Alteration of Receptor/G-protein Interaction by Putative Endogenous Protein Kinase Activity in Dictyostelium discoideum Membranes
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Membranes of Dictyostelium discoideum cells were incubated under phosphorylation conditions and washed, and the effects on cAMP binding to chemotactic receptors in the absence and presence of guanosine 5'-O-(3-thiotriphosphate) (GTPγS) were investigated. Most experiments were done with adenosine 5'-O-(3-thiotriphosphate) (ATPγS), which is a good substrate for many kinases, but the product, protein phosphothioate, is not easily hydrolyzed by phosphatases.

 Pretreatment of membranes under phosphorylating conditions with MgATPγS alters the site heterogeneity of the cAMP-binding forms, without a significant effect on the total number of binding sites. A similar effect was induced by GTPγS under nonphosphorylation conditions.

 The effects of MgATPγS were rapid (τ0 = 1 min), irreversible, and not induced by Mg2+ or ATPγS alone or by magnesium adenylyl imidodiphosphate and magnesium adenylyl (β,γ-methylene)diphosphate.

 MgATP induced a smaller inhibition than MgATPγS, which was potentiated by the addition of exogenous cAMP-dependent protein kinase. The effect of MgATP was rapidly reversible; reversibility was reduced by the phosphatase inhibitor NaF. These results suggest that the effects of MgATPγS are mediated by an endogenous protein kinase.

 The major 35S-thiophosphorylated band detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis was a protein with Mr = 36,000. The phosphorylation of a protein with the molecular weight of the cAMP receptor (Mr = 40,000–45,000) was not observed.

 Extracellular cAMP functions as a signal molecule in Dictyostelium discoideum during chemotaxis (1), morphogenesis (2), and cell differentiation (3). cAMP is detected by highly specific surface receptors, which results in several cellular responses such as the activation of adenylyl and guanylyl cyclase (these and other responses have been reviewed (4–6)). The stimulation of guanylyl and adenylyl cyclase terminate within a few seconds and a few minutes, respectively, even when cAMP remains present at constant levels (7–10). Desensitization of adenylyl cyclase stimulation has been studied extensively by Dinnaeur et al. (11, 12).

 Recent results (13, 14) suggest the presence of two subpopulations of cell surface cAMP receptors, A and B sites, that have been implicated in the stimulation of adenylyl and guanylyl cyclase, respectively. Binding of cAMP to both subpopulations is complex, showing interconversions of binding states in vitro (16–19). This may suggest the involvement of guanine nucleotide regulatory proteins in the transduction pathways of adenylyl and guanylyl cyclase. This view is further supported by the recent observations (20, 21) that guanosine triphosphates stimulate adenylyl cyclase in vitro.

 Desensitization of cAMP-stimulated guanylyl cyclase occurs within a few seconds after cAMP addition (9) and may be caused by an impairment of receptor/G-protein interaction (16). Desensitization of adenylyl cyclase is much slower and requires the presence of constant cAMP concentrations during several minutes (12). This desensitization process may be caused by a receptor modification, presumably by phosphorylation (22–24). cAMP also induces the loss of detectable cAMP-binding sites (down-regulation) which occurs with similar kinetics as desensitization of adenylyl cyclase, but which requires 10-fold higher cAMP concentrations and recovers more slowly (25).

 The role of phosphorylation of signal transducing components in desensitization has been extensively studied in vertebrates (26–33). These studies make use of the well-characterized functional coupling between receptors, G-proteins, and adenylyl cyclase in native membranes or after reconstitution of purified components in artificial membranes. It has been shown that phosphorylation of receptor or G-protein diminishes their functional interaction (29–31).

 Conditions for the functional reconstitution of isolated receptor, G-protein(s), and adenylyl cyclase have not yet been found for the components from D. discoideum. Therefore, a study of the role of phosphorylation in transmembrane signaling in this organism is limited to experiments in vivo (22–24) or to receptor/G-protein/adenylate cyclase interaction in isolated membranes. In this study, the receptor/G-protein interaction was investigated by detecting the effects of guanine nucleotides on cAMP binding to membranes that were preincubated under conditions where protein kinases are active. The results show that phosphorylation conditions alter the receptor/G-protein interaction, and evidence is presented which suggests that this is due to an endogenous protein kinase.

 Experimental Procedures

 Materials—[2,8-3H]cAMP (1.5 TBq/mmol), [γ-32P]ATP (115 TBq/mmol), [γ-35S]ATPγS (24 TBq/mmol), and carrier-free or-

1 P. J. M. Van Haastert, manuscript in preparation.
2 The abbreviations used are: ATPγS, adenosine 5'-O-(3-thiotriphosphate); GTPγS, guanosine 5'-O-(3-thiotriphosphate); AppNHp, adenylyl imidodiphosphate; AppChp, adenylyl (β,γ-methylene)imidophosphate; HEPES, N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
the[32P]phosphate were obtained from Amersham Corp. cAMP, ATP, ATPyS, AppNHp, AppChp, and GTPyS were from Boehringer Munchen. Dithiothreitol was purchased from Sigma; cAMP-depe-
dendent protein kinase type I was isolated from bovine muscle by DEAE-cellulose chromatography as described (34).

**Culture Conditions and Membrane Isolation—**D. discoideum cells (strain NC-4) were grown on a buffered glucose/peptone medium (9). Cells were frozen in 10 mM KH₂PO₄/Na₂HPO₄, pH 6.5 (buffer A), freed from bacteria by repeated centrifugations at 100 × g for 2 min, and starred in buffer A by shaking at a density of 10⁷ cells/ml. After 5–6 h, cells were collected by centrifugation and washed twice with buffer B, and the pellet was resuspended in buffer B (40 mM HEPES/NaOH, 0.5 mM EDTA, 250 mM sucrose, pH 7.7) to a density of 10⁷ cells/ml. Cells were lysed by pressing them through 3-μm pores of a Nucleopore filter (35). The lysate was centrifuged at 10,000 × g for 5 min, the pellet was washed once with buffer B, and the final pellet was resuspended in buffer B to the equivalent of 2 × 10⁶ cells/ml.

**Incubation with ATP and Analogs—**Membranes (300 μl) were incubated for 5 min at 20 °C with 3% of 100 × concentrated solutions yielding final concentrations of 10 mM NaF, 5 mM MgCl₂, and 1 mM ATP or one of its analogs. In one experiment, membranes were incubated in the presence of cAMP-depended protein kinase. This enzyme (1 μmol of holoenzyme in 10 μl) was preactivated with 3 μM (S₅)-cAMP at 20 °C for 15 min; the amount of enzyme was estimated from its cAMP binding activity as described (34).

Incubations were terminated by addition of 1200 μl of ice-cold buffer A and immediately centrifuged at 0 °C for 2 min at 10,000 × g. The pellet was washed once with 1 ml of buffer B, and the final pellet was resuspended in 600 μl of buffer A.

**cAMP binding**—cAMP binding was measured in a total volume of 100 μl containing buffer A, 5 nM [3H]cAMP, 10 mM dithiothreitol, GTPyS (30 μM when present), and 80-μl membranes. The incubation period was 5 min at 0 °C. Samples were centrifuged for 3 min at 10,000 × g, the supernatant was aspirated, and the pellet was dissolved in 75 μl of 1 M acetic acid. Radioactivity was determined by liquid scintillation counting after the addition of 1.4 ml of Emulsi-fer (Packard Instrument Co.). NonSpecific binding was determined in the presence of 1 mM cAMP and was subtracted from all data shown. Typical binding data for control membranes at 5 nM [3H]cAMP are input = 18,000 cpm, specific binding = 3,058 ± 112 cpm, and nonspecific binding = 186 ± 21 cpm (both n = 3).

**Scatchard analysis of cAMP-binding** (Fig. 1) was determined by incubating membranes with different cAMP concentrations at 20 °C. The binding reaction was terminated by centrifugation of membranes through silicon oil (16).

**[3S]Thiophosphorylation and [32P]Phosphorylation in Vitro—**Membranes (10 μl) were incubated for 5 min at 20 °C in buffer B with 5 mM MgCl₂, 10 mM NaF, and 1 μM [γ-32P]ATP or [γ-32P]ATP (4 TBq/mmol, 10 μCi/incubation). The reaction was terminated by the addition of 25 μl of a mixture yielding 2% SDS, 2.5% dithiothreitol, and 10% glycine (w/v; final concentrations; sample buffer); SDS-PAGE was run as described above. SDS-PAGE was carried out as described in the previous section.

**RESULTS**

The aim of the present study was to investigate the effects of endogenous protein kinase activity on cell-surface cAMP receptors and on the interaction between receptor and puta-
tive G-protein(s). D. discoideum cells contain high levels of protein phosphophosphatase activity (36). To by-pass this phosphatase activity, a derivative of ATP was used, ATPγS, which is a relatively good substrate of many protein kinases. However, the product, protein phosphorothioate, is a poor substrate for protein phosphophosphatases (reviewed in Ref. 37). As a result, the phosphorylation reaction becomes irre-
versible.

**Scatchard Analysis of cAMP Binding—**D. discoideum cells contain different forms of cAMP-binding sites. A form designated as B₄₈ has high affinity for cAMP (Kₐ = 15 nm) and shows a very slow rate of dissociation (t₁/₂ = 15 s). B₉ has the same high affinity for cAMP, but the complex dissociates 10-fold faster (t₁/₂ = 15 s). The major part of the receptors are fast dissociating (Fast, t₁/₂ = 1 s) and are composed of at least two forms, A₁ and A₂, with high (Kₐ = 60 nm) and low (Kₐ = 450 nm) affinity, respectively (15, 16). These binding forms are also present in membranes from D. discoideum cells; their proportioning is affected by guanine nucleotides, resulting in an increase of A₁ and a decrease of the other forms.

The influence of pretreatment of membranes with MgATPγS on the binding heterogeneity was investigated. Membranes were incubated with 1 nM ATPγS and 5 mM Mg²⁺ for 5 min at 20 °C and then extensively washed at 0 °C. Control and MgATPγS-treated membranes were incubated with different concentrations [³²P]cAMP in the absence and presence of 50 μM GTPyS. Incubation of [³²P]cAMP was detected at equilibrium (b(0)), at 10 s after the onset of dissociation (b(10)), and at 2 min after the onset of dissociation (b(120)). Binding to the different receptor forms was calculated with the following set of equations (see Ref. 16).

\[
\begin{align*}
b(0) &= \text{Fast} + B^5 + B^{18} \\
b(10) &= 0.65 B^5 + 0.95 B^{18} \\
b(120) &= 0.56 B^{18}
\end{align*}
\]

The results are shown in Fig. 1. The binding of cAMP to B₉ or B₄₈ is apparently homogeneous, whereas binding to Fast is clearly composed of at least two components. The kinetic data of B₉ and B₄₈ were calculated by linear regression analysis, whereas the data of the fast dissociating components were calculated by a hyperbolic regression analysis.

The obtained kinetic data are presented in Table I. The site distribution of control membranes is 26% Aᵣ with Kₐ = 50 nm, 59% A₁ with Kₐ = 545 nm, 10% B₁ with Kₐ = 6.4 nm, and 5% B₈ with Kₐ = 7.7 nm. GTPyS strongly alters this distribution: about 98% of the sites have the properties of A₁; Aᵣ and B₈ are reduced to 1%, whereas B₅ is virtually absent (<0.2%). It should be noted that the low abundance of Aᵣ complicates the determination of its affinity.

cAMP binding to MgATPγS-treated membranes in the absence of GTPγS strongly resembles the binding to control membranes in the presence of GTPγS. Binding of cAMP to Aᵣ, B₁, and B₈ is strongly decreased with a concomitant increase of binding to A₁. It should be noted that binding to B₅ is still detectable in MgATPγS-treated membranes. As in control membranes, this form is undetectable after the addition of GTPγS.

The results of Fig. 1 and Table I indicate that GTPγS or pretreatment with MgATPγS predominantly alters the proportioning of binding forms, whereas the total number of binding sites and the affinities are not strongly altered. The effect of MgATPγS is a shift of high to low affinity binding forms, which is most easily detected by equilibrium binding of low cAMP concentrations.

**Kinetics of Modification of cAMP Binding by MgATPγS—**Membranes were preincubated for various time periods with MgATPγS at 20 °C and washed, and cAMP binding was

\[
\begin{align*}
\text{ Kinetics of Modification of cAMP Binding by MgATPγS— } & \\
\text{ Membranes were preincubated for various time periods with MgATPγS at 20°C and washed, and cAMP binding was }
\end{align*}
\]
Branes with M$^+$ and ATPyS resulted in a 60% inhibition of phosphorylation reactions, activity as control membranes. In contrast, treatment of membranes were washed at 0°C, and cAMP binding was detected in the absence of GTPyS and essentially no inhibition of binding in the presence of ATPyS or divalent cations, showed a 75% inhibition of binding in the absence or presence of GTPyS. The data are derived from the results presented in Fig. 1.

Effect of MgATPyS pretreatment on cAMP binding heterogeneity
Membranes were preincubated in the absence or presence of MgATPyS. cAMP binding was performed in the absence or presence of GTPyS. The data are derived from the results presented in Fig. 1.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MgATPyS</th>
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<tbody>
<tr>
<td></td>
<td>−GTPyS</td>
<td>+GTPyS</td>
</tr>
<tr>
<td></td>
<td>−GTPyS</td>
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</tr>
<tr>
<td>$B_{max}$</td>
<td>2.45</td>
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<td>$K_d$</td>
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<td></td>
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<td>0.10</td>
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<tr>
<td>$A'$</td>
<td>9.30</td>
<td>9.48</td>
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<tr>
<td>$A''$</td>
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<td>507</td>
</tr>
<tr>
<td>$B''$</td>
<td>545</td>
<td>507</td>
</tr>
<tr>
<td>$B''''$</td>
<td>7.4</td>
<td>7.2</td>
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</table>

FIG. 1. Scatchard analysis of cAMP binding. Membranes were incubated with or without 5 mM MgCl$_2$ and 1 mM ATPyS for 5 min at 20°C and washed at 0°C, and cAMP binding was detected at 20°C in the absence or presence of 30 μM GTPyS. Binding equilibrium was reached after 75 s; at this moment, excess cAMP (0.1 mM) was added. Membranes were centrifuged through silicon oil just before or 10 or 120 s after the addition of excess cAMP. Membrane-associated radioactivity was determined, and binding to the Fast (A), B$^{+}$ (B), and B$^{SS}$ (C) forms was calculated using Equation a-c. These curves were subjected to hyperbolic (A) or linear (B and C) curve fitting. The kinetic data are shown in Table I. O and •, control cells; Δ and △, MgATPyS-treated cells; O and Δ, and • and ◇, cAMP binding in the absence or presence of GTPyS, respectively. The binding of 10 nM cAMP is equal to 1.5 pmol of cAMP/mg of protein and to 75,000 binding sites/cell equivalent.

Modification of cAMP Binding Requires Phosphorylating Conditions—Membranes were preincubated with 1 mM ATPyS and/or 5 mM Mg$^{2+}$ for 5 min at 20°C. Then, membranes were washed at 0°C, and cAMP binding was detected in the absence or presence of 30 μM GTPyS (Table II). Control membranes, which were incubated at 20°C in the absence of ATPyS or divalent cations, showed a 75% inhibition of cAMP binding by 30 μM GTPyS. Membranes treated with either ATPyS or Mg$^{2+}$ had identical cAMP binding activity as control membranes. In contrast, treatment of membranes with Mg$^{2+}$ and ATPyS resulted in a 60% inhibition of cAMP binding in the absence of GTPyS and essentially no inhibition of binding in the presence of GTPyS.

Analogs of ATP which cannot donate a phosphate group in phosphorylation reactions, i.e. MgAppNHp and MgAppCHp,

FIG. 2. Kinetics of MgATPyS-induced modification of cAMP binding. Membranes were incubated for 10 min at 20°C, the last part of the incubation period was with 5 mM MgCl$_2$ and 1 mM ATPyS (Thus, the incubation at t = 2 min was for the first 8 min without and then for 2 min with MgATPyS; this protocol allows the simultaneous processing of all samples.) Membranes were washed, and cAMP binding was detected in the absence (C) or presence (•) of 30 μM GTPyS. The percent inhibition by GTPyS is indicated (+).

Modification of cAMP binding by ATP analogs
Membranes were preincubated in the absence (control) or presence of 5 mM Mg$^{2+}$, 1 mM nucleotides, and preactivated cAMP-dependent protein kinase (cAK) as indicated for 5 min at 20°C. Then, membranes were extensively washed, and cAMP binding was measured in the absence or presence of 30 μM GTPyS. The results shown are the means ± S.D. of three independent experiments.

<table>
<thead>
<tr>
<th>Preincubation</th>
<th>cAMP binding</th>
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<tbody>
<tr>
<td></td>
<td>−GTPyS</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>ATPyS</td>
<td>102 ± 7</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>97 ± 8</td>
</tr>
<tr>
<td>MgATPyS</td>
<td>38 ± 8</td>
</tr>
<tr>
<td>MgAppNHp</td>
<td>93 ± 8</td>
</tr>
<tr>
<td>MgAppCHp</td>
<td>97 ± 8</td>
</tr>
<tr>
<td>MgATP</td>
<td>68 ± 6</td>
</tr>
<tr>
<td>MgATP + cAK</td>
<td>55 ± 6$^*$</td>
</tr>
</tbody>
</table>

$^*$Significantly below control (p < 0.05).
$^*$The difference is significant (p < 0.05, paired t test).
did not alter cAMP binding (Table II). The replacement of ATP\_S by ATP yielded qualitatively similar results. However, the effect was less pronounced than with MgATP\_S, possibly due to rapid reversion of the reaction by protein phosphatase. The addition of an exogenous protein kinase activity may shift the equilibrium of phosphorylation/dephosphorylation to higher levels of phosphorylation. We have used mammalian cAMP-dependent protein kinase because it has been shown that this enzyme alters receptor/G-protein/adenylate cyclase interaction in vertebrate cells (26, 32). It was observed that the effect of MgATP is significantly ($p < 0.05$, paired t test) potentiated by exogenous protein kinase (Table II).

**Reversibility of Modification of cAMP Binding**—Membranes were preincubated with MgATP or MgATP\_S, washed, and resuspended in buffer at 0 °C. cAMP binding was detected immediately at 5 min or at 45 min after resuspending the membranes (Fig. 3). Membranes treated with MgATP\_S showed the usual decrease of cAMP binding, which did not reverse. The effect of MgATP was almost reversed at 5 min and completely reversed at 45 min after resuspending treated membranes in phosphate buffer. The reversibility was considerably decreased in the presence of the phosphatase inhibitor NaF (38). This and duplicate experiments suggest that the effects of MgATP reverse with half-times of about 3 and 20 min in the absence and presence of NaF, respectively.

**Phosphorylation of Receptor**—It has been shown that cAMP induces the phosphorylation of the cAMP receptor in vivo, which is associated with a shift of the apparent molecular weight of the receptor from 40,000 to 43,000 (22-24). The cAMP-induced shift of the mobility of a \[^{32}P\] phosphorylated protein is shown in Fig. 4. The question was addressed whether MgATP\_S or MgATP may phosphorylate the receptor in vitro. Therefore, membranes were incubated with \[^{γ-32}P\]ATP\_S or \[^{γ-32}P\]ATP, and phosphorylated proteins were separated by SDS-PAGE. The results (Fig. 4) reveal that the major phosphorylated protein has a molecular weight of 36,000 and clearly does not co-migrate with the phosphorylated protein whose mobility changes in stimulated cells. It should be noted that cells contain a phosphorylated protein which co-migrates with the protein that is phosphorylated in vitro.

**DISCUSSION**

Phosphorylation reactions play an important role in the desensitization of hormone-stimulated adenylate cyclase (26-33) and possibly also in the desensitization of phospholipase C (39-41). The substrate of the putative or identified kinases has been shown to be the agonist-occupied receptor or the G-protein. In *D. discoideum*, at least three desensitization processes take place: (i) the rapid ($t_0 \sim 4$ s) desensitization of guanylate cyclase (9), (ii) the slower ($t_0 \sim 2$ min) desensitization of adenylate cyclase (11, 12), and (iii) the slow ($t_0 \sim 2$ min)\(^1\) down-regulation of surface cAMP receptors (25). Desensitization of adenylate and guanylate cyclase reverses with $t_0 \sim 2-3$ min upon removal of the cAMP stimulus (9, 11), whereas down-regulation reverses with a $t_0 \sim 1$ h (25). Recently, it has been shown that desensitization of adenylate cyclase *in vivo* may be due to the phosphorylation of the cAMP receptor (22-24).

The binding of cAMP to membranes is modified by guanine nucleotides (16-19), which may suggest that the receptor(s) interact with a G-protein(s). This view is substantially supported by the recent findings that guanosine triphosphates activate adenylate cyclase *in vitro* (20, 21).

In this report, experiments are shown which probe the hypothesis that phosphorylation reactions modify the functional interaction between cell-surface cAMP receptors and a putative guanine nucleotide regulatory protein in *D. discoideum* membranes. An ATP derivative, ATP\_S, has been used. It has been reported that this analog is a potent substrate in many kinase reactions, but that the product, protein phosphorothioate, is not easily hydrolyzed by phosphatases (37).

Therefore, little attention has to be paid to protein phosphatase activity which is high in *D. discoideum* (36).
The main results of this study are the following. 1) MgATPyS rapidly (t0 ∼ 1 min) and irreversibly alters cAMP binding. The number of binding sites is slightly (∼15%) reduced. In control membranes, GTPγS induces a transition of high affinity binding to a low affinity form. This low affinity binding form is induced by pretreatment of membranes with MgATPyS, and GTPγS does not strongly affect cAMP binding any further. 2) These effects are not induced by Mg2+ or ATPγS alone or by MgAppNHp or MgAppCHp. 3) MgATP induces qualitatively similar results as MgATPyS, which are, however, less pronounced. 4) The effect of MgATPyS is potentiated by exogenous cAMP-dependent protein kinase and is readily reversible. Reversibility is slowed down by NaF, a phosphatase inhibitor (38). 5) The major phosphorylated protein in membranes is a protein with M, = 36,000.

These results suggest that the effects of MgATPyS and MgATP on cAMP binding are mediated by endogenous protein kinase activity. The substrate of this reaction could be the cAMP receptor, the G-protein, or any other component that affects the receptor/G-protein interaction. The major phosphorylated protein in isolated membranes has an apparent molecular weight of 36,000 and does not co-migrate on SDS-PAGE with the phosphorylated receptor. This suggests that the receptor is not phosphorylated in vitro or that phosphorylation is below the detection limit. A definite proof whether phosphorylation of the receptor, G-protein, or another component is responsible for the observed effects can be given only after a functional analysis of purified phosphorylated and nonphosphorylated components that are reconstituted in artificial membranes. Unfortunately, conditions for the isolation and reconstitution of signal transducing components that have been used in vertebrates (31) are not effective in D. discoideum, and no alternative conditions have been found yet.2

Recently, it has been shown that guanosine triphosphate stimulates adenylate cyclase of D. discoideum in vitro (20, 21). We observed only a modest degree of stimulation (70%). Interestingly, however, this stimulation was lost in membranes derived from desensitized cells (adenylate and guanylate cyclases were desensitized, and receptors were partly down-regulated). In addition, when membranes from control cells were preincubated with MgATPyS, the 70% stimulation of adenylate cyclase by GTPγS was converted to a 30% inhibition. These observations suggest that MgATPyS alters the receptor/G-protein as well as the G-protein/adenylate cyclase interactions. MgATPyS does not alter the number of binding sites. Interestingly, 40% of the binding sites are lost in a reversible manner upon further incubation of MgATPyS-treated membranes with cAMP and micromolar concentrations of Ca2+.3 These observations may suggest that the action of MgATPyS could be related to the mechanism of down-regulation of cAMP receptors.

The present and previous (42) results that a putative endogenous protein kinase activity modifies receptor/G-protein interactions allow the investigation of many potentially interesting questions such as whether occupation of the receptor or G-protein affects the rate of phosphorylation and dephosphorylation and concerning the identities of the kinase, the phosphatase, and their substrates. The notion that ATPγS irreversibly and ATP reversibly alter the cAMP binding activity could facilitate these experiments.

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REFERENCES

Footnotes:
2 P. M. W. Janssens and R. Van Driel, personal communication.
3 P. J. M. Van Haastert, unpublished results.