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Phosphorylation and Functional Desensitization of the α_{2A}-Adrenergic Receptor by Protein Kinase C

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ABSTRACT

We have investigated the potential for protein kinase C (PKC) to phosphorylate and desensitize the α_{2A}-adrenergic receptor (α_{2A}AR). In whole-cell phosphorylation studies, recombinantly expressed human α_{2A}AR displayed an increase in phosphorylation after short-term exposure to 100 nM phorbol 12-myristate-13-acetate (PMA) that was blocked by preincubation with a PKC inhibitor. This increase in receptor phosphorylation over basal amounted to 172 ± 40% in COS-7 cells and 201 ± 40% in Chinese hamster ovary cells. In permanently transfected Chinese hamster fibroblast cells, PKC activation by brief exposure of the cells to PMA resulted in a marked desensitization of α_{2A}AR function, amounting to a 68 ± 4% decrease in the maximal agonist (UK14304)-stimulated intracellular calcium release. Such desensitization was blocked by the PKC inhibitor bisindolylmaleimide I and was not evoked by an inactive phorbol ester. The desensitization of this agonist response was not caused by PKC-mediated augmentation of G protein-coupled receptor kinase activity, because PMA-promoted desensitization of a mutated α_{2A}AR that lacked G protein-coupled receptor kinase phosphorylation sites was identical to that of wild-type α_{2A}AR. To test whether PKC phosphorylation is a mechanism by which α_{2A}AR can be regulated by other receptors, the α_{1b}AR was co-expressed with the α_{2A}AR in Chinese hamster ovary cells. Upon selective activation of α_{1b}AR, the function of α_{2A}AR underwent a 53 ± 5% desensitization. Thus, cellular events that result in PKC activation promote phosphorylation of the α_{2A}AR and lead to substantial desensitization of receptor function. This heterologous regulation also represents a mechanism by which rapid crosstalk between the α_{2A}AR and other receptors can occur.

The α_2ARs regulate several effector systems including adenylyl cyclase, potassium channels, calcium channels, and inositol phosphate-mediated intracellular calcium release (Limbird, 1988; Liggett, 1996; Akerman *et al.* 1997; Dorn *et al.* 1997). Studies in intact organisms and cell culture systems have indicated that the function of G protein-coupled receptors, including α_2ARs, can be dynamically regulated under various physiologic and pathophysiologic conditions (Liggett and Lefkowitz, 1993; Liggett, 1997). We have recently delineated one pathway whereby persistent agonist activation results in a dampening of α_2AR signaling, termed desensitization, which is due to receptor phosphorylation by GRKs (Eason and Liggett, 1992; Liggett *et al.,* 1992; Eason *et al.,* 1995). GRK-mediated desensitization is evoked by agonist occupancy of the receptor, is independent of the generation of second messengers, and represents one mechanism of homologous desensitization of the α_2AR. The molecular basis of heterologous regulation of α_2AR has been largely unexplored. In the current study, we investigated the regulation of α_2AR signaling by PKC. This kinase was found to phosphorylate the α_{2A} subtype, which resulted in a rapid desensitization of receptor function. These effects represent a mechanism by which crosstalk between α_2AR and other G protein-coupled receptors can occur.

Experimental Procedures

Constructs and transfections. The human α_{2A}AR cDNA and a construct encoding a mutated α_{2A}AR lacking the four GRK phosphorylation sites in the third intracellular loop were in the mammalian expression vector pBC12BI as described previously (Eason *et al.,* 1995). The hamster α_{1b}AR cDNA was inserted into pRK5. For transient expression of wild-type α_2AR, COS-7 cells in monolayers at ~30–50% confluence were transfected with 10 μg of the α_{2A}AR construct via the DEAE-dextran method as described previously (Jewell-Motz and Liggett, 1996). Cells were then used for experiments 48 hr after transfection. COS-7 cells were maintained in

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ABBREVIATIONS: AR, adrenergic receptor; βARK, β-adrenergic receptor kinase; $[Ca^{2+}]_i$, intracellular calcium; CHO, Chinese hamster ovary; EGTA, ethylene glycol bis(β-aminoether)-*N,N,N′,N′*-tetraacedic acid; GRK, G protein-coupled receptor kinase; [^{125}I]HEAT, 2-[β-(4-hydroxy-3-[^{125}I]iodophenyl)ethylaminomethyl] tetralone; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol-12-myristate-13-acetate; SDS, sodium dodecyl sulfate; AM, acetoxymethyl ester.

Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37° in a 5% CO_2 atmosphere. For permanent expression of α_2AR, CHO cells in monolayers at ~30% confluence were cotransfected with the use of a calcium phosphate precipitation method. Cells were transfected with 3 μg of pSV$_2$neo, which provides G-418 resistance, and 20 μg of the α_2AR construct. A similar approach was used to co-express the α_{2A}AR and α_{1b}AR receptor by simultaneous transfections with 10 μg of each receptor construct. Screening for α_{2A}AR expression was by a [^{3}H]yohimbine binding assay and screening for the α_{1b}AR by a [^{125}I]HEAT binding assay, as described below. CHO cells were maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 80 μg/ml G-418 (to maintain selection pressure) at 37° in a 5% CO_2 atmosphere.

Desensitization of calcium signaling. CHO cells were detached, washed, and loaded with Fura-2/AM as described previously (Dorn *et al.,* 1997). Cells in 3.0 ml aliquots (~4 × 10^6 cells) at 37° were then added to cuvettes and after baseline measurements were obtained, the increases in intracellular calcium in response to the indicated concentrations of α_2AR agonists or 0.3 units/ml thrombin were determined over the ensuing 2 min by using standard methods exactly as described previously (Dorn *et al.,* 1997). Routinely, cells were incubated with PMA at the indicated concentrations for 1 min before the addition of the agonist. α_{2A}AR desensitization was defined as follows:

$$\text{Desensitization} = \left[1 - \frac{[Ca^{2+}]_i\text{post}}{[Ca^{2+}]_i\text{pre}}\right] \times 100\%$$

where $[Ca^{2+}]_i$ is the change in intracellular free calcium concentration evoked by the agonist in untreated cells (pre) or in cells after exposure to desensitizing agent (post). For cells expressing α_{2A}AR and α_{1b}AR, we were interested in the effects of selective activation of α_{1b}AR on α_{2A}AR function. These studies were carried out with a 1.0-μM concentration of the agonist phenylephrine, which was incubated with the cells for 10 min in the presence of 10 μM yohimbine (to block any activation of α_2AR by the phenylephrine). Control cells were incubated with yohimbine alone. Cells were then washed three times with 30 volumes of cold buffer to remove both agents, and then challenged with 1.0 μM UK14304 to assess α_{2A}AR stimulation of intracellular calcium release as above.

α_2AR phosphorylation. Whole cell phosphorylation studies were carried out in a manner similar to that described previously (Eason *et al.,* 1995; Jewell-Motz and Liggett, 1996). Briefly, COS-7 cells transiently co-expressing α_{2A}AR were incubated with [^{32}P]orthophosphate (~2.4 mCi/150-cm^2 plate) for 2 hr at 37° in 5% CO_2. Cells were then incubated for the indicated times with the medium alone or the medium plus the indicated concentrations of PMA or the agonist UK14304, washed five times with ice-cold phosphate-buffered saline, and scraped in buffer containing 10 mM Tris·HCl, pH 7.4, 5 mM EDTA, 5 mM EGTA, 10 mM NaF, 10 mM sodium pyrophosphate, and 5 μg/ml of the protease inhibitors benzamidine, soybean trypsin inhibitor, and leupeptin, which were included in this and all subsequent steps. Particulates were centrifuged at 40,000 × *g* for 10 min at 4°, and the resulting pellet was resuspended in the previously mentioned buffer, sonicated for 15 sec, and centrifuged once again. The receptor was then purified by immunoprecipitation as described previously (Eason *et al.,* 1995; Jewell-Motz and Liggett, 1996). Briefly, membranes were solubilized by stirring in phosphate-buffered saline containing 1% Triton X-100, 0.05% SDS, 1 mM EDTA, and 1 mM EGTA for 2 hr at 4°. Unsolubilized material was removed by centrifugation at 40,000 × *g* for 20 min at 4°, and the solubilized material was incubated with preimmune serum and protein A-Sepharose beads for 30 min at room temperature. The beads containing nonspecific immunoprecipitant were removed by brief centrifugation, and the remaining supernatant was incubated with a 1:200 dilution of polyclonal α_{2A}AR antisera (Kurose *et al.,* 1993) and protein A-Sepharose beads for 16 hr at 4°. The beads were washed five times, sonicated in SDS-sample buffer, and removed by centrifugation. The released immunoprecipitates containing equal amounts of receptor were fractionated on 10% SDS-polyacrylamide gels. Autoradiography was used to detect phosphorylation of receptors, and the amount of radioactivity was quantified on a PhosphorImager with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). For presentation purposes, autoradiograms were produced by exposing the gels to X-ray film for ~16 hr.

Radioligand binding. Expression levels of α_{2A}AR were determined using a [^{3}H]yohimbine binding assay. Membranes prepared as described above were incubated with 25 nM [^{3}H]yohimbine in the absence or presence of 10 μM phentolamine, which was used to define nonspecific binding, in a buffer containing 75 mM Tris, pH 7.4, 12.5 mM $MgCl_2$, and 2 mM EDTA for 30 min at 25°. For determination of

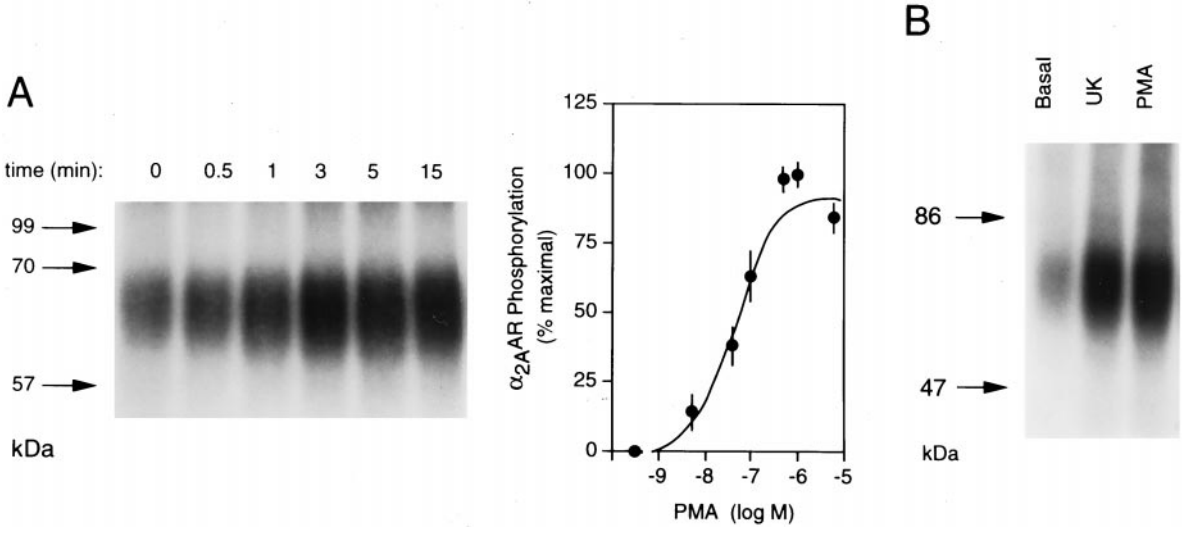

Fig. 1. Phosphorylation of α_{2A}AR by PMA and UK14304 in COS-7 cells. Cells were pre-incubated with [^{32}P]orthophosphate and exposed to the indicated agents, and the α_{2A}AR was purified as described in Experimental Procedures. A, COS-7 cells expressing α_{2A}AR were treated with 0.1 μM PMA for the indicated times or for 15 min with the indicated concentrations of PMA. Phosphorylation was maximal by ~3 min at concentrations of ~0.1–0.5 μM PMA. Results are representative of three experiments. B, COS-7 cells were treated with 1 μM UK14304 or 0.1 μM PMA for 15 min. Results are representative of 15 independent experiments.

α_{1b}AR expression, binding studies were carried out with 350 pM [^{125}I]HEAT in the absence or presence of 10 μM phentolamine for 20 min at 25°. Reactions were terminated by dilution with ice-cold 10 mM Tris, pH 7.4, followed by vacuum filtration through GF/C glass fiber filters (Whatman, Clifton, NJ). Specific binding was normalized for protein. For the current studies, CHO cells expressing wild-type α_{2A}AR at 1081 ± 80 fmol/mg and the GRK deletion mutant at 1770 ± 126 fmol/mg were used. For the CHO co-expression studies α_{2A}AR density was 3.1 ± 0.2 pmol/mg and α_{1b}AR density was 355 ± 75 fmol/mg. In COS-7 cells, transient expression of α_{2A}AR at levels of 5–7 pmol/mg was attained.

Miscellaneous. Western blots of cytosolic and membrane fractions of CHO cells using antisera agonist PKC isoforms α, β, δ, ϵ, and ζ were carried out as described (D'Angelo *et al.,* 1997) and visualized using the enhanced chemiluminescence system from New England Nuclear (Boston, MA). Protein concentrations were determined by the copper bicinchoninic method (Smith *et al.,* 1985).

Materials. PMA and the inactive phorbol ester 4α-phorbol-12,13-didecanoate were purchased from Sigma (St. Louis, MO). Bisindolylmaleimide I was obtained from Calbiochem (San Diego, CA). The isoform-specific PKC antisera were from Santa Cruz Biotechnology (Santa Cruz, CA) and Transduction Laboratories (Lexington, KY). The hamster α_{1b}AR construct was provided by D. Schwinn (Duke University Medical Center, Durham, NC). Sources for all other reagents were as referenced elsewhere (Eason *et al.,* 1995; Dorn *et al.,* 1997).

Results and Discussion

To assess the potential for PKC to regulate α_{2A}AR function, the receptor was transiently overexpressed in COS-7 cells and intact cell phosphorylation studies were carried out. As shown in Fig. 1A, exposure of cells to the PKC activator PMA indeed resulted in receptor phosphorylation that was rapid (maximal response occurred at ~3 min), with concentrations of 0.1–0.5 μM giving the maximal response. The extent of PMA-promoted receptor phosphorylation under optimal conditions was found to be 172 ± 14% (n = 38, where n indicates the number of experiments) above basal levels, whereas phosphorylation by the α_2AR agonist UK14304 was 219 ± 30% (n = 15) over basal (Fig. 1B). Because we planned to assess the functional consequences of PKC-mediated phosphorylation in CHO cells that permanently expressed the receptor at lower levels, additional phosphorylation studies were carried out in these cells as well to confirm that the pathway is also intact in CHO cells. As shown in Fig. 2, PMA did promote receptor phosphorylation in these cells to 201 ± 40% over basal. Again, the extent of PMA phosphorylation was less than that of UK14304-promoted phosphorylation, which amounted to 336 ± 31% over basal. As is shown, PMA-promoted phosphorylation of α_{2A}AR was completely abolished by pre-exposure to 1 μM of the PKC inhibitor staurosporine. Although the basal levels of phosphorylation were lowered, the fold stimulation of phosphorylation over basal induced by UK14304 was not altered by staurosporine.

To investigate whether PKC-mediated receptor phosphorylation altered α_2AR function, we examined α_2AR-mediated stimulation of intracellular calcium release. This signaling pathway is due to receptor coupling to G_i with subsequent $\beta\gamma$ release that activates PLC (Dorn *et al.,* 1997). As shown in Fig. 3A, 100 nM PMA exposure for 1 min results in a significant decrease in α_{2A}AR-mediated calcium signaling. In dose-response studies, maximum desensitization occurred with 100 nM of PMA, and the calculated concentration of PMA that evoked a half maximal desensitization response was ~10 nM (Fig. 4). PMA desensitization was blocked by pretreatment of the cells for 10 min with the PKC inhibitor bisindolylmaleimide I (1 μM), whereas treatment with the inactive phorbol ester 4α-phorbol-12,13-didecanoate (100 nM) had no effect on α_2AR-calcium signaling (n = 4; Fig. 5). In 15 studies, 100 nM pretreatment with PMA was found to evoke a 68 ± 4% desensitization of α_2AR-calcium signaling (Figs. 3A, 5). In contrast, thrombin-mediated stimulation of intracellular calcium release, which occurs via a $G_{q/11}$-stimulated PLC pathway in CHO cells (Dorn *et al.,* 1997), displayed 26 ± 6% desensitization by PMA (Figs. 3B, 5). The desensitization by PMA was also observed when α_2ARs were subsequently activated by the endogenous catecholamines epinephrine and norepinephrine. The extent of desensitization under these conditions was virtually the same (53% ± 3 with epinephrine and 66% ± 9 with norepinephrine; n = 6) as when the agent UK14304 was used for activation.

Upon phosphorylation by PKC, βARK activity (Deblasi *et al.,* 1995) and translocation (Freund *et al.,* 1996) are enhanced. Thus, we considered that an alternative explanation for the desensitization of the agonist responsiveness of α_2AR induced by PMA exposure could be these effects on βARK. To address this, studies were carried out with a mutated α_{2A}AR lacking the four serines in the third intracellular loop that are phosphorylated by βARK (Eason *et al.,* 1995). PMA-induced desensitization of agonist stimulation of intracellular calcium release occurred to the same extent (65 ± 2%, n = 4) with this mutated receptor as with the wild-type α_{2A}AR (Fig. 5).

Given these results, we considered that activation of PKC by another receptor should induce desensitization of the α_{2A}AR. This potential crosstalk was assessed by co-expression of the Gq-coupled α_{1b}AR with the α_{2A}AR in CHO cells and selective activation of the α_1AR. As shown in Fig. 5, activation of the α_{1b}AR indeed resulted in depressed maximal α_{2A}AR stimulation of intracellular calcium release. The extent of this desensitization amounted to 53 ± 5% (n = 4). Again, thrombin signaling under the same conditions was minimally desensitized.

The PKC isoforms expressed in CHO cells were determined with Western blots using antisera directed against the α, β,

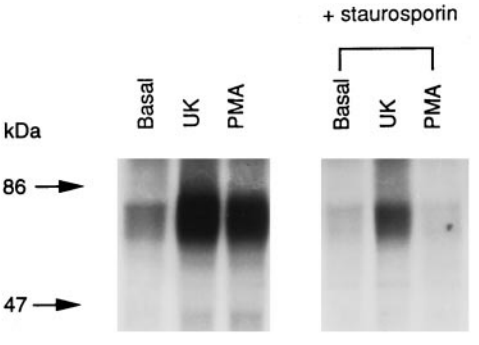

Fig. 2. Phosphorylation of α_{2A}AR by PMA and UK14304 in CHO cells. Cells were pre-incubated with [^{32}P]orthophosphate and exposed to 0.1 μM PMA or 10 μM UK14304 for 15 min, and the α_{2A}AR was purified as described in Experimental Procedures. Shown are representative experiments from seven performed. Pre-incubation for 15 min with the PKC inhibitor staurosporine (1 μM) completely inhibited PMA-promoted phosphorylation. Although basal levels of phosphorylation were decreased by staurosporine, the fold increase in phosphorylation induced by agonist (2.9 ± 0.8 versus 2.0 ± 0.6 fold, n = 3) was not significantly altered.

δ, ϵ, and ζ isoforms (Fig. 6). PKC α, ϵ, and ζ, but not β or δ, were expressed, and consistent with the known sensitivity of conventional and novel PKCs, but not atypical PKCs, to diacylglycerol analogues (Hug and Sarre, 1993), the α and ϵ isoforms translocated to the membrane upon exposure of the cells to PMA. Thus, in regard to the PMA effects on α_{2A}AR function observed in CHO cells, it seems that these are mediated by PKC_{α} and/or PKC_{ϵ}. We also assessed which PKC isoforms were translocated as a result of α_{2A}AR and α_{1b}AR activation. All three isoforms were translocated by α_{2A}AR, whereas α_{1b}AR activation was associated with translocation of the α and ζ isoforms.

Recent studies have shown that α_{2A}AR expressed in CHO, COS-7, and human embryonic kidney 293 cells undergo rapid phosphorylation and homologous desensitization during agonist occupancy (Eason and Liggett, 1992; Liggett *et al.,* 1992; Kurose and Lefkowitz, 1994; Eason *et al.,* 1995; Jewell-Motz and Liggett, 1996) With the use of several different approaches, homologous desensitization of α_{2A}AR has been shown to be caused by rapid phosphorylation of the receptor. One kinase that has been implicated in this process is the βARK or related G protein-coupled receptor kinases. Some evidence, however, indicates that α_2AR function can be regulated by other kinases. For the rapid form of heterologous desensitization, PKC phosphorylation has been considered a likely candidate mechanism for the α_2AR and several other G protein-coupled receptors. Convents *et al.* (1989) have shown that PMA exposure to NG108 cells, which express the α_{2B} subtype, results in a loss of α_2AR inhibition of cAMP production, but no change in the response to carbachol. For the α_1AR, PMA pretreatment results in phosphorylation of the receptor and decreased coupling to phosphoinositide hydrolysis (Leeb-Lundberg *et al.,* 1987). β_2AR function has also been shown to be decreased by phorbol esters, an effect associated with receptor phosphorylation (Bouvier *et al.* 1991). One of the most well characterized G protein-coupled receptor signaling pathways that is altered by PKC-mediated receptor phosphorylation is that of the $5HT_{1A}$ receptor (Raymond, 1991), where the sites of this phosphorylation recently have been mapped (Lembo and Albert, 1995).

A possible confounding factor in our current work has been the potential for phorbol esters to modify signal transduction at multiple levels, from the receptor to the measured outcome (intracellular calcium release). PKC has been reported to phosphorylate G_i (Katada *et al.,* 1985), and if this occurred in the intact cell experiments of our study, it would make interpretation of receptor-specific desensitization difficult. However, this has been addressed in the aforementioned studies of PKC phosphorylation and desensitization of the G_i coupled $5HT_{1A}$ receptor (Lembo and Albert, 1995). In these studies, when all PKC sites were ablated in this receptor, phorbol esters had minimal effects on receptor signaling, which suggests that G_i function remains relatively intact with experiments such as those in the current report involving brief exposure (minutes) to phorbol esters. Phorbol esters have also been reported to phosphorylate the effector PLCβ (Ryu *et al.,* 1990). We therefore used thrombin signaling as a control, because this receptor ultimately activates PLC as well. Under the conditions used here, thrombin-stimulated release of intracellular calcium was desensitized only 15–25% by PMA although the α_{2A}AR response was desensitized by ~70%. Similarly, desensitization of the inositol phosphate receptor or depletion of calcium stores would result in de-

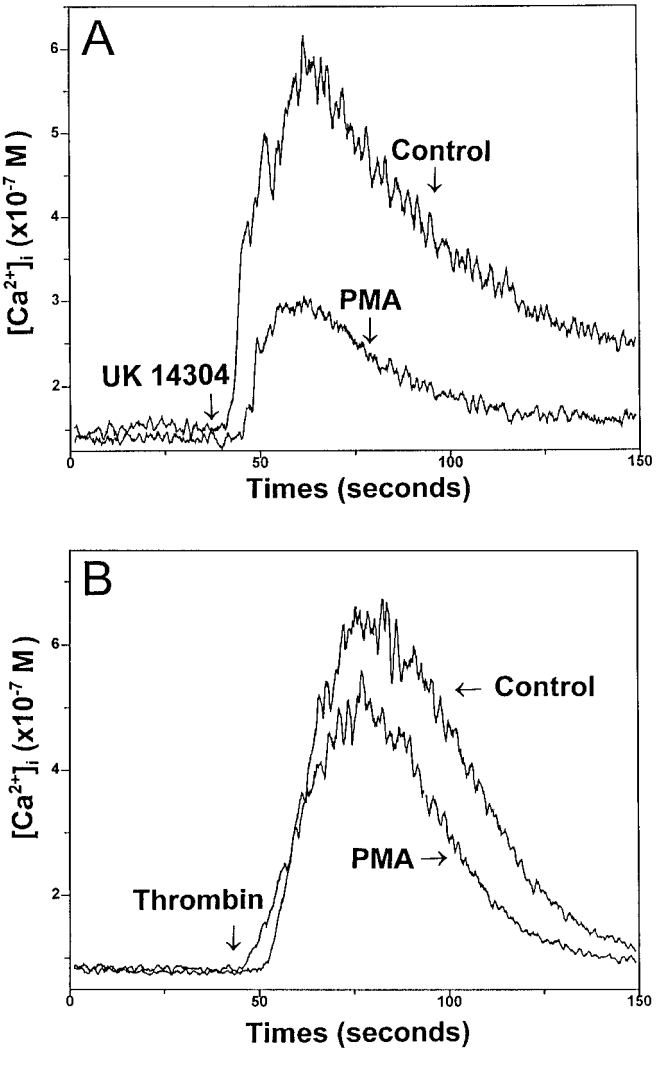

Fig. 3. Desensitization of α_{2A}AR-mediated stimulation of intracellular calcium release by PMA. CHO cells were loaded with Fura-2/AM, treated with 0.1 μM PMA for 1 min, followed by vehicle alone; 1.0 μM UK14304 (A); or 0.3 units/ml thrombin (B). Shown are tracings of intracellular calcium concentrations from a single experiment representative of 15 performed.

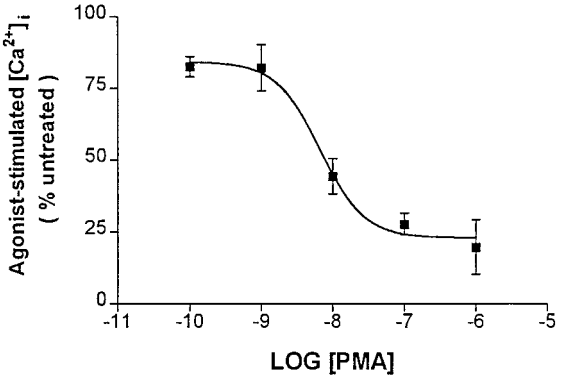

Fig. 4. Desensitization of α_{2A}AR by PMA. CHO cells expressing α_{2A}AR were studied as in Fig. 3, by using the indicated concentrations of PMA. The concentration that evoked half maximal desensitization was 8.9×10^{-9} M (pK_i = 8.05 ± 0.07; n = 3). Shown are the results of three experiments.

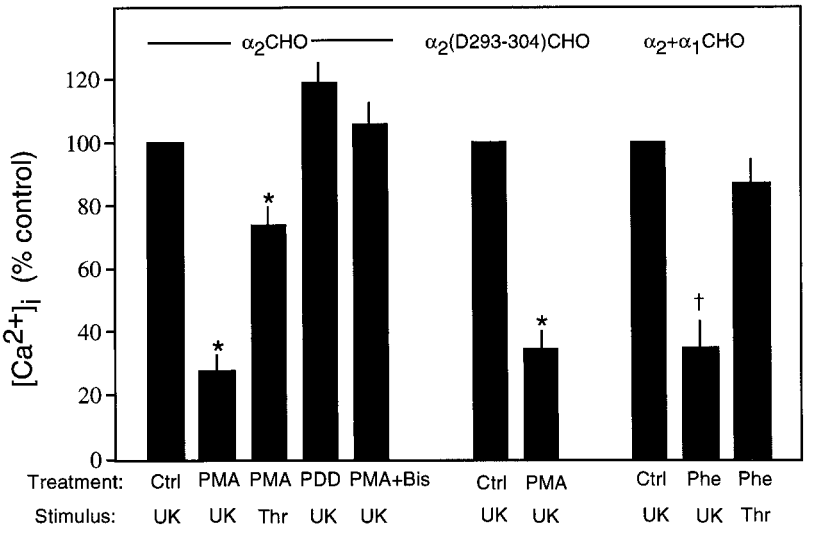

Fig. 5. Characterization of PKC-mediated desensitization of α_{2A}AR. CHO cells expressing wild-type α_{2A}AR, those expressing the D(293–304) receptor lacking βARK phosphorylation sites, and those expressing both the α_{2A}AR and the α_{1b}AR were exposed to the indicated agents and subsequently challenged with the indicated agonists to stimulate intracellular calcium release. See text for drug concentrations, exposure times, and number of experiments performed. *UK*, UK14304; *Thr*, thrombin; *Phe*, phenylephrine; *, $p < 0.001$; †, $p < 0.01$, versus untreated.

creased intracellular calcium release. Again, however, such desensitization would also be expected with thrombin receptor signaling, which was not desensitized to nearly the same extent as was α_2AR signaling. The possibility that PKC could alter a portion of the α_2AR signal-transduction pathway that would not be accounted for by our controls must nevertheless be considered.

We initially investigated PKC-mediated α_{2A}AR phosphorylation by expressing the receptor in COS-7 cells. The use of this transient-expression approach allowed for high levels of receptor expression and facilitated receptor purification. A concentration- and time-dependent PMA-induced phosphorylation of the α_{2A}AR over basal was observed. Although stoichiometry cannot be accurately determined in these small-scale preparations, we were able to compare the extent of PMA-promoted phosphorylation to agonist-promoted receptor phosphorylation. [The latter process has a stoichiometry thought to be 4 mol of phosphate/mol of receptor (Eason *et al.* 1995; Benovic *et al.,* 1987)]. PMA-promoted phosphorylation was found to be ~80% of agonist phosphorylation. Because evaluation of α_{2A}AR signaling and its rapid desensitization is not feasible in COS-7 cells, we subsequently studied receptors permanently expressed at lower levels in CHO cells where PMA promoted phosphorylation of the α_{2A}AR was also demonstrated.

For functional studies, we chose to study α_{2A}AR-mediated stimulation of intracellular calcium release. The choice of this α_2AR signaling pathway was based on several factors. First, we have found that quantitative analysis of desensitization is more readily observed in a stimulatory pathway as compared with an inhibitory pathway (such as inhibition of cAMP) because inhibition assays typically require a concomitant stimulus. Secondly, calcium responses are readily observed and quantified in real time. Also, because we have found no evidence for receptor reserve in these transfected cells when examining this signal (unpublished data), there is less concern about receptor overexpression masking desensitization of this response. The desensitization of this α_{2A}AR function by PKC was substantial, with maximal agonist-stimulated calcium release blunted by 68%. The residues within the receptor protein that are phosphorylated by PKC are presently not known, but the third intracellular loop of the α_{2A}AR has several serines and threonines in a favorable milieu for PKC phosphorylation. This is consistent with the fact that this loop is known to be important for functional G protein coupling (Eason and Liggett, 1996).

It may be concluded from this study, then, that PKC-mediated phosphorylation of the α_{2A}AR results in functional desensitization and is one mechanism for heterologous desensitization of the receptor. Such regulation can be evoked by activation of receptors such as the α_1AR that couple to PLC/PKC, or by any other mechanism that activates PKC. Interestingly, because in some cells α_{2A}AR can stimulate PLC via $\beta\gamma$ and thus activate PKC, phosphorylation by PKC may also play a role in agonist-dependent desensitization. This may be analogous to the desensitization of β_2AR by agonist, which is caused by both GRK phosphorylation and phosphorylation by the second messenger-dependent kinase protein kinase A (Hausdorff *et al.,* 1989). Finally, in that activation of PKC is a widespread signaling event, delineation of PKC-mediated desensitization of α_2AR function defines a mechanism by which receptor function is dynamically regulated by homeostatic and pathophysiologic processes.

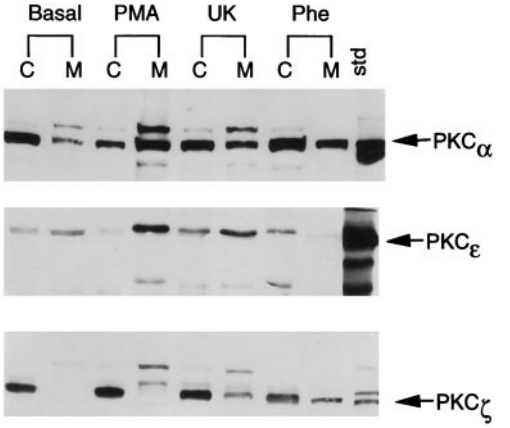

Fig. 6. Expression and translocation of PKC isoforms in CHO cells. Western blots were carried out as described in Experimental Procedures, and revealed expression of the α, ϵ, and ζ isoforms. Activation by PMA or receptor agonists was assessed by determining the increase in the ratio of membrane to cytosolic expression of the kinase.

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