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Phorbol ester induces desensitization of adenylate cyclase and phosphorylation of the β-adrenergic receptor in turkey erythrocytes

(tumor promoter/receptor regulation)

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ABSTRACT **Incubation of turkey erythrocytes with the phorbol ester phorbol 12-myristate 13-acetate (PMA) results in a dose- and time-dependent desensitization of isoproterenol-stimulated adenylate cyclase activity. Compared to controls, membranes from PMA-treated cells have an isoproterenol-stimulated adenylate cyclase activity that is decreased 20%–40%, with little effect on forskolin or fluoride activation of adenylate cyclase. No change in β-adrenergic receptor number is observed after PMA treatment, indicating that the major effect of PMA is to uncouple receptor interactions with N_s, the stimulatory guanine nucleotide regulatory protein of adenylate cyclase. Purification of β-adrenergic receptors from $^{32}P_i$-labeled turkey erythrocytes, incubated in the presence or absence of PMA, indicates that the phorbol ester is capable of inducing a 3-fold increase in phosphorylation of the β-adrenergic receptor. The PMA effect is similar to the phosphorylation of the β-adrenergic receptor during isoproterenol- and dibutyryl cAMP-induced desensitization of adenylate cyclase in turkey erythrocytes. The findings indicate that decreased receptor–N_s coupling is correlated with receptor phosphorylation and that phorbol esters can influence the responsiveness of hormone-sensitive adenylate cyclase in certain cell types.**

Phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), are well-known tumor promoting agents that induce a variety of functional and biochemical changes in cells (1–8). Several recent reports suggest that the high-affinity binding site for phorbol esters is the Ca^{2+}/phospholipid-dependent protein kinase C (9–11). Phorbol esters have also been shown to influence several plasma membrane-associated hormone receptor systems. For example, PMA treatment of cells decreases the affinity of epidermal growth factor (12–17) and insulin (18) for their respective receptors. Recently, PMA was shown to stimulate the phosphorylation of insulin and somatomedin C receptors (19).

PMA has been reported to induce a desensitization of isoproterenol-stimulated adenylate cyclase activity in C6 glioma cells (20) and mouse skin and epidermis (21), although the properties of the desensitization have not been characterized. In this communication, we show that PMA induces phosphorylation of β-adrenergic receptors and uncoupling of receptor activation of adenylate cyclase in turkey erythrocytes. The influence of phorbol esters on hormone-sensitive adenylate cyclase and other cell-surface hormone receptors may well be an important component in the action of these compounds.

METHODS

PMA and Isoproterenol-Induced Desensitization of Turkey Erythrocytes. Fresh washed turkey erythrocytes in Eagle's minimal essential medium containing 50 mM Hepes (pH 7.4) were incubated 60 min at 37°C and then treated with 4 μM PMA or 1 μM isoproterenol for 30 min at 37°C unless stated otherwise in the figure legends. After chilling and washing, the cells were resuspended in 2 vol of 5 mM Tris·HCl/3 mM $MgCl_2$, pH 8.0, and broken by nitrogen cavitation (22). Membranes isolated from the 15%–40% sucrose interface of sucrose step gradients (100,000 × *g*; 20 min) were washed twice (35,000 × *g*; 30 min) in the gradient buffer minus sucrose (20 mM Tris·HCl/2 mM EDTA/10 mM $MgCl_2$/100 mM NaCl, pH 8) and stored at −70°C in the same buffer.

^{32}P-Labeling of Turkey Erythrocyte β-Adrenergic Receptor. Turkey erythrocytes were labeled with $^{32}P_i$, using a modification of the procedure of Alper *et al.* (23, 24). Turkey erythrocytes (5–8 ml) were incubated 19 hr at 35–37°C in 25 ml of buffer containing 150 mM NaCl/2.5 mM KCl/11.1 mM glucose/10 mM Hepes/0.05 mM $CaCl_2$/0.1 mM $MgCl_2$, pH 7.4/$^{32}P_i$ (0.5–1.0 mCi/ml; 1 Ci = 37 GBq). In control experiments, radioactivity was omitted and 0.2 μM NaH_2PO_4 was added. PMA (10 μM) or isoproterenol (1 μM) was then added and incubation continued for 30 min at 37°C. Subsequent procedures were carried out at 4°C. The cells were then washed 3 times with 30 vol of buffer (150 mM NaCl/2.5 mM KCl/11.1 mM glucose/10 mM Hepes, pH 7.4) followed by a 20-min incubation in 30 vol of lysis buffer (5 mM Tris·HCl/2 mM EDTA/5 mM $MgCl_2$, pH 7.4) containing sodium fluoride (1 mM), aprotinin (0.2 units/ml), and phenylmethylsulfonyl fluoride (100 μg/ml). After washing twice with 30 vol of lysis buffer containing sodium fluoride, the nucleated ghosts were extracted with 2% digitonin for 60 min at 4°C. β-Adrenergic receptor was purified 1,000- to 4,000-fold by alprenolol Sepharose chromatography in digitonin as described (22), using bovine serum albumin-treated alprenolol Sepharose; all manipulations were carried out at 4°C.

Other Procedures. Photoaffinity labeling using [^{125}I]iodoazidobenzylpindolol (IABP), [^{125}I]iodocyanopindolol (ICYP) binding assays and $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography were done as described (22). Standard concentrations of [^{125}I]IABP and [^{125}I]ICYP used were 1.5 nM and 0.2 nM, respectively. Adenylate cyclase assays were done as described (25).

Materials. $^{32}P_i$ (9000 Ci/mmol) and [^{125}I]ICYP were from New England Nuclear. [^{125}I]IABP and digitonin were prepared as described (22). PMA was obtained from P-L Biochemicals.

RESULTS

Fig. 1 shows a time course of isoproterenol-stimulated adenylate cyclase activity in the presence of GTP, using membranes prepared from control turkey erythrocytes and after

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Abbreviations: ICYP, iodocyanopindolol; IABP, iodoazidobenzylpindolol; PMA, phorbol 12-myristate 13-acetate; N_s, stimulatory guanine nucleotide regulatory protein of adenylate cyclase; GTP[S], guanosine 5'-[γ-thio]-triphosphate.

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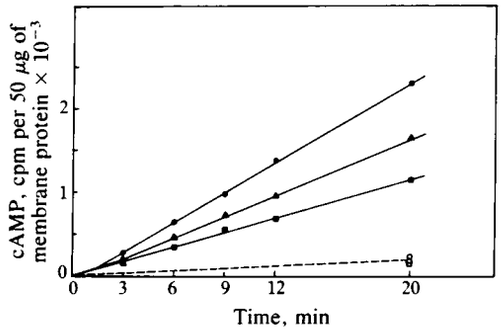

FIG. 1. Time course of isoproterenol-stimulated adenylate cyclase activity. Membranes prepared from control (●) turkey erythrocytes or after a 30-min incubation of cells with either 10 μM isoproterenol (■) or 25 μg/ml PMA (▲) were used to measure adenylate cyclase activity in the presence of 20 μM GTP in the presence (solid lines) or absence (broken line) of 10 μM isoproterenol. Values represent the mean of duplicate determinations, which varied by <5%.

incubation with PMA or isoproterenol. After a 30-min incubation with either PMA or isoproterenol, there is a significant desensitization of agonist-stimulated adenylate cyclase activity. The approximate 50% decrease in adenylate cyclase stimulation in membranes prepared from isoproterenol-treated turkey erythrocytes is similar to that reported by workers in our laboratory (25) and others (26, 27) for avian erythrocytes. Exposure of turkey erythrocytes to PMA for 30 min resulted in an approximate 30% decrease in isoproterenol-stimulated adenylate cyclase activity. The degree of desensitization after PMA treatment was generally about one-half that observed with isoproterenol treatment of the turkey erythrocytes. Table 1 confirms the desensitization of isoproterenol-stimulated adenylate cyclase activity after PMA or isoproterenol treatment of turkey erythrocytes. After pretreatment of cells with either isoproterenol or PMA, the cyclase activity in isolated membranes in the presence of isoproterenol and GTP is significantly decreased, while the isoproterenol-stimulated activity in the presence of guanosine 5'-[γ-thio]-triphosphate (GTP[S]) is less affected. The ability of forskolin or fluoride to activate adenylate cyclase is slightly, but reproducibly, diminished in membranes prepared from cells incubated with isoproterenol or PMA. This heterologous-type desensitization is somewhat less than reported by others (26, 27), and it is probably due in part to our relatively short incubation intervals of cells with isoproterenol or PMA. Our data show that a 30-min exposure of intact turkey erythrocytes to isoproterenol or PMA has the primary effect of uncoupling β-adrenergic receptor stimulation of adenylate cyclase activity. No effect of isoproterenol or PMA was observed on receptor number (not shown).

Fig. 2 shows the time course and dose dependency of the PMA-induced desensitization of isoproterenol-stimulated adenylate cyclase activity. PMA at a relatively high concentration (4 μM) induces a maximal desensitization of isoproterenol-stimulated adenylate cyclase activity within 20 min at 37°C with an apparent $t_{1/2}$ of 7–8 min. A similar time course for isoproterenol-induced desensitization is also observed (not shown). Exposure of erythrocytes to PMA for 30 min at 37°C induces maximal desensitization at 1–4 μM PMA with a half-maximal effect at ≈100 nM PMA. The dose–response relationship shown here for PMA-induced desensitization of isoproterenol-stimulated adenylate cyclase activity is similar to that for PMA-induced phosphorylation of a M_r 40,000 protein during platelet activation (9). PMA (1 μM) added directly to control membranes had no effect on adenylate cyclase activity in the presence of isoproterenol and GTP or fluoride, indicating that PMA was not in some way nonspecifically interacting with adenylate cyclase components to decrease agonist stimulation (not shown). Furthermore, the non-tumor-promoting compound 4α-phorbol did not induce desensitization of hormone-sensitive adenylate cyclase at concentrations as high as 10 μM (not shown).

Our findings indicate that PMA is capable of decreasing the ability of isoproterenol to activate adenylate cyclase. Fig. 3 shows that the presence of 4 μM PMA during a 30-min treatment of turkey erythrocytes with isoproterenol does not significantly alter the EC_{50} for isoproterenol-induced desensitization of adenylate cyclase. The maximal desensitization observed with 0.01–10 μM isoproterenol was always greater in the presence of PMA. The difference, however, was consistently less than additive, suggesting that the effects of PMA and isoproterenol are different, but may be affecting a common component of the adenylate cyclase system. Work done in several laboratories (25–29) indicates the initial event in agonist-induced desensitization is an uncoupling of β-adrenergic receptor interactions with the stimulatory guanine nucleotide regulatory protein of adenylate cyclase (N_s). Stadel *et al.* (30) have also recently demonstrated that isoproterenol-induced desensitization of turkey erythrocytes results in an increased phosphorylation of the β-adrenergic receptor. These findings suggest that regulation of coupling between receptor and N_s could be regulated by phosphorylation–dephosphorylation mechanisms. Furthermore, a high-affinity receptor for phorbol esters has recently been shown to be the Ca^{2+}/phospholipid-dependent protein kinase C (9–11), suggesting that PMA-induced desensitization may involve a phosphorylation reaction.

Evidence that the β-adrenergic receptor is phosphorylated in response to PMA is shown in Fig. 4. Turkey erythrocytes labeled with $^{32}P_i$ were exposed to PMA or isoproterenol and the receptors were purified on alprenolol-Sepharose. After gel filtration to remove alprenolol, the amount of β-adrenergic receptor was quantitated and equal amounts of specific [^{125}I]ICYP binding activity were loaded on NaDodSO$_4$ gels

Table 1. Adenylate cyclase activity in membranes prepared from turkey erythrocytes incubated in the presence or absence of isoproterenol or PMA

Assay addition	Control, pmol of cAMP $mg^{-1}\cdot min^{-1}$	Isoproterenol, pmol of cAMP $mg^{-1}\cdot min^{-1}$	PMA, pmol of cAMP $mg^{-1}\cdot min^{-1}$
GTP	2.4 ± 0.45	1.6 ± 0.71	1.8 ± 0.33
ISO/GTP	21 ± 0.5	12 ± 1.1 (42.9)	15 ± 0.6 (28.6)
ISO/GTP[S]	220 ± 7.7	164 ± 10.6 (25.5)	196 ± 10.0 (10.9)
Forskolin	36 ± 0.2	34 ± 1.0 (5.6)	34 ± 1.0 (5.6)
Fluoride	177 ± 2.5	173 ± 8.3 (2.3)	164 ± 6.5 (7.3)

Turkey erythrocytes were treated for 30 min at 37°C with 100 μM isoproterenol (ISO) or PMA (25 μg/ml), then washed, and membranes were isolated. Adenylate cyclase activity was then measured in the presence of various combinations of 10 μM GTP, 10 μM ISO, 100 μM GTP[S], 10 mM fluoride, or 100 μM forskolin. Assays were for 20 min at 30°C. Values represent mean ± SD (n = 3). Numbers in parentheses represent the percentage difference in adenylate cyclase activity of isoproterenol or PMA-treated preparations relative to control membranes.

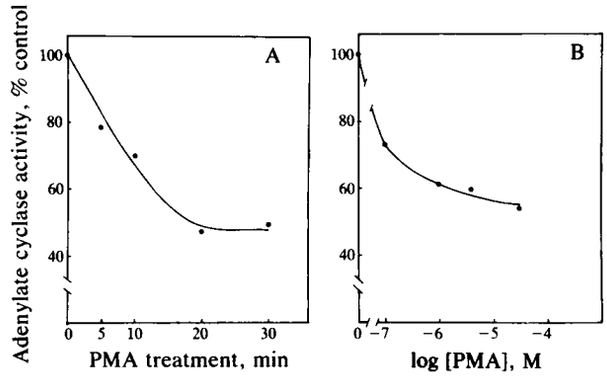

FIG. 2. (*A*) Time course of PMA-induced desensitization. Turkey erythrocytes were incubated 60 min at 37°C and then treated with 4 μM PMA for the times shown. The cells were then chilled 15 min in ice prior to centrifugation and preparation of membranes. The adenylate cyclase activity of membranes from control cells treated with vehicle only (0.025% ethanol) was 7.0 ± 0.22 pmol of cAMP $mg^{-1} \cdot min^{-1}$. (*B*) Dose dependence of PMA-induced desensitization. Turkey erythrocytes were incubated as in *A* and then treated for 30 min at 37°C with 0.1, 1, 4, or 40 μM PMA. Membranes were then isolated and assayed for adenylate cyclase activity. Activity of membranes from control cells was 6.5 ± 0.17 pmol of cAMP $mg^{-1} \cdot min^{-1}$. Adenylate cyclase activity in *A* and *B* represents activity assayed in the presence of 20 μM (−)-isoproterenol and 20 μM GTP and is expressed relative to that of control membranes.

and electrophoresed. Autoradiograms of the $NaDodSO_4$ gels, containing equal amounts of specific receptor binding, show a significant increase in the phosphorylation of a M_r 39,000 peptide in response to both PMA and isoproterenol. The M_r 39,000 peptide corresponds to the same M_r as the major form of the β-adrenergic receptor purified from turkey erythrocytes (22, 30, 31). A second receptor peptide of M_r 52,000, which is variable in amount in different preparations (22, 30, 31), was not abundant after purification of the β-adrenergic receptor in these two experiments. Thus, both PMA and isoproterenol induce desensitization of adenylate cyclase and phosphorylation of the M_r 39,000 β-adrenergic receptor peptide. Quantitation of the receptor phosphorylation in response to PMA and isoproterenol is shown in Table 2. Densitometry of the M_r 39,000 band in lanes 1–3 and lanes 4–6 of Fig. 4, in which the amount of receptor loaded on each lane was the same as determined by [^{125}I]ICYP binding, indicates an approximately 2- to 3-fold increase in the phosphorylation of the β-adrenergic receptor peptide by either agent.

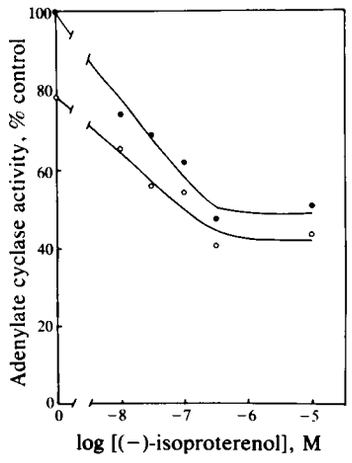

FIG. 3. Dose dependence of isoproterenol-induced desensitization in the presence or absence of PMA. Turkey erythrocytes were incubated without additions for 60 min at 37°C. The cells were then treated for an additional 30 min at 37°C with 0, 0.01, 0.03, 0.1, 0.3, or 10 μM (−)-isoproterenol either in the absence (●) or presence (○) of 4 μM PMA. Membranes were then isolated and assayed for adenylate cyclase activity. Activity in the presence of 20 μM (−)-isoproterenol and 20 μM GTP is shown expressed relative to control activity (10.8 ± 0.11 pmol of cAMP $mg^{-1} \cdot min^{-1}$).

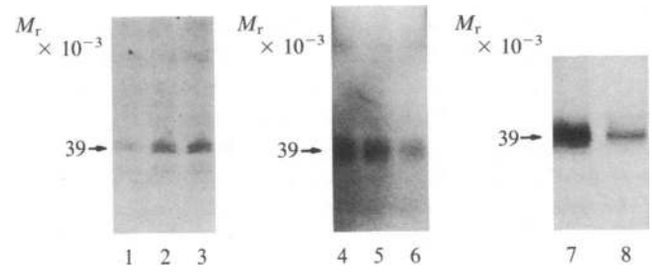

FIG. 4. Autoradiograms of $NaDodSO_4$/10% polyacrylamide gels of ^{32}P-labeled preparations containing β-adrenergic receptors partially purified by alprenolol-Sepharose chromatography. Preparations were from ^{32}P-labeled turkey erythrocytes treated for 30 min at 37°C with vehicle only (control, lanes 1, 6, and 8), 10 μM PMA (lanes 3, 4, and 7), or 1 μM (−)-isoproterenol (lanes 2 and 5). Samples in lanes 1–6 represent ≈200-μl aliquots of 2.8 ml G-50 void volume fractions containing peak [^{125}I]ICYP binding activity and represent 0.7 fmol of β-adrenergic receptor per lane. Samples in lane 7 (PMA-treated) and lane 8 (control) represent 200-μl aliquots of 3.6-ml eluates from alprenolol-Sepharose taken prior to G-50 column chromatography. The results of two separate experiments are shown (exp. 1, lanes 1–3; exp. 2, lanes 4–8). Exposure of autoradiographs was for 8–10 days. The bromophenol blue tracking dye was allowed to run just off the bottom of the gels.

Evidence that the M_r 39,000 peptide whose phosphorylation is enhanced by both PMA and isoproterenol, is in fact the β-adrenergic receptor, is shown in Fig. 5*A*. It has been previously demonstrated that the β-adrenergic receptor photoaffinity probe [^{125}I]IABP specifically labels a predominant M_r 39,000 peptide and a M_r 52,000 peptide of low abundance in turkey erythrocyte membranes and with the alprenolol-Sepharose purified receptor (22). Photolabeling of both peptides is specifically blocked by propranolol. Similar results have been reported by Stadel *et al.* (30) using [^{125}I]iodoazidocarazolol. Lanes 1 and 2 of Fig. 5*A* show the [^{125}I]IABP photolabeling profile of a partially purified β-adrenergic receptor preparation in the absence (lane 2) and presence (lane 1) of propranolol. The predominant M_r 39,000 and less abundant M_r 52,000 receptor peptides are specifically labeled by [^{125}I]IABP, and the labeling is blocked by propranolol. When ^{32}P-labeled cells are used as a source of β-adrenergic receptor, alprenolol specifically elutes the M_r 39,000 peptide from an alprenolol-Sepharose column (Fig. 5*A*, lane 3). Substitution of an equimolar concentration of NaCl for alprenolol in the elution buffer results in failure to elute this band (lane 4). The ^{32}P-labeled M_r 39,000 band eluted by alprenolol (lane 3) can be specifically photolabeled with [^{125}I]IABP (lane 5). The photolabeling is specifically blocked by propanolol (lane 6). In this preparation, there is phosphorylation to a small degree of the M_r 52,000 peptide (lane 3), which is also photolabeled with [^{125}I]IABP (lane 5). The remaining label in the receptor bands in lane 6, which is [^{125}I]IABP photolabeling in the presence of propranolol, is primarily due to the ^{32}P incorporated into the peptides as observed in lane 3. Thus, the bands specifically eluted from the affinity column by alprenolol are specifically photolabeled using [^{125}I]IABP and represent the β-adrenergic receptor binding protein.

Further evidence that the phosphorylated M_r 39,000 and M_r 52,000 bands are the β-adrenergic receptor is shown in Fig. 5*B*. β-Adrenergic receptors from ^{32}P-labeled turkey erythrocytes were purified on an alprenolol-Sepharose column. The fraction eluted from the affinity column by alpren-

Table 2. ^{32}P phosphorus content of M_r 39,000 β-adrenergic receptor peptide from PMA- or isoproterenol-treated turkey erythrocytes relative to control

	Exp. 1	Exp. 2
Control	1.0	1.0
Isoproterenol	3.3	1.6
PMA	3.2	2.2

β-Adrenergic receptors were partially purified by alprenolol-Sepharose chromatography from ^{32}P-labeled turkey erythrocytes treated for 30 min with either PMA (10 μM), isoproterenol (1 μM), or vehicle only (control). β-Receptor content of the M_r 39,000 band was estimated as the β-receptor content of the samples loaded onto $NaDodSO_4$/10% polyacrylamide gels based on specific [^{125}I]ICYP binding, and ranged from 0.4 to 1.4 fmol of β-receptor per lane. Phosphorus content of the M_r 39,000 band was estimated by densitometry of ^{32}P autoradiographs developed after 8–10 days and is expressed in arbitrary units. For each condition, ^{32}P phosphorus content per unit of β-receptor was then calculated and is expressed relative to the control.

olol was chromatographed on a Sephadex G-50 column to remove the alprenolol, and void volume fractions were assayed for specific [^{125}I]ICYP binding or run on $NaDodSO_4$ gels and autoradiographed. Specific [^{125}I]ICYP binding corresponds to 0.1, 1.3, 1.8, and 1.0 fmol (lanes 1–4, respectively). There is a positive correlation of [^{125}I]ICYP binding and purification of the phosphorylated M_r 39,000 and M_r 52,000 peptides. By several criteria, including specific elution by alprenolol, photolabeling of the phosphorylated peptides analyzed on $NaDodSO_4$ gels (Fig. 5*A*), and specific binding with [^{125}I]-ICYP in fractions eluted from a gel filtration column (Fig. 5*B*) indicate, that the phosphorylated peptides are β-adrenergic receptor ligand binding proteins.

DISCUSSION

Our findings demonstrate two effects of PMA: (*i*) desensitization of isoproterenol activation of adenylate cyclase and (*ii*) increased phosphorylation of the β-adrenergic receptor. The results also support the findings of Stadel *et al.* (30) that isoproterenol-induced desensitization of adenylate cyclase in turkey erythrocytes results in phosphorylation of the β-adrenergic receptor. Phorbol ester-induced effects on the hormone-sensitive adenylate cyclase system may be a general phenomenon. PMA induces desensitization of hormone-stimulated adenylate cyclase in turkey erythrocytes (this report), C6 glioma (20), and mouse skin and epidermis (21). We have preliminary evidence that phorbol esters alter the regulation of hormone-sensitive adenylate cyclase in A431 human carcinoma cells (unpublished data).

The PMA-induced desensitization that we observe in turkey erythrocytes is similar in magnitude to the desensitization observed with dibutyryl cAMP (26). However, our studies in turkey erythrocytes and those of others in different cell types (32) suggest no major or consistent effect of PMA on increasing cAMP levels. We have also found that the β-adrenergic receptor in membranes or purified in a soluble or liposome reconstituted form is a poor substrate for the purified catalytic subunit of cAMP-dependent protein kinase from bovine heart (unpublished observation). The work of Stadel *et al.* (30) also suggested the β-adrenergic receptor is a poor substrate for cAMP-dependent protein kinase. Using a genetic approach, two other independent lines of evidence suggest that activation of cAMP-dependent protein kinase does not have to occur during desensitization. Shear *et al.* (33), using a cAMP-dependent protein kinase-deficient mutant (*kin*$^-$) of S49 mouse lymphoma cells, demonstrated that isoproterenol-induced desensitization is essentially identical in the *kin*$^-$ mutants compared to wild-type cells. Green and Clark (34), using membrane reconstitution procedures, also

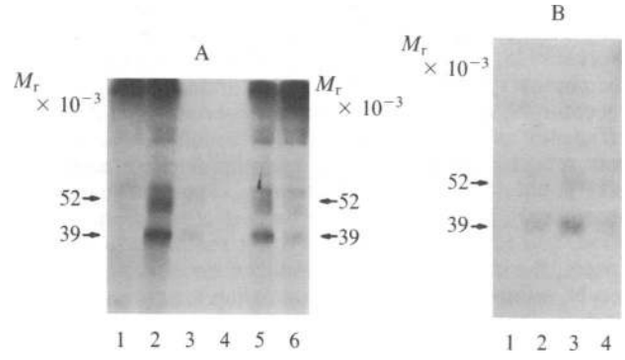

FIG. 5. Autoradiograph of $NaDodSO_4$/10% polyacrylamide gel of ^{32}P-labeled and [^{125}I]IABP photoaffinity-labeled partially purified β-adrenergic receptor. (*A*) Turkey erythrocytes labeled with ^{32}P were treated with 1 μM isoproterenol for 30 min at 37°C. A digitonin extract of washed nucleated ghosts was incubated with alprenolol-Sepharose. After washing, the affinity resin was divided into two equal portions. One portion was eluted with 200 μM alprenolol in 0.3 M NaCl; the other portion was eluted with 200 μM NaCl added to the 0.3 M NaCl containing wash buffer. Both eluates were chromatographed on Sephadex G-50 columns and [^{125}I]ICYP binding was assayed throughout the void volume fractions. Alprenolol specifically eluted the receptor compared to the control salt elution. Aliquots from peak fractions electrophoresed on $NaDodSO_4$/10% polyacrylamide gels are shown in *A* for elution by alprenolol (lane 3) or control (lane 4). A M_r 39,000 band and a less prominent M_r 52,000 band are detectable in lane 3. A control partially purified β-adrenergic receptor preparation [^{125}I]IABP photolabeled in the absence (lane 2) or presence (lane 1) of 10 μM propranolol is shown for comparison. [^{125}I]IABP photolabeling of the alprenolol-eluted ^{32}P-labeled preparation shown in lane 3 gives a similar specific labeling pattern compared to control in the absence (lane 5) or presence (lane 6) of 10 μM propranolol. The remaining label at M_r 39,000 and M_r 52,000 in lane 6 is due to ^{32}P-labeling in the bands. (*B*) Lanes 1–4 are the G-50 void volume fractions of the ^{32}P-labeled β-adrenergic receptor preparation eluted from alprenolol-Sepharose using 200 μM alprenolol. Based on [^{125}I]ICYP binding, 0.1, 1.3, 1.8, and 1.0 fmol of β-adrenergic receptor was loaded on lanes 1–4, respectively. Exposure of autoradiographs was for 6 days (*A*) and 8 days (*B*).

demonstrated isoproterenol-induced desensitization in *cyc*$^-$ S49 mutants, which lack a functional N_s protein and do not activate adenylate cyclase in response to isoproterenol.

How then could desensitization of hormone-sensitive adenylate cyclase occur in the turkey erythrocyte? It is tempting to hypothesize that protein kinase C is involved; however, our data do not demonstrate that protein kinase C phosphorylates the β-adrenergic receptor. Certainly, other kinases or phosphatases may be involved in the change in phosphorylation state of the receptor. The correlation between phosphorylation of the β-adrenergic receptor and desensitization by PMA, isoproterenol, and dibutyryl cAMP is compelling evidence to hypothesize that this is an important mechanism to regulate receptor–N_s coupling. Phosphorylation of the receptor at two different sites, depending on agonist occupancy and the resulting conformation of the receptor, could explain the partial desensitization observed with PMA compared to isoproterenol.

Agonist-induced desensitization of adenylate cyclase is admittedly different in turkey erythrocytes compared to many other cell types. Most notably is the failure of β-adrenergic receptors to be internalized by turkey erythrocytes during desensitization. In other cell types, internalization of receptors appears to be a component of the desensitization process for hormone-sensitive adenylate cyclase. Since phosphorylation of the β-adrenergic receptor has been observed only in the turkey erythrocyte, the question that arises is whether receptor phosphorylation is unique to the avian erythrocyte. We are not aware of any reports that di-

rectly address this question. There are several studies that may bear on this question indirectly. Birnbaumer and coworkers (35, 36) and others (37, 38) have described isolated membrane systems in which agonist-induced uncoupling of receptor–N_s interactions could be observed. The decreased efficiency of receptor–N_s coupling usually required ATP, was receptor mediated, and imidodiphosphate analogs of ATP could not induce the uncoupling. The data suggest that receptor–N_s uncoupling might be mediated by phosphorylation, and furthermore, cAMP is not required. In many respects, the degree of agonist-induced uncoupling of receptor–N_s interactions in these mammalian membrane preparations is similar to the desensitization phenomenon observed in avian erythrocytes. Therefore, phosphorylation of receptors could conceivably be an early step in agonist-induced desensitization of adenylate cyclase in many cell types. Endocytosis of the receptor could result in rapid dephosphorylation due to phosphatases present in the cytoplasm of the cell, making detection of phosphorylated receptor difficult. The ability to purify components and reconstitute receptor–N_s interactions in liposomes (22, 39, 40) or cells (41, 42) should allow us to develop *in vitro* systems to directly address the questions of phosphorylation and the regulation of receptor–N_s interactions.

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