0026-895X/87/060737-06$02.00/0

MOLECULAR PHARMACOLOGY, 32:737-742

Agonists and Phorbol Esters Desensitize β-Adrenergic Receptors by Different Mechanisms

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Received May 27, 1987; Accepted August 11, 1987

SUMMARY

Exposure of 1321N1 human astrocytoma cells to the protein kinase C (PKC) activator phorbol 12-myristate, 13-acetate (PMA) led to a rapid and concentration-dependent decrease in isoproterenol (ISO)-stimulated adenylate cyclase (AC) activity in cell lysates. This desensitization of β-adrenergic receptor (BAR) function was mimicked by mezerein, which also activates PKC, but not by 4-O-methyl-PMA, which is a very weak activator of PKC. Pretreatment with PMA led to desensitization of AC activity stimulated by ISO and by prostaglandin E_1, in contrast to the β-receptor-specific desensitization induced by ISO. Stimulation of AC activity by forskolin and by fluoride remained unaltered. The extent of desensitization observed with PMA plus ISO was greater than with either agent alone. Desensitization with PMA did not result in internalization of BAR, as assessed by sucrose density gradient centrifugation assays and by assays of competition by the hydrophilic ligand ISO for radioligand binding to intact cell receptors. PMA pretreatment did not alter the apparent affinity of the agonist ISO for intact cell BAR, nor was the potency of ISO for stimulation of AC activity altered. The protein kinase inhibitor H7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine] inhibited the desensitization induced by PMA but not that induced by ISO. These results indicate that activation of PKC can lead to desensitization of receptor-stimulated AC activity but that agonist-induced desensitization of BAR-stimulated AC activity occurs by a different mechanism.

Phorbol esters such as PMA have been shown to induce a myriad of morphological and functional changes in a variety of cells and tissues. These effects are thought to result from activation of a Ca^{2+}- and phospholipid-dependent protein kinase (PKC). PMA and related compounds are thought to mimic the activation of this enzyme by diacylglycerol that occurs after activation of receptors coupled to turnover of polyphosphoinositides (1–3).

Among the many reported effects of phorbol esters are marked changes in a variety of cell surface receptors (3), including BAR (4–14). In a number of previous studies, treatment of cells with phorbol esters led to a decrease in the ability of BAR agonists to activate the enzyme AC (4–12). In other studies, phorbol esters led to an increase in BAR-stimulated AC activity (13, 14). A recent study in S49 cells found that either increased or decreased activity of BAR-AC was observed, depending on the exact conditions of the phorbol ester treatment and subsequent assay (14). These results suggest the possibility that the BAR-AC system can be regulated by PKC-mediated phosphorylation(s). The relationship, if any, of these PKC-mediated changes in BAR-AC to the agonist-induced desensitization of BAR-AC that is known to occur, or to changes in BAR-AC that may occur after activation of receptors coupled to turnover of polyphosphoinositides, remains to be determined. Thus, additional studies in well-characterized cell systems seem warranted.

The 1321N1 human astrocytoma cell line is one of the best characterized model systems in terms of the multiple steps involved in agonist-induced desensitization of BAR-AC (15–18). Exposure of these cells to catecholamines or other BAR agonists leads to rapid uncoupling of BAR from the enzyme AC (19, 20). Uncoupling is rapidly followed by changes in the properties of BAR that are thought to reflect receptor internalization (21–25). These changes include a shift of BAR from the plasma membrane fraction to the light vesicle fraction in sucrose density gradient centrifugation assays (21, 22) and a decrease in accessibility of BAR to hydrophilic ligands in short-time competition binding assays at 37° (23, 24) or in equilibrium binding assays on ice (25, 26). Uncoupling does not result simply from physical separation of BAR from AC caused by internalization, because uncoupling occurs in the absence of

This work was supported in part by National Institutes of Health Research Grants GM34500 and GM36254 and by Research Career Development Award HL01593 to M. L. T. A preliminary report of these results was presented at the Federation of American Societies of Experimental Biology Annual Meeting in Washington, D.C., April, 1987.

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ABBREVIATIONS: PMA, phorbol 12-myristate, 13-acetate; PKC, protein kinase C; BAR, β-adrenergic receptor(s); AC, adenylate cyclase; H7, [1-(5-isoquinolinesulfonyl)-2-methylpiperazine]; ISO, isoproterenol; PGE_1, prostaglandin E_1; IPIN, ^{125}I-iodopindolol; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

internalization in cells pretreated with concanavalin A or with phenylarsineoxide before agonist exposure (22, 26). With more prolonged exposure of cells to agonist, there is a loss of BAR as determined by radioligand binding assays, a phenomenon referred to as down-regulation (20, 27). Agonists also induce changes in the binding properties of BAR measured in assays with intact cells (28). The relationship of this change in binding properties to the other aspects of desensitization in these cells outlined above has not been established. In the studies reported here, we have used PMA to investigate the effects of PKC activation on the binding and functional properties of BAR in 1321N1 cells. In addition, we have used the protein kinase inhibitor H7 [1-(5-isoquinolinesulfonyl)-2-methylpiperazine] (11, 29, 30) to differentiate between the mechanisms of desensitization induced by PMA and by BAR agonists.

Materials and Methods

Chemicals. PMA, 4-*O*-methyl-PMA, mezerein, H7, ISO, and prostaglandin E_1 (PGE_1) were purchased from Sigma Chemical Co. Forskolin and concanavalin A were purchased from Calbiochem. ^{32}P-ATP and ^{3}H-cAMP were obtained from ICN Biochemicals. Sodium^{125}I-iodide was obtained from Amersham; (−)-pindolol was a generous gift from Dr. G. Engel (Sandoz). ^{125}I-iodopindolol (IPIN) was prepared as previously described (31). Tissue culture medium, serum, and trypsin were from Gibco.

Cell culture. 1321N1 human astrocytoma cells were grown in monolayer culture at 37° in a humidified atmosphere containing 8% CO_2. Growth medium was Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum. Confluent monolayers were removed from tissue culture flasks with 0.05% trypsin and plated at a density of 10,000 cells/cm^2 in plastic tissue culture dishes. Experiments were performed on cells 4 days after plating (just confluent).

Drug treatments. Most drugs were added directly to the growth medium bathing cells as 100 × dilutions of stock solutions dissolved in dimethyl sulfoxide (DMSO). Thus the final concentration of DMSO in all pretreatments was 1%. The effects of pretreatment of cells with ISO in the presence of DMSO were qualitatively and quantitatively similar to those previously observed when DMSO was not present. H7 was dissolved in 20 mM HCl to prepare a 10-mM stock solution. Appropriate dilutions of H7 were added to cells 5 min before addition of ISO or PMA.

Cell lysis. After exposure of cells to various drugs to induce desensitization, pretreatment medium was aspirated and cell sheets were rinsed two times on ice with ice-cold lysis buffer (1 mM Tris, pH 7.4, 2 mM EDTA). After 20 min incubation on ice in lysis buffer, cells were scraped from the dish with a rubber policeman and then homogenized with a Tekmar Tissuemizer (5 sec at full power).

AC assays. AC activity in cell lysates was determined in 30-min assays at 37° using previously described assay conditions (32). cAMP was separated from ATP by the standard procedure of sequential chromatography over Dowex and alumina columns. Radioactivity in the eluates was determined by liquid scintillation counting using BudgetSolve scintillation cocktail. ^{32}P-cAMP values were corrected for column recovery based on recoveries of ^{3}H-cAMP standard included in each sample.

Sucrose density gradient centrifugation. Cells were incubated on ice with 0.5 mg/ml concanavalin A for 20 min before lysis to improve subsequent separation of plasma membrane and light vesicle fractions, as described previously (19). Lysates (3 ml) were then layered onto step gradients (26) consisting of 3.2 ml of 5%, 4 ml of 38%, and 4 ml of 60% sucrose (w/v in 20 mM Tris, pH 7.4). Samples were centrifuged for 60 min at 35,000 rpm in a Beckman SW40Ti rotor at 4°. The 5–38% interface was collected as the light vesicle fraction (internalized BAR), and the 38–60% interface was collected as the plasma membrane fraction (cell surface BAR).

Binding assays on broken cell preparations. Gradient fractions were diluted to about 1.5 ml with 20 mM Tris buffer (pH 7.4) containing 2 mM $MgCl_2$ and 140 mM NaCl. Binding assays were performed as previously described (23) using IPIN (100 pM) to label BAR. Tissue (180 μl) was added to polypropylene tubes containing 50 μl of radioligand in the above buffer and 20 μl of 1 mM HCl ± 100 μM ISO to define nonspecific binding. After incubation for 60 min at 37°, assays were terminated by dilution with 20 mM Tris buffer (pH 7.4) containing 140 mM NaCl, followed by rapid filtration over Schleicher and Schuell No. 30 glass fiber filters with a Brandel cell harvester modified for receptor binding assays. The filters were washed an additional time with the same buffer and then transferred to tubes for gamma counting.

Intact cell binding assays. Assays of BAR on intact cells were performed as previously described (23, 28). Cells on 35-mm dishes were washed twice at 37° with 2 ml of DMEM buffered to pH 7.4 with 20 mM Hepes (DMEM-Hepes). Cells were then incubated for 30 sec at 37° in DMEM-Hepes medium containing 1 mM sodium ascorbate, IPIN (100 pM), and varying concentrations of ISO. After incubation, the cell sheets were rinsed twice at 37° with DMEM-Hepes medium containing 100 μM propranolol. Radioactivity associated with the cells was then determined after removal from the dishes with 1 ml of 0.2 N NaOH. Nonspecific binding was defined as that occurring in the presence of 1 μM propranolol.

Data analysis. Nonlinear least squares curve fitting of the raw data from activation and competition binding curves to one- or two-site models was performed using the Graph-Pad program (H. Motulsky, University of California, San Diego) on an IBM PC-XT. Data from competition binding experiments are expressed as the percentage of maximal specific binding measured in the absence of competing ligand. The IC_{50} is defined as the concentration of competing ligand that reduces the amount of radioligand specifically bound to one half of the amount specifically bound in the absence of competing ligand. As described previously (23), the model used is an empirical one based on equations for equilibrium binding to either one or two classes of specific sites. Because this model is technically correct only in those cases where the competing ligand is at equilibrium with both sites, IC_{50} values are generated rather than K_D (equilibrium dissociation constants) values. The absolute values for AC activity were somewhat variable among different experiments; values are therefore expressed as percentage of control values from the same experiment. Typical values are given in the legends to Figs. 1 and 3. Values in the text and figures are presented as means ± SE from at least three experiments.

Results

Exposure of 1321N1 cells to PMA at 37° caused a decrease in the subsequent ability of the BAR agonist ISO to stimulate AC activity in lysates from pretreated cells (Fig. 1). The decrease in ISO-stimulated AC activity was relatively rapid, with half-maximal decrease after 10-min incubation with 1 μM PMA, and was dependent on the concentration of PMA used, with half-maximal effect occurring at 1 nM PMA.

The ability of various agents to decrease subsequent ISO-stimulated AC activity was compared (Fig. 2A). Pretreatment of cells with a maximally effective concentration of PMA (1 μM) led to a 34 ± 4% decrease, compared with a 56 ± 2% decrease after pretreatment with a maximally effective concentration of ISO (1 μM). The PMA analog 4-*O*-methyl-PMA, which is structurally similar to PMA but is a very weak activator of PKC (2), did not mimic the effect of PMA. On the other hand, mezerein, a strong activator of PKC that is structurally dissimilar to PMA (33), decreased ISO-stimulated AC activity to a similar extent as did PMA. These results are

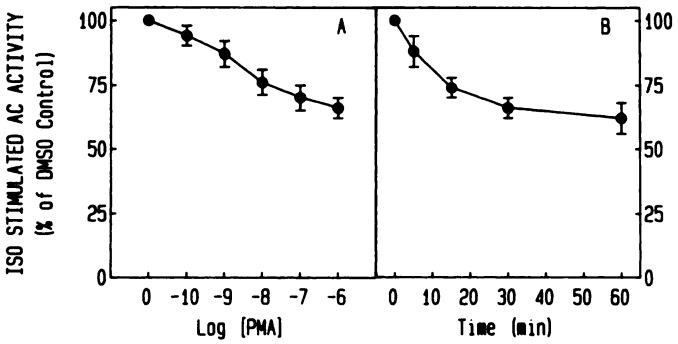

Fig. 1. Time and concentration dependence of PMA-induced desensitization. 1321N1 cells were incubated at 37° with the indicated concentrations of PMA for 30 min (A) or with 1 μM PMA for the indicated times (B) and then lysed. AC activity stimulated by 100 μM ISO was then determined in the lysates. Values are presented as percentage of activity in control cells and are the averages of four experiments. Typical basal and ISO-stimulated activities in control cells were 15 and 120 pmol/min/mg protein, respectively.

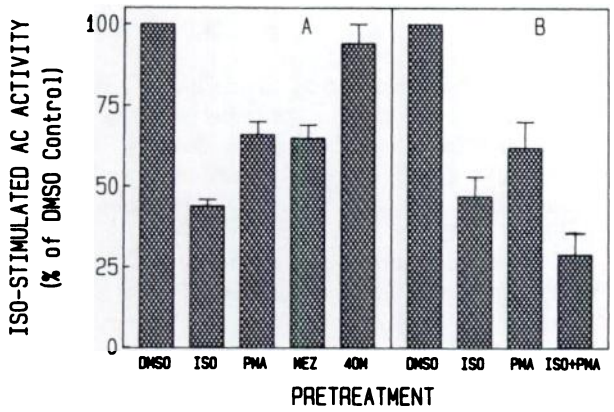

Fig. 2. Desensitization of ISO-stimulated AC activity by various agents. 1321N1 cells were incubated for 30 min at 37° with the indicated drugs and then lysed. AC activity stimulated by 100 μM ISO was then determined in the lysates. Values are expressed as percentage of activity in control cells and are averages of three to six experiments. *DMSO*, control; *ISO*, 1 μM; *PMA*, 1 μM; *MEZ*, 1 μM mezerein; 4OM, 4-*O*-methyl-PMA. (In some of the experiments in B, the concentration of PMA used was 4 μM).

consistent with the effect of PMA being mediated by activation of PKC.

In a separate set of experiments, the decrease in ISO-stimulated AC activity caused by pretreatment with maximally effective concentrations of ISO plus PMA was compared with that from pretreatment with each agent alone (Fig. 2B). Pretreatment with ISO alone led to 53 ± 6% decrease, pretreatment with PMA alone led to 38 ± 8% decrease, and pretreatment with ISO plus PMA led to 71 ± 6% decrease. The greater (but less than additive) effect of the two agents together is the result expected if ISO and PMA are acting by independent pathways, either of which can functionally desensitize ISO-stimulated AC activity.

The specificity of the changes in AC activity induced by PMA were compared with those induced by ISO (Fig. 3). As in previous studies, ISO pretreatment decreased AC activity stimulated by ISO with little effect on that stimulated by prostaglandin E_1 or by forskolin or fluoride. In contrast, pretreatment with PMA caused decreases in AC activity stimulated by ISO and by prostaglandin E_1, but did not decrease activity stimulated by forskolin or fluoride. For PMA pretreatment, as for ISO pretreatment, the decrease in ISO-stimulated AC activity results form a decrease in maximal activity with only small changes in the potency of ISO (Fig. 4).

Sucrose density gradient centrifugation assays were performed to compare changes in the subcellular distribution of BAR for cells pretreated with ISO and with PMA (Fig. 5). As demonstrated previously, pretreatment of cells with ISO led to a marked shift of BAR from the plasma membrane fraction to the light vesicle fraction, consistent with receptor internalization. In contrast, pretreatment of cells with PMA did not lead to changes in the distribution of BAR between these two fractions. Neither pretreatment led to changes in the total number of BAR detected, indicating that BAR down-regulation does not occur under these conditions.

Short-time assays of ISO competition for binding of IPIN to BAR of intact cells were compared for control cells and for cells pretreated with ISO and with PMA (Fig. 6). Two-component competition curves were obtained in all cases. In control

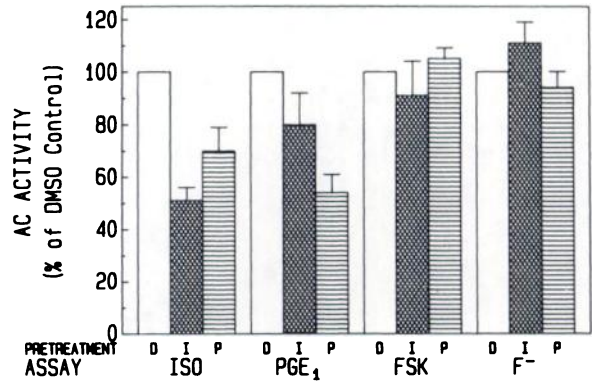

Fig. 3. Specificity of AC desensitization induced by ISO and by PMA. 1321N1 cells were incubated for 30 min at 37° in the absence (D) or presence of 1 μM ISO (*I*) or 1 μM PMA (*P*) and then lysed. AC activity stimulated by 100 μM ISO, 150 μM PGE_1, 10 μM forskolin (*FSK*), and 10 mM fluoride (F^-) was then determined in lysates. Results are expressed as percentage of activity in control cells and are averages of 5–6 experiments. Typical stimulations (fold over basal) in control cells were as follows: ISO, 8-fold; PGE_1, 6-fold; FSK, 10-fold; and F^-, 12-fold.

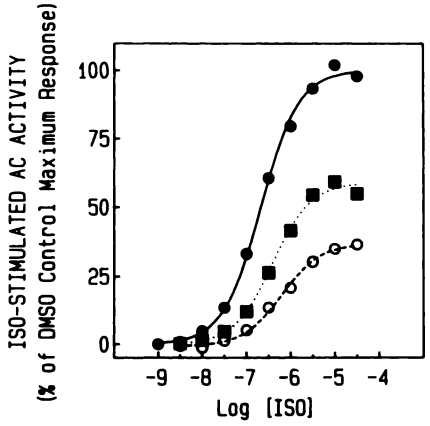

Fig. 4. Concentration-effect curves for ISO stimulation of AC. 1321N1 cells were incubated for 30 min at 37° in the absence (●) or presence of 1 μM ISO (○) or 1 μM PMA (■). Cells were lysed and AC activity in the lysates was determined in the presence of the indicated concentrations of ISO. Values are expressed as percentage of maximal response obtained with control cells. Half-maximal effects of ISO were obtained at the following concentrations (nM): DMSO, 210 ± 10; ISO, 610 ± 70; PMA 390 ± 50.

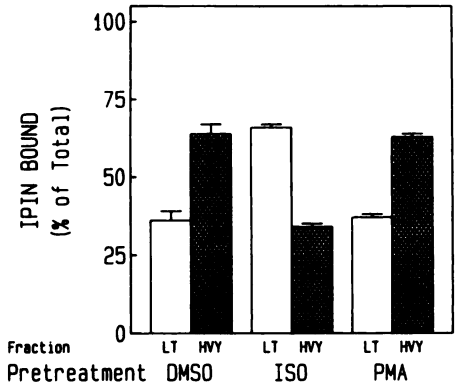

Fig. 5. Sucrose density gradient assays of BAR internalization. 1321N1 cells were incubated for 30 min at 37° in the absence (DMSO) or presence of 1 μM ISO or 1 μM PMA. Sucrose density step gradient centrifugation of the lysates was then performed, and BAR in the light vesicle fraction and the plasma membrane fraction were determined. Binding to each fraction is expressed as percentage of total specific binding to both fractions (DMSO, 2670 cpm; ISO, 2320 cpm; PMA, 2940 cpm). Results are averages of three experiments.

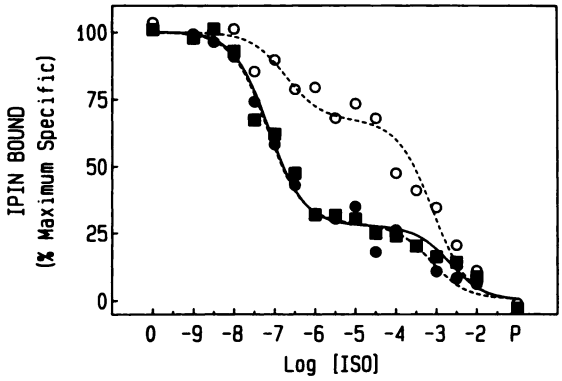

Fig. 6. Effects of ISO and PMA on ISO binding properties of intact cell BAR. Short-time (30-sec) assays of ISO competition for ^{125}I-iodopindolol binding to intact 1321N1 cells were performed with cells incubated for 30 min at 37° in the absence (●) or presence of 1 μM ISO (○) or 1 μM PMA (■). Values are the averages of three experiments. Specific and nonspecific bindings were 2800 and 800 cpm/dish for control cells and were not significantly altered by pretreatment with ISO or PMA.

cells, 72 ± 4% of binding was inhibited with an IC_{50} of 72 ± 14 nM and 28% with a IC_{50} of 2.2 ± 1.3 mM. ISO pretreatment caused a dramatic increase in the fraction of BAR in the very low affinity form in agreement with previous results (23). In ISO-pretreated cells, 33 ± 5% of binding was inhibited with an IC_{50} of 190 ± 70 nM and 67% with an IC_{50} of 0.80 ± 0.29 mM. In contrast, PMA pretreatment did not lead to changes in the fractions of BAR in the two states. In PMA-pretreated cells, 71 ± 2% of binding was inhibited with an IC_{50} of 63 ± 9 nM and 29% with an IC_{50} of 0.70 ± 0.23 mM. Neither pretreatment led to major changes in the IC_{50} values for ISO competition for binding to either state or in the binding of IPIN observed in the absence of ISO. These results provide additional evidence that desensitization by ISO leads to receptor internalization, whereas desensitization by PMA does not.

The results presented above demonstrate that PMA, presumably by activation of PKC, can lead to desensitization of BAR-stimulated AC activity. However, the pattern of changes induced by PMA is different from that induced by the BAR agonist ISO. To more directly assess the possible relationship

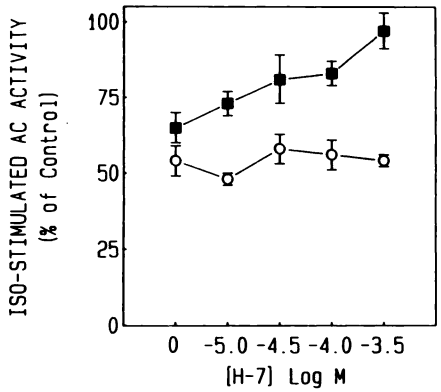

Fig. 7. Effects of H7 on desensitization induced by ISO and by PMA. 1321N1 cells were incubated for 5 min at 37° with the indicated concentrations of H7 and then incubated for 30 min at 37° in the presence of DMSO (100% values), 1 μM ISO (○), or 1 μM PMA (■), in the continued presence of H7. Cells were then lysed and AC activity stimulated by 100 μM ISO was determined in the lysates. Values are presented as percentage of activity in the lysates from cells incubated with the indicated concentrations of H7 in the absence of ISO or PMA and are the averages of 10 experiments.

of PMA-induced desensitization to mechanisms involved in agonist-induced desensitization, the effects of the protein kinase inhibitor H7 on desensitization induced by PMA and by ISO were investigated (Fig. 7). Incubation of cells with H7 alone caused only a small decrease (15%, not shown) in ISO-stimulated AC activity. However, H7 caused a complete inhibition of PMA-induced desensitization that was dose-dependent, with half-maximal inhibition observed at 30 μM H7. Recently, inhibition by H7 of PMA-induced desensitization of BAR has also been reported for rat reticulocytes (11). We also determined the effect of H7 on ISO-induced desensitization of BAR. In contrast to the essentially complete inhibition of PMA-induced desensitization observed with H7, this inhibitor did not alter ISO-induced desensitization over this same concentration range.

Discussion

Exposure of 1321N1 human astrocytoma cells to ISO or other BAR agonists leads to a decrease in the subsequent ability of BAR agonists to stimulate AC activity, a phenomenon referred to as desensitization (15–18). The studies presented here demonstrate that exposure of these cells to the PKC activator PMA also leads to desensitization of AC activity. However, the changes in BAR and AC activity induced by these two agents are markedly different. Desensitization by BAR agonists is homologous (BAR-specific), because PGE_1, acting through a different receptor, and fluoride and forskolin, which act distal to receptors (34, 35), remain able to stimulate AC activity. In contrast, desensitization by PMA is not BAR-specific, because the ability of PGE_1 and ISO to stimulate AC activity were both decreased to a similar extent. However, PMA pretreatment did not decrease AC activity stimulated by fluoride or by forskolin. These results suggest that PMA-induced desensitization results either from changes in multiple specific receptors coupled to AC activation or from a change in a site on the guanine nucleotide-binding protein G_s involved in receptor-stimulated activation of AC but not in activation of AC by fluoride or forskolin. Phorbol esters have been shown to enhance phosphorylation of BAR in avian erythrocytes (7, 8), and this is an

obvious and likely possibility for the mechanism of PMA effects in 1321N1 cells as well.

Agonist-induced desensitization is accompanied by an apparent internalization (sequestration) of BAR, as demonstrated by a shift of BAR from the plasma membrane fraction to the light vesicle fraction in sucrose density gradient centrifugation assays. In contrast, pretreatment of cells with PMA under conditions where desensitization of BAR-stimulated AC was observed did not lead to internalization of BAR in these assays. Short-time assays of competition by hydrophilic ligands for binding of ^{125}I-iodopindolol to intact cell BAR can also be used to monitor receptor internalization (23, 24). Pretreatment of cells with ISO leads to a marked increase in the fraction of BAR exhibiting very low affinity for ISO in these assays. The low apparent affinity of these BAR for ISO is thought to reflect slow equilibration of this hydrophilic ligand with receptors that are present within endocytotic vesicles in the cell (23, 24). Pretreatment of cells with PMA did not increase the fraction of BAR in the very low affinity form in short-time assays, again indicating that PMA-induced desensitization of BAR-AC does not result from internalization of BAR. The lack of changes in apparent affinity for agonist of intact cell BAR after PMA pretreatment also indicates that changes in receptor affinity for agonist are not involved in the observed desensitization of BAR-AC.

The desensitization induced by PMA most likely results from activation of PKC. Thus the concentration range over which PMA exerts its effect is similar to that for other PKC-mediated effects of PMA. Furthermore, the ability of mezerein, but not 4-O-methyl-PMA, to mimic the effect of PMA is consistent with the known effects of these agents on activation of PKC. Desensitization of BAR-stimulated AC by PMA has been reported in a number of previous studies in various cell types (4–14). These observations have led to the suggestion that PKC might be involved in agonist-induced desensitization of BAR-AC as well. Our results from additivity experiments suggest that ISO and PMA are acting through different mechanisms. In addition, our studies with the protein kinase inhibitor H7 provide strong evidence against the involvement of PKC in agonist-induced desensitization. H7 was able to completely prevent desensitization induced by PMA, presumably because of its ability to inhibit PKC, whereas no inhibition of agonist-induced desensitization was observed. Thus PKC-mediated phosphorylation is probably not involved in desensitization of BAR-AC by agonists. Recent studies of phorbol ester effects on BAR-AC in C6 rat glioma cells led to the same conclusion. Again, the pattern of changes in BAR-AC induced by PMA and by agonist were found to be different (9). Furthermore, down-regulation of PKC by prolonged exposure of cells to PMA did not prevent subsequent agonist-induced desensitization (36). The conclusion that PKC activation is not involved in agonist-induced desensitization is consistent with the recent identification of a novel protein kinase with relatively high selectivity for agonist-occupied BAR *in vitro* (37, 38). This so-called BAR kinase has been postulated to mediate agonist-induced desensitization by phosphorylating and thus inactivating BAR.

Although recent studies have provided evidence suggesting the involvement of BAR phosphorylation in the mechanism of agonist-induced desensitization (18, 37, 38), direct evidence that phosphorylation is responsible for desensitization rather than being secondary to desensitization is lacking. In fact, it has been shown that desensitization of BAR-AC can occur in cells expressing modified BAR that lack most of the postulated phosphorylation sites (39). Accordingly, we propose two alternate explanations for the failure of H7 to inhibit agonist-induced desensitization. The most obvious is that H7 does not inhibit BAR kinase and, thus, both phosphorylation and desensitization of BAR are unaltered by H7. However, H7 apparently exerts its inhibitory effects at the ATP binding site of kinases and not at regulatory sites (such as the PMA binding site), and therefore H7 inhibits a wide range of protein kinases (29, 30). Accordingly, an alternate explanation for the failure of H7 to inhibit agonist-induced desensitization of BAR is that BAR phosphorylation is in fact inhibited by H7 but that phosphorylation of BAR is not required for desensitization. Direct assays of the effects of H7 on agonist-stimulated BAR phosphorylation will be required to distinguish between these possibilities. Regardless of which explanation is correct, our results indicate that H7 may be an important tool for elucidating the specific role(s), if any, of BAR phosphorylation in the overall process of agonist-induced desensitization of BAR.

No consistent pattern of effects of PMA on BAR-AC emerges from the studies to date in a variety of cell types. In most studies a decrease in BAR-AC has been observed (4–12), although in other studies an increase (13, 14) or no effect (40) has been reported. In those systems where decreased BAR-AC has been observed, the mechanisms involved appear to be different. Thus, in C6 cells, PMA caused internalization of BAR, but the internalized receptors were functional for stimulation of AC in reconstitution assays (9). Internalization of BAR was also implicated in phorbol ester-induced desensitization of cardiac myocytes (10). In contrast, in our studies with 1321N1 cells PMA apparently causes a decrease in receptor function (uncoupling from stimulation of AC) without causing internalization of BAR. The studies by Johnson *et al.* (14) with S49 mouse lymphoma cells demonstrated that the effects observed after PMA pretreatment were highly dependent on the specific conditions of the pretreatment and subsequent assays. Thus, studies under identical conditions in the same laboratory may be required to determine whether the observed differences in the effects of PMA on BAR in various cells are real or are caused by differences in experimental conditions.

In summary, treatment of 1321N1 cells with PMA results in heterologous desensitization of BAR-AC that is not accompanied by internalization of BAR, in contrast to the homologous desensitization of BAR-AC and internalization of BAR observed during agonist-induced desensitization. The protein kinase inhibitor H7 inhibits PMA-induced desensitization but not that induced by agonists, indicating that activation of PKC is probably not the mechanism of agonist-induced desensitization as it presumably is for PMA-induced desensitization. Nonetheless, the effects of PMA may be of physiological relevance, because they may reflect changes in BAR-AC that might occur after activation of PKC by agonists acting at receptors coupled to turnover of polyphosphoinositides and generation of the endogenous PKC activator diacylglycerol. Studies are in progress to determine the effects of agonists acting at polyphosphoinositide-coupled receptors on subsequent activity of BAR-AC in these cells.

Acknowledgments

The authors wish to thank Michael R. Barnes for his expert technical assistance throughout the course of these studies.

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