Activation of cAMP-dependent protein kinase is required for heterologous desensitization of adenylyl cyclase in S49 wild-type lymphoma cells

(β-adrenergic receptor)

Richard B. Clark*, Mark W. Kunkel, Jacqueline Friedman, Thomas J. Goka, and John A. Johnson

The University of Texas Health Science Center at Houston, Graduate School of Biomedical Sciences, Houston, TX 77030

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ABSTRACT We report here that, contrary to previously reported findings, treatment of S49 wild-type (WT) lymphoma cells with 0–50 nM epinephrine resulted in a heterologous desensitization of adenylyl cyclase (EC 4.6.1.1)—that is, epinephrine and prostaglandin E₃ (PGE₃) stimulations of adenylyl cyclase were reduced. Observation of this heterologous desensitization required the assay of adenylyl cyclase with submillimolar concentrations of Mg²⁺ and low concentrations of epinephrine. Also, whereas previously there had been no evidence for any role of cAMP-dependent protein kinase in the desensitization of the WT β-adrenergic receptor, our data comparing the characteristics of the desensitization in WT, kin⁻, and cyc⁻ lymphoma cells [where kin⁻ and cyc⁻ refer to variants of S49 WT cells lacking cAMP-dependent protein kinase activity (kin⁻) and the α subunit of the stimulatory guanine nucleotide-binding regulatory protein (cyc⁻)] now suggest that cAMP-dependent protein kinase mediates the heterologous desensitization of adenylyl cyclase. Specifically, we found that only the WT cells exhibited epinephrine-induced heterologous desensitization. The kin⁻ and cyc⁻ cells exhibited only homologous desensitization, and much higher concentrations of epinephrine were required to elicit the homologous desensitization in the variants relative to the heterologous desensitization of the WT. Treatment of WT and cyc⁻ cells with dibutyryl cAMP or treatment of WT with forskolin or PGE₃ caused the heterologous desensitization of adenylyl cyclase, indicating that neither receptor occupancy nor activation of adenylyl cyclase was necessary for the heterologous desensitization.

For some time now it has been widely thought that epinephrine-induced desensitization of the β-adrenergic receptor in wild-type (WT) S49 lymphoma cells was homologous and that it did not require the α subunit of the stimulatory guanine nucleotide-binding regulatory protein (Gₐα), cAMP, or cAMP-dependent protein kinase (1–9). Support for this was derived in large part from studies of the various mutants of the WT defective in components of the cAMP second messenger system. In particular, we demonstrated that homologous, agonist-induced desensitizations of either the β-adrenergic or the prostaglandin E₃ (PGE₃)-responsive adenylyl cyclase (EC 4.6.1.1) required rather high levels of receptor occupancy and were indistinguishable in the WT and cyc⁻ mutant (the cyc⁻ S49 mutant lacks Gₐα) (4–7).

Recently we presented data that, in contrast to earlier studies, revealed a major difference in epinephrine-induced desensitization between WT and cyc⁻ cells and raised once again the possibility that either cAMP, cAMP-dependent protein kinase, or Gₐα was involved in the desensitization of S49 lymphoma cells (10). We found that incubation of WT cells with 5–50 nM epinephrine desensitized epinephrine stimulation of the WT adenylyl cyclase but that even 50 nM epinephrine had no effect on the response of cyc⁻ cells. Observation of this difference in the desensitization of adenylyl cyclase between the WT and cyc⁻ cells was dependent on the use, in our adenylyl cyclase assays, of submillimolar concentrations of Mg²⁺, which appear to reflect the intracellular concentration of free Mg²⁺ in these cells (11, 12).

The original intent of the present experiments was to clarify the role of cAMP and Gₐα in the homologous desensitization of the β-adrenergic receptor in response to the treatment of WT cells with low concentrations of epinephrine. In the process we discovered, to our surprise, that the desensitization of WT cells in response to low concentrations of epinephrine was heterologous, not homologous, and that it required increased levels of cAMP and activation of cAMP-dependent protein kinase. This has led us to propose a model in which the desensitization of the WT β-adrenergic receptor is mediated by two separate pathways. One, which is triggered by low concentrations of hormones or other agents that raise cAMP levels, appears to be mediated by cAMP-dependent protein kinase and leads to heterologous desensitization. The other pathway, which requires much higher levels of epinephrine, appears to be independent of cAMP and leads to homologous desensitization.

METHODS

Cell Culture and Membrane Preparation. WT, kin⁻ (S49 WT cell variant lacking cAMP-dependent protein kinase activity), and cyc⁻ lymphoma cells were grown at 37°C in Dulbecco's modified Eagle's medium with 5% or 10% horse serum (13). The kin⁻ cell line, 24.6.1.1, was obtained from Henry Bourne (University of California School of Medicine, San Francisco), and the kin⁻ clone U200/19 was from Ted Van Daalen Wetters (University of California, San Francisco). kin⁻ cells were routinely assayed for cAMP-dependent protein kinase activity as described (14) to confirm that they had none.

Cells were collected with a 5-min centrifugation at 600 x g and were resuspended in medium buffered with 20 mM Hepes (pH 7.4) at 30–35 x 10⁶ cells per ml. The cells were then incubated at 37°C for 15 min with various concentrations of epinephrine in ascorbate/thioura at final concentrations of 0.1 and 1.0 mM, respectively; controls received

Abbreviations: WT, wild-type S49 lymphoma cells; cyc⁻, variant of S49 WT cells lacking the α subunit of the stimulatory guanine nucleotide-binding regulatory protein (Gₐ); UNC, variant of S49 WT cells with a defective G, that uncouples hormonal stimulation; kin⁻, variant of S49 WT cells lacking cAMP-dependent protein kinase activity; p[NH]ppG, guanylyl imidophosphate; PGE₃, prostaglandin E₃.

*To whom reprint requests should be addressed.

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ascorbate/thiourea alone. For experiments involving pre-treatment of cys- cells with dibutyryl cAMP, the time of incubation was 20 min with dibutyryl cAMP; this was followed by a 10-min incubation with ascorbate/thiourea or epinephrine in ascorbate/thiourea. For treatments with forskolin and PGE1, stock solutions were made in ethanol and diluted into the cell mixture such that the final concentration of ethanol was <0.1%

Following the pretreatments, the cells were rapidly cooled in an ice bath, collected by centrifugation, washed one time in ice-cold buffer A (137 mM NaCl/5.56 mM KCl/1.1 mM KH2PO4/1.08 mM Na2HPO4, pH 7.2), and resuspended in ice-cold buffer B (150 mM NaCl/20 mM Hepes ph 7.4) containing 5 mM NaH2PO4 and 10 mM NaF as phosphatase inhibitors and 1.0 mM benzamidine as a protease inhibitor.

Following resuspension in buffer B, cells were lysed by nitrogen cavitation in a Parr cell disruption bomb on ice at 500 psi (1 psi = 6.89 kPa) of nitrogen for 20 min. The cell lysate was collected and centrifuged at 600 x g to remove nuclei and cell debris. Alternatively, for most of the WT and kin- experiments shown in Fig. 3, the cells were lysed by 20 strokes of a Dounce homogenizer in a buffer composed of 20 mM Hepes, 1 mM EDTA, 1 mM benzamide, 10 μg of leupeptin per ml, 5 mM Na2HPO4, and 10 mM tetrasodium pyrophosphate (ph 7.2). This allowed processing of a greater number of samples. Similar results were obtained for the % desensitization of adenylyl cyclase with the two methods of cell breakage, although we consistently found a higher Kdes for epinephrine stimulation of adenylyl cyclase with the homogenization technique.

The 600 x g supernatant fraction resulting from either method of lysis was layered over a step gradient containing 43%, 35%, 31%, 27%, 23%, and 19% sucrose in 20 mM Hepes/1 mM EDTA, pH 8. The gradients were centrifuged for 80 min at 25,000 rpm in a Beckman SW27 rotor, and the membrane fractions were removed and immediately frozen.

Reconstitution of cys- Adenylyl Cyclase. Adenylyl cyclase activity in cys- membranes was reconstituted by the procedure of Sternweis and Gilman (15), modified as described (10).

Adenylyl Cyclase Assay. Adenylyl cyclase was assayed by the method of Salomon et al. (16) as modified (7). The assay solution consisted of 50 mM Hepes (pH 7.7), 1 mM EDTA, 1.47 mM MgCl2, 8 mM creatine phosphate, 16 units of creatine phosphokinase per ml, 0.2 mM ATP, 10 μM GTP, 0.1 mM 1-methyl-3-isobutylxanthine, ~2 μCi (1 Ci = 37 GBq) of [α-32P]ATP (~25 Ci/mmol, from ICN), and 50 μl of membranes (30–60 μg) in a final volume of 100 μl. Each assay was carried out for 10 min at 30°C. The free Mg2+ concentration in this standard assay was 0.3 mM calculated by the method of Iyengar and Birnbaumer (17). Percent desensitization was calculated as follows: [1 - (treated activity/control activity)] x 100. Basal activities, which were not altered as a result of the various pretreatments, were subtracted from hormone-stimulated activities. To determine the Kdes and Vmax, for either epinephrine or PGE1 stimulation of adenylyl cyclase, membranes were stimulated with a minimum of six concentrations and the data were analyzed by linear regression analysis of Eadie–Hofstee plots. In no case were data presented for which the linear regression coefficient was <0.97.

Measurement of cAMP Accumulation. Intracellular accumulation of cAMP was measured with the [3H]adenine prelabeling technique as discussed (13, 18).

RESULTS

Epinephrine Treatment of WT Cells Leads to Heterologous Desensitization of Adenylyl Cyclase. We had previously demonstrated that the desensitization of WT and cys- adenylyl cyclase by either epinephrine or PGE1 was homologous. This was based for the most part on experiments involving treatment of cells with relatively high concentrations of hormones and on cell-free assays of adenylyl cyclase in the presence of high Mg2+ concentrations (4–7). We have recently shown that increasing Mg2+ in the range of 0.1–10 mM progressively obscures epinephrine-induced desensitization of the WT adenylyl cyclase (10). Use of the low Mg2+ concentrations in our adenylyl cyclase assays enabled us to observe desensitization of epinephrine-stimulated adenylyl cyclase following treatment of cells with 5–20 nM epinephrine (10), concentrations that had previously been shown to cause desensitization in intact WT cells (19, 20).

Originally we presumed that the low Mg2+ assay conditions affected only our observation of the extent of desensitization of epinephrine stimulation; however, many experiments such as that shown in Fig. 1 demonstrated that PGE1 stimulation of adenylyl cyclase was desensitized as well. In this experiment WT cells were pretreated with various low concentrations of epinephrine. Membranes prepared from control and pretreated cells were assayed for adenylyl cyclase in the presence of either epinephrine (50 nM or 10 μM) or 200 nM PGE1. The free Mg2+ concentration in the assay was 0.3 mM. The extent of desensitization of adenylyl cyclase stimulated by either 50 nM epinephrine or 200 nM PGE1 was similar following treatment with 5 or 10 nM epinephrine. Thus, the desensitization induced by low concentrations of epinephrine was heterologous, not homologous. When cells were desensitized with 50 nM epinephrine the loss of epinephrine stimulation of adenylyl cyclase was somewhat greater relative to the loss of PGE1 stimulation. Further increase of the concentration of epinephrine led to additional desensitization of the epinephrine stimulation of adenylyl cyclase but not of the PGE1 stimulation. Pretreatment of WT cells with 1.0 μM epinephrine for 15 min led to an 87.5% ± 4.8% desensitization of 50 nM epinephrine stimulation of adenylyl cyclase and only a 34% ± 10% desensitization of 5.0 μM PGE1 stimulation (mean ± range, n = 2). These differences between the extent of epinephrine and PGE1 desensitization were observed consistently following pretreatment with ≅50 nM epinephrine and reflect the increasing contribution of homologous desensitization that appears to be half-maximal in WT cells at an epinephrine concentration of about 200 nM (5, 7).

The experiment in Fig. 1 demonstrated a much lower estimate of desensitization when the adenylyl cyclase was assayed with 10 μM epinephrine. This observation reflects the role of pretreatment with epinephrine or PGE1.
the fact that, in S49 lymphoma cells, heterologous desensitization induced by 20 nM epinephrine causes a 1.9-fold ± 0.2 increase in the $K_{act}$ for epinephrine stimulation of adenyl cyclase but only a small (13.6% ± 10.4%) decrease in the $V_{max}$ (mean ± range, $n = 2$).

In the experiment shown in Fig. 1, we found that the various epinephrine pretreatments of WT cells did not cause any change in the stimulation of adenyl cyclase by 50 µM forskolin (activities of membranes from control vs. 50 nM epinephrine-treated cells were 209 ± 1.7 and 204 ± 2.8 pmol/min per mg of protein, respectively). In other experiments we have demonstrated repeatedly that neither guanyl imidodiphosphate (p[NH]ppG) nor NaF stimulation was affected by epinephrine pretreatment. These data demonstrate that the nature of this heterologous desensitization of adenyl cyclase is quite different from other reported types of heterologous desensitization of adenyl cyclase (see review, ref. 1), which invariably affected either p[NH]ppG-, NaF-, or forskolin-stimulated activities.

Forskolin and Dibutyryl cAMP Cause the Heterologous Desensitization of WT Adenyl Cyclase. The epinephrine-induced heterologous desensitization suggested that ligand occupancy of the receptor was not required since the PGE$_1$-stimulated activity was reduced in the absence of any exogenous PGE$_1$. This was confirmed by the experiment shown in Table 1 in which WT cells were pretreated for 15 min with either 10 µM forskolin or 50 µM dibutyryl cAMP. Both of these agents caused the heterologous desensitization of adenyl cyclase. Epinephrine- and PGE$_1$-stimulated activities were reduced 50% and 51%, respectively, by 10 µM forskolin, and 40% and 55%, respectively, by dibutyryl cAMP. Treatment with 50 µM forskolin produced no additional desensitization, indicating that the maximal desensitization was about 50–60%, similar to the maximal heterologous desensitization caused by epinephrine, at least under these conditions (i.e., assay with 50 nM epinephrine and 0.3 mM free Mg$^{2+}$). We have also found that the pretreatment with 10 µM forskolin for 15 min caused a 2.34-fold ± 0.15 increase in the $K_{act}$ for epinephrine stimulation of adenyl cyclase and a 12.3% ± 8.4% decrease in the $V_{max}$ (mean ± the range of two experiments). This result is in good agreement with the increased $K_{act}$ caused by pretreatment of WT cells with 20 nM epinephrine. The effects of 10 µM forskolin pretreatment on the $V_{max}$ (from 289 to 226 pmol/min per mg) and $K_{act}$ (from 1.2 to 2.6 µM) for PGE$_1$ stimulation of adenyl cyclase were similar to those for epinephrine stimulation.

Heterologous desensitization was also observed when WT cells were pretreated with low concentrations of PGE$_1$ (0–20 nM). The PGE$_1$-induced heterologous desensitization resem-bled the epinephrine- and forskolin-induced heterologous desensitization in that the extent of desensitization of PGE$_1$ and epinephrine stimulations of adenyl cyclase was comparable and forskolin stimulation was unaltered. In one experiment in which WT cells were pretreated with 20 nM PGE$_1$ for 15 min, we found that the $K_{act}$ for subsequent epinephrine stimulation of adenyl cyclase was increased from 106 to 178 nM, whereas the $V_{max}$ was decreased from 246 to 220 pmol/min per mg.

In summary, the changes in the kinetic parameters of epinephrine stimulation of adenyl cyclase were remarkably similar in membranes from WT cells that were pretreated with either 20 nM epinephrine, 20 nM PGE$_1$, or 10 µM forskolin. This heterologous desensitization was characterized by about a 2-fold increase in the $K_{act}$ for epinephrine stimulation of adenyl cyclase and a slight decrease in the $V_{max}$.

Dibutyryl cAMP Causes the Heterologous Desensitization of Adenyl Cyclase in cyc$^{-}$ Cells. We have recently demonstrated (10) that pretreatment of cyc$^{-}$ cells with 50 nM epinephrine does not desensitize epinephrine stimulation of adenyl cyclase, whereas concentrations of epinephrine >50 nM cause homologous desensitization (4–7), as measured following reconstitution of cyc$^{-}$ membrane preparations with cholate extracts of WT Gs.

To help resolve the role of cAMP in the desensitization of this mutant we performed experiments such as that shown in Fig. 2. cyc$^{-}$ cells were incubated for 30 min at 37°C with either no additions (0.1 mM ascorbate/1 mM thioare), 50 µM dibutyryl cAMP, 50 nM epinephrine in ascorbate/thioare, or dibutyryl cAMP in combination with epinephrine. Epinephrine was present for the last 10 min of the 30-min incubation. Following the intact cell treatment, membranes were prepared, reconstituted with cholate extracts of WT membranes, and assayed for adenyl cyclase activity with either 30 nM, 60 nM, or 10 µM epinephrine. As previously reported (10), the pretreatment with 50 nM epinephrine alone caused no desensitization, whereas treatment with dibutyryl cAMP with or without epinephrine caused about a 35–45% desensitization of the 30 and 60 nM epinephrine stimulations but had almost no effect on the stimulation of adenyl cyclase by 10 µM epinephrine. In separate experiments we have found that the dibutyryl cAMP-induced desensitization of 200 nM PGE$_1$ stimulation of adenyl cyclase was actually greater than that of 50 nM epinephrine.

![Fig. 2. Pretreatment of cyc$^{-}$ cells with dibutyryl cAMP desensitizes adenyl cyclase activity. cyc$^{-}$ cells were pretreated for 20 min at 37°C with no additions or 50 µM dibutyryl cAMP (DibCa), after which time 50 nM epinephrine (Epi) was added as indicated for another 10 min. cyc$^{-}$ membranes were then prepared and reconstituted. Adenyl cyclase activity was measured in response to stimulation with 30 nM, 60 nM, or 10 µM epinephrine. The free Mg$^{2+}$ concentration was 0.125 mM. Values shown are the means ± SEM of triplicate determinations.](image-url)

<table>
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<th>Addition to assay</th>
<th>Control</th>
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<th>Dibutyryl cAMP (50 µM)</th>
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<td>None</td>
<td>9.0 ± 0.8</td>
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<td>(47.4)</td>
<td>(9.1)</td>
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S49 WT cells were pretreated for 15 min at 37°C with either 10 µM forskolin or 50 µM dibutyryl cAMP. Membranes were prepared and assayed for basal, epinephrine-, PGE$_1$-, and forskolin-stimulated adenyl cyclase activities. The free Mg$^{2+}$ concentration in the assay was 0.3 mM. Values in parentheses are the % desensitization.
stimulation (59% ± 3%, n = 3, and 27% ± 5%, n = 4, respectively); thus, the desensitization was heterologous. A similar pattern of heterologous desensitization was caused by dibutyryl cAMP treatment of UNC cells. Since these cells lack hormonal stimulation of adenylyl cyclase due to a defective G,α, membranes were reconstituted prior to adenylyl cyclase assays by the same procedure used for the cyclase assay. We have also shown that 50 μM butyrate caused no desensitization of adenylyl cyclase following treatment of either the cyclase− or the WT cells.

**Epinephrine Pretreatment Does Not Induce Heterologous Desensitization in cyclase− Cells**. The dibutyryl cAMP- and forskolin-induced heterologous desensitization in the WT and cyclase− cells suggested that activation of cAMP-dependent protein kinase was essential for the heterologous desensitization of adenylyl cyclase. A variant of the S49 WT lymphoma cell designated cyclase−, which lacks detectable cAMP-dependent protein kinase activity, provided us with a means of directly testing the role of cAMP-dependent protein kinase in heterologous desensitization. It had been shown previously that cyclase− cells undergo homologous desensitization in response to relatively high concentrations of catecholamines (8, 21).

Our first approach was to compare the extent of desensitization of adenylyl cyclase in WT and cyclase− cells following pretreatment with 5–50 nM epinephrine. The data shown in Fig. 3, the compilation of >15 experiments, demonstrated that about 4-fold higher concentrations of epinephrine were required to observe desensitization in the cyclase− cells. Pretreatment of WT cells with 5 and 10 nM epinephrine resulted in 22% and 28% desensitization of 50 nM epinephrine stimulation of adenylyl cyclase, and yet these same concentrations of epinephrine did not desensitize the cyclase− cells. These results were confirmed by intact cell studies in which the accumulations of cAMP in WT and cyclase− cells were measured following stimulation with 10 nM epinephrine. We found that 10 nM epinephrine rapidly desensitized the WT cells—that is, it caused the expected rapid rise and fall in cAMP consistent with earlier work (data not shown). In contrast, the extent of desensitization was much less in the cyclase− cells. Even though the initial rates of cAMP synthesis in the two lines were similar, as indicated by cAMP accumulations after 2 min, the cyclase− cells were able to maintain much higher cAMP levels after 10 min. In fact, accumulation of cAMP in the cyclase− cells was 12- to 15-fold higher than in the WT cells at 20 min. Since the desensitization in response to the incubation of WT cells with low epinephrine concentrations was heterologous (see Fig. 1), the cyclase− results suggested that there may be no heterologous desensitization of adenylyl cyclase in the cyclase− cell line in response to treatment with the very low concentrations of epinephrine.

The lack of heterologous desensitization of the cyclase− cells was confirmed by experiments such as that shown in Fig. 4 in which cyclase− cells were pretreated with 80 nM epinephrine for 15 min. This treatment caused about a 50% desensitization of 50 nM epinephrine stimulation of adenylyl cyclase but no significant desensitization of the PGE1 stimulation, thus demonstrating that the desensitization of the cyclase− adenylyl cyclase was purely homologous. Higher epinephrine concentrations, though increasing the extent of the homologous desensitization (e.g., 1.0 μM epinephrine induced an 85% desensitization of epinephrine stimulation of adenylyl cyclase), still did not result in any heterologous desensitization of PGE1 stimulation. Similar results have been obtained with another cyclase− clone, U200/19, that was isolated from the WT cells independently and also lacks measurable cAMP-dependent protein kinase. These results suggested that cAMP-dependent protein kinase mediates the heterologous desensitization of adenylyl cyclase.

**DISCUSSION**

Recently Lefkowitz's group reported the isolation of an apparently unique protein kinase (β-adrenergic receptor kinase) from cyclase− cells whose capacity to phosphorylate the purified lung β-adrenergic receptor in liposomes was dependent on agonist occupancy of the receptor but apparently independent of cAMP, Ca2+, or calmodulin (22). The dependency of its in vitro action on a high level of receptor occupancy suggested that it was a possible candidate for the mediator of homologous desensitization of WT cells, and this group proposed that β-adrenergic receptor kinase mediated the desensitization of the β-adrenergic receptor in mammalian cells. At the time this hypothesis was proposed, homologous desensitization was the only type of desensitization thought to occur in response to β-adrenergic agonist treatment of the S49 WT cells. The requirement for a high level of receptor occupancy made it unlikely that β-adrenergic receptor kinase was involved in the more physi-

![Fig. 3. Comparison of the desensitization of WT and cyclase− adenylyl cyclase in response to the pretreatment with 5–50 nM epinephrine. S49 WT (●) and cyclase− (●) cells were treated for 15 min at 37°C with or without various concentrations of epinephrine. Membranes were prepared, and their response to 50 nM epinephrine stimulation in the adenylyl cyclase assay was assessed. Values shown are the means ± SEM. Free Mg2+ concentrations in these assays were between 0.3 and 0.75 mM. For the WT cells, n = 5 for 5 and 10 nM, n = 2 for 20 nM, and n = 1 for 50 nM, where n is the number of separate experiments, each assayed in triplicate. For the cyclase− cells, n = 2 for 5 nM, n = 4 for 10 nM, n = 5 for 20 nM, n = 2 for 40 nM, and n = 2 for 50 nM.](image1)

![Fig. 4. Epinephrine pretreatment of cyclase− cells results in homologous desensitization of cyclase− adenylyl cyclase. cyclase− cells were treated for 15 min at 37°C with or without 80 nM epinephrine (Epi). Membranes were prepared and adenylyl cyclase activity was measured in response to the indicated concentrations of PGE1 and epinephrine. The free Mg2+ concentration in the assay was 0.3 mM. Data are plotted as mean ± SEM for triplicate determinations.](image2)
Heterologous desensitization induced by low concentrations of hormones (10, 19, 20).

The data we have reported here have led us to modify previous hypotheses concerning the mechanisms of desensitization of the S49 WT lymphoma cells and to suggest that the rapid, agonist-induced desensitization of hormonal stimulation of adenylyl cyclase in WT cells involves two separate pathways, as shown in Fig. 5. The heterologous pathway requires cAMP and cAMP-dependent protein kinase and is active when WT cells are pretreated with low concentration of either epinephrine, PGE$_1$, or forskolin. It remains to be determined whether there are other steps in a cascade analogous to cAMP-dependent protein kinase, regulation of phosphorylation by means of phosphorylase kinase, or inactivation of a phosphatase by means of activation of an inhibitor. The second pathway, which leads to homologous desensitization, is independent of either cAMP or cAMP-dependent protein kinase and requires much higher levels of hormones. Homologous desensitization may be synonymous with sequestration–internalization, since they appear to occur over a similar range [effective concentration for half-maximal desensitization (EC$_{50}$) $= 200$ nM] of epinephrine concentrations in the WT (5, 7). The requirement for high ligand occupancy of the $\beta$-adrenergic receptor engenders speculation that this process may be triggered by $\beta$-adrenergic receptor kinase as previously suggested (22). In our model we suggest that the phosphorylation by $\beta$-adrenergic receptor kinase involves sites different from those for cAMP-dependent protein kinase and does not desensitize the $\beta$-adrenergic receptor but rather is the signal that leads to sequestration–internalization. Physical isolation of the receptor then prevents receptor–adenyl cyclase coupling.

One of the questions that surfaces based on our results is how general the role of cAMP-dependent protein kinase will be in heterologous desensitization of adenylyl cyclase. There has been ample evidence (1–3) that cAMP causes desensitization of the $\beta$-adrenergic receptor in other systems, most notably the turkey erythrocyte (3), the C6-2B glioma cell line (23), and the astrocytoma cell line (24). In cultured mammalian cells there has been a real problem with the detection of heterologous desensitization of adenylyl cyclase in cell-free preparations. There is a distinct possibility that the heterologous desensitization in the mammalian cells may have been obscured in previous work by the use of only high concentrations of hormones in the pretreatment and the assay of epinephrine stimulation of adenylyl cyclase, as well as in the use of high Mg$^{2+}$ concentrations in the adenylyl cyclase assays, which we have recently shown obscures heterologous desensitization (10).

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