Phosphorylation and Functional Desensitization of the α2A-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 α2A-adrenergic receptor (α2AAR). In whole-cell phosphorylation studies, recombinantly expressed human α2AAR 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 α2AAR 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 phospholipase C, potassium channels, calcium channels, and adenyl 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 α2ARs, 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 α2AR 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 α2AR. The molecular basis of heterologous regulation of α2AR has been largely unexplored. In the current study, we investigated the regulation of α2AR signaling by PKC. This kinase was found to phosphorylate the α2A subtype, which resulted in a rapid desensitization of receptor function. These effects represent a mechanism by which crosstalk between α2AR and other G protein-coupled receptors can occur.

The α2ARs regulate several effector systems including adenyl 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 α2ARs, 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 α2AR 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 α2AR. The molecular basis of heterologous regulation of α2AR has been largely unexplored. In the current study, we investigated the regulation of α2AR signaling by PKC. This kinase was found to phosphorylate the α2A subtype, which resulted in a rapid desensitization of receptor function. These effects represent a mechanism by which crosstalk between α2AR and other G protein-coupled receptors can occur.

Experimental Procedures

Constructs and transfections. The human α2A AR cDNA and a construct encoding a mutated α2AAR 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 α1bAR cDNA was inserted into pRK5. For transient expression of wild-type α2AR, COS-7 cells in monolayers at ~30–50% confluence were transfected with 10 µg of the α2AAR 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

Abbreviations: AR, adrenergic receptor; βARK, β-adrenergic receptor kinase; [Ca2+]i, intracellular calcium; CHO, Chinese hamster ovary; EGTA, ethylene glycol bis(β-aminoethoxy)-N,N,N',N'-tetraacetic acid; GRK, G protein-coupled receptor kinase; [35S]HEAT, 2-[β-(4-hydroxy-3-ethylphenyl)ethylaminomethyl]-tetraol; 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% CO₂ atmosphere. For permanent expression of α₂AR, 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 pSVNeo, which provides G-418 resistance, and 20 μg of the α₂AR construct. A similar approach was used to co-express the α₂AR and α₁AR receptor by simultaneous transfections with 10 μg of each receptor construct. Screening for α₂AR expression was by a [³²P]yohimbine binding assay and screening for the α₁AR by a [¹²⁵I]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% CO₂ 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 × 10⁶ 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 α₂AR 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. α₂AR desensitization was defined as follows:

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

where [Ca²⁺] 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 α₂AR and α₁AR, we were interested in the effects of selective activation of α₁AR on α₂AR 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 α₂AR 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 α₂AR stimulation of intracellular calcium release as above.

α₂AR 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 α₂AR were incubated with [³²P]orthophosphate (~2.4 mCi/150-cm² plate) for 2 hr at 37° in 5% CO₂. 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 α₂AR 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 α₂AR were determined using a [³²P]yohimbine binding assay. Membranes prepared as described above were incubated with 25 nM [³²P]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 MgCl₂, and 2 mM EDTA for 30 min at 25°. For determination of

**Fig. 1.** Phosphorylation of α₂AR by PMA and UK14304 in COS-7 cells. Cells were pre-incubated with [³²P]orthophosphate and exposed to the indicated agents. A, COS-7 cells expressing α₂AR were purified as described in Experimental Procedures. A, COS-7 cells expressing α₂AR 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.
α2AAR expression, binding studies were carried out with 350 pm [32P]HEAT in the absence or presence of 10 μM phentolamine for 20 min at 25°C. 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 α2AAR at 1081 ± 80 fmol/mg and the GRK deletion mutant at 1770 ± 126 fmol/mg were used. For the CHO co-expression studies α2AR density was 3.1 ± 0.2 pmol/mg and α1AR density was 355 ± 75 fmol/mg. In COS-7 cells, transient expression of α2AAR at levels of 5–7 pmol/mg was attained.

**Miscellaneous.** Western blots of cytosolic and membrane fractions of CHO cells using antisera against 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 4a-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 α1AR construct was provided by D. Schwin (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 α2AAR 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 α1AR 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 α2AAR 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 α1AR function, we examined α2AAR-mediated stimulation of intracellular calcium release. This signaling pathway is due to receptor coupling to G1, with subsequent βγ release that activates PLC (Dorn et al., 1997). As shown in Fig. 3A, 100 nM PMA exposure for 1 min resulted in a significant decrease in α2AAR-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 4a-phorbol-12,13-didecanoate (100 nM) had no effect on α2AAR-calcium signaling (n = 4; Fig. 5). In 15 studies, 100 nM pretreatment with PMA was found to evoke a 68 ± 4% desensitization of α2AR-calcium signaling (Figs. 3A, 5). In contrast, thrombin-mediated stimulation of intracellular calcium release, which occurs via a Gq/11-stimulated PLC pathway in CHO cells (Dorn et al., 1997), displayed 26 ± 5% desensitization by PMA (Figs. 3B, 5). The desensitization by PMA was also observed when α2ARs 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 α2AR induced by PMA exposure could be these effects on βARK. To address this, studies were carried out with a mutated α2AR 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 α2AAR (Fig. 5).

Given these results, we considered that activation of PKC by another receptor should induce desensitization of the α2AR. This potential crosstalk was assessed by co-expression of the Gq-coupled α1AR with the α2AAR in CHO cells and selective activation of the α1AR. As shown in Fig. 5, activation of the α1AR indeed resulted in depressed maximal α2AAR 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 using Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC. As shown in Fig. 5, the PKC isoforms expressed in CHO cells were α1, β1, γ1, δ1, ε1, and ζ1. The PKC isoforms were determined by Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC. As shown in Fig. 5, the PKC isoforms expressed in CHO cells were α1, β1, γ1, δ1, ε1, and ζ1. The PKC isoforms were determined by Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC. As shown in Fig. 5, the PKC isoforms expressed in CHO cells were α1, β1, γ1, δ1, ε1, and ζ1. The PKC isoforms were determined by Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC. As shown in Fig. 5, the PKC isoforms expressed in CHO cells were α1, β1, γ1, δ1, ε1, and ζ1. The PKC isoforms were determined by Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC. As shown in Fig. 5, the PKC isoforms expressed in CHO cells were α1, β1, γ1, δ1, ε1, and ζ1. The PKC isoforms were determined by Western blots using antisera directed against the α, β, δ, ε, and ζ isoforms of PKC.
isoforms were translocated as a result of the activation. All three isoforms were translocated by diacylglycerol analogues (Hug and Sarre, 1993), the 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 α2AAR function observed in CHO cells, it seems that these are mediated by PKCa and/or PKCc. We also assessed which PKC isoforms were translocated as a result of α2AAR and α1aAR activation. All three isoforms were translocated by α2AAR, whereas α11AR activation was associated with translocation of the α and ε isoforms.

Recent studies have shown that α2AAR 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 α2AAR 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 α2AR 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 α2AR and several other G protein-coupled receptors. Convents et al. (1989) have shown that PMA exposure to NG108 cells, which express the α2c subtype, results in a loss of α2AR inhibition of cAMP production, but no change in the response to carbachol. For the α1AR, PMA pretreatment results in phosphorylation of the receptor and decreased coupling to phosphoinositide hydrolysis (Leeb-Lundberg et al., 1987). β2AR 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 5HT1A receptor (Raymond, 1991), where the sites of this phosphorylation recently have been mapped (Leombo 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 Gi (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, coupled 5HT1A receptor (Leombo 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 Gi 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 α2AAR response was desensitized by ~70%. Similarly, desensitization of the inositol phosphate receptor or depletion of calcium stores would result in detranslocation of the receptor.

**Fig. 3.** Desensitization of α2AAR-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 α2AAR by PMA. CHO cells expressing α2AAR were studied as in Fig. 3, by using the indicated concentrations of PMA. The concentration that evoked half maximal desensitization was $8.9 \times 10^{-6}$ M ($pK_d = 8.05 \pm 0.07; n = 3$). Shown are the results of three experiments.
increased 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 $\alpha_2$AR signaling. The possibility that PKC could alter a portion of the $\alpha_2$AR signal-transduction pathway that would not be accounted for by our controls must nevertheless be considered.

We initially investigated PKC-mediated $\alpha_2$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 $\alpha_{2A}$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 $\alpha_{2A}$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 $\alpha_{2A}$AR was also demonstrated.

For functional studies, we chose to study $\alpha_{2A}$AR-mediated stimulation of intracellular calcium release. The choice of this $\alpha_{2}$AR 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 $\alpha_{2A}$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 $\alpha_{2A}$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 $\alpha_{2A}$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 $\alpha_1$AR that couple to PLC/PKC, or by any other mechanism that activates PKC. Interestingly, because in some cells $\alpha_{2A}$AR can stimulate PLC via $\beta_2$AR agonist and, 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 $\alpha_{2A}$AR function defines a mechanism by which receptor function is dynamically regulated by homeostatic and pathophysiologic processes.

**Fig. 5.** Characterization of PKC-mediated desensitization of $\alpha_{2A}$AR. CHO cells expressing wild-type $\alpha_{2A}$AR, those expressing the D(293–304) receptor lacking $\beta$ARK phosphorylation sites, and those expressing both the $\alpha_{2A}$AR and the $\alpha_{2A}$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.

**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 $\alpha$, $\epsilon$, and $\zeta$ isoforms. Activation by PMA or receptor agonists was assessed by determining the increase in the ratio of membrane to cytosolic expression of the kinase.


References

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