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Phorbol-ester-induced phosphorylation of the β_2-adrenergic receptor decreases its coupling to G_s

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Phorbol-esters have been shown to modulate the β-adrenergic-stimulated adenylyl cyclase in a number of cell lines. Here, using site directed mutagenesis, we investigate the role of the β_2-adrenergic receptor phosphorylation by protein kinase C in this regulatory process. Mutation of the serine-261, -262, -344 and -345 of the β_2-adrenergic receptor prevented the phorbol-ester-induced phosphorylation of the receptor. This mutation also abolished the phorbol-ester-induced decrease in high-affinity agonist binding and potency of the β_2-adrenergic receptor. We suggest that protein kinase C mediated phosphorylation of the receptor promotes its functional uncoupling.

β_2-Adrenergic receptor; Phosphorylation; Protein kinase C; Adenylyl cyclase; Desensitization; Phorbol-ester; Receptor uncoupling

1. INTRODUCTION

Cross-regulation among transmembrane signalling systems has recently attracted considerable attention (for a review see [1]). In particular, modulation of the adenylyl cyclase reactivity by the stimulation of receptors coupled to the phospho-inositides (PI) turnover pathway has been reported [2,3]. The observation that the protein kinase C (PKC) activators, phorbol-esters, mimick these modulating effects in many cell lines [4–15] has led to the proposal of a role for PKC in this inter-system regulatory mechanism. However, the effects of PKC-mediated phosphorylation on the adenylyl cyclase reactivity appear to be rather complex, and both desensitization [10–15] and supersensitization [4–9] have been reported following phorbol-ester treatments.

In many mammalian cell lines, hormone-, fluoride- and GTP-stimulated adenylyl cyclase activities have been shown to be enhanced by phorbol-ester treatment [4–9]. Phosphorylation by PKC of the inhibitory GTP-binding protein (G_i) [16,17] and of the adenylyl cyclase catalytic unit itself [18,19] have been proposed to contribute to this sensitization effect of the phorbol-esters.

In sharp contrast, phorbol-ester treatment has been shown to induce a desensitization of the β-adrenergic-stimulated adenylyl cyclase in avian erythrocytes [14,15]. Desensitization was accompanied by an increased phosphorylation of the β_2-adrenergic receptor (β_2AR). It was suggested that PKC-mediated phosphorylation of the receptor could contribute to this desensitization. The mammalian β_2AR receptor has been shown to be an in vitro substrate for PKC [20], and potential PKC phosphorylation sites are apparent in its primary structure [21,22]. Thus, questions arise concerning the role of the PKC-mediated phosphorylation of the β_2AR in mammalian cellular systems. To evaluate the effects of the phosphorylation in such a system, a β_2AR lacking potential PKC phosphorylation sites was constructed by site directed mutagenesis and expressed in Chinese hamster fibroblasts (CHW-1102). The influence of 4β-phorbol 12β-myristate 13 α-acetate (PMA) treatment on the phosphorylation level of the receptor and on the β-adrenergic-stimulated adenylyl cyclase activity were then evaluated in cells expressing either the wild type (WT) or the mutant (PKC$^-$$\beta_2$AR).

2. MATERIALS AND METHODS

2.1. *Materials*

Carrier-free $^{32}P_i$, [^{125}I]CYP, [α-^{32}P]ATP and [^{3}H]cAMP were obtained from New England Nuclear. Isoproterenol, ATP, GTP, cAMP, phosphoenol pyruvate, myokinase were purchased from Sigma. Pyruvate kinase and isobutylmethylxanthine were from Calbiochem. Digitonin was purchased from Gallard-Schlessinger. Geneticin (G418), DMEM, fetal calf serum, penicillin, streptomycin, amphotericin B and trypsin were purchased from Gibco. The site directed mutagenesis kit was obtained from Amersham. The expres-

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Abbreviations: AR, adrenergic receptor; PMA, 4β-phorbol 12β-myristate 13α-acetate; PKC, protein kinase C; G_s, stimulatory guanine-nucleotide-binding protein; G_i, inhibitory guanine-nucleotide-binding protein; PI, phospho-inositides; [^{125}I]CYP, [^{125}I]cyanopindolol; CHW, Chinese hamster fibroblast; DMEM, Dulbecco's minimum Eagle's medium

sion vector pBC12BI was generously provided by Dr B. Cullen (Duke University). Alprenolol was a generous gift from Hassle pharmaceutical (Sweden).

2.2. *Site directed mutagenesis and cell transfection*

A mutant human β_2AR was constructed where serine-261, -262, -344, and -345 have been replaced by alanines. The *Eco*RI-*Hin*dIII fragment of pSPNAR [23] containing the β_2AR coding sequence was cloned into the *Eco*RI-*Hin*dIII sites of pTZ18R (Pharmacia). Single stranded DNA was generated using VCS-M13 helper phage (Stratagene) and served as a template for oligonucleotide directed mutagenesis (Amersham kit). For eucaryotic expression, the *Eco*RI-*Hin*dIII fragment of the mutant and wild type constructs were subcloned in the *Hin*dIII-*Bam*I site of pBC12BI [24] and cotransfected with PSV2-neo [25] into Chinese hamster fibroblasts 1102 (CHW) by calcium phosphate precipitation [26]. Positive clones selected for their resistance to neomycin (G418; 150 μg/ml) were then screened for β_2AR expression in a radio-ligand binding assay, using 400 pM [^{125}I]CYP as the radioligand and 10 μM alprenolol to define specific binding.

2.3. *Cell culture*

The transfected CHW cells were grown as monolayers in 75 cm^2 flasks containing DMEM supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), amphotericin B (0.25 μg/ml), and glutamine (1 mM) in an atmosphere of 95% air and 5% CO_2 at 37°C.

2.4. *Membrane preparation*

Cells were incubated for various periods of time at 37°C with DMEM supplemented as above and with or without PMA at the specified concentrations. Cells were washed with PBS and lysed with a polytron homogenizer (2 bursts of 5 s) in 10 ml of ice-cold 5 mM Tris-HCl (pH 7.4), 2 mM EDTA. The lysate was centrifuged at 45 000 × g for 20 min and washed twice in the same buffer. The pelleted membranes were resuspended in 0.6 ml of a buffer containing 75 mM Tris-HCl (pH 7.4), 12.5 mM $MgCl_2$ and 2 mM EDTA and used immediately. Protein content was determined according to the method of Bradford [27] (Biorad).

2.5. *Adenylyl cyclase assay and radio-ligand binding assay*

Adenylyl cyclase activity was measured by the method of Salomon et al. [28] as previously described [29] using ~20 μg of membrane protein in a total volume of 0.05 ml. The incubation mixture included 0.12 mM ATP, 1 μCi [α-^{32}P]ATP, 0.1 mM cAMP, 0.053 mM GTP, 2.8 mM phosphoenolpyruvate, 0.2 U of pyruvate kinase, 1 U of myokinase, 30 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ and 0.8 mM EDTA. Enzyme activity was determined in triplicate in the absence (i.e. basal activity) or in the presence of activators (isoproterenol 0–100 μM, forskolin 0–100 μM). Radio-ligand binding assays were conducted essentially as described [29] using ~10 μg of membrane protein in a total volume of 0.5 ml. For saturation experiments, duplicate assay tubes contained 2–400 pM [^{125}I]CYP in the presence and absence of 10 μM alprenolol. For competition experiments duplicate assay tubes contained ~50 pM [^{125}I]CYP and 0–100 mM isoproterenol. The binding reactions were terminated by rapid filtration on Whatman GF/C glass fiber filters. Data from competition and saturation experiments were analyzed by non-linear least-squares regression using the computer program LIGAND [30].

2.6. *Whole cell phosphorylation*

Cells were detached, washed twice with phosphate-free DMEM and preincubated in this medium for 60 min at 37°C. Carrier-free ^{32}P$_i$ (0.5 mCi/ml) was then added to the medium and the cells incubated for an additional 60 min at 37°C. At the end of this equilibration period, PMA (10 μM) or the vehicle was added to the cells and incubated at 37°C for 15 min. The cells were then lysed by sonication in ice-cold buffer containing 20 mM Tris-HCl (pH 7.4), 5 mM EDTA, 10 mM Na_2HPO_4, leupeptin 5 μg/ml, soybean trypsin inhibitor 5 μg/ml and benzamidine 10 μg/ml. The membranes were then centrifuged at 40 000 × g and washed twice in the same buffer. The washed membrane preparations were solubilized in 100 mM NaCl, 10 mM Tris-HCl (pH 7.4), 5 mM EDTA, 2% digitonin at 4°C for 2 h and the β_2AR purified by alprenolol-Sepharose affinity chromatography as described elsewhere [31].

2.7. *SDS-polyacrylamide gel electrophoresis*

Gel electrophoresis was performed according to the method of Laemmli [32] with 10–12% slab gels. The amount of receptor loaded on the gel was monitored by radio-ligand binding using 400 pM [^{125}I]CYP and 10 mM alprenolol to define the specific binding. The binding reactions were terminated by passing the samples through Sephadex G-25 columns at 4°C. After electrophoresis, the gels were dried and autoradiographed at −90°C using Kodak XAR-5 film.

3. RESULTS AND DISCUSSION

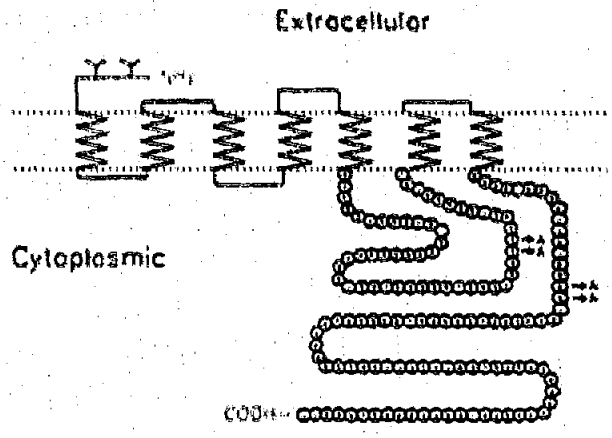
A mutant β_2AR lacking potential phosphorylation sites for PKC was constructed by site direct mutagenesis of the human β_2AR cDNA. Serine and threonine residues flanked on both sides by basic amino acids have been proposed as potential PKC phosphorylation sites in many peptides and proteins [33]. The serines-261, -262, -344 and -345 of the human β_2AR fulfill this criterion and were replaced by alanine residues (PKC$^-$ β_2AR) (Scheme 1). Both wild type (WT) and mutant receptor cDNA constructs were transfected in CHW-1102 cells (see section 2). Cell lines expressing comparable levels of receptors were used for the study (WTβ_2AR: 1.2 ± 0.1 pmol/mg protein, vs PKC$^-$ β_2AR: 1.4 ± 0.2 pmol/mg protein).

Table I

Adenylyl cyclase activity (pmol/min/mg)

Cell line	Basal		Isoproterenol-stimulated		Forskolin-stimulated	
	Ctrl	PMA	Ctrl	PMA	Ctrl	PMA
Wtβ_2AR (n = 7)	20 ± 3	48 ± 13	34 ± 4	101 ± 9	94 ± 14	179 ± 57
PKC$^-$ β_2AR (n = 4)	16 ± 4	34 ± 7	28 ± 5	80 ± 11	98 ± 18	166 ± 28

Cells expressing either WTβ_2AR or PKC$^-$ β_2AR were pretreated (PMA) or not (Ctrl) with 10 μM PMA for 30 min. The basal, isoproterenol-stimulated (100 μM) and forskolin-stimulated (100 μM) adenylyl cyclase activities were measured as described in section 2. Values represent the mean ± SEM.



Scheme 1. Schematic representation of the postulated transmembrane organization of the human β_2AR. The arrows indicate the serine residues 261, 262, 344 and 345 which were mutated to alanines by the directed mutagenesis to generate PKC$^-$ β_2AR.

The mutation did not affect the affinity of the receptor for the antagonist [^{125}I]CYP (data not shown) nor did it significantly change the ability of the receptor to mediate isoproterenol-stimulation of the adenylyl cyclase (Table I). However, the mutation completely abolished the PMA-induced phosphorylation of the receptor. Indeed as has been reported in other cell types [14,15], an incubation of 15 min with 10 μM PMA induced a significant increase in the phosphorylation level of the WTβ_2AR expressed in CHW-1102 cells whereas the same treatment did not alter the phosphorylation level of PKC$^-$ β_2AR (Fig. 1). These results suggest that one or more of the mutated serines is the major PKC phosphorylation site(s) of the β_2AR.

In cells expressing WTβ_2AR PMA induces a time- and dose- dependent increase in basal, isoproterenol-stimulated and forskolin-stimulated adenylyl cyclase activities. As illustrated in Fig. 2, the maximal effects of the PMA treatment on the adenylyl cyclase activities were observed following a 30 min incubation with 10 μM PMA, and therefore these conditions were used in subsequent experiments. Table I summarizes the effects of this PMA treatment on the basal and stimulated adenylyl cyclase activities in cells expressing WTβ_2AR and PKC$^-$ β_2AR. In both cell lines, PMA induces an increase in the basal activity as well as in the maximum activity when stimulated by isoproterenol (100 μM) and forskolin (100 μM). The sensitizing effect of the PMA was virtually identical in the two cell lines. However, when the effect of PMA was assessed on full dose-response curves in cells expressing the WTβ_2AR, the PMA treatment induced a 5-fold increase in the EC_{50} of adenylyl cyclase stimulation by isoproterenol (Ctrl: 60 ± 8 nM vs PMA treated 300 ± 30 nM, $n = 5$). This PMA-induced decrease in potency was abolished by mutation of the 4 serines. Indeed, the EC_{50} of the adenylyl cyclase stimulation by isoproterenol was not affected by the PMA treatment in cells expressing PKC$^-$ β_2AR (Ctrl: 110 ± 20 nM vs PMA treated: 100 ± 10 nM; $n = 5$). This effect on the potency appears receptor specific since PMA treatment did not affect the EC_{50} of adenylyl cyclase stimulation by forskolin (data not shown).

The total amount of β_2AR, as assessed by [^{125}I]CYP binding was not affected by the PMA treatment in either cell line (**WTβAR**: Ctrl; 1.2 ± 0.1 pmol/mg vs PMA; 1.3 ± 0.2 pmol/mg **PKC$^-$ β_2AR**: Ctrl; 1.4 ± 0.2 pmol/mg vs PMA; 1.4 ± 0.2 pmol/mg). However, the

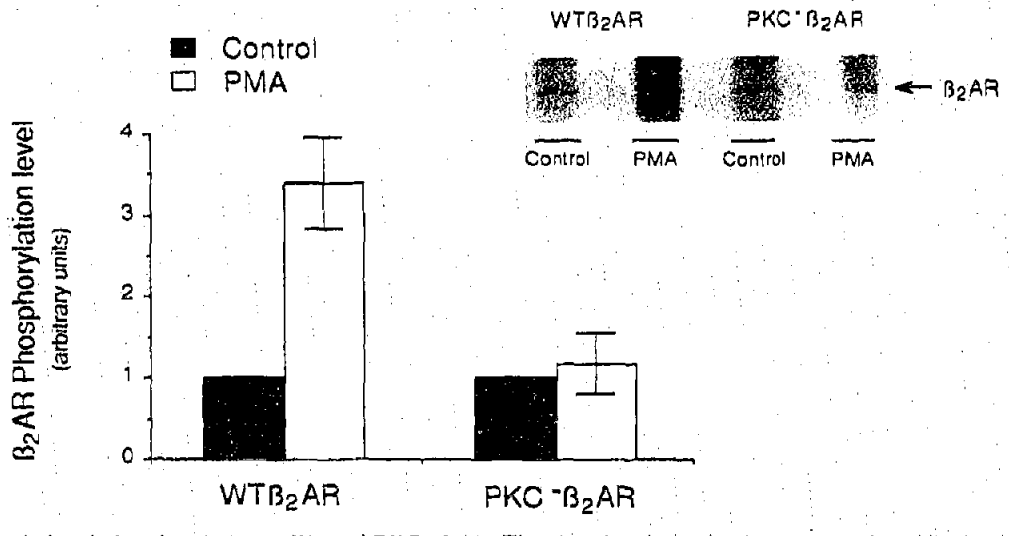


Fig. 1. Phorbol-ester-induced phosphorylation of WT and PKC$^-$ β_2AR. The phosphorylation levels were quantitated by densitometric analysis of SDS-PAGE autoradiographs. The level of PMA induced phosphorylation of β_2AR is expressed as a ratio of the basal level and represents the mean ± SEM of 4 separate experiments. (inset) A representative autoradiograph of a SDS-PAGE of the purified receptor is shown.

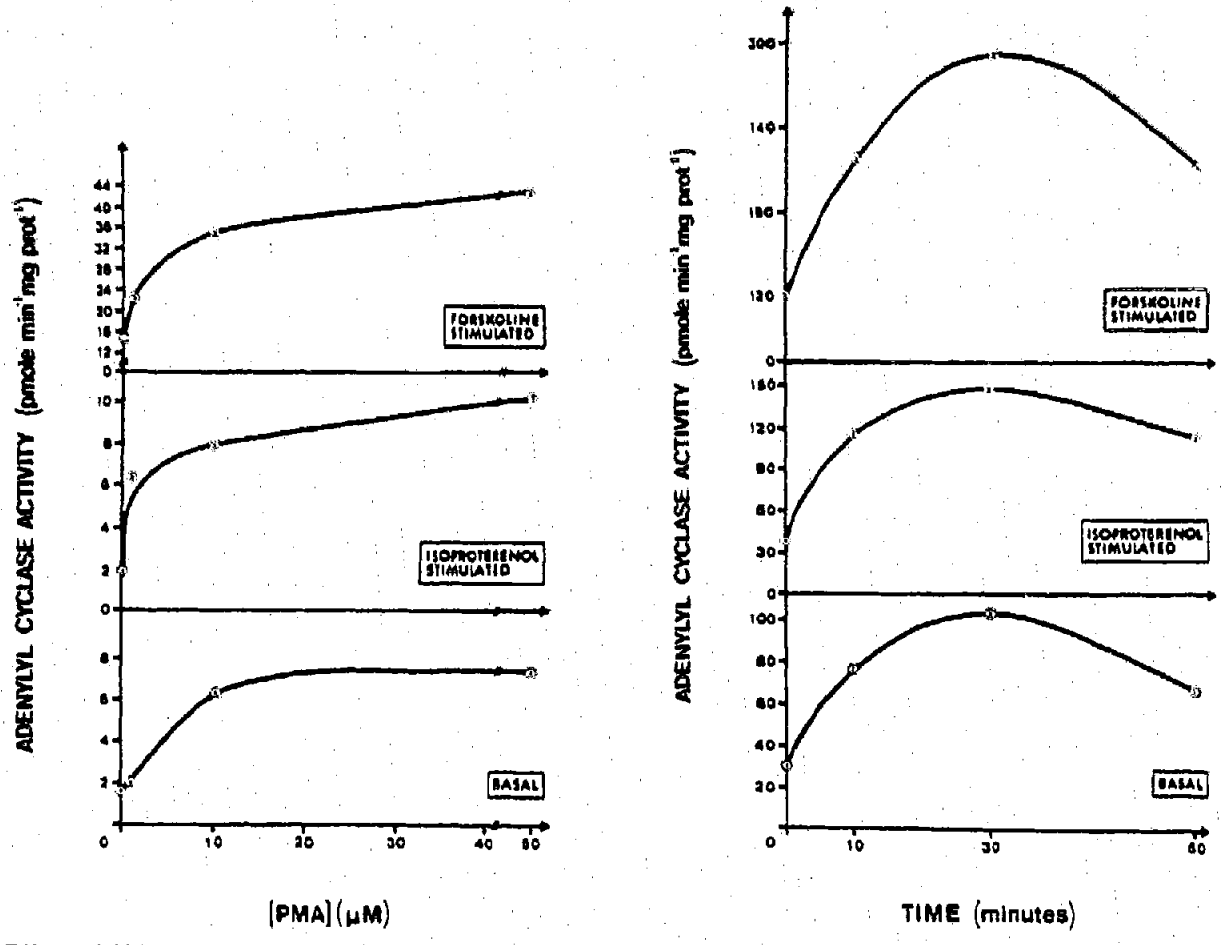


Fig. 2. Effects of PMA treatment on the basal, isoproterenol-stimulated and forskolin-stimulated adenylyl cyclase activity in cells expressing WTβ_2AR. Left panel: cells were incubated for 30 min with increasing concentrations of PMA. Right panel: cells were incubated with 10 μM PMA for various periods of time. The adenylyl cyclase activities were determined in membranes derived from these cells as described in section 2. The data shown are representative of 2 or 3 separate experiments.

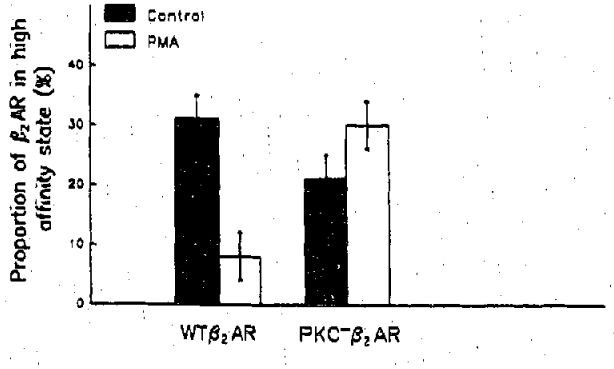


Fig. 3. Effects of PMA treatment on the proportion of β_2AR in the high affinity state for the agonist isoproterenol. The proportions were determined from displacement curves of [^{125}I]CYP binding by isoproterenol (10^{-10} M to 10^{-4} M) as described in section 2. The data are expressed as % of the total receptor number and represent the mean ± SEM of three separate experiments. The high and low affinities are as follows: WT: K_H: 1.5 ± 0.7 nM, K_L: 248 ± 39 nM; PKC⁻: K_H: 6.2 ± 1.5 nM, K_L 274 ± 10 nM.

agonist binding properties of WTβ_2AR were significantly affected by the tumor promoter. Isoproterenol competition of [^{125}I]CYP binding revealed that WTβ_2AR and PKC⁻β_2AR exhibit the characteristic two-affinity state binding for agonist (data not shown). Using the computer program LIGAND [30], 31 ± 4% and 21 ± 4% of the total receptor contingent in cells expressing WTβ_2AR and PKC⁻β_2AR respectively, are found to be in the guanine-nucleotide-sensitive high-affinity state for isoproterenol (Fig. 3). When WTβ_2AR expressing cells were treated with 10 μM PMA for 30 min the proportion of β_2AR in high affinity was dramatically reduced (Fig. 3). In contrast, the same treatment in cells expressing PKC⁻β_2AR did not reduce the proportion of receptor in the high affinity state. It is generally accepted that the proportion of receptor in the guanine-nucleotide-sensitive high-affinity state for its agonist represents the ability of the receptor to couple to G_s [34]. These results

therefore suggest that PKC-mediated phosphorylation of the β_2AR reduces its capacity to couple to G_s. Uncoupling of a significant proportion of the receptor, which is not observed with PKC⁻ β_2AR, could be responsible for the PMA-induced decrease in isoproterenol potency observed in WTβ_2AR expressing cells. Similarly, it has recently been shown that cAMP-promoted phosphorylation of the β_2AR also leads to a decrease in the potency with no change in the efficacy of adenylyl cyclase stimulation by β-adrenergic agonists [35-37].

The results presented here also suggest that the PMA-induced increase in the efficacy of adenylyl cyclase stimulation by isoproterenol is independent of the receptor phosphorylation. Most likely, this increase results from the phosphorylation of other components of the signalling pathway [16-18], which in turn leads to the increase in basal and forskolin-stimulated activities. In this respect, it is noteworthy that PMA induces very similar increases in basal and stimulated adenylyl cyclase activities (basal: 2.4-fold, isoproterenol-stimulated: 2.9-fold, forskolin-stimulated: 1.9-fold; Table I).

The hypothesis that PKC phosphorylation of the receptor decreases its coupling to G_s while the phosphorylation of distinct components of the adenylyl cyclase pathway increases the reactivity of the enzyme itself is supported by several observations. Patya et al. [37] reported that PMA treatment of murine thymocytes reduced the isoproterenol stimulated cAMP accumulation while potentiating the cAMP production induced by cholera toxin. More recently, Johnson et al. [39] reported that PMA treatment of 1321N1 human astrocytoma cells induced a desensitization of the β-adrenergic-stimulated adenylyl cyclase activity in a membrane preparation whereas a marked increase of the forskolin-stimulated adenylyl cyclase activity was observed in intact cells.

The results presented here suggest that PMA treatment modulates the β-adrenergic-stimulated adenylyl cyclase activity by affecting distinct components of the signalling pathway. The PMA treatment increases the efficacy of both forskolin and isoproterenol to stimulate the adenylyl cyclase, most likely via the PKC-mediated phosphorylation of the adenylyl cyclase and/or G_i [16-18]. In contrast, the phosphorylation of the β_2AR by PKC results in a reduced ability of the receptor to couple to G_s.

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REFERENCES

[1] Bouvier, M. (1990) Ann. NY Acad. Sci. 564, 120–129.
[2] Nabika, T., Nara, Y., Yamori, Y., Lovenberg, W. and Endo, J. (1985) Biochem. Biophys. Res. Commun. 131, 30–36.
[3] Vanecek, J., Sugden, D., Weller, J. and Klein, D.C. (1985) Endocrinology 116, 2167–2173.
[4] Sugden, D., Vanacek, J., Klein, D.C., Thomas, T.P. and Anderson, W.B. (1985) Nature 314, 359–361.
[5] Bell, J.D., Buxton, I.L.O. and Brunton, L.L. (1985) J. Biol. Chem. 260, 2625–2628.
[6] Sibley, D.R., Jeffs, R.A., Daniel, K., Nambi, P. and Lefkowitz, R.J. (1986) Arch. Biochem. Biophys. 244, 273–381.
[7] Summers, S.T. and Cronin, M.J. (1986) Biochem. Biophys. Res. Commun. 135, 276–281.
[8] Hollingsworth, E.B., Sears, E.B. and Daly, J.W. (1985) FEBS Lett. 184, 339–342.
[9] Cronin, M.J. and Canonico, P.L. (1985) Biochem. Biophys. Res. Commun. 128, 404–410.
[10] Garte, S. and Belman, S. (1980) Nature 284, 171–173.
[11] Mallorga, P., Tallman, J.F., Henneberry, R.C., Hirata, F., Strittmatter, W.T. and Axelrod, J. (1980) Proc. Natl. Acad. Sci. USA 77, 1341–1345.
[12] Brostrom, M.A., Brostrom, C.O., Brotman, L.A., Lee, C.S., Wolff, D.J. and Geller, H.M. (1982) J. Biol. Chem. 257, 6758–6765.
[13] Kassis, S., Zaremba, T., Patel, J. and Fishman, P.H. (1985) J. Biol. Chem. 260, 8911–8917.
[14] Sibley, D.R., Nambi, P., Peters, J.R. and Lefkowitz, R.J. (1984) Biochem. Biophys. Res. Commun. 121, 973–979.
[15] Kelleher, D.J., Pessin, J.E., Ruoho, A.E. and Johnson, G.L. (1984) Proc. Natl. Acad. Sci. USA 81, 4316–4320.
[16] Jakobs, K.H., Bauer, S. and Watanabe, Y. (1985) Eur. J. Biochem. 151, 425–430.
[17] Katada, T., Gilman, A.G., Watanabe, Y., Bauer, S. and Jacobs, K.H. (1985) Eur. J. Biochem. 151, 431–437.
[18] Yoshimasa, T., Sibley, D.R., Bouvier, M., Lefkowitz, R.J. and Caron, M.G. (1987) Nature 327, 67–70.
[19] Yoshimasa, T., Bouvier, M., Benovic, J.L., Amlaiky, N., Lefkowitz, R.J. and Caron, M.G. (1988) in: Molecular Biology of Brain and Endocrine Peptidergic System (K.W. McKern and M. Chretien, eds) pp. 123–139. Plenum, New York.
[20] Bouvier, M., Leeb-Lunberg, L.M.F., Benovic, J.L., Caron, M.B. and Lefkowitz, R.J. (1987) J. Biol. Chem. 262, 3106–3113.
[21] Kobilka, B.K., Dixon, R.A.F., Frielle, T., Dohlman, H.G., Bolanowski, M.A., Sigal, I.S., Yang-Feng, T.L., Trancke, U., Caron, M.G. and Lefkowitz, R.J. (1987) Proc. Natl. Acad. Sci. USA 84, 46–50.
[22] Dixon, R.A.F., Kobilka, B.K., Strader, D.J., Benovic, J.L., Dohlman, H.G., Frielle, T., Bolanowski, M.A., Bennett, C.D., Rands, E., Diehl, R.E., Mumford, R.A., Slater, E.E., Sigal, I.R., Caron, M.G., Lefkowitz, R.J. and Strader, C.D. (1986) Nature 321, 75–80.
[23] Kobilka, B.K., MacGregor, C., Daniel, K., Kobilka, T.S., Caron, M.G. and Lefkowitz, R.J. (1987) J. Biol. Chem. 262, 15796–15802.
[24] Cullen, B.R. (1987) Methods Enzymol. 152, 684–704.
[25] Southern, P.S. and Berg, P. (1982) J. Mol. Appl. Genet. 1, 327–341.
[26] Mellon, P.L., Parker, J., Gluyman, P. and Maniatis, T. (1981) Cell 27, 279–288.
[27] Bradford, M.M. (1976) Anal. Biochem. 72, 248–259.
[28] Salomon, P., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541–548.
[29] Bouvier, M., Hnatowich, M., Collins, S., Kobilka, B.K., Deblasi, A., Lefkowitz, R.J. and Caron, M.G. (1987) Mol. Pharmacol. 33, 133–139.
[30] Munson, P.J. and Rodbard, D. (1980) Anal. Biochem. 107, 220–239.
[31] Shorr, R.G.L., Lefkowitz, R.J. and Caron, M.G. (1981) J. Biol. Chem. 256, 5820–5826.
[32] Laemmli, U.K. (1970) Nature 227, 680–686.

[33] Kishimoto, A., Nishiyama, K., Nakanishi, H., Uratsuji, Y., Nomura, H., Takeyama, Y. and Nishizuka, Y. (1985) J. Biol. Chem. 260, 12492–12499.
[34] DeLean, A., Stadel, J.M. and Lefkowitz, R.J. (1980) J. Biol. Chem. 255, 7108–7117.
[35] Clark, R.B., Kunkel, M.W., Friedman, J., Goka, T.S. and Johnson, J.A. (1988) Proc. Natl. Acad. Sci. USA 85, 1442–1446.
[36] Hausdorff, W.P., Bouvier, M., O'Dowd, B.F., Irons, G.P., Caron, M.G. and Lefkowitz, R.J. (1989) J. Biol. Chem. 264, 12657–12665.
[37] Clark, R.B., Friedman, J., Dixon, R.A.F. and Strader, C.D. (1989) Mol. Pharmacol. 36, 343–348.
[38] Palya, M., Stenzel, K.H. and Novogrodsky, A. (1986) Biochem. Biophys. Res. Commun. 133, 904–910.
[39] Johnson, R.A. and Toews, M.L. (1990) Mol. Pharmacol. 37, 296–303.