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Phosphorylation/dephosphorylation of the β-adrenergic receptor regulates its functional coupling to adenylate cyclase and subcellular distribution

(homologous desensitization/hormone receptor/receptor sequestration/protein phosphatase)

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ABSTRACT **Prolonged exposure of cells or tissues to drugs or hormones such as catecholamines leads to a state of refractoriness to further stimulation by that agent, known as homologous desensitization. In the case of the β-adrenergic receptor coupled to adenylate cyclase, this process has been shown to be intimately associated with the sequestration of the receptors from the cell surface through a cAMP-independent process. Recently, we have shown that homologous desensitization in the frog erythrocyte model system is also associated with increased phosphorylation of the β-adrenergic receptor. We now provide evidence that the phosphorylation state of the β-adrenergic receptor regulates its functional coupling to adenylate cyclase, subcellular translocation, and recycling to the cell surface during the process of agonist-induced homologous desensitization. Moreover, we show that the receptor phosphorylation is reversed by a phosphatase specifically associated with the sequestered subcellular compartment. At 23°C, the time courses of β-adrenergic receptor phosphorylation, sequestration, and adenylate cyclase desensitization are identical, occurring without a lag, exhibiting a $t_{1/2}$ of 30 min, and reaching a maximum at ≈3 hr. Upon cell lysis, the sequestered β-adrenergic receptors can be partially recovered in a light membrane vesicle fraction that is separable from the plasma membranes by differential centrifugation. The increased β-adrenergic receptor phosphorylation is apparently reversed in the sequestered vesicle fraction as the sequestered receptors exhibit a phosphate/receptor stoichiometry that is similar to that observed under basal conditions. High levels of a β-adrenergic receptor phosphatase activity appear to be associated with the sequestered vesicle membranes. The functional activity of the phosphorylated β-adrenergic receptor was examined by reconstituting purified receptor with its biochemical effector the guanine nucleotide regulatory protein (N_s) in phospholipid vesicles and assessing the receptor-stimulated GTPase activity of N_s. Compared to controls, phosphorylated β-adrenergic receptors, purified from desensitized cells, were less efficacious in activating the N_s GTPase activity. These results suggest that phosphorylation of the β-adrenergic receptor leads to its functional uncoupling and physical translocation away from the cell surface into a sequestered membrane domain. In the sequestered compartment, the phosphorylation is reversed thus enabling the receptor to recycle back to the cell surface and recouple with adenylate cyclase.**

Prolonged exposure of target cells to hormone agonists results in diminished cellular responsiveness to further hormonal stimulation through a process known as desensitization. Hormone-induced desensitization has been extensively investigated in a variety of cells that contain β-adrenergic receptors coupled to the stimulation of adenylate cyclase activity. Desensitization of β-adrenergic receptor-coupled adenylate cyclase is generally divided into two major categories. One form is referred to as homologous and is distinguished by the fact that only stimulation by β-adrenergic agonists is attenuated (1–3). Conversely, heterologous desensitization is characterized by a diminished responsiveness to additional hormones and to nonhormonal activators such as guanine nucleotides and fluoride ion (1–3).

Recent information has indicated that heterologous desensitization is associated with phosphorylation and functional uncoupling of the β-adrenergic receptor, which is mediated, at least in part, by the cAMP-dependent protein kinase (4–7). In contrast, homologous desensitization appears to involve the sequestration or removal of the β-adrenergic receptors away from the cell surface through a cAMP-independent process (1–3). The location of the sequestered β-adrenergic receptors in desensitized cells is not yet known with certainty. Evidence has accumulated that the sequestered β-adrenergic receptors can be recovered in small vesicular membrane particles, which, under basal conditions, are nearly devoid of receptors (1–3). It is not yet clear whether these light membrane particles actually represent endocytotic vesicles or whether they might be a sequestered domain of the plasma membrane (8, 9). Whether or not the β-adrenergic receptors become functionally modified or uncoupled during homologous desensitization in addition to their physical sequestration is also currently a point of controversy (10–15). Recently, we have demonstrated that homologous desensitization is associated with increased phosphorylation of the β-adrenergic receptor (16, 17). This phosphorylation process is pharmacologically specific, stoichiometric, and may be mediated by a β-adrenergic receptor kinase (16–18). In the present investigation, we demonstrate a direct correlation between the phosphorylation state of the β-adrenergic receptor and both its functionality and subcellular location. Our results further suggest that dephosphorylation and reactivation of the receptor are functions specifically accomplished in the sequestered compartment of the cell.

MATERIALS AND METHODS

Materials. [γ-^{32}P]GTP and $^{32}P_i$ (carrier free) were obtained from New England Nuclear. ^{3}H-labeled 4-{3-[1,1-dimethyl(ethyl)amino]-2-hydroxypropoxy}-1,3-dihydro-2*H*-benzimidazol-2-one ([^{3}H]CGP12177) (30–50 Ci/mmol; 1 Ci = 37 GBq) was from Amersham. Soybean phosphatidylcholine, bovine serum albumin (fraction V), polyethylene glycol, and

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Abbreviations: [^{3}H]CGP12177, ^{3}H-labeled 4-{3-[1,1-dimethyl(ethyl)amino]-2-hydroxypropoxy}-1,3-dihydro-2*H*-benzimidazol-2-one; N_s, stimulatory nucleotide regulatory protein.

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p-nitrophenyl phosphate were from Sigma. Extracti-Gel D was from Pierce. Octyl β-D-glucopyranoside (octyl glucoside) was from Calbiochem-Behring. All other materials were from sources described (5).

^{32}P Incorporation into Frog Erythrocyte β-Adrenergic Receptors. Washed frog erythrocytes were suspended to 20% hematocrit in 110 mM NaCl/10 mM glucose/20 mM sucrose/17 mM Tris·HCl, pH 7.4 (buffer A), with 300 units of penicillin per ml and 1 mg of streptomycin per ml. The cells were incubated with carrier-free $^{32}P_i$ (0.5 mCi/ml) at 23°C for 20 hr to allow ^{32}P incorporation into the intracellular ATP. After the ^{32}P incorporation, 0.2 mM sodium metabisulfite was added to each flask of cells, which were then further incubated in the absence (control) or presence (desensitized) of 10 μM (−)-isoproterenol for 3 hr or as indicated. The cells were then washed three times with buffer A and lysed either by hypotonic shock in 5 mM $MgCl_2$/5 mM Tris·HCl, pH 7.4, or by Dounce homogenization in ice-cold H_2O as described below.

Membrane Preparations and Receptor Purification. The location of the sequestered β-adrenergic receptors in desensitized cells is not known with certainty. The observation that upon cell lysis the sequestered receptors appear to be translocated into light membrane particles has suggested that they reside in cytoplasmic vesicles (1–3). However, we have recently demonstrated that in frog erythrocytes the production of these vesicular particles is dependent on the method of cell lysis (8, 16, 19). If the desensitized cells are lysed in H_2O using a Potter–Elvehjem homogenizer, then a portion of the sequestered receptors can be recovered in a light membrane fraction obtained by high-speed centrifugation of the cell cytosol (8, 19). In contrast, if the cells are lysed by freezing and thawing or by hypotonic shock in the absence of homogenization, then the sequestered receptors can be found associated with the plasma membrane fraction (8, 16).

In the present investigation, we have used two different membrane preparations. One involves hypotonic lysis of the cells in 5 mM $MgCl_2$/5 mM Tris·HCl, pH 7.4, without homogenization followed by centrifugation at 30,000 × *g* for 10 min. This results in a membrane pellet containing the total cellular pool of β-adrenergic receptors—that is, the cell-surface receptors in addition to the receptors sequestered from the cell surface. In the second method, the cells are lysed by Dounce homogenization in ice-cold H_2O followed by centrifugation at 30,000 × *g* for 10 min. The low-speed pellet contains plasma membranes that are contaminated with some sequestered vesicle membranes (ref. 8; unpublished observations). The supernatant fraction is further centrifuged at 158,000 × *g* for 1 hr. The resulting high-speed pellet consists solely of the sequestered vesicular membrane receptors (8, 19). The Dounce homogenization was less efficient than that of the Potter–Elvehjem for separating the sequestered vesicle particles from the plasma membranes but was necessary when using ^{32}P-labeled cells because of the high levels of radioactivity.

The subsequent solubilization, affinity chromatography, and concentration of the β-adrenergic receptors were performed exactly as described (5, 16). After desalting on Sephadex G-50 columns, each sample was assayed for maximal receptor binding with [^{125}I]iodocyanopindolol followed by lyophilization and resuspension in $NaDodSO_4$/PAGE sample buffer. $NaDodSO_4$/PAGE and autoradiography were performed as described (5, 16).

Preparation of the Stimulatory Nucleotide Regulatory Protein (N_s). N_s was purified by a procedure similar to that of Sternweis (20). Briefly, bovine brain membranes were prepared and solubilized with sodium cholate. Solubilized N_s was then successively chromatographed on DEAE-Sephacel, Ultrogel AcA-34, octyl-Sepharose, and hydroxylapatite, followed by a final purification step on DEAE-Sephacel.

Insertion of β-Adrenergic Receptors and N_s into Phospholipid Vesicles. β-Adrenergic receptors from control or desensitized erythrocytes were purified by affinity chromatography and inserted into phospholipid vesicles with purified N_s as described (13, 21). β-Adrenergic receptor (5–10 pmol in <150 μl) and N_s (15 pmol in <15 μl) were incubated with bovine serum albumin (2 mg/ml)/1.5 mM sonicated soybean phosphatidylcholine/0.85% (wt/vol) octyl glucoside on ice for 30 min. The detergent concentration was then reduced by chromatography on 1 ml of Extracti-Gel D columns, which were eluted with 100 mM NaCl/20 mM $MgCl_2$/10 mM Tris·HCl, pH 7.4. The eluates (2 ml) were added to 0.6 ml of polyethylene glycol 8000 [final concentration, 12.5% (wt/vol)] and incubated for 10 min at 22°C followed by dilution and centrifugation at 250,000 × *g* for 90 min at 4°C. The protein–lipid pellets were suspended in 100 mM NaCl/10 mM Tris·HCl, pH 7.4, and assayed for radioligand binding with [^{125}I]iodocyanopindolol or GTP hydrolytic activity.

GTP Hydrolytic Activity (GTPase). The GTPase activity was assayed by incubating 20 μl of the phospholipid vesicles containing β-adrenergic receptors and N_s in a total vol of 100 μl containing 10 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM EDTA, 0.2% bovine serum albumin, 1 mM adenyl-5′-yl-imidodiphosphate, and 100–200 nM [γ-^{32}P]GTP (≈40,000 cpm/pmol). The assay was stopped by the addition of 10 μl of cold 50% trichloroacetic acid and centrifugation at 1000 × *g* for 20 min. The supernatant was assayed for inorganic $^{32}P_i$ as described (21).

Radioligand Binding and Adenylate Cyclase Assays. Soluble β-adrenergic receptor binding assays were performed as described (5) using a saturating (300 pM) concentration of [^{125}I]iodocyanopindolol. Intact cell [^{3}H]CGP12177 binding assays were performed as described (17) using 2.5 × 10^7 erythrocytes and 10 nM [^{3}H]CGP12177. Adenylate cyclase assays were performed as described (5) using frog erythrocyte membranes prepared by hypotonic lysis of the cells. Protein was assayed by the method of Lowry *et al.* (22).

RESULTS

Recent data (23) have suggested that in astrocytoma cells, agonist-induced desensitization can be temporally dissociated from receptor sequestration with the former event preceding the latter. We thus wished to initially compare the rates of β-adrenergic receptor phosphorylation, receptor sequestration, and adenylate cyclase desensitization in frog erythrocytes. Fig. 1 shows that isoproterenol-induced receptor phosphorylation occurs without a lag, achieving a maximal level at 2.5–3 hr with a $t_{1/2}$ of ≈30 min. Similarly, the agonist-induced loss of isoproterenol-stimulated adenylate cyclase activity and loss of cell-surface receptor binding (monitored with the membrane-impermeable β-adrenergic antagonist [^{3}H]CGP12177) both proceed without a lag and reached maximal levels at ≈3 hr. The rates of β-adrenergic receptor phosphorylation, sequestration, and desensitization are thus identical in frog erythrocytes. This is consistent with the notion that receptor phosphorylation is intimately associated with the processes of homologous desensitization and sequestration of the β-adrenergic receptor.

It should be noted (Fig. 1) that the maximum extent of receptor sequestration (≈35%) is not as great as the extent of desensitization (≈50%). This might suggest that sequestration of receptors from the cell surface may not fully explain the desensitization and that phosphorylation may additionally produce a functional alteration or uncoupling of the receptors. To test this hypothesis, we purified the total cellular pool of β-adrenergic receptors from control and desensitized cells and directly tested their ability to couple with N_s in phospholipid vesicles. The purified N_s protein has

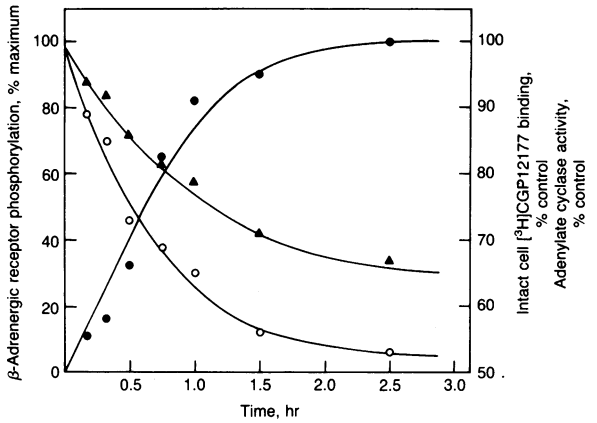

FIG. 1. Time course of isoproterenol-induced β-adrenergic receptor phosphorylation, receptor sequestration, and adenylate cyclase desensitization. Frog erythrocytes were ^{32}P-labeled and then incubated in the absence (control) or presence of 10 μM isoproterenol for the indicated times at 23°C. The cells were then lysed hypotonically in 5 mM $MgCl_2$/5 mM Tris·HCl, pH 7.4, and membranes were prepared as described. The β-adrenergic receptors were purified and assessed for ^{32}P incorporation on $NaDodSO_4$/PAGE. The phosphorylation data (●) are expressed as percentage of maximum increase in the phosphate/receptor stoichiometry (mol/mol) over the basal level (0.57 mol/mol). Adenylate cyclase activities (○) were determined and expressed as a percentage of the control value, which was 98 pmol·min^{-1}·mg^{-1}. Unlabeled frog erythrocytes were incubated with 10 μM isoproterenol in parallel, washed, and assessed for [^{3}H]CGP12177 binding (▲) by using intact cells. [^{3}H]CGP12177 is a hydrophilic β-adrenergic antagonist that is membrane impermeable and restricted to binding only those receptors present on the cell surface (9, 24). The control [^{3}H]CGP12177 binding activity was 3240 receptor sites per cell. The time course experiment shown is representative of three such experiments.

been shown to exhibit GTPase activity elicited by its interaction with the β-adrenergic receptor, which presumably terminates hormone and GTP-induced adenylate cyclase activation (21, 25). Fig. 2 shows the GTPase activity exhibited by reconstituted control or desensitized β-adrenergic

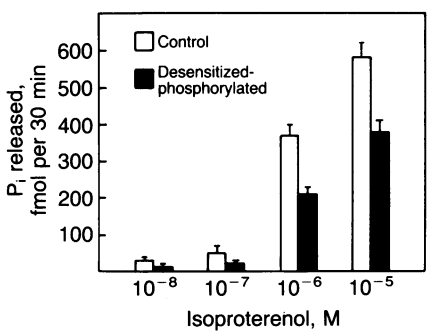

FIG. 2. GTPase activity of phospholipid vesicles containing N_s and β-adrenergic receptors purified from control or desensitized cells. Frog erythrocytes were incubated in the absence (control) or presence of 10 μM isoproterenol (desensitized) for 3 hr at 23°C. The cells were washed and lysed hypotonically with 5 mM $MgCl_2$/5 mM Tris·HCl, pH 7.4, as described. The total cellular pool of β-adrenergic receptors was solubilized and purified by affinity chromatography. After purification, the β-adrenergic receptors were inserted into phospholipid vesicles with N_s as described. Equal amounts of control and desensitized receptor were reconstituted (1.5 pmol) as assessed with [^{125}I]iodocyanopindolol binding after reconstitution. GTPase activity, as a function of the isoproterenol concentration, was then measured at 30°C for 30 min. The basal GTPase activity was subtracted from all isoproterenol-promoted GTPase activities. Data represent mean ± SD (n = 3) from a single experiment, which was performed three times with similar results.

receptors and N_s as a function of isoproterenol concentration. As shown, isoproterenol markedly stimulates GTPase activity; however, the phosphorylated receptors, purified from desensitized cells, are impaired in their ability to couple with N_s when compared to controls. This is evident despite the fact that equal amounts of control or desensitized β-adrenergic receptor are reconstituted into the phospholipid vesicles as assessed with [^{125}I]iodocyanopindolol binding subsequent to the reconstitution. Phosphorylation of the β-adrenergic receptors during homologous desensitization is thus associated with functional alterations in the receptors in addition to their physical sequestration.

In contrast, Strulovici *et al.* (10) found that the sequestered β-adrenergic receptors from frog erythrocytes were functionally active in reconstituting catecholamine-sensitive adenylate cyclase in *Xenopus laevis* erythrocytes. Similar results were obtained by Clark *et al.* (15) in characterizing the sequestered β-adrenergic receptors in S49 lymphoma cells. These results indicate that the sequestered receptors are in fact functionally normal. To reconcile these previous observations with our current data using the total cellular pool of desensitized receptors, we attempted to fractionate the receptors from desensitized cells into plasma membrane and sequestered membrane components and determine the phosphorylation states of each. For technical reasons (see *Materials and Methods*), however, it is not possible to prepare a plasma membrane fraction that is devoid of sequestered receptors. One can, however, obtain purified sequestered membrane vesicles that are, in fact, devoid of plasma membranes. We utilized this latter approach and the results are shown in Fig. 3. Lane 1 of the autoradiogram shows a purified β-adrenergic receptor preparation from control ^{32}P-labeled frog erythrocytes. As we previously demonstrated (16), the frog erythrocyte β-adrenergic receptor is phosphorylated under basal conditions, exhibiting a phosphate/re-

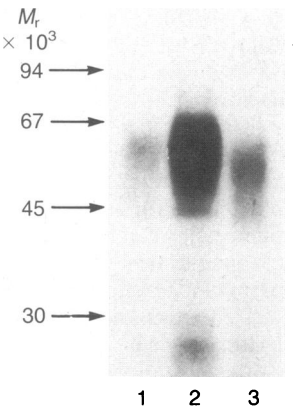

FIG. 3. $NaDodSO_4$/PAGE of ^{32}P-labeled β-adrenergic receptors from control and desensitized frog erythrocytes. Cells were preincubated with $^{32}P_i$ prior to incubation in the absence (control) or presence (desensitized) of 10 μM isoproterenol for 3 hr at 23°C. The desensitized cells were then split into two groups. One group, along with the control cells, was lysed hypotonically in 5 mM $MgCl_2$/5 mM Tris·HCl, pH 7.4, membranes were prepared, and the receptors were solubilized and purified. The second group of desensitized cells was lysed by Dounce homogenization in H_2O followed by preparation of a sequestered vesicular membrane fraction as described. The receptors in these membranes were subsequently purified and characterized on $NaDodSO_4$/PAGE. Lanes: 1, receptor preparation from control cells; 2, receptor preparation from the hypotonically lysed desensitized cells consisting of the total cellular pool of receptors (see *Materials and Methods*); 3, receptors purified from the sequestered vesicle membranes obtained from the Dounce-homogenized desensitized cells. Equal amounts (2.6 pmol) of receptor were loaded onto each lane of the gel. The experiment shown was performed three times with similar results.

ceptor stoichiometry (mol/mol) of 0.62 ± 0.15 (n = 5). Lane 2 shows a purified receptor preparation from isoproterenol-desensitized erythrocytes. This preparation consists of the total pool of β-adrenergic receptors in the desensitized cells, including both the cell surface and the sequestered receptors. As can be seen, the amount of phosphate incorporated into the receptor is substantially increased. The average stoichiometric value (mol of phosphate per mol of receptor) for the total pool of receptors from desensitized cells is 2.1 ± 0.19 (n = 7). Lane 3, however, shows a receptor preparation purified from the sequestered vesicle membranes obtained from desensitized cells. In this case, the level of receptor phosphorylation is low (0.75 ± 0.08 mol of phosphate per mol of receptor; n = 3), similar to that observed under basal conditions (Fig. 3, lane 1). This indicates that the major increase in phosphorylation of the β-adrenergic receptor upon desensitization (lane 2) is due predominately to an increase in the phosphorylation state of the cell-surface receptors. Moreover, this observation suggests that the increased receptor phosphorylation is reversed either during or rapidly after receptor sequestration from the cell surface. This dephosphorylation event may enable the receptor to eventually recycle back to the cell surface.

To explore the possibility that receptor dephosphorylation actually occurs in the sequestered vesicle compartment, we examined the ability of various cellular fractions to promote dephosphorylation of ^{32}P-labeled β-adrenergic receptor. Fig. 4 shows that incubation of ^{32}P-labeled receptor with an enriched plasma membrane fraction results in partial receptor dephosphorylation. However, incubation with a purified sequestered vesicle fraction results in receptor dephosphorylation, which occurs considerably more rapidly and to a greater extent (Fig. 4). Incubation with cell cytosol did not result in appreciable receptor dephosphorylation (data not shown). We further compared the ability of the plasma membrane and vesicle membrane fractions to dephosphorylate *p*-nitrophenyl phosphate, a general phosphatase substrate. Interestingly, the enriched plasma membrane fraction exhibited ≈10-fold greater activity toward this substrate (data not shown). Thus, even though the vesicle membranes possess ≈1/10th of the total phosphatase activity of the plasma membranes, they exhibit much higher levels of phosphatase activity when using phosphorylated β-adrenergic receptor as a substrate. This suggests that the phosphatase responsible for reversing the increased receptor phosphorylation likely resides in the sequestered vesicle compartment.

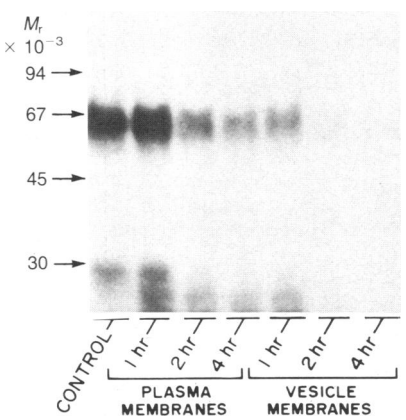

FIG. 4. Dephosphorylation of ^{32}P-labeled β-adrenergic receptors using plasma membranes or vesicle membranes as sources of phosphatase activity. Frog erythrocyte membrane fractions were prepared as described (19). Equal amounts of the plasma membrane-enriched and the sequestered vesicle membrane fractions were incubated with ^{32}P-labeled receptor in 12.5 mM $MgCl_2$/1.5 mM EDTA/75 mM Tris·HCl, pH 7.0, for the indicated times followed by freezing, lyophilization, and characterization on $NaDodSO_4$/PAGE. ^{32}P-labeled receptor used as the substrate for the phosphatase assay was prepared by phosphorylating purified hamster lung β-adrenergic receptor, reconstituted into phospholipid vesicles with partially purified β-adrenergic receptor kinase as described (18). The data shown are representative of three such experiments.

DISCUSSION

The present results clearly establish a functional role for the phosphorylation state of the β-adrenergic receptor in homologous desensitization of adenylate cyclase. One consequence of receptor phosphorylation is a functional alteration in the receptor protein that renders it less efficacious in coupling to its effector, the guanine nucleotide binding regulatory protein, N_s. The issue of whether or not a functional modification occurs in the receptor during homologous desensitization has recently been controversial. Evidence has been presented both for (11, 12, 15, 23) and against (10, 13–15) a functional receptor alteration. The latter investigations, however, have primarily involved characterizing the receptors after they had been sequestered from the cell surface. It was shown that the sequestered receptors appeared to be functionally normal (10, 15). Our present observations linking increased phosphorylation of the cell-surface receptors to functional uncoupling and showing that the sequestered receptors exhibit decreased phosphorylation thus suggest a rational explanation for these previously discrepant findings.

The phosphorylation state of the β-adrenergic receptor also appears to be intimately related to its expression on the cell surface. Increased receptor phosphorylation in response to agonist occupancy is shown here to directly correlate with sequestration of the receptors from the cell surface. Once the receptors are sequestered, the increased phosphorylation is reversed, perhaps enabling the receptors to translocate back to the cell surface. The steady-state subcellular distribution of the β-adrenergic receptors can thus be viewed as a reflection of the opposing rates of receptor phosphorylation and dephosphorylation. Agonist occupancy leads to an increase in the rate of receptor phosphorylation and thus to a decrease in the steady-state expression of receptors on the cell surface. A number of other cell-surface receptors that exhibit sequestration and recycling phenomena, such as those for insulin, epidermal growth factor, and transferrin, also undergo phosphorylation (26). It is thus tempting to speculate that phosphorylation/dephosphorylation represents a general mechanism through which recycling of receptors can be regulated.

The nature of the protein kinase and phosphatase involved in homologous desensitization of the β-adrenergic receptor is not yet known with certainty. Recently, Benovic *et al.* (18) have identified a β-adrenergic receptor kinase that phosphorylates only the agonist-occupied form of the receptor and that may be involved in homologous desensitization. In contrast, very little is known concerning the receptor phosphatase. Our present results indicate that it is highly concentrated in the sequestered vesicle compartment. Although small amounts of β-adrenergic receptor dephosphorylating activity were observed in the enhanced plasma membrane fraction (Fig. 4), it is likely that this low activity is due to the presence of contaminating membranes from the sequestered compartment (see *Materials and Methods*). Preliminary evidence has indicated that the action of the phosphatase on the β-adrenergic receptor is not affected by agonist occupancy or by the prior exposure of cells to agonists (data not shown). This phosphatase might represent a specific β-adrenergic receptor phosphatase or a previously unrecognized activity of one of the known protein phosphatases. Protein phosphatases have generally been divided into four major

classes referred to as types 1, 2A, 2B, and 2C (27). It is interesting to note that type 1 has been shown to be associated with liver microsomes, while type 2B has been shown to be localized at postsynaptic densities in the brain (27).

Our current working hypothesis for the molecular mechanisms involved in homologous desensitization of adenylate cyclase is shown in Fig. 5. Agonist occupancy of the β-adrenergic receptor leads to its phosphorylation by a β-adrenergic receptor kinase. This phosphorylation results in the functional uncoupling of the receptors and triggers their sequestration from the cell surface. In the frog erythrocyte, these events are not temporally separable, although in some cells (23) they appear to be. Sequestration from the cell surface is associated with dephosphorylation of the β-adrenergic receptors. The relevant phosphatase responsible for dephosphorylation of the receptor likely resides in the sequestered membrane compartment. Dephosphorylation of the receptor restores its functionality and enables it to recycle back to the cell surface and recouple with adenylate cyclase.

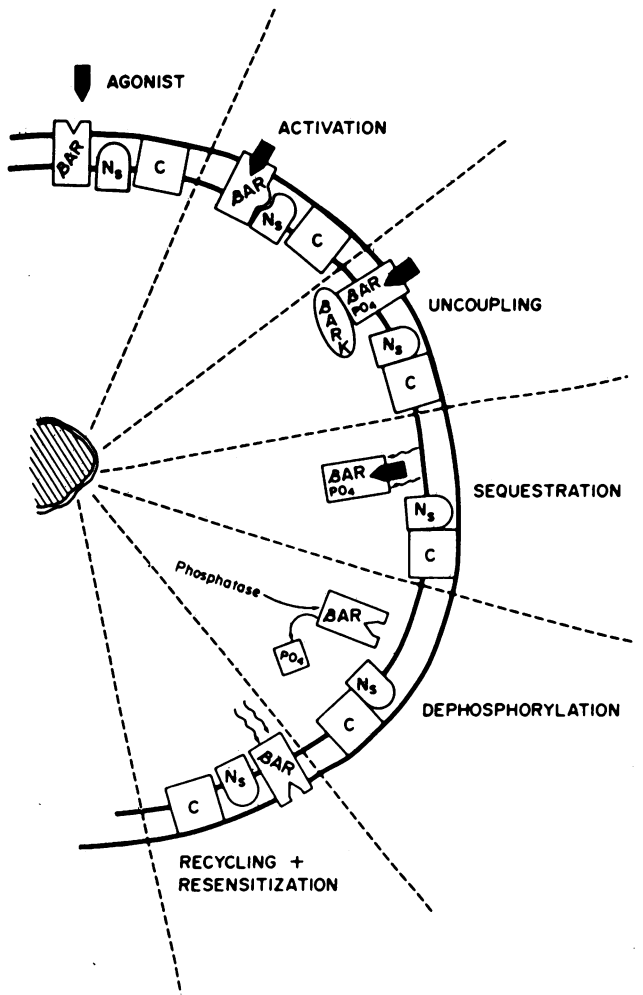

FIG. 5. Molecular mechanisms of homologous desensitization of β-adrenergic receptor-coupled adenylate cyclase. βAR, β-adrenergic receptor; β-ARK, β-adrenergic receptor kinase; C, catalytic unit.

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