and thus they may present a novel strategy to target double-stranded DNA to achieve gene modulation and to construct artificial restriction enzymes. Strand displacement may be a general principle yet to be demonstrated for other oligonucleotide analogs with a neutral backbone, and such complexes may serve as valuable models in studies of the DNA structure of transcription complexes in which strand displacement by the nascent RNA chain is a central process. The strand displacement complexes would also be analogous to three-strand DNA complexes that can be induced by the DNA recombination protein RecA.

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8. We used a Gilford response apparatus; solutions contained 10 mM phosphate, 10 mM $MgCl_2$, and 140 mM NaCl at pH 7.4. M. Egholm, O. Buchardt, P. E. Nielsen, R. H. Berg, in preparation.
9. The plasmid, pT10, containing the dA_{10}-dT_{10} target sequence was constructed by cloning of oligonucleotides **1** and **2** into the Bam HI site of pUC19 with *Escherichia coli* strain JM 101 as host. The pT10 plasmid was cleaved with Eco RI, labeled at the Eco RI site at the 3′ or the 5′ end with standard techniques (*14*), and cleaved with Pvu II, and the 248-bp fragment containing the target sequence was isolated. Complexes for probing were prepared by mixing 100.000 cpm (~1 pmol) of ^{32}P-labeled fragment with 0.5 μg of calf thymus DNA and the desired amount of PNA-1 (diluted from a stock solution of 10 mg/ml in H_2O) in 100 μl of the desired probing buffer (see below). The mixture was incubated at 37°C for 60 min before probing. Affinity photocleavage was performed in TE buffer by irradiating the sample with 300-nm radiation (Philips TL 20 W/12 fluorescent light tube, ~24 J m^{-2} s^{-1}) for 30 min. Photofootprinting was performed in TE buffer by adding 50 ng (~100 pmol) of "diazohexyl-linked–acridine" (DHA) to the sample and irradiating for 30 min at 365 nm as described (*10*). Potassium permanganate probing was done in TE buffer as described (*15*). Staphylococcus nuclease probing was done in 25 mM tris-HCl (pH 7.4), 1 mM $MgCl_2$, and 0.1 mM $CaCl_2$ with 750 U/ml of nuclease for 5 min at 20°C. The reaction was stopped by addition of EDTA to a concentration of 25 mM. S_1-nuclease probing was performed for 5 min at 20°C in 50 mM sodium acetate (pH 4.5), 200 mM NaCl, 0.5% glycerol, and 1 mM $ZnCl_2$ with 0.005, 0.05, or 0.5 U/ml of S_1. The reaction was stopped with EDTA as above. Samples from affinity photocleavage, photofootprinting, and permanganate probing were treated with piperidine (1 M, 90°C, 20 min) before polyacrylamide gel electrophoresis (PAGE). All of the samples were analyzed by 10% PAGE in 7 M urea and TBE buffer, and ^{32}P bands were visualized by autoradiography as below (*16*). One microgram of oligonucleotide **1** was included in the samples for analysis of the A strand before gel electrophoresis to avoid retardation of the fragments in the gel due to complexing with PNA-1.
10. DHA: 9-{[6-(2-diazacyclopentadienylcarbonyloxy)-hexyl]amino}acridine; C. Jeppesen and P. E. Nielsen, *Eur. J. Biochem.* **182**, 437 (1989).
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16. Oligonucleotides were synthesized on a Biosearch 7500 DNA synthesizer and labeled with [γ^{32}P]ATP (adenosine triphosphate) by standard procedures (*14*). We performed experiments by mixing 3000 cpm (~10 pmol) of ^{32}P-labeled oligonucleotide with the desired amount (0 or 180 pmol) of oligonucleotide **2** in 20 μl of 10 mM tris-HCl and 1 mM EDTA at pH 7.4 (TE buffer). After incubation at 20°C for 30 min the desired amount of PNA-1 was added, and the samples were incubated for a further 30 min at 20°C and 15 min at 0°C. The samples were divided into two: (i) to a 10-μl aliquot was added 1 μl of glycerol and 1 μl of 10 × TBE (1 × TBE = 90 mM tris-borate, 1 mM EDTA, pH 8.3), and it was analyzed by 15% PAGE in TBE buffer at 4°C; (ii) another 10-μl aliquot was evaporated to dryness, redissolved in 10 μl of 80% formamide in TBE buffer, heated to 90°C for 5 min, and analyzed by 15% PAGE with 7 M urea. Radioactive bands were visualized by autoradiography (Agfa curix RP1 film, −80°C, exposed overnight with intensifying screens).
17. The thymine monomer was synthesized by alkylation of thymine with methyl bromoacetate, subsequent hydrolysis, and conversion to the pentafluorophenyl ester with dicyclohexylcarbodiimide (DCC) before attachment to *N*-(2-Boc-aminoethyl)-glycine (Boc = butoxycarbonyl). The Boc-protected monomer was activated by conversion to the pentafluorophenyl ester. The PNA oligomers were synthesized by standard Merrifield synthesis with the Boc-benzyl strategy on a 4-methylbenzhydrylamine resin. All couplings but one proceeded with an efficiency of ≥99%, and in a typical synthesis, 24 mg of PNA-1 (80% purity) was obtained after HF cleavage of 76 mg of PNA resin. The crude product was purified by reversed-phase high-pressure liquid chromatography (>98% pure) and characterized by plasma-desorption mass spectrometry.

18. The financial support of the Alfred Benzon Foundation and the Danish Natural Science Research Council is gratefully acknowledged.

27 June 1991; accepted 20 September 1991

Type-Specific Regulation of Adenylyl Cyclase by G Protein βγ Subunits

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Heterotrimeric guanine nucleotide–binding regulatory proteins (G proteins) dissociate into guanosine triphosphate (GTP)–bound α subunits and a complex of β and γ subunits after interaction with receptors. The GTP-α subunit complex activates appropriate effectors, such as adenylyl cyclase, retinal phosphodiesterase, phospholipase C, and ion channels. G protein βγ subunits have been found to have regulatory effects on certain types of adenylyl cyclase. In the presence of $G_{s\alpha}$, the α subunit of the G protein that activates adenylyl cyclase, one form of adenylyl cyclase was inhibited by βγ, some forms were activated by βγ, and some forms were not affected by βγ. These interactions suggest mechanisms for communication between distinct signal-transducing pathways.

G PROTEINS ACT AS TRANSDUCERS BY coupling membrane-bound receptors to intracellular effectors. G proteins are heterotrimers and are believed to dissociate to liberate a nucleotide-bound α subunit and a complex of β and γ subunits when the proteins are activated by the binding of GTP (*1*). Functional characterization provided the first basis for classification of G proteins: G_s is the G protein that activates adenylyl cyclase, and G_t (transducin) is the retinal G protein that activates a guanosine 3′,5′-monophosphate–specific phosphodiesterase. In each of these cases, the dissociated GTP-α subunit complex activates the effector enzyme (cyclase or phosphodiesterase). Thus, the concept arose that each G protein oligomer contains a functionally specific α subunit in association with mixtures of a small number of different β and γ subunits. Nearly 20 distinct α subunits have now been described, as well as four β subunits and a similar number of γ polypeptides (*2*).

Although interest has centered on the idea that α subunits are the elements that provide specificity in G protein–mediated signal transduction systems, it was suggested that

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subunit dissociation provided an opportunity for a branch point in signaling pathways (*3*). Thus, it was hypothesized that inhibition of adenylyl cyclase by G_i proteins was mediated by βγ subunits, albeit indirectly by means of their capacity to interact with and deactivate the α subunit of G_s ($G_{s\alpha}$). This suggestion and others about the role of the βγ subunit complex in signal transduction have generated controversy. For example, some investigators have argued that βγ activates myocardial K^+ channels (*4*), whereas others have proposed that α subunits provide this function (*5*). Genetic evidence implicates βγ as the primary mediator of the response to mating factors in budding yeast (*6*). Disruption of the gene that encodes the G protein α subunit in this pathway causes constitutive signaling, presumably as a result of the uncontrolled activity of βγ. Disruption of β or γ eliminates the response. Unfortunately, the subsequent effector in this pathway is unknown, and there is no biochemical evidence to support these mechanistic interpretations.

Calmodulin-activated (type-I) adenylyl cyclase can be inhibited by G protein βγ subunits. βγ was hypothesized to interact with calmodulin and sequester the activator (*7*). We have cloned (*8*) and expressed (*9*) a cDNA that encodes type-I adenylyl cyclase and shown that this mechanism is incorrect. However, βγ does inhibit type-I adenylyl cyclase activity when the enzyme is assayed after expression in Sf 9 cells infected with recombinant baculovirus. It seems likely that this inhibitory effect of βγ is exerted directly. The cDNAs for additional forms of adenylyl cyclase have been cloned, and we have examined the generality of this response. The effect of βγ depends on the type of adenylyl cyclase under study.

Type-I and type-II adenylyl cyclases are structural homologs. Both are expressed predominantly in the central nervous system, although the type-II protein is found in some peripheral tissues as well (*10*). Type-I adenylyl cyclase can be stimulated by calmodulin; type-II cannot. Both of these proteins were expressed in insect ovarian Sf 9 cells infected with recombinant baculoviruses (B-rACI and B-rACII) to approximately the same specific activity (*11*). More than 95% of the adenylyl cyclase activity in these membranes was contributed by the recombinant enzyme. The activities of both forms of adenylyl cyclase were stimulated to approximately the same extent by recombinant (*Escherichia coli*–derived) $G_{s\alpha}$ (GTP-γ-S–$rG_{s\alpha}$) activated with guanosine-5′-O-(3-thiotriphosphate) (GTP-γ-S) (Fig. 1, A and B). Bovine brain βγ itself had essentially no effect on adenylyl cyclase activity. However, as described (*9*), βγ inhibited type-I adenylyl cyclase activity by approximately 60% in the presence of GTP-γ-S–$rG_{s\alpha}$ (Fig. 1A). (GTP-γ-S binds to $G_{s\alpha}$ with high affinity and under these conditions βγ cannot reassociate with $G_{s\alpha}$.) Under identical conditions, βγ stimulated type-II adenylyl cyclase activity in the presence of activated $G_{s\alpha}$ (Fig. 1B). The capacity of βγ to activate type-II adenylyl cyclase was exerted rapidly (with a lag time of less than 1 min) (*12*). The concentration of βγ required for this stimulatory effect [median effective concentration (EC_{50}) ~5 to 10 nM] was similar to that required for inhibition of the type-I enzyme. Thus, after exposure of the two adenylyl cyclases to both activated $G_{s\alpha}$ and βγ, their activities differed by approximately 20-fold (Fig. 1C).

To confirm that both the inhibitory and the stimulatory modulation of adenylyl cyclase activity was due to βγ, we tested the capacity of guanosine diphosphate (GDP)–bound bovine brain $G_{o\alpha}$ to reverse the effects of endogenous or exogenous βγ subunits (Fig. 2). As anticipated, $G_{o\alpha}$ slightly increased or decreased type-I or type-II adenylyl cyclase activity, respectively, in the absence of

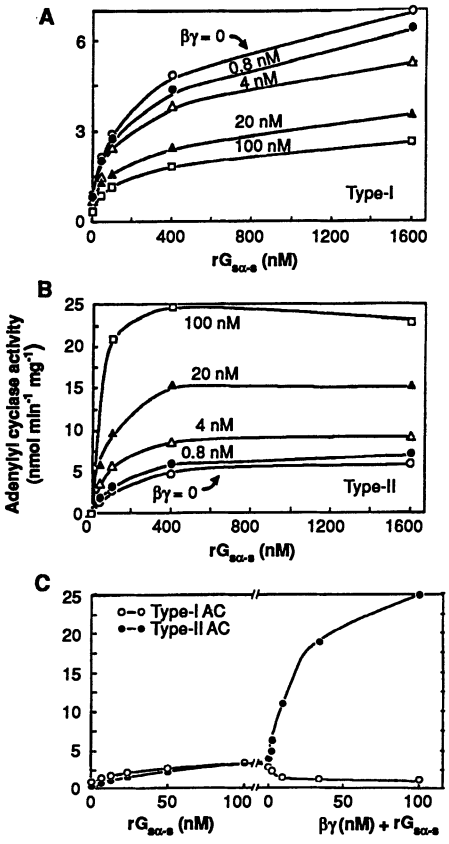

Fig. 1. Effects of GTP-γ-S–$rG_{s\alpha\text{-}s}$ and bovine brain βγ on type-I (**A**) and type-II (**B**) adenylyl cyclase activity in membranes from Sf 9 cells infected with B-rACI or B-rACII. (**C**) Comparison of the effect of GTP-γ-S–$rG_{s\alpha\text{-}s}$ (left) and bovine brain βγ in the presence of 100 nM GTP-γ-S–$rG_{s\alpha\text{-}s}$ (right) on type-I and type-II adenylyl cyclase. The $rG_{s\alpha\text{-}s}$ was activated with 100 μM GTP-γ-S for 30 min at 30°C in 50 mM sodium Hepes (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol, and 5 mM $MgSO_4$. Free GTP-γ-S was removed by gel filtration. The concentration of $rG_{s\alpha\text{-}s}$ was determined by GTP-γ-S binding, and the concentration of βγ was determined by staining with amido black. GTP-γ-S–$rG_{s\alpha\text{-}s}$ was incubated with 5 μg of Sf 9 cell membranes for 10 min at 30°C before the assay. βγ was added immediately before the assay. Solutions of βγ contained lubrol PX, and the concentration of detergent in the assay was held fixed at 0.025%. The duration of the adenylyl cyclase assay was 10 min at 30°C in the presence of 10 mM $MgCl_2$, as described (*24*). Data shown are a representative experiment of more than 20 similar experiments.

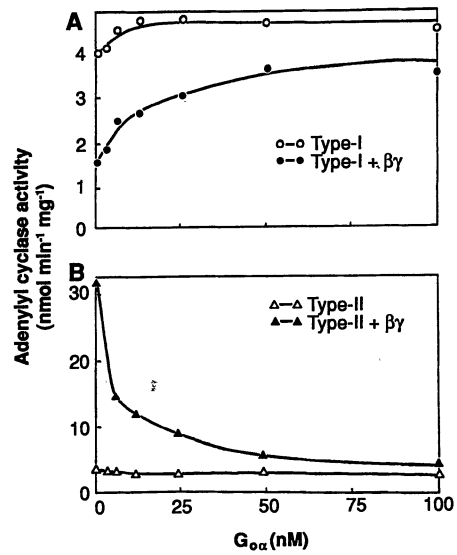

Fig. 2. Effects of GDP-$G_{o\alpha}$ on adenylyl cyclase activity in the presence of GTP-γ-S–$rG_{s\alpha}$ and βγ. (**A**) Type-I adenylyl cyclase; (**B**) type-II adenylyl cyclase. βγ (100 nM) was incubated on ice for 10 min with $G_{o\alpha}$ in 10 μM GDP. GTP-γ-S–$rG_{s\alpha\text{-}s}$ (100 nM) was incubated with 5 μg of Sf 9 cell membranes at 30°C for 10 min before the addition of βγ or βγ and $G_{o\alpha}$. The concentration of bovine brain $G_{o\alpha}$ was determined by GTP-γ-S binding. Data shown are representative of two experiments.

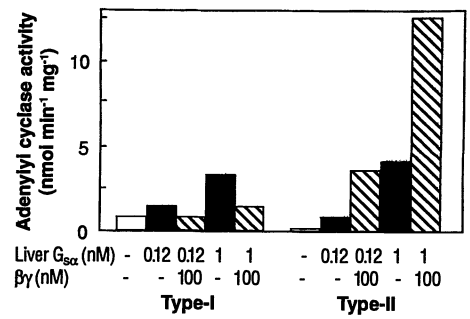

Fig. 3. The effect of various concentrations of rabbit liver $G_{s\alpha}$ and βγ on type-I and type-II adenylyl cyclase activity. Liver G_s was purified as described (*25*). $G_{s\alpha}$ was then separated from βγ by fractionation on immobilized βγ agarose (*26*). $G_{s\alpha}$ was eluted from the immobilized βγ column with 10 μM GTP-γ-S. Unbound GTP-γ-S was removed by gel filtration. Assays were performed in the presence of 0.05% lubrol PX. Data shown are representative of two experiments.

Table 1. Capacity of G protein α subunits to support activation of type-II adenylyl cyclase by βγ. G protein α subunits were activated with GTP-γ-S. Adenylyl cyclase activity was assayed in membranes from Sf 9 cells infected with B-rACII. Data shown are representative of two experiments. $rG_{s\alpha\text{-}s}$ (FT), $rG_{s\alpha\text{-}s}$ (LR268,269FT); $rG_{s\alpha\text{-}s}$ (CFT), $rG_{s\alpha\text{-}s}$ (WLR263,268,269CFT).

Subunit (100 nM)	Adenylyl cyclase activity (nmol min^{-1} mg^{-1})	
	Without βγ	With βγ (100 nM)
None	0.2	0.2
$rG_{s\alpha\text{-}s}$	3.6	25
$rG_{s\alpha\text{-}l}$	2.6	26
$rG_{s\alpha\text{-}s}$ (FT)	0.6	2.6
$rG_{s\alpha\text{-}s}$ (CFT)	0.3	0.7
$rG_{i\alpha}$	0.3	0.4

exogenous βγ. $G_{o\alpha}$ increased or decreased type-I or type-II adenylyl cyclase activity, respectively, when 100 nM βγ was present (*13*) (Fig. 2). Twelve different batches of βγ were tested, including preparations from brain and retina and some preparations that were enriched for the β_{35} or the β_{36} isoform of the β subunit (*14*). All influenced adenylyl cyclase activity as anticipated, except for retinal βγ, which has a single isoform of both β and γ. Aside from this observation, the relative efficacies of different β and γ subunits are unknown. Heat-inactivated βγ had no effect.

Although $G_{s\alpha}$ synthesized in *E. coli* appears normal in most of its properties, it has a reduced affinity for adenylyl cyclase (*15*). Thus, the concentrations of $rG_{s\alpha}$ and βγ required for the results shown in Fig. 1 are approximately equal. Mammalian $G_{s\alpha}$ activates adenylyl cyclase at lower concentrations than does the *E. coli*–derived recombinant protein. To demonstrate this fact and to rule out the possibility that the effects of βγ were somehow dependent on the use of $rG_{s\alpha}$, we repeated the experiments with $G_{s\alpha}$ purified from rabbit liver. The inhibitory and stimulatory effects of βγ on type-I and type-II adenylyl cyclase, respectively, were also observed in the presence of rabbit liver $G_{s\alpha}$ (Fig. 3). However, $G_{s\alpha}$ from rabbit liver activated adenylyl cyclase in the 0.1 to 1 nM range, whereas effects of βγ required concentrations in the 1 to 20 nM range.

We tested the capacity of other G protein α subunits to support activation of type-II adenylyl cyclase by βγ (Table 1). Two different splice variants of $rG_{s\alpha}$ [a short form ($rG_{s\alpha\text{-}s}$) and a long form ($rG_{s\alpha\text{-}l}$)] were equally effective. Mutants of $rG_{s\alpha}$ with impaired ability to activate adenylyl cyclase have been defined that appear to have a reduced affinity for the enzyme (*16*). The activities of $rG_{s\alpha}$ mutants LR268,269FT and WLR263,268,269CFT were approximately 8% and 1% of that of $G_{s\alpha}$, respectively (Table 1) (*16*). These mutant proteins were similarly ineffective when tested in combination with βγ. The α subunit of rG_i ($rG_{i\alpha}$) had no effect on type-II adenylyl cyclase activity in the absence or presence of βγ. The βγ subunit complex activated adenylyl cyclase only weakly in the presence of forskolin (*12*).

The structures of mammalian membrane-bound adenylyl cyclases are complex, although there is a rough symmetry between the NH_2 and COOH halves of the molecules. Each half of these molecules is thought to contain six putative transmembrane helices and a large (~40 kD) cytosolic domain. The two cytosolic domains show 55% sequence homology with each other. Each is similar to the catalytic domain of guanylyl cyclase, and each may contain a nucleotide-binding site. No enzymatic activity is apparent when each half of type-I adenylyl cyclase (designated I-NM_1C_1 and I-M_2C_2) is expressed separately (Table 2) (*9*). However, an active enzyme is formed when the two halves of the molecule are expressed concurrently. This provided us the opportunity to assemble noncovalent chimeras (*17*). As expected, the chimera formed from I-NM_1C_1 and I-M_2C_2 was inhibited by βγ after activation with $G_{s\alpha}$. Less activity was detected when II-NM_1C_1 and II-M_2C_2 were coexpressed, but

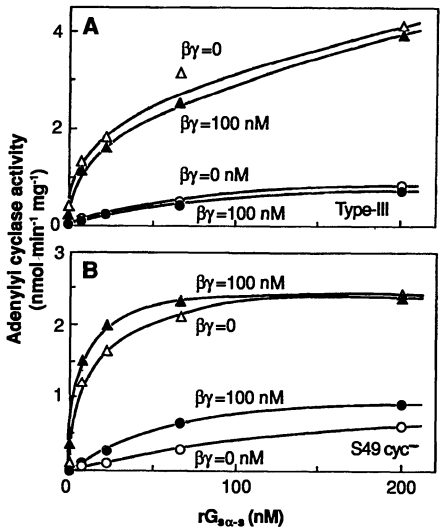

Fig. 4. Effects of GTP-γ-S–$rG_{s\alpha}$, forskolin, and bovine brain βγ on type-III adenylyl cyclase (membranes from Sf 9 cells infected with B-rACIII) (**A**) and adenylyl cyclase in cyc^- S49 cell membranes (**B**). Sf 9 cell membranes (12 μg) or cyc^- S49 cell membranes (60 μg) were incubated with GTP-γ-S–$rG_{s\alpha}$ (circles) or GTP-γ-S–$rG_{s\alpha}$ plus 10 μM forskolin (triangles) for 10 min at 30°C. βγ (100 nM) (filled symbols) was added immediately before the assay. The duration of the adenylyl cyclase assay was 10 min for Sf 9 cell membranes and 20 min for cyc^- membranes. Data are representative of two experiments.

βγ potentiated the activation by $G_{s\alpha}$. I-NM_1C_1 and II-M_2C_2 formed an active enzyme, and addition of βγ to this complex resulted in activation in the presence of $G_{s\alpha}$. Thus, we tentatively assign the site for activation of adenylyl cyclase by βγ (whether the effect is direct or indirect) to the COOH half of the molecule. Unfortunately, the combination of II-NM_1C_1 and I-M_2C_2 was inactive.

We do not as yet know the mechanism of the inhibitory and stimulatory effects of βγ because the assays have been performed with adenylyl cyclase in Sf 9 cell membranes. Inhibition of type-I adenylyl cyclase and stimulation of the type-II enzyme by βγ was observed after solubilization of the enzymes with detergents and activation with GTP-γ-S–$rG_{s\alpha}$ (*18*). We were unable to observe the inhibitory effect of βγ on the type-I enzyme after its purification by forskolin-Sepharose chromatography. However, only small quantities of purified protein were obtained, and manipulations were technically difficult. Purification of type-II adenylyl cyclase on forskolin-Sepharose is not efficient; only a tenfold increase in specific activity was obtained. However, this preparation retained the capacity to be stimulated by βγ in the presence of activated $G_{s\alpha}$. Solubilization and purification of type-II adenylyl cyclase removed immunoreactive, but catalytically inactive, protein from the preparation. Thus, renaturation of such material is not the basis for the stimulatory effect of βγ.

Table 2. Effect of GTP-γ-S–$rG_{s\alpha}$ and βγ on truncated forms of adenylyl cyclase. Sf 9 cells were infected with the viruses indicated, and membranes were prepared as described (*9*). Assays were performed without activators or with 100 μM forskolin, 80 nM GTP-γ-S–$rG_{s\alpha\text{-}s}$, or 80 nM GTP-γ-S–$rG_{s\alpha\text{-}s}$ and 300 nM βγ. Data shown are representative of three experiments.

Virus	Adenylyl cyclase activity (nmol min^{-1} mg^{-1})			
	No activator	Forskolin	GTP-γ-S–$rG_{s\alpha}$	GTP-γ-S–$rG_{s\alpha}$ + βγ
I-NM_1C_1	0.02	0.1	0.04	0.04
I-M_2C_2	0.04	0.1	0.08	0.09
II-NM_1C_1	0.04	0.1	0.07	0.07
II-M_2C_2	0.04	0.1	0.1	0.07
I-NM_1C_1 + I-M_2C_2	0.5	3.1	2.8	1.4
I-NM_1C_1 + II-M_2C_2	0.05	3.6	2.1	4.0
II-NM_1C_1 + I-M_2C_2	0.04	0.13	0.11	0.11
II-NM_1C_1 + II-M_2C_2	0.02	0.13	0.18	0.6

Full-length cDNAs for two additional mammalian adenylyl cyclases have been described (*19, 20*). The type-III enzyme is abundant in olfactory tissue. We expressed this adenylyl cyclase in Sf9 cells (*17*); activity is stimulated synergistically by $G_{s\alpha}$ and forskolin (Fig. 4A). There was little or no effect of bovine brain $\beta\gamma$ on type-III adenylyl cyclase activity in the presence of $G_{s\alpha}$, forskolin, or a combination of the two activators (Fig. 4A). The type-IV enzyme is found in brain and several peripheral tissues. Its sequence most resembles that of type II, and its activity can be stimulated by a combination of $G_{s\alpha}$ and $\beta\gamma$ in much the same manner as type-II adenylyl cyclase can be (*20*). S49 cells express two other adenylyl cyclases, designated V and VI (*21*). Much of the initial work on the effects of G protein α and $\beta\gamma$ subunits on adenylyl cyclase activity was performed with S49 cell membranes (*1, 22*). In keeping with other results, we observed only weak potentiation of cyc^- ($G_{s\alpha}$-deficient) S49 cell adenylyl cyclase activity by $\beta\gamma$ in the presence of $G_{s\alpha}$ (Fig. 4B). Thus, it may be possible to classify adenylyl cyclases as being potentiated or inhibited by $\beta\gamma$ or as relatively immune to the subunits. It will be of interest to see if any non–calmodulin-sensitive form of the enzyme can be inhibited by $\beta\gamma$.

The major question is the physiological significance of these phenomena. An obvious prediction is that agents that interact with receptors that are coupled to G proteins other than G_s might alter the effects of G_s-linked receptors on adenosine 3′,5′-monophosphate (cAMP) accumulation (by releasing $\beta\gamma$) while having no primary effect on adenylyl cyclase themselves. G_o in particular could serve as a large pool of $\beta\gamma$ because this G protein represents 1 to 2% of brain membrane protein. Such a mechanism might explain the effects of combinations of neurotransmitters on cAMP concentrations in brain slices (*23*). Agents such as glutamate and α-adrenergic agonists, which do not stimulate adenylyl cyclase by themselves, potentiate the actions of compounds such as histamine, β-adrenergic agonists, and adenosine, which can interact with G_s-linked receptors. It will be of interest to discover if effects of this sort can be ascribed to regulation of the concentration of the free $\beta\gamma$ subunit complex.

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11. Sf9 cells were maintained in suspension culture as described (*9*). Cell membranes were prepared from 1-liter cultures after infection with either B-rACI or B-rACII (one plaque-forming unit per cell) as described (*9*).
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13. The concentrations of $G_{o\alpha}$ required to reverse the effects of $\beta\gamma$ were less than anticipated, based on stoichiometric interaction with $\beta\gamma$. $G_{o\alpha}$ was quantified by nucleotide binding; $\beta\gamma$ was quantified by protein assay. Inactive $\beta\gamma$ in the preparation may explain this discrepancy. In addition, bovine brain $\beta\gamma$ contains several species of both β and γ polypeptides. The relative affinities of different $\beta\gamma$ complexes for $G_{o\alpha}$ and adenylyl cyclase are not known.
14. Nine different preparations of bovine brain $\beta\gamma$ were provided by P. Casey, E. Lee, L. Quarmby, and G. Berstein and were purified as described [P. J. Casey, M. P. Graziano, A. G. Gilman, *Biochemistry* **28**, 611 (1989)]. Transducin $\beta\gamma$ and fractions enriched in $\beta_{35}\gamma$ and $\beta_{36}\gamma$ were provided by P. Casey. These preparations were in three different detergents: cholate (0.05 to 0.1%), 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS) (0.07%), or lubrol PX (0.025 to 0.05%). The type of the detergent had little effect on the results.
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17. For mutant II-M_2C_2, the Afl II fragments of plasmid p1-4, which contained bovine type-II adenylyl cyclase cDNA, were isolated and ligated with phosphorylated adapters (5′-GATCCACCATGGGC-3′ and 5′-TTAAGCCCATGGTG-3′) and pBluescript SK^- (Stratagene), which had been digested with Bam HI. For II-NM_1C_1, plasmid prII, which contained rat type-II adenylyl cyclase cDNA, was digested with Afl II and linked with phosphorylated adapters (5′-TTAAAC-TAGGATCCTAGT-3′). For type-III adenylyl cyclase, the Eco RI fragment that contained the full-length type-III cDNA was transferred from the pCMV vector (*19*) to pBluescript SK^-. The resulting DNAs were then transferred to the baculovirus expression vector pVL1392 (into the Bam HI site for II-M_2C_2 and into the Eco RI site for II-NM_1C_1 and type-III). These DNAs were cotransfected with linearized AcRP23-*lacZ* viral DNA by lipofectin-mediated transfection. Positive viral clones were selected by plaque assay, and production of protein was confirmed either by protein immunoblotting or by visualizing the [^{35}S]methionine-labeled products [R. D. Possee and S. C. Howard, *Nucleic Acids Res.* **15**, 10233 (1987); D. R. Groebe, A. E. Chung, C. Ho, *ibid.* **18**, 4033 (1990); P. Kitts, M. D. Ayres, R. D. Possee, *ibid.*, p. 5667]. II-NM_1C_1 terminates and II-M_2C_2 is initiated 55 amino acid residues before the beginning of the second set of transmembrane spans of type-II adenylyl cyclase. Infection of Sf9 cells and preparation of cell membranes were performed as described (*9*).
18. Solubilization of proteins from Sf9 cell membranes with lubrol PX and purification of type-I adenylyl cyclase by forskolin-Sepharose (Pharmacia) chromatography were performed as described (*9*). Similar procedures were followed for the type-II enzyme.
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27. We thank R. Reed for type-II and type-III adenylyl cyclase cDNA and B-rACII; M. Linder, H. Itoh, and P. Middleton for $rG_{s\alpha}$ and $rG_{i\alpha}$; T. Higashijima for bovine brain $G_{o\alpha}$; P. Kitts for AcRP23-*lacZ* viral stock; M. Stanzel and L. Ortlepp for technical assistance; and T. Nguyen for the II-M_2C_2 construct. Supported by USPHS grant GM34497, American Cancer Society grant BE-30M, the Raymond and Ellen Willie Chair of Molecular Neuropharmacology, the Lucille P. Markey Charitable Trust, and the Perot Family Foundation.

18 June 1991; accepted 23 August 1991

A Requirement for the Intercellular Messenger Nitric Oxide in Long-Term Potentiation

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Long-term potentiation (LTP) of synaptic transmission is a widely studied model of neuronal plasticity. The induction of LTP is known to require processes in the postsynaptic neuron, while experimental evidence suggests that the expression of LTP may occur in the presynaptic terminal. This has led to speculation that a retrograde messenger travels from the post- to the presynaptic cell during induction of LTP. Extracellular application or postsynaptic injection of two inhibitors of nitric oxide synthase, *N*-nitro-L-arginine or N^G-methyl-L-arginine, blocks LTP. Extracellular application of hemoglobin, which binds nitric oxide, also attenuates LTP. These findings suggest that nitric oxide liberated from postsynaptic neurons may travel back to presynaptic terminals to cause LTP expression.

IN THE SCHAFFER COLLATERAL–CA1 synapses of the hippocampus, LTP is induced by a series of postsynaptic events including activation of the *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor channel (*1*) and the influx of Ca^{2+} (*2*). The site of the expression of LTP is controversial (*3–5*), but recent studies with quantal analysis (*4, 5*) have strengthened the evidence that, after postsynaptic induction, LTP is expressed by an increase in transmitter release from presynaptic terminals (*6, 7*). If the mechanisms underlying the induction and expression of LTP reside