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Antagonists of the Receptor-G Protein Interface Block G_i-coupled Signal Transduction*

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The carboxyl terminus of heterotrimeric G protein α subunits plays an important role in receptor interaction. We demonstrate that peptides corresponding to the last 11 residues of $G\alpha_{i1/2}$ or $G\alpha_{o1}$ impair agonist binding to A_1 adenosine receptors, whereas $G\alpha_s$ or $G\alpha_t$ peptides have no effect. Previously, by using a combinatorial library we identified a series of $G\alpha_t$ peptide analogs that bind rhodopsin with high affinity (Martin, E. L., Rens-Domiano, S., Schatz, P. J., and Hamm, H. E. (1996) *J. Biol. Chem.* 271, 361–366). Native $G\alpha_{i1/2}$ peptide as well as several analogs were tested for their ability to modulate agonist binding or antagonist-agonist competition using cells overexpressing human A_1 adenosine receptors. Three peptide analogs decreased the K_i, suggesting that they disrupt the high affinity receptor-G protein interaction and stabilize an intermediate affinity state. To study the ability of the peptides to compete with endogenous $G\alpha_i$ proteins and block signal transduction in a native setting, we measured activation of G protein-coupled K^+ channels through A_1 adenosine or γ-aminobutyric acid, type B, receptors in hippocampal CA1 pyramidal neurons. Native $G\alpha_{i1/2}$, peptide, and certain analog peptides inhibited receptor-mediated K^+ channel gating, dependent on which receptor was activated. This differential perturbation of receptor-G protein interaction suggests that receptors that act on the same G protein can be selectively disrupted.

Hormones and neurotransmitters that bind to G protein-coupled receptors control a myriad of physiological functions. Transduction of these extracellular signals involves receptor-mediated activation of specific G proteins by catalysis of GDP/GTP exchange. These receptors are the target for many pharmaceutical products and are the focus of intense drug discovery efforts. Traditionally, the agonist binding site is the point of intervention, but in some cases receptor subtype-selective drugs have been difficult to achieve. Another possible target for inhibition is the receptor-G protein interface, which has been defined in some detail and involves the intracellular loops of the seven-transmembrane helix receptors with several regions on heterotrimeric G proteins (1–3). It is important to assess whether inhibitors of this interface can be found or designed and whether specific inhibition can be achieved.

The carboxyl-terminal region of the $G\alpha$ subunits represents an important site of interaction between heterotrimeric G proteins and their cognate receptors. Within this region mutations (4–6), covalent modification by pertussis toxin-catalyzed ADP-ribosylation (7), or binding of specific antibodies (8) all uncouple G proteins from their receptors. In particular, the last 4 residues of the $G\alpha$ carboxyl terminus play an important role in determining the fidelity of receptor activation (9, 10). Moreover, synthetic peptides from various portions of the $G\alpha_s$ carboxyl terminus inhibit β-adrenergic receptor-G_s coupling (11, 12). Two of these peptides, $G\alpha_s$-(384–394) and $G\alpha_s$-(354–372), also stabilize the high affinity state of the receptor (12). A synthetic peptide corresponding to the last 11 residues of $G\alpha_t$, $G\alpha_t$-(340–350), both inhibits rhodopsin G_t coupling and mimics G_t by stabilizing the active metarhodopsin II conformation (13). By screening a combinatorial peptide-on-plasmid library based on the carboxyl terminus of $G\alpha_t$, we previously identified numerous peptides that can also mimic the effects of heterotrimeric G protein with a much greater affinity than the native sequence by both binding to rhodopsin and stabilizing it in its active conformation, metarhodopsin II (14).

The similarity between the carboxyl terminus of $G\alpha_t$ and $G\alpha_i$ led us to test the $G\alpha_t$ peptide analogs, which bound to rhodopsin, for their ability to bind other G_i-coupled receptors. In this study we have investigated whether these peptides can 1) bind to G_i-coupled A_1 adenosine receptors and induce the high affinity binding of the receptor; 2) block the ability of G_i proteins to stabilize the high affinity state of agonists; or 3) inhibit signal transduction through G_i by two different G_i-coupled receptors, A_1 adenosine and $GABA_B$[1] receptors. Whereas some receptors activate multiple G proteins (reviewed in Ref. 15), the A_1 adenosine receptor (16, 17) and $GABA_B$ receptor (18, 19) are preferentially coupled to G_i/G_o proteins in many cellular systems.

The extracellular nucleoside adenosine regulates a variety of metabolic functions through the activation of specific cell mem-

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[1] The abbreviations used are: $GABA_B$, γ-aminobutyric acid, type B; ABA, N^6-(3-iodo,4-amino)benzyladenosine; ADA, adenosine deaminase; CHA, (cyclohexyl)adenosine; CHO, Chinese hamster ovary; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; GIRK, G protein-coupled inwardly rectifying K^+ channel; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate; HPLC, high performance liquid chromatography; MBP, maltose-binding protein; R-PIA, (*R*)-N^6-(phenylisopropyl)adenosine.

brane receptors. Adenosine receptors, which exhibit the presumed seven transmembrane-spanning topography typical of almost all G protein-coupled receptors, are currently classified into four subtypes, A_1, A_{2a}, A_{2b} and A_3, based on the pharmacological profile for agonist and antagonist ligands and their effects on intracellular cAMP accumulation (reviewed in Refs. 20–23). The A_1 adenosine receptor is widely distributed in several tissues such as heart, kidney, epididymal fat, and testis, and it is especially prominent in the central nervous system (24–27). In the brain, the highest expression is observed in cortex, cerebellum, hippocampus, and thalamus (25, 26). In the cortex it represents a primary signaling target for adenosine and is thought to tonically inhibit neuronal activity. In the hippocampus the highest density is in the dendritic region of the CA1 area (28–30) where A_1 adenosine receptors are located at synaptic and extrasynaptic sites (31, 32).

Originally, signaling through the A_1 adenosine receptor subtype was linked to inhibition of adenylyl cyclase activity in a pertussis-sensitive manner (33, 34). Since then, A_1 adenosine receptors have been shown to modulate phospholipase C activity in some systems (35, 36), as well as activate K^+ currents and inhibit voltage-gated Ca^{2+} channels (37–40) through the mediation of pertussis-sensitive G proteins (G_i family) (41, 42). In reconstituted systems, human and bovine A_1 adenosine receptors appear to interact preferentially with recombinant $G\alpha_i$ rather than $G\alpha_o$ (43, 44). The bovine A_1 adenosine receptor couples selectively to the $G\alpha_{i3}$ subunit, whereas the human receptor is able to activate each $G\alpha_i$ subtype with similar potency (45). Other researchers (46), using purified bovine brain G proteins, have shown that G_{i2} is more potent than G_{i1} or G_o at restoring high affinity agonist binding to bovine brain A_1 adenosine receptors. However, the ability of A_1 adenosine receptors to preferentially interact with a specific $G\alpha$ subunit does not preclude their ability to interact with other G protein subunits in an intact system.

Here, we study the effect of synthetic peptides corresponding to the last 11 residues of $G\alpha_{i1/2}$, $G\alpha_o$, $G\alpha_t$, and $G\alpha_s$ or the $G\alpha_t$ peptide analogs on agonist binding to the A_1 adenosine receptor in rat cortical membranes or CHO-K_1 cell membranes overexpressing the human receptor. Our findings indicate that in contrast to other receptors (12, 13, 47), the carboxyl-terminal region of the $G\alpha_{i1/2}$ subunit was not capable of stabilizing the high affinity state of A_1 adenosine receptors either in rat cortex membranes or CHO-K_1 cell membranes overexpressing the human receptor. However, the native peptide $G\alpha_{i1/2}$-(344–354) as well as some $G\alpha_t$ peptide analogs can negatively modulate agonist binding and compete with heterotrimeric G protein for binding to the A_1 adenosine receptor. Moreover, $G\alpha_i$ carboxyl-terminal peptides blocked signal transduction to activation of K^+ channels. Depending on whether the activation was through the A_1 adenosine or $GABA_B$ receptor, different $G\alpha_t$ peptide analogs were most effective. Thus, it appears that certain $G\alpha_t$ peptide analogs can selectively disturb the molecular interface that occurs between G_i proteins and G_i protein-coupled receptors.

EXPERIMENTAL PROCEDURES

Materials—[^{3}H]N^6-(cyclohexyl)adenosine (CHA) (27.7 Ci/mmol) and [^{3}H]1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (80–120 Ci/mmol) were obtained from NEN Life Science Products, and [^{125}I]N^6-(3-iodo,4-amino)benzyladenosine (ABA) (2000 Ci/mmol) was synthesized by Dr. J. Linden (Department of Internal Medicine, Cardiovascular Division, University of Virginia, Charlottesville, VA) (48). Adenosine deaminase (ADA) and (*R*)-N^6-(phenylisopropyl)adenosine (R-PIA) were purchased from Sigma, and GTPγS was obtained from Boehringer Mannheim. Other reagents were from standard commercial sources.

Synthetic Peptides and MBP Fusion Proteins—Peptides were purchased from Peptidogenics (Livermore, CA). The native $G\alpha$ peptides tested for their effect on agonist binding to rat cortical membranes had a free amino terminus, whereas all other peptides were synthesized with an acetyl group at the amino terminus. All peptides were purified by reversed-phase high performance liquid chromatography (HPLC), and their purity was checked by fast atom bombardment-mass spectrometry, analytical HPLC, and amino acid analysis.

Plasmid pELM3 (provided by P. J. Schatz, Affymax Research Institute, Palo Alto, CA) was used for expression of maltose-binding protein (MBP) and fusion proteins as described by Martin *et al.* (14). The controls were the vector alone, which expressed the TGGG linker only fused to MBP (pELM 6), and pELM 17, which expressed the peptide $G_{\alpha t}$-(340–350K341R) with the TGGG linker fused to MBP. All frozen cell stocks were kept in 25% glycerol at −80 °C.

Preparation of Rat Cortical Membranes—The brain cortices from young male Sprague-Dawley rats (150–200 g) that had been subjected to cervical dislocation were rapidly removed and homogenized in 10 volumes of 0.25 M sucrose prepared in 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 100 μg/ml bacitracin). The membrane homogenate was centrifuged at 1,000 × *g* for 10 min at 4 °C. The supernatant was centrifuged at 46,000 × *g* for 20 min at 4 °C. The resulting pellet was resuspended in 10 volumes of 50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$ (TEM1 buffer) containing protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 100 μg/ml bacitracin), and the homogenate was centrifuged again. The pellet was resuspended in 5 volumes of TEM1 buffer containing protease inhibitors and 2 units/ml ADA and incubated at 37 °C for 30 min to remove endogenous adenosine. The membrane homogenate was centrifuged, and the final pellet was stored as aliquots at −80 °C until needed. Protein concentration was determined by the method of Lowry *et al.* (49) using bovine serum albumin as the standard.

Preparation of Hippocampal Slices—Young male Sprague-Dawley rats (200–220 g) were decapitated and the brains rapidly removed. The hippocampi were cut into 400-μm thick transverse slices on a Sorvall tissue chopper. Slices were submerged in a recording chamber and continuously perfused with artificial cerebral spinal fluid containing 1.2 mM NaH_2PO_4, 25.9 mM $NaHCO_3$, 126 mM NaCl, 3 mM KCl, 1.5 mM $MgCl_2$, 2.4 $CaCl_2$, 11 mM glucose, oxygenated with 95% O_2, 5% CO_2.

Cell Culture—Chinese hamster ovary (CHO-K_1) cells stably overexpressing the human A_1 adenosine receptor were grown to confluence in Ham's F12 media (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (Life Technologies, Inc.), 100 units/ml penicillin (Life Technologies, Inc.), 0.1 mg/ml streptomycin (Life Technologies, Inc.), and 0.5 mg/ml G418 (Sigma) in an atmosphere of 95% air, 5% CO_2 at 37 °C. Cells were seeded at 1 × 10^5 cells/ml and subcultured after detachment with trypsin/EDTA (0.05%, 0.5 mM).

Preparation of CHO-K_1 Cell Membranes—Confluent CHO-K_1 cells were lysed with hypotonic buffer (10 mM HEPES, pH 7.4, 10 mM EDTA) and scraped from the plate. The lysates were homogenized, centrifuged, and washed twice in washing buffer (10 mM HEPES, pH 7.4, 5 mM EDTA). The homogenized lysates were then stored at −80 °C in storage buffer (10 mM HEPES, pH 7.4, 1 mM EDTA, 10% (w/v) sucrose) until needed. Protein concentration was measured using the Coomassie Blue binding method (50) (Bio-Rad) with bovine serum albumin as the standard.

Rat Cortex Binding Assays—Rat cortical membranes (100–150 μg of proteins) and [^{3}H]CHA (1.3 nM) were incubated in 0.5 ml of TEM1 buffer containing 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 2.7 μg/ml leupeptin, and 1 unit/ml ADA for 60 min at 25 °C. Binding reactions were terminated by filtration through Whatman GF/C filters (Hillsboro, OR) under reduced pressure using a Millipore apparatus (Bedford, MA). Nonspecific binding was defined in the presence of 15 μM R-PIA. Specific binding was 85–90% of total binding for all experiments. For saturation studies, membranes were incubated in TEM1 buffer containing protease inhibitors and ADA with eight different concentrations of [^{3}H]CHA ranging from 0.1 to 46 nM. For studying the effect of GTPγS, rat cortical membranes and [^{3}H]CHA (1.2 nM) were incubated in the absence and presence of varying concentrations of the guanine nucleotide ranging from 1 nM to 100 μM.

The effects of the following $G\alpha$ peptides were tested as follows: $G\alpha_{i1/2}$-(344–354), $G\alpha_{o1}$-(344–354), $G\alpha_t$-(340–350), and $G\alpha_s$-(384–394). The $G\alpha_{i1/2}$, $G\alpha_{o1}$, and $G\alpha_t$ peptides were dissolved in 50 mM Tris-HCl, pH 8.5, and the $G\alpha_s$ peptide was dissolved in 50 mM Tris-HCl, pH 7.5. Solubility of peptides was checked as described by Rarick *et al.* (51). The effect of $G\alpha$ peptides was investigated by incubating rat cortical membranes and [^{3}H]CHA (~1.3 nM) in TEM1 buffer containing protease inhibitors and ADA with different concentrations of the peptide (0.1 to 300 μM).

CHO-K_1 Binding Assays—Membranes (10 μg/ml proteins) from

CHO-K_1 cells overexpressing the human A_1 adenosine receptor were added to tubes containing HEM buffer (50 mM HEPES, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$), 2.5 units/ml ADA, [^{125}I]ABA (0.5 nM) and either the MBP fusion proteins (50 μM) or synthetic peptide (100 μM). The Gα_t peptide analogs were dissolved in HEM buffer, and their solubility was checked as described by Rarick *et al.* (51). The reaction was allowed to proceed at 30 °C for 2 h. The bound and free radioligands were separated by filtration through Whatman GF/C filter paper (soaked in 0.3% polyethyleneimine) using a Brandel tissue harvester. Filters were washed twice with ice-cold TEM2 buffer (20 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.5 mM EDTA). Binding assays were performed in duplicate, and nonspecific binding was determined by adding 15 μM R-PIA at the same time as the radioligand to some samples. Specific binding was 85–90% of total binding for all experiments.

For the competition assays, CHO-K_1 cell membranes (10 μg/ml proteins) were added to tubes containing HEM buffer, 2.5 units/ml ADA, 0.8 nM [^{3}H]DPCPX, and increasing concentrations of R-PIA (0 to 10 μM) in the presence and absence of peptide analogs (100 μM). The reaction was allowed to proceed at 25 °C for 2 h. Then the reaction mixtures were filtered through Whatman GF/C filter paper using a Brandel tissue harvester. Filters were washed twice with ice-cold TEM2 buffer. Binding assays were performed in duplicate, and nonspecific binding was determined by adding 15 μM R-PIA at the same time as the radioligand. Specific binding was 85–90% of total binding for all experiments.

Dilution and saturation experiments using either the agonist, [^{125}I]ABA, or the antagonist, [^{3}H]DPCPX, were performed in order to determine the binding parameters (B_{max} and K_D) and thus to estimate the number of G protein-coupled receptors in CHO-K_1 cell membranes overexpressing the human A_1 adenosine receptor. Based on the binding of [^{125}I]ABA and [^{3}H]DPCPX the number of coupled receptors was approximately 4.5 pmol/mg protein (K_D = 1 nM), and the total number of receptors was approximately 10 pmol/mg protein (K_D = 2.5 nM).

Electrophysiological Recording—Recording electrodes for whole cell recording were pulled from borosilicate glass (outer diameter 1.5 mm and inner diameter 0.86 mm, with filament; Sutter Instrument Co., Novato, CA) on a Flaming/Brown Micropipette puller (Sutter) and had tip resistances of 5–10 MΩ when filled with a solution containing 125 mM potassium gluconate, 11 mM KCl, 0.1 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM K-EGTA, 2 mM Mg-ATP, 0.3 mM Tris-GTP, 10 mM HEPES, pH adjusted to 7.2–7.3 with KOH, osmolarity adjusted to 280–290 mOsm. Peptides were dissolved directly into the electrode filling solution to obtain a final concentration of 1 mM.

Whole cell electrophysiological recordings were made using the "blind" patch recording technique (52). Briefly, the patch pipette was attached to a hydraulic microdrive and advanced into the stratum pyramidal layer of the CA1 region of the hippocampus in 2-μm steps. Positive pressure was applied while the electrode was advanced through the slice, and once contact between the electrode and a cell body was achieved (indicated by a small increase in the electrode resistance), suction was then applied to the electrode to form a gigaohm seal with the membrane. Further suction was used to rupture the membrane patch, providing low resistance access between the electrode filling solution and the cytoplasm of the cell. Neurons were voltage-clamped at −65 mV immediately upon rupturing the membrane using the continuous single electrode voltage clamp mode of an Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA). The average membrane potential of the cells was approximately −70 mV after correction for the liquid junction potential.

To ensure that the peptides had adequate time to diffuse into the cells, experiments were begun a minimum of 15 min following patch rupture. During the experiment, the holding current necessary to maintain the voltage clamp was sampled every 15 s, and membrane resistance was determined every 30 s by the current response to a −4-mV voltage command step. The access resistance between the electrode and the cytoplasm of the cell was continually monitored by observation of the cell membrane capacitive currents in response to a brief voltage step and was below 30 MΩ in all experiments. All responses were digitized with an R.C. Electronics ISC-16 analog-to-digital card and analyzed by computer with software developed in our laboratory. Bath-applied drugs were delivered into the superfusion line by a syringe pump (Razel Scientific Instruments, Inc., Stamford, CN) from stock solutions that were 100 × the final concentration of drug. Nearly all neurons were tested with both adenosine and baclofen, and when possible, cells were retested with either one or both drugs (*e.g.* Fig. 3*C*).

Data Analysis—A nonlinear multipurpose curve-fitting computer program (GraphPad Prism, version 2.0, GraphPad Software, San Diego, CA) was used for analysis of saturation and competition binding data.

TABLE I

Amino acid sequences of native Gα peptides and analogs and their ability to stabilize metarhodopsin II

Peptides were synthesized and purified as described under "Experimental Procedures." Their purity was checked by fast atom bombardment-mass spectrometry, analytical HPLC, and amino acid analysis. Native Gα peptides tested for their effect on agonist binding to rat cortical membranes had a free amino terminus, whereas all other peptides were synthesized with an acetyl group at the amino terminus. Metarhodopsin II stabilization was measured as described by Martin *et al.* (14), and the EC_{50} values were derived by fitting the dose-response curves with a nonlinear regression program (GraphPad Prism, version 2.0). ND, not determined. Bold letters indicate those amino acid residues that differ with respect to those in the G$\alpha_{i1/2}$ carboxyl-terminal sequence. Underlined letters indicate amino acid residues that are conserved change with respect to those in the G$\alpha_{i1/2}$ carboxyl-terminal sequence.

	Amino acid sequence	Meta II stabilization EC_{50}
		μM
G$\alpha_{i1/2}$-(344–354)	IKNNLKDCGLF	ND
Gα_s-(384–394)	**Q**R**MH**LR**QYE**L**L**	ND
Gα_{o1}-(344–354)	I**A**NNLR**G**CGL**Y**	ND
Gα_t-(340–350)	IK**E**NLKDCGLF	807[a,b]
Peptide 8	L**LE**NLRDCG**ML**	0.3[a]
Peptide 9	L**QQV**LKDCGL**L**	1.6[a]
Peptide 15	IR**E**NL**E**DCGL**L**	66.6[a]
Peptide 19	IR**ET**LKDCGL**L**	13.8[a]
Peptide 23	**VLED**LK**S**CGLF	0.5[a]
Peptide 24	**MLK**NLKDCG**M**F	9.6[a]

[a] S. Rens-Domiano, L. Aris, E. Dratz, and H. E. Hamm, unpublished data.

[b] The EC_{50} value for heterotrimeric G_t was 0.3 μM.

A partial F test evaluated whether the data were best fit by a one- or two-site model. The IC_{50} values calculated from the competition curves were converted to K_i values by the Cheng and Prusoff equation (53). The dose-response curves for the Gα peptides were fit using nonlinear regression analysis and IC_{50} values were derived (GraphPad Prism, version 2.0). Data are presented as mean ± S.E. of at least three experiments, unless otherwise noted. The statistical differences were determined using the unpaired *t* test (GraphPad Prism, version 2.0).

RESULTS

Table I shows the amino acid sequences of all peptides used for the experiments and indicates the EC_{50} values of their ability to stabilize rhodopsin in its active conformation, metarhodopsin II. Synthetic or MBP-fused peptides were tested for their ability to modulate agonist binding to A_1 adenosine receptors from two different species, rat and human. The human receptor was expressed in stably transfected cells, whereas the rat receptor was in its native cellular background.

Effects of Native Gα Carboxyl-terminal Peptides on Agonist Binding to Rat A_1 Adenosine Receptors—The A_1 adenosine receptor agonist, [^{3}H]CHA, binds specifically to a single class of binding sites in rat cortical membranes with K_D and B_{max} values of 1.1 nM and 418 fmol/mg protein, respectively (data not shown). Specific binding was decreased by the non-hydrolyzable GTP analog, GTPγS, in a dose-dependent fashion with an approximate IC_{50} value of 200 nM. At 100 μM GTPγS-specific [^{3}H]CHA binding was inhibited by 95%, indicating that the majority of A_1 adenosine receptors are coupled to G proteins. The effect of synthetic peptides corresponding to the carboxyl-terminal sequence of several Gα subunits (Table I) on [^{3}H]CHA binding was evaluated. Peptides G$\alpha_{i1/2}$-(344–354) and Gα_{o1}-(344–354) inhibited [^{3}H]CHA-specific binding in a dose-dependent fashion with IC_{50} values of 31.29 and 30.02 μM, respectively (Fig. 1). In contrast, neither Gα_s-(384–394) nor Gα_t-(340–350) inhibited agonist binding to A_1 adenosine receptors at concentrations of 200 μM (Fig. 1). Therefore, under these conditions, both G$\alpha_{i1/2}$ and Gα_{o1} peptides appear to compete with heterotrimeric G proteins for interaction with the A_1

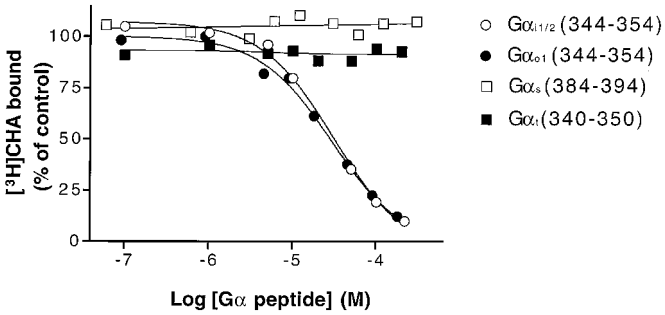

FIG. 1. **Peptide inhibition of specific [^{3}H]CHA binding to rat cortical membranes.** Rat cortical membranes were incubated in TEM1 buffer containing protease inhibitors and ADA (see "Experimental Procedures") with [^{3}H]CHA (1.3 nM) and increasing concentrations of native Gα peptides (○, $G\alpha_{1/2}$344–354; ●, $G\alpha_{o1}$344–354; □, $G\alpha_s$384–394; ■ $G\alpha_t$340–350). The amount of [^{3}H]CHA that specifically bound to the membranes was determined as described under "Experimental Procedures." Nonspecific binding was always <15% of total binding. Specific binding of [^{3}H]CHA is expressed as percentage of that achieved in the absence of any peptide (control) and ranged from 190 to 210 fmol/mg protein. Values are the average of duplicates from a representative experiment which was repeated twice with similar results.

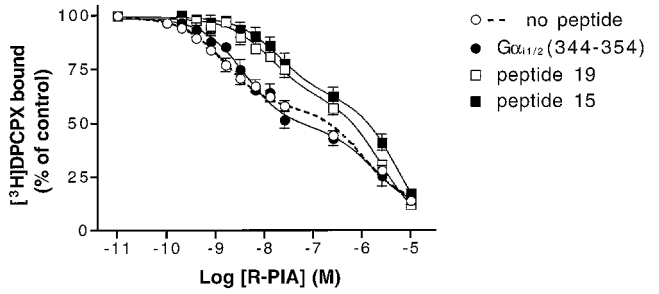

FIG. 2. **Displacement of [^{3}H]DPCPX from human A_1 adenosine receptors by R-PIA in the presence and absence of $G\alpha_t$ peptide analogs.** Membranes from CHO-K_1 cells expressing the human A_1 adenosine receptor were incubated with [^{3}H]DPCPX (0.8 nM) and the indicated concentrations of R-PIA in the presence and absence of $G\alpha_t$ peptide analogs (○, no peptide; ●, $G\alpha_{i1/2}$344–354; □, peptide 19; ■, peptide 15) as described under "Experimental Procedures." Binding of [^{3}H]DPCPX is expressed as a percentage of that achieved in the absence of competing R-PIA (control). In the absence of any peptide, control value was 22.30 ± 0.86 pmol/mg protein (n = 15), whereas in the presence of peptide 15 and 19, control values were 22.61 ± 0.09 and 20.99 ± 1.82 pmol/mg protein, respectively. The data are the means ± S.E. of 3–15 experiments performed in duplicate. The curves were generated using the nonlinear regression analysis of GraphPad Prism, version 2.0. All data were significantly better fit by a two-site model ($p < 0.001$).

adenosine receptors in rat cortical membranes, but they are unable to stabilize high affinity agonist binding.

Effects of $G\alpha_t$ Peptide Analogs from the Combinatorial Library—By using a combinatorial approach, we previously identified a series of $G\alpha_t$ peptide analogs which can both bind to rhodopsin and stabilize the receptor in its active conformation, metarhodopsin II, with higher affinity than the native $G\alpha_t$ carboxyl-terminal peptide (14). There is a high degree of homology between $G\alpha_t$ and $G\alpha_i$ carboxyl-terminal regions, with only one amino acid difference between $G\alpha_t$ and $G\alpha_{i1/2}$ (Table I) and two amino acid differences between $G\alpha_t$ and $G\alpha_{i3}$. To determine whether these peptides could stabilize the high affinity agonist binding state of G_i-coupled receptors, we tested MBP-fused peptides or synthetic peptide analogs in agonist binding assays of human A_1 adenosine receptors overexpressed in CHO-K_1 cells. As reported under "Experimental Procedures," approximately 50% of A_1 adenosine receptors in CHO-K_1 cell membranes are coupled to G proteins. Therefore, if any of the MBP fusion proteins or peptide analogs were able to mimic the heterotrimeric G protein, we should detect an increase of agonist binding. None of the MBP fusion proteins or peptide analogs tested resulted in a significant increase of specific [^{125}I]ABA binding to CHO-K_1 cell membranes expressing human A_1 adenosine receptors (data not shown). However, a few of the analogs either as MBP fusion proteins or synthetic peptides inhibited agonist binding to CHO-K_1 cell membranes. MBP fusion proteins 19 and 24 (50 μM) inhibited [^{125}I]ABA-specific binding by 24 and 23%, respectively. The corresponding synthetic peptides (100 μM) resulted in 21 and 33% decrease of specific binding, respectively.

Since we could not detect a significant effect of either MBP fusion proteins or peptide analogs on direct agonist binding to human A_1 adenosine receptors, an alternative approach that allows identification of the two affinity states of the receptor was used to detect variations of its agonist affinity states. Inhibition of radiolabeled antagonist binding, [^{3}H]DPCPX, by the full agonist R-PIA in the presence and absence of native peptide $G\alpha_{i1/2}$-(344–354) or $G\alpha_t$ analogs was studied. We focused our interest on the activity of six peptide analogs (peptides 8, 9, 15, 19, 23, and 24) which demonstrated different abilities to stabilize metarhodopsin II (Table I). All competition curves showed biphasic patterns. Analysis of data using a nonlinear curve-fitting program revealed that they were better represented by a two-site rather than a one-site model, indicating the presence of two affinity states of the receptor. Fig. 2 shows the competition binding isotherms obtained in the absence (control) or presence of peptide $G\alpha_{i1/2}$-(344–354) as well as the $G\alpha_t$ peptide analogs 15 or 19. The native peptide $G\alpha_{i1/2}$-(344–354) did not induce any significant modification of the displacement curve (Table II). This result indicates that under these conditions the native peptide $G\alpha_{i1/2}$-(344–354) is unable to mimic the effect of heterotrimeric G_i proteins and thus stabilize the high affinity state of the receptor. Both peptides 15 and 19 demonstrated significant right-shifts of the displacement curves (Fig. 2). The percentage of receptors in the high affinity state decreased significantly in the presence of peptide 15 compared with control (47% to 33%; $p < 0.05$). These findings indicate that peptide 15 partially disrupts the high affinity interaction between A_1 adenosine receptors and G_i proteins. Interestingly, in the presence of peptide 15, the K_i values for the high and low affinity states of the receptor showed a 13- and 5-fold increase, respectively (Table II). Both values were significantly different from control K_i values ($p < 0.0001$ and $p < 0.01$, respectively). This suggests that this peptide is able to modulate the high and low affinity states of the receptor. In the presence of peptide 19, the K_i values for the high affinity and low affinity states of the receptor increased 9- ($p < 0.0001$) and 2-fold, respectively, whereas the percentage of the high affinity receptors decreased by approximately 10% (Table II). Thus, peptide 19 also appears to disrupt the high affinity interaction between A_1 adenosine receptors and G_i proteins, but it modulates only the high affinity state of the receptor. Another $G\alpha_t$ peptide analog, peptide 8, also affected the K_i values for both the high and low affinity state of the A_1 adenosine receptor. In the presence of this peptide, the K_i values for the high and low affinity state of the receptor increased 10- and 5-fold compared with control values ($p < 0.0001$ and $p < 0.005$, respectively), whereas the percentage of receptors in the high affinity state did not change (Table II). Therefore, peptide 8 has the ability to modulate both the high and low affinity state of the A_1 adenosine receptor, but it does not change the relative distribution of these two affinity states. All other peptide analogs that were tested had no significant effects on binding parameters (Table II).

Functional Effects of $G\alpha_i$- and $G\alpha_t$-related Synthetic Peptides on Inhibition of Signal Transduction—To study the ability of

TABLE II

Effects of native peptide $G\alpha_{i1/2}$-(344–354) and $G\alpha_t$ analogs on agonist (R-PIA) displacement of [^{3}H]DPCPX binding to human A_1 adenosine receptors in CHO-K_1 cell membranes

Competition of [^{3}H]DPCPX specifically bound by R-PIA in the presence and absence of synthetic peptides was carried out as described under "Experimental Procedures." A nonlinear multipurpose curve-fitting computer program (GraphPad Prism, version 2.0) was used for analysis of competition binding data. A partial F test evaluated whether the data were best fitted by a one- or two-site model. The IC_{50} values calculated from the competition curves were converted to K_i values by the Cheng and Prusoff equation (53). Values are means ± S.E. for 3–15 independent experiments each performed in duplicate.

	K_i high	High affinity receptors	K_1 low
	nM	%	*μM*
Control	1.15 ± 0.61	47.94 ± 2.43	0.80 ± 0.52
$G\alpha_{i1/2}$-(344–354)	2.89 ± 0.61	56.28 ± 3.64	1.36 ± 0.43
Peptide 8	11.73 ± 0.63[a]	47.39 ± 4.41	4.03 ± 0.45[b]
Peptide 9	4.05 ± 0.58	45.85 ± 3.27	1.16 ± 0.50
Peptide 15	15.17 ± 0.66[a]	33.76 ± 1.88[c]	4.48 ± 0.59[d]
Peptide 19	9.98 ± 0.61[a]	37.16 ± 2.53	1.86 ± 0.58
Peptide 23	1.30 ± 0.56	55.78 ± 3.86	0.41 ± 0.05
Peptide 24	2.17 ± 0.55	49.25 ± 3.69	0.69 ± 0.05

[a] Significantly different from control value ($p < 0.0001$).
[b] Significantly different from control value ($p < 0.005$).
[c] Significantly different from control value ($p < 0.05$).
[d] Significantly different from control value ($p < 0.01$).

these peptides to compete with endogenous G proteins in the native setting and block signaling through G_i, we introduced the peptides into hippocampal neurons through a patch pipette and subsequently determined the extent to which G protein-coupled inwardly rectifying K^+ channels (GIRKs) could be activated by either A_1 or $GABA_B$ receptors. Superfusion of hippocampal brain slices with 100 μM adenosine or 50 μM baclofen elicit outward currents in CA1 pyramidal neurons, a reflection of the activation of GIRK by A_1 adenosine or $GABA_B$ receptors, respectively. This effect is mediated via a pertussis toxin-sensitive G protein (54). An example of recording under standard conditions is shown in Fig. 3*A*. The maximal outward current induced by adenosine was 50 ± 5.5 pA ($n = 38$ cells). Internal dialysis of these neurons with the carboxyl-terminal $G\alpha_{1/2}$ peptide completely eliminated the adenosine response (Fig. 4; $p < 0.0001$ *versus* control). Synthetic peptides 19 and 24 (Figs. 3*C* and 4) were also able to completely block the response to adenosine ($p < 0.0001$ and $p < 0.002$ *versus* control, respectively), whereas other peptides (8, 9, and 15) appeared to partially block the adenosine response, although these effects were not statistically significant. Peptide 23 had no effect on the response to adenosine (Fig. 3*B*). Thus, the native $G\alpha_{i1/2}$ peptide as well as peptides 19 and 24 are effective inhibitors of G protein-coupled signal transduction through A_1 adenosine receptors.

Specificity of Functional Blockade of G_i-coupled Receptors by Gα Peptides and Analogs—One would expect that a peptide corresponding to the carboxyl terminus of $G\alpha_i$ would block signaling through all G_i-coupled receptors. It is of great interest to determine whether there can be any selectivity at the receptor-G protein interface. To evaluate whether the $G\alpha_t$ peptide analogs show a pattern of specificity for different G_i-coupled receptors, we measured the effect of these peptides on $GABA_B$ receptor mediated activation of GIRK in CA1 pyramidal neurons, an effect which is also mediated via a pertussis toxin-sensitive G protein (55). Superfusion of hippocampal brain slices with 50 μM baclofen elicited large outward currents (Fig. 3*A*) with an average maximal response of 83 ± 8.8 pA ($n = 29$). Internal dialysis of CA1 pyramidal neurons with carboxyl-terminal Gα peptides either had no effect upon current response to baclofen (peptide 23; Fig. 3*B*) or reduced the baclofen

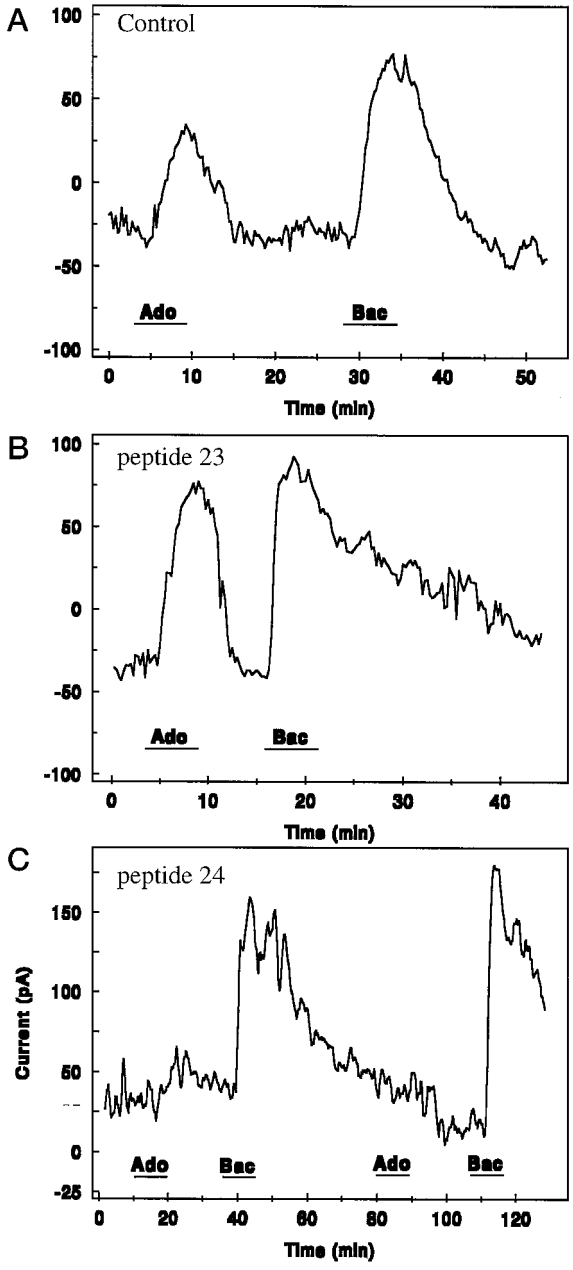

FIG. 3. **Effects of intracellular dialysis with $G\alpha_t$ peptide analogs on current responses to adenosine and baclofen.** Each panel shows the holding current required to clamp a cell to −65 mV during superfusion with 100 μM adenosine and 50 μM baclofen (indicated by *horizontal bars* below the records). In control cells, adenosine responses were usually smaller than the responses to baclofen (*A*). Dialysis with peptide 23 (*B*) did not appear to have any significant effect on the adenosine or baclofen responses (in this cell the responses were, if anything, larger than the control responses in *A*, whereas dialysis with peptide 24 markedly attenuated the adenosine response relative to the baclofen response *C*). Each panel represents data from a different cell. In all cases, patch rupture occurred at least 15 min prior to the beginning of adenosine superfusion. In cases such as that illustrated in *C*, where adenosine and baclofen could be tested repeatedly, no significant differences were observed between initial drug tests and subsequent responses, suggesting that the peptide effects were near maximal within the 15-min waiting period following patch rupture.

response (the native $G\alpha_{i1/2}$, peptides 8, 9, 15, 19, and 24 (Fig. 4)). No synthetic peptide completely blocked baclofen-induced activation of GIRK. However, peptides 8, 15, and 24 produced significant reduction of the current response compared with control ($p < 0.005$, $p < 0.007$, and $p < 0.05$).

In some individual cases (native $G\alpha_{i1/2}$, peptides 19 and 24),

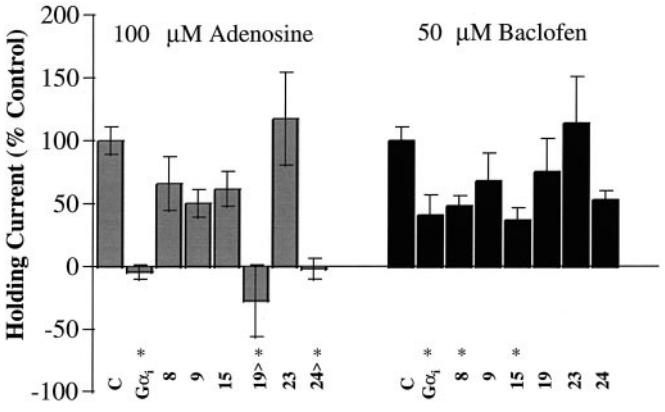

FIG. 4. **Effects of the native peptide $G\alpha_{i1/2}$-(340–350) and $G\alpha_t$ peptide analogs on outward current responses to adenosine and baclofen.** Holding current responses are shown as a percentage of the current responses in control cells (*C*, 50 pA for 100 μM adenosine and 83 pA for 50 μM baclofen). Between 3 and 12 cells were tested with each of the peptides ($G\alpha_{i1/2}$-(355–354) ($G\alpha_i$) or peptide analogs 8, 9, 15, 19, 23, 24), and the *asterisks* indicate points that are significantly different from the corresponding control responses ($p < .05$). *Bars* denoted with the symbols are those where the peptide inhibited the adenosine response to a significantly greater extent than the baclofen response ($p < 0.05$).

the adenosine response was virtually abolished, whereas the baclofen response was only reduced, and in each of these cases the effect of the peptides on adenosine responses was significantly greater than the effect on the $GABA_B$ response ($p < 0.05$ in each case). These differences were readily observed in individual cells (*e.g.* Fig. 3*C*); thus, the failure to antagonize the responses to baclofen could not be attributed to inadequate dialysis of the cells with the peptide, because the adenosine responses in many such cases were completely blocked. The overall results are shown in Fig. 4, in which the holding current for each condition is expressed as percentage of control. A similar pattern of effects on changes in the presence of peptides was observed for whole cell conductance elicited by either adenosine or baclofen, although the changes in conductance appeared to be somewhat more variable than the changes in holding current (data not shown). Clearly, the ability of some peptides to inhibit receptor-mediated K^+ channel gating was different depending on which receptor was activated. This finding suggests that in the same rat hippocampal neurons A_1 adenosine and $GABA_B$ receptor G_i protein interaction can be differentially disrupted by peptide analogs of the carboxyl terminus of $G\alpha_i$.

DISCUSSION

Recently, a variety of studies have focused on finding new agents that selectively uncouple receptors from G proteins (14, 56, 57) and thus disrupt cellular responses. The carboxyl-terminal region of Gα subunits provides the molecular basis for receptor-mediated activation of G proteins and plays a crucial role in determining the fidelity of this activation (13, 47). Synthetic peptides corresponding to the last 11 residues of the $G\alpha_t$ and $G\alpha_s$ subunit are able to mimic the conformational effects of heterotrimeric G proteins on their cognate receptors, rhodopsin and β-adrenergic receptors, by stabilizing their active conformation, although with low potency (12, 13, 47). By using a random "peptides-on-plasmids" library approach (14), we identified several analogs of the native peptide $G\alpha_t$-(340–350) that bind with high affinity to rhodopsin and stabilize its active form, metarhodopsin II. As the carboxyl-terminal sequences of $G\alpha_{i1/2}$ and $G\alpha_{i3}$ diverge just by one and two amino acids from $G\alpha_t$ carboxyl-terminal sequence, respectively, we evaluated the effects of native Gα and analog peptides on receptor coupling to G_i proteins.

No Gα Carboxyl-terminal Peptides Stabilize the High Affinity State of Rat A_1 Adenosine Receptors—The effects of synthetic peptides $G\alpha_{i1/2}$-(344–354), $G\alpha_{o1}$-(344–354), $G\alpha_t$-(340–350), and $G\alpha_s$-(384–394) on agonist binding to A_1 adenosine receptors were studied using rat cortical membranes. None of the native peptide sequences increased agonist binding, implying that they are unable to stabilize the high affinity state of the receptor. On the contrary, peptide $G\alpha_{i1/2}$-(344–354) and $G\alpha_{o1}$-(344–354) inhibited specific binding in a dose-dependent fashion. The result suggests that synthetic peptides corresponding to $G\alpha_{i1/2}$ and $G\alpha_{o1}$ carboxyl-terminal sequence disrupt the interaction between A_1 adenosine receptors and G_i proteins. Since in our assay conditions, agonist binding was also sensitive to inhibition by GTPγS, indicating that most receptors were effectively interacting with G proteins, probably these peptides compete with G_i/G_o for binding to the receptor. Such an outcome implies that peptide $G\alpha_{i1/2}$-(344–354) and $G\alpha_{o1}$-(344–354) are not able to mimic heterotrimeric G protein in stabilizing the high affinity state of the rat A_1 adenosine receptor. The inability of peptide $G\alpha_t$-(340–350) to inhibit agonist binding was also quite surprising since there is only one amino acid difference between it and peptide $G\alpha_{i1/2}$-(344–354). This difference may be due to $G\alpha_t$ peptide having decreased affinity for the rat A_1 adenosine receptor, which would be critical for its competition with G_i/G_o proteins.

No $G\alpha_t$ Peptide Analogs Stabilize the High Affinity State of Human A_1 Adenosine Receptors—These peptide analogs were selected for their ability to bind with high affinity to metarhodopsin II. All analogs stabilized metarhodopsin II with higher affinity than the native $G\alpha_t$ peptide (14). It is possible that if another receptor is used to screen the combinatorial library, high affinity peptides that selectively bind and stabilize this receptor in its active state might be found. The structural basis of this idea is that receptors have different amino acid sequences and thus perhaps some differences in structure in their G protein binding region(s). The combinatorial approach should be able to find such differences. These considerations motivated us to examine the effects of $G\alpha_t$-(340–350) analogs on agonist binding to human A_1 adenosine receptors overexpressed in CHO-K_1 cell membranes. Under our cell culture and binding assay conditions, approximately 50% of total receptors appeared to be in the high affinity state and thus effectively coupled to G proteins. Therefore, peptides did not need to compete with the heterotrimeric G proteins for binding to the receptor and modulating agonist affinity. However, none of the $G\alpha_t$-(340–350) peptide analogs either as MBP fusion proteins or synthetic peptides were able to increase agonist binding to human A_1 adenosine receptors, suggesting they were unable to mimic the effects of heterotrimeric G_i/G_o proteins.

To confirm this finding, a delineation of receptor affinity states was carried out by competing the radiolabeled antagonist bound, [^{3}H]DPCPX, with an agonist (R-PIA) in the presence and absence of the native peptide $G\alpha_{i1/2}$-(344–354) or $G\alpha_t$-(340–350) analogs. High and low affinity states of the receptor are detectable in this assay condition. None of the synthetic peptides resulted in a significant increase of the receptor number in the high affinity state confirming that they are not able to mimic the allosteric effect of heterotrimeric G proteins on receptor conformation.

Modulation of Agonist Affinity by $G\alpha_t$ Peptide Analogs—Although Gα peptides could not mimic heterotrimeric G proteins and stabilize the high affinity state of the A_1 adenosine receptor, we found evidence that the peptides bind to the receptors and compete with heterotrimeric G proteins, as indi-

cated by a decrease of agonist high affinity binding. Interestingly, three $G\alpha_t$-(340–350) analogs, peptides 8, 15 and 19, increased the K_i value of the high affinity state of the receptor, indicating that these peptides not only disrupt the high affinity interaction between agonist-activated receptors and G_i proteins, but they also stabilize the receptor at an intermediate affinity state. Thus, these peptides appear to be affecting the receptor conformation in a subtle way. One might speculate that there is a continuum of conformations between R and R*, and these peptides may stabilize an intermediate conformation. The existence of multiple distinct active receptor states differing in their G protein-coupling abilities has been suggested for both rhodopsin-(58–61) and the human thyroid-stimulating hormone receptor (15).

The K_i value for the receptor in the low affinity state increased significantly in the presence of peptide 8 and 15. One possibility is that the peptides bind to uncoupled receptors in the low affinity state and determine conformational changes leading to a further decrease of receptor affinity for the agonist. However, the significance of this variation must be interpreted with caution since a filtration binding assay is not very suitable for studying low affinity binding sites. That the native peptide $G\alpha_{i1/2}$-(344–354) and the other $G\alpha_t$-(340–350) analogs do not result in any significant modification of the displacement curves may be due to their ability to bind mainly to the uncoupled receptor without changing its conformation and thus the affinity state.

Eleven amino acid peptides from the carboxyl terminus of $G\alpha_t$ and $G\alpha_s$ bind to rhodopsin and β-adrenergic receptor, respectively, and mimic G_t and G_s in stabilizing their active conformation (12, 13, 47). The carboxyl-terminal region of $G\alpha_i$ subunits may have the necessary sequence for specific and efficient signal transduction from the A_1 adenosine receptor but by itself may be not be able to modulate the high affinity state of the receptor. This raises the question of whether other parts of the $G\alpha_i$ subunit participate in forming the binding site for the A_1 adenosine receptor. Peptide mapping and proteolytic digestion studies have shown that both rhodopsin and β-adrenergic receptor contact at least one other region on $G\alpha_t$ and $G\alpha_s$, respectively, which is located in the α4-β6 and β6-α5 loops of Gα subunits (12, 13, 62). Recently, Bae *et al.* (63) have reported that the presence of $G\alpha_{i1}$ residues 299–318 in $G\alpha_{i1/t}$ chimeras is required both to increase agonist binding to the 5-HT_{1B} receptor and obtain receptor-stimulated GTPγS binding. This study has pointed out the importance of the α4 helix and α4-β6 loop region for specific recognition between the 5-HT_{1B} receptor and $G\alpha_{i1}$ subunit. Thus, evidence is accumulating that depicts the receptor binding site on the Gα subunits as a mosaic with each piece playing a distinct role depending on the type of receptor and G protein. It may be that to stabilize the high affinity state of the A_1 adenosine receptor, a multiple interaction between the receptor and the Gα subunit or even the heterotrimeric G protein is required.

Functional Effects of the Native $G\alpha_i$ Carboxyl-terminal Peptide and $G\alpha_t$ Analogs in Intact Cells—To test the hypothesis that some peptides that disrupt the interaction between the A_1 adenosine receptor and the carboxyl-terminal region of G_i proteins are also able to impair signal transduction, we measured the effects of synthetic peptides on the activation of GIRKs by A_1 adenosine receptors in rat hippocampal CA1 pyramidal neurons. Except for peptide 23 which did not show any significant effect on receptor-mediated opening of K^+ channels, all other peptides reduced the K^+ current with different activity. Thus, peptides that modulated the high affinity agonist binding to the receptor also disrupted signal transduction between A_1 adenosine receptors and G_i proteins. The native peptide $G\alpha_{i1/2}$-(344–354) as well as peptide analogs 19 and 24 completely blocked the adenosine-activated response. There did not appear to be a strict correspondence between the ability of the peptides to modulate agonist binding or antagonist displacement by an agonist and their potencies as inhibitors of A_1 adenosine receptor-mediated activation of K^+ current. However, the efficacy of the native peptide $G\alpha_{i1/2}$-(344–354) was consistent with its activity as inhibitor of agonist binding to A_1 adenosine receptors in rat cortical membranes.

Specificity of the Functional Disruption of Receptor G_i Protein Coupling by Gα Peptides—One would expect that a peptide corresponding to the carboxyl terminus of $G\alpha_i$ would block signaling through all G_i-coupled receptors. Although most receptors show specificity for a particular class of G proteins, it is much less clear whether drugs that target the receptor-G protein interface could be highly specific for a particular receptor. Thus, it was important to evaluate the efficacy of Gα peptides on activation of GIRKs by a different G_i-coupled receptor. $GABA_B$ receptors modulate the activity of several downstream effectors (64) mainly through the activation of pertussis toxin-sensitive G proteins (17, 55). However, this receptor differs structurally from other G protein-coupled neurotransmitter receptors and forms a separate gene family together with the metabotropic receptor for L-glutamate (64). In this context, it was of interest that three of the peptides (the native peptide $G\alpha_{i1/2}$-(344–354) and peptide analogs 19 and 24) were more effective blockers of A_1 than $GABA_B$ receptor responses, whereas peptide 15 was more effective in blocking $GABA_B$ than A_1 receptor responses. Although both A_1 and $GABA_B$ receptors in rat hippocampal CA1 pyramidal neurons activate what appears to be a common population of GIRKs (65, 66), such that outward current responses to baclofen occlude responses to adenosine, there is no definitive evidence that the same G proteins mediate these actions. Thus, the differential effects of these peptides could reflect either the specificity of the interaction of the peptides with homologous but not identical regions of the A_1 and $GABA_B$ receptors or could reflect mediation of these responses by different G proteins. In this context, it should also be noted that these peptides are all analogs of the carboxyl terminus of Gα subunits, whereas it is the βγ dimer that is thought to activate GIRKs (67, 68). Thus, although it is possible that these peptides interact directly with GIRKs to inhibit their function, as has been shown for $G_{\alpha i1}$ (69), this could not explain their selectivity in blocking actions mediated via A_1 receptors *versus* $GABA_B$ receptors. The most probable explanation for these results is that the peptides interact instead with specific elements of the A_1 and $GABA_B$ receptors in slightly different ways to disrupt their interaction with the corresponding G proteins. This is also suggested by comparing the potency of the peptides for rhodopsin and A_1 adenosine receptors. Peptide 15, which was the most potent at decreasing A_1 adenosine receptor agonist affinity (Table II), was the least potent of the analogs at stabilizing metarhodopsin II (Table I).

In conclusion, we have found that peptide analogs based on the carboxyl terminus of $G\alpha_t$-(340–350) uncouple A_1 adenosine receptors from G_i proteins, but they do not stabilize the high affinity state of the receptor. Peptide analogs with different abilities to modulate agonist binding and signal transduction activation have been also identified. Evidence for selective effects of the analog peptides on different G_i-coupled receptors was also demonstrated. Thus, the receptor-G protein interface is a possible target for inhibition of G protein-coupled receptor activation. Future studies will assess whether inhibitors of this interface can be found or designed and whether specificity of inhibition can be achieved.

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