunits of both G_i and G_o interacted with the δ opioid receptor (17, 18) and subtypes of G_i were implicated in the coupling of this receptor type to adenylyl cyclase (21), evidence for a functional role of G_o in opioid signal transduction was hitherto limited to its participation in the inhibition of Ca^{2+} channels by opioids (10). Coupling of the μ opioid receptor to G_o was also demonstrated in reconstitution studies (13, 14), but its involvement in transduction of the signal from receptor to effector has not been shown. In addition to describing a function for G_o in mediating the cAMP signal, the results of this study indicate

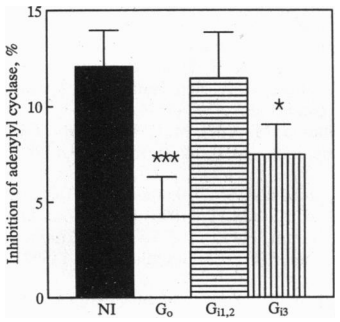

FIG. 4. Opioid inhibition of adenylyl cyclase in brain membranes in the presence of G-protein antibodies. Membranes from rat brain striatum were preincubated with either nonimmune serum (NI) or antisera raised against the α subunits of G_o (GC/2), $G_{i1,2}$ (AS/7), or G_{i3} (EC/2). Subsequently, the membranes were incubated with 1 μM DAMGO in the presence of 100 nM ICI 174,864, and the cAMP concentration was determined as described in *Materials and Methods*. The inhibition of adenylyl cyclase was related to the enzyme activity in membranes treated with nonimmune serum [498 ± 90 pmol cAMP per min per mg of protein (mean ± SEM, n = 12)]. The basal activity in the membranes preincubated with nonimmune serum was not significantly different from that in the untreated (control) membranes. The mean values and SEM obtained in five experiments are shown. The stars indicate statistical significance compared to the results obtained with nonimmune serum: ★★★, $P < 0.02$; ★, $P < 0.09$ (based on an unpaired, two-tailed t test).

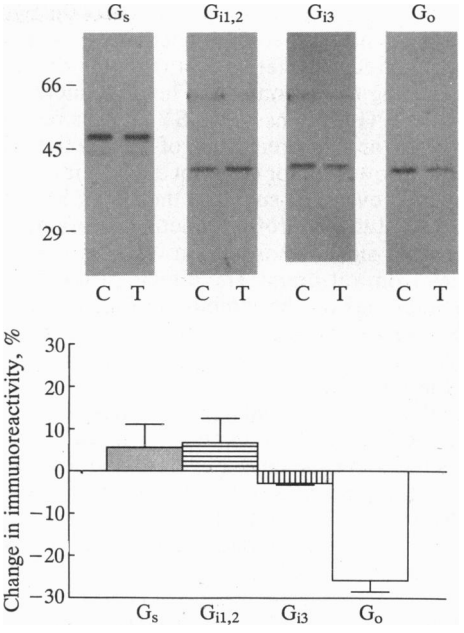

FIG. 5. Western blot of membrane proteins from SH-SY5Y cells exposed to opioid. The cells were incubated for 24 hr at 37°C in the presence and absence of 10 μM DAMGO in the culture medium. The proteins in isolated membranes from treated (T) and control (C) cells were separated by SDS/PAGE and probed with a 1:1000 dilution of antisera raised against the α subunits of either G_o (GC/2), $G_{i1,2}$ (AS/7), G_{i3} (EC/2), or $G_{s\alpha}$ (RM/1). Immunoreactivity was visualized by autoradiography using ^{125}I-labeled secondary antibody (*Upper*), and the spots were excised and quantified by scintillation counting (*Lower*). The changes in treated cells relative to control cells are plotted. The results of a representative experiment (*Upper*) replicated four to six times and the mean values and SEM obtained in five experiments (*Lower*) are shown.

differential regulation of multiple receptors coupling to the same effector in a given cell based on transducer specificity: μ and δ opioid receptors couple to adenylyl cyclase by preferential, although not exclusive, interaction with different subtypes of G protein. The preferential coupling of the two receptor types to distinct G proteins provides a mechanism for differential cellular response to opioid action, while their ability to interact with both G_o and G_i allows for cross talk between these two opioid systems.

The evidence presented here is supported by several methodological approaches utilizing multiple probes: the role of G_o was substantiated with two antisera raised against either the N or C terminus of the protein, the attenuation of the μ signal by the G_o antisera was virtually identical for both an opioid peptide and alkaloid, and the involvement of G_i was assessed with antibodies to three of its subtypes. The results of the reconstitution experiments, carried out with two separate preparations of G_o purified from the same tissue, showed close agreement with those obtained with the immunological approach: preferential coupling of the μ signal was clearly demonstrated, and maximal reconstitution of both the μ and δ signal equaled the respective maximal inhibition caused by the antisera. The antibodies used in these experiments have been well characterized, including by ELISA with purified proteins, and shown to be highly selective (32) except for a minor cross-reactivity between EC/2 (anti-G_{i3}) and GO/1 (anti-G_o). It is, therefore, possible that the slight attenuation of the DAMGO signal produced by the G_{i3} antisera (Fig. 3) reflects its action at G_o. It is interesting that antibodies to either the N or C terminus of the G protein disrupted μ receptor–effector coupling. As reported, the C terminus of transducin interacts with rhodopsin (40), while the amino end of the $G_{o\alpha}$ is essential for association with the

$\beta\gamma$ subunit (41). Thus, the observed effects of the two antisera used here may have been produced by two independent mechanisms: direct interference in receptor–G-protein coupling or blocking formation of the heterotrimer.

The profile of G proteins in SH-SY5Y cells resembles that of human brain, and differentiation of these cells with retinoic acid or nerve growth factor does not alter their content of $G_{o\alpha}$ (42). While this evidence supports the use of SH-SY5Y cells as a model to study G-protein function in brain, the modulation of opioid signal transduction was also investigated in membranes from rat brain. The effects of the antibodies to G-protein subtypes on the inhibition of adenylyl cyclase by DAMGO were virtually identical to those observed with the neural cells: G_o was the primary transducer of the μ opioid signal to the effector in brain.

The development of opioid tolerance in SH-SY5Y cells has been described, including receptor down-regulation (27) and the desensitization of adenylyl cyclase and low K_m GTPase (27, 43). In the present study, prolonged exposure of SH-SY5Y cells to DAMGO produced a typical 4.5-fold shift of its dose–response curve to the right, and Western blots revealed a specific reduction in the cellular content of G_o. Considering the evidence for compartmentation of G proteins (44, 45), the observed decrease of 26% could reflect a larger change in a discrete region of the plasma membrane. Previous studies have described altered levels of G protein following chronic opioid treatment: the effects were tissue dependent and resulted in different patterns of G-protein content in various regions of rat brain (46, 47). Our findings show that the G protein whose quantity was altered by chronic exposure to a selective opioid agonist was that mediating the coupling of the agonist-occupied receptor to effector.

The molecular mechanism of receptor-induced inhibition of adenylyl cyclase has yet to be fully understood (1, 48). Whereas previous models have proposed either a direct action of $G_{i\alpha}$ on the enzyme or the complexing of $G_{\beta\gamma}$ from G_i with $G_{s\alpha}$ to prevent the stimulatory effect of the latter, recent evidence suggests that the $\beta\gamma$ subunit may interact directly with some forms of adenylyl cyclase to produce inhibition (49). Howsoever, the identity of G proteins involved in this pathway is of intense interest: recently the G_z subtype was implicated in the inhibition of adenylyl cyclase (50), and regulation of enzyme activity by G_o has been indirectly implied; in rat atrial tissue, muscarinic agonists inhibited adenylyl cyclase and opened K^+ channels, and muscarinic receptors were found to associate exclusively with G_o (51). By providing direct evidence for the coupling of μ opioid receptor to adenylyl cyclase through G_o, the results presented here describe a signaling pathway for this G protein present at a high concentration in neural tissue, whereby the identity of respective isoforms remains unresolved at this time.

Note Added in Proof. In a just published paper, the differential coupling of μ and δ opioid receptors to G protein subtypes in membranes from SH-SY5Y cells has been described (52).

We are grateful to Drs. A. G. Gilman, M. E. Linder, R. R. Neubig, and A. E. Remmers for purified preparations of G_o. This work was supported by Grant DA 04087 from the United States Public Health Service.

1. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–650.
2. Medzihradsky, F. & Carter, B. D. (1991) in *Biochemistry and Physiology of Substance Abuse, Vol. III*, ed. Watson, R. R. (CRC, Boca Raton, FL), pp. 229–245.
3. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259**, 13806–13813.
4. Suki, W., Abramowitz, J., Mattera, R., Codina, J. & Birnbaumer, L. (1987) *FEBS Lett.* **220**, 187–192.
5. Inanobe, A., Shibasaki, H., Takahashi, K., Kobayashi, I., Tomita, U., Ui, M. & Katada, T. (1990) *FEBS Lett.* **263**, 369–372.
6. Milligan, G., Carr, C., Gould, G. W., Mullaney, I. & Lavan, B. E. (1991) *J. Biol. Chem.* **266**, 6447–6455.
7. Gerhardt, M. A. & Neubig, R. R. (1991) *Mol. Pharmacol.* **40**, 707–711.
8. Van Dongen, A. M. J., Codina, J., Olate, J., Mattera, R., Joho, R., Birnbaumer, L. & Brown, A. M. (1988) *Science* **242**, 1433–1436.
9. Moriarity, T. M., Padrell, E., Carty, D. J., Omri, G., Landau, E. M. & Iyengar, R. (1990) *Nature (London)* **343**, 79–82.
10. Hescheler, J., Rosenthal, W., Trautwein, W. & Schultz, G. (1987) *Nature (London)* **325**, 445–447.
11. Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., Padrell, E., Iyengar, R., Brown, A. M. & Birnbaumer, L. (1988) *Nature (London)* **336**, 680–682.
12. Childers, S. R. (1991) *Life Sci.* **48**, 1991–2003.
13. Ueda, H., Misawa, H., Katada, T., Ui, M., Takagi, H. & Satoh, M. (1990) *J. Neurochem.* **54**, 841–848.
14. Ueda, H., Harada, H., Nozaki, M., Katada, T., Ui, M., Satoh, M. & Takagi, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7013–7017.
15. Ueda, H., Uno, S., Harada, J., Kobayashi, I., Katada, T., Ui, M. & Satoh, M. (1990) *FEBS Lett.* **266**, 178–182.
16. Wong, Y. H., Demoliou-Mason, C. D. & Barnard, E. (1989) *J. Neurochem.* **52**, 999–1009.
17. Offermanns, S., Schultz, G. & Rosenthal, W. (1991) *J. Biol. Chem.* **266**, 3365–3368.
18. Roerig, S. C., Loh, H. H. & Law, P. Y. (1992) *Mol. Pharmacol.* **41**, 822–831.
19. Sharma, S. K., Nirenberg, M. & Klee, W. A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 590–594.
20. North, R. A. (1990) in *Opioid Peptides and Receptor Biochemistry, Physiology and Pharmacology*, eds. Almeida, O. F. X. & Shippenberg, T. S. (Springer, Heidelberg), pp. 141–150.
21. McKenzie, F. R. & Milligan, G. (1990) *Biochem. J.* **267**, 391–398.
22. Kennedy, C., Seward, E. P. & Henderson, G. (1990) in *New Leads in Opioid Research*, eds. Van Ree, J. M., Mulder, A. H., Wiegant, V. M. & Van Wimersma Greidanus, T. B. (Excerpta Medica, Amsterdam), pp. 302–304.
23. Clark, M. J. & Medzihradsky, F. (1987) *Neuropharmacology* **26**, 1763–1770.
24. Clark, M. J., Nordby, G. L. & Medzihradsky, F. (1989) *J. Neurochem.* **52**, 1162–1169.
25. Remmers, A. E. & Medzihradsky, F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2171–2175.
26. Carter, B. D. & Medzihradsky, F. (1992) *J. Neurochem.* **58**, 1611–1619.
27. Carter, B. D. & Medzihradsky, F. (1993) *Mol. Pharmacol.* **43**, 465–473.
28. Carter, B. D., Lee, S. L. & Medzihradsky, F. (1992) *Trans. Am. Soc. Neurochem.* **23**, 257.
29. Hawkins, K. N., Knapp, R. J., Lui, G. K., Gulya, K., Kazmierski, W., Wan, Y.-P., Pelton, J., Hruby, V. J. & Yamamura, H. I. (1989) *J. Pharmacol. Exp. Ther.* **248**, 73–80.
30. Clark, M. J., Carter, B. D. & Medzihradsky, F. (1988) *Eur. J. Pharmacol.* **148**, 343–351.
31. Yu, V. C. & Sadee, W. (1988) *J. Pharmacol. Exp. Ther.* **245**, 350–355.
32. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G. & Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7809–7813.
33. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
34. Probiner, B. F., Northrup, J. K., Bauer, P. H., Fraser, E. D. & Garrison, J. C. (1991) *Mol. Pharmacol.* **40**, 156–167.
35. Kim, M. H. & Neubig, R. R. (1987) *Biochemistry* **26**, 3664–3672.
36. Senogles, S. E., Spiegel, A. M., Padrell, E., Iyengar, R. & Caron, M. (1990) *J. Biol. Chem.* **265**, 4507–4514.
37. Katada, T., Oinuma, M. & Ui, M. (1986) *J. Biol. Chem.* **261**, 5215–5221.
38. Roof, D. J., Applebury, M. L. & Sternweis, P. (1985) *J. Biol. Chem.* **260**, 16242–16249.
39. Kobayashi, I., Shibasaki, H., Takahashi, K., Tohyama, K., Kurachi, Y., Ito, H., Ui, M. & Katada, T. (1990) *Eur. J. Biochem.* **191**, 499–506.
40. Deretic, D. & Hamm, H. E. (1987) *J. Biol. Chem.* **262**, 10839–10847.
41. Denker, B. M., Neer, E. J. & Schmidt, C. J. (1992) *J. Biol. Chem.* **267**, 6272–6277.
42. Klinz, F.-J., Yu, V. C., Sadee, W. & Costa, T. (1987) *FEBS Lett.* **224**, 43–48.
43. Yu, V. C., Eiger, S., Duan, D.-S., Lameh, J. & Sadee, W. (1990) *J. Neurochem.* **55**, 1390–1396.
44. Ott, S., Costa, T. & Herz, A. (1989) *J. Neurochem.* **52**, 619–626.
45. Mochly-Rosen, D. & Gordon, A. S. (1990) *Biochem. Biophys. Res. Commun.* **173**, 388–395.
46. Vogel, Z., Attali, B. B. & Simantov, R. (1990) *J. Neurosci. Res.* **27**, 106–111.
47. Terwilliger, R. Z., Beitner-Johnson, D., Sevarino, K. A., Crain, S. M. & Nestler, E. J. (1991) *Brain Res.* **548**, 100–110.
48. Simon, M. I., Strathmann, M. P. & Gautam, N. (1991) *Science* **252**, 802–808.
49. Tang, W.-J. & Gilman, A. G. (1991) *Science* **254**, 1500–1503.
50. Wong, Y. H., Conklin, B. R. & Bourne, H. R. (1992) *Science* **255**, 339–342.
51. Matesic, D. F., Manning, D. R. & Luthin, G. R. (1991) *Mol. Pharmacol.* **40**, 347–353.
52. Langwitz, K.-L., Offermanns, S., Spicher, K. & Schultz, G. (1993) *Neuron* **10**, 233–242.