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# PHORBOL 12-MYRISTATE 13-ACETATE INDUCES BETA-ADRENERGIC RECEPTOR UNCOUPLING AND NON-SPECIFIC DESENSITIZATION OF ADENYLATE CYCLASE IN HUMAN MONONUCLEAR LEUKOCYTES*

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**Abstract**—Tumour-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA) have been reported to modulate beta-adrenergic receptor responses in various cell types, presumably by the activation of protein kinase C. In the present investigation we assessed the effect of PMA on the beta-adrenergic receptor–adenylate cyclase system of human mononuclear leukocytes (MNL). It was found that incubation of MNL with PMA resulted in a time- and concentration-dependent desensitization of isoproterenol-induced adenylate cyclase activity. However, the effect of PMA was not restricted to the beta-adrenergic receptor system, since basal adenylate cyclase activity and histamine-, prostaglandin $E_1$-, 5′-guanylylimidodiphosphate (GppNHp)-, and NaF-stimulated values were also reduced. By contrast, no effect was found on the forskolin-induced adenylate cyclase activity. The inactive phorbol ester, 4α-phorbol 12,13-didecanoate had no effect on adenylate cyclase at all, suggesting that the observed PMA effect was specifically mediated by activation of protein kinase C. The reduced beta-adrenergic response induced by PMA was not associated with a reduced beta-adrenergic receptor number, indicating uncoupling of this receptor from adenylate cyclase. Isoproterenol competition curves for $^3$H-dihydroalprenolol binding to membranes from untreated and PMA-treated cells demonstrated that the uncoupling was due to a reduced ability of the agonist to promote formation of the guanine nucleotide-sensitive high affinity state of the receptor. The results indicate that PMA may cause desensitization of catecholamine-responsive adenylate cyclase in MNL, and that the major locus of alteration is the guanine nucleotide regulatory protein.

Phorbol esters, such as phorbol 12-myristate 13-acetate (PMA), are well known tumour-promoting agents that may induce a great variety of effects in different cell types, including activation of human mononuclear leukocytes (MNL) [1–4]. These actions of phorbol esters appear to be mediated by specific binding to and stimulation of the $Ca^{2+}$- and phospholipid-dependent protein kinase C [5, 6]. Due to structural similarities, phorbol esters may substitute for diacylglycerol which is produced from hormone-induced turnover of polyphosphoinositides [7, 8]. Phorbol esters have also been shown to influence various plasma membrane-associated receptor systems, including those for epidermal growth factors [9], insulin [10], transferrin [11], hCG [12], and alpha- and beta-adrenergic agonists [13–21].

Depending on the cell type studied, different effects of phorbol esters on beta-agonist-induced adenylate cyclase activity have been found in animal cells [14–21]. Thus, phorbol ester caused augmentation of the beta-adrenergic response in mouse S49 lymphoma cells, presumably by an increased interaction between the stimulatory guanine nucleotide regulatory protein ($N_s$) and the catalytic unit of adenylate cyclase (C) [14]. By contrast, phorbol esters had no effect on the beta-adrenergic response in rat hepatocytes [15] and caused beta-adrenergic desensitization in mouse epidermis cells [16, 17], rat $C_6$ glioma cells [18], and avian erythrocytes [18–21]. In mouse epidermis [16, 17] and in turkey erythrocytes [19], the phorbol ester-induced desensitization was associated with uncoupling of the beta-adrenergic receptor from adenylate cyclase with no change in receptor number. In the avian erythrocytes it was further demonstrated that the reduced beta-adrenergic responsiveness and beta-receptor uncoupling were correlated with hyperphosphorylation of the receptor, which could directly or indirectly result from activation of protein kinase C [19–21].

In man, MNL have frequently been used as an easily obtainable model to study mechanisms of beta-adrenergic receptor regulation, both in health [22–24] and in disease [25, 26]. The aim of the present study was to extend these investigations by assessing the effect of PMA on the beta-receptor–adenylate cyclase system of normal human MNL *in vitro*.

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## MATERIALS AND METHODS

*Mononuclear leukocyte preparation.* MNL were isolated from heparinized blood from healthy donors by density gradient centrifugation on Ficoll-Paque as described by Bøyum [27]. The cells at the interphase were washed three times in cold 25 mM Tris–HCl, pH 7.4, containing 120 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 0.6 mM $CaCl_2$, 5 mM glucose, and 0.1% human serum albumin (Tris buffer). One centrifugation step was performed at 160 *g* for 10 min in order to reduce platelet contamination. The mononuclear cell fraction contained approximately 90% lymphocytes, 10% monocytes, and <1% polymorphonuclear leukocytes with a viability >95% as determined by trypan blue exclusion.

*Incubation conditions.* MNL were suspended in 25 mM HEPES-buffered RPMI-1640 medium, supplemented with 10% foetal calf serum, 125 μg/ml streptomycin, and 125 IU/ml sodium benzylpenicillin, at a density of 0.6–1 × $10^7$ cells/ml. The cells (3–5 × $10^7$) were incubated at 37° in the presence of various concentrations of phorbol ester during different periods of time as indicated in the results. Phorbol esters were dissolved in dimethylsulphoxide (DMSO). The final DMSO concentration during the incubation was 0.00125%. Incubations with 0.00125% DMSO in HEPES-buffered RPMI-1640 medium served as controls.

*Preparation of membranes.* After the incubation, the cells were centrifuged and washed twice in 5 ml Tris buffer. For determination of adenylate cyclase activity and beta-adrenergic receptor density, crude membranes were prepared according to Williams *et al.* [22].

The cells were swollen in 5 ml 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 8.1 for 20 min at 0° and homogenized in an ice-cooled Potter-Elvehjem glass homogenizer with a motor-driven Teflon pestle (25 strokes). The homogenate was centrifuged at 36,000 *g* for 15 min at 4°, and the pellet was washed with 10 ml of 50 mM Tris–HCl and 10 mM $MgCl_2$, pH 7.4. The final pellet was resuspended in the same buffer, resulting in a suspension containing 1–2 mg protein/ml.

For competition binding studies, membranes were prepared according to a slight modification of the method of Feldman *et al.* [24]. The cells were suspended in 5 ml ice-cold distilled water and homogenized twice for 15 sec using a Polytrom homogenizer (Kinematica, Kriens-Luzern, Switzerland; setting 5). After homogenization, 5 ml 100 mM Tris–HCl, 20 mM $MgCl_2$, pH 7.4 was added and the homogenate was centrifuged at 36,000 *g* for 15 min at 4°. The pellet was resuspended in 10 ml 50 mM Tris–HCl, 12.5 mM $MgCl_2$, 1.5 mM EDTA, homogenized by Polytron action for 5 sec, and centrifuged at 36,000 *g* for 15 min. The final pellet was suspended in the same buffer by manual use of a Potter Elvehjem glass–Teflon homogenizer (20 strokes), to a protein concentration of 0.5–1 mg/ml. Protein was determined according to Lowry *et al.* [28], using bovine serum albumin as a standard. All membrane preparations were immediately used for assay.

*Adenylate cyclase assay.* Adenylate cyclase activity was determined as described previously [25]. In short, 20 μl of membrane suspension was added to a final incubation volume of 100 μl, containing 50 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, 20 mM creatine phosphate, 0.5 mg/ml creatine phosphokinase, 0.5 mM 1-methyl-3-isobutylxanthine, 1 mM ascorbic acid, and various stimuli at maximally responsive concentrations as indicated in the results. For agonist stimulations, 100 μM GTP was included in the incubation buffer. Incubations were carried out at 30° for 10 min. The reaction was stopped by addition of 400 μl 50 mM Tris–HCl, 4 mM EDTA, pH 7.5 to the samples and heating the tubes for 10 min in a boiling water bath. Precipitated protein was centrifuged and cAMP was determined by a protein-binding assay as described before [29]. cAMP production was linear for at least 15 min and proportional to the amount of protein.

*Determination of beta-adrenergic binding sites.* Beta-adrenergic binding sites were determined as described previously [30]. Aliquots of 100 μl membrane suspension were incubated in 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.4 with various concentrations of $^3$H-DHA (0.25–8 nM) in the absence or presence of 1 μM l-propranolol, for 15 min at 37° (final volume of 150 μl). Incubations were terminated by rapidly diluting the samples with 5 ml of ice-cold 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.4, immediately followed by vacuum filtration through Whatman GF/C glass fiber filters. Subsequently, the filters were rapidly washed with 10 ml of the same buffer. After washing, the filters were counted in 10 ml Plasmasol (Packard, U.S.A.), in a liquid scintillation spectrometer. All results were corrected for counting efficiency (about 45%). Specific receptor binding was defined as the difference between total $^3$H-DHA binding, as determined in the absence of l-propranolol, and non-specific $^3$H-DHA binding, as determined in the presence of 1 μM l-propranolol. Beta-receptor number and $K_d$ for $^3$H-DHA were derived from the saturation data of specific $^3$H-DHA binding as analyzed by the method of Scatchard [31].

*Competition binding studies.* Isoproterenol competition curves for $^3$H-DHA binding were constructed to assess the affinity of the beta-receptor for the agonist. Samples of 100 μl membrane suspension were incubated for 15 min at 37° in 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$, 1 mM ascorbic acid, approximately 2.5 nM $^3$H-DHA, and various concentrations of isoproterenol as indicated in the results (final volume 150 μl). Maximal $^3$H-DHA binding was obtained in the presence of buffer instead of isoproterenol. The effect of guanine nucleotides on the agonist binding was determined in the presence of 100 μM 5′-guanylylimidodiphosphate (GppNHp), the hydrolysis-resistant analogue of GTP.

The experimental binding data were analyzed for high- and low-affinity agonist binding states of the receptor by using the non-linear least-squares curve fitting program LIGAND [32] on a Zenith Z-100 microcomputer. This iterative fitting program was run interactively and provided the best estimates (with their standard error) for the affinity constants of the agonist and the concentrations of the corresponding receptor states, corrected for non-specific binding and real ligand concentrations. The data

were repeatedly fitted for a one-, two- or three-affinity state model. For each model the best fit was chosen on the basis of the lowest value of mean squares of residuals. A multiple state model was accepted only if it statistically significantly improved the fit over the simpler model(s) by an F-ratio test. According to the current concept of agonist binding to beta-adrenergic receptors [33], only a one- or a two-affinity state model was revealed.

*Statistics.* Statistical analysis of the data obtained from PMA-treated and non-treated cells was performed by the Student's *t*-test for paired observations.

*Materials.* Ficoll-Paque was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Foetal calf serum (heat-inactivated) and 25 mM HEPES-buffered RPMI-1640, supplemented with l-glutamine were from GIBCO Europe, Hoofddorp, The Netherlands. Forskolin was obtained from Calbiochem, La Jolla, CA. Phorbol 12-myristate 13-acetate, 4α-phorbol 12,13-didecanoate, l-isoproterenol, histamine, prostaglandin $E_1$, ATP, GTP, GppNHp, creatine phosphokinase, creatine phosphate, dithiothreitol and 1-methyl-3-isobutylxanthine were purchased from Sigma Chemical Co., St. Louis, MO. $(-)^3$H-dihydroalprenolol ($^3$H-DHA; 105 Ci/mmol) was obtained from New England Nuclear, Boston, MA. Reagents for cAMP determinations were from the Radiochemical Center, Amersham, U.K. l-Propranolol was a kind gift from ICI, Macclesfield, U.K.

## RESULTS

Figure 1 shows that MNL exposed to 1 μM PMA at 37° developed a rapid desensitization of isoproterenol-stimulated adenylate cyclase activity with an essentially maximal effect within 30 min and an apparent $t_{\frac{1}{2}}$ of 2–3 min. A similar pattern was observed for basal and GppNHp-stimulated adenylate cyclase activity, indicating a mechanism of non-specific desensitization. The dose-dependence of the PMA-induced desensitization after 30 min of incubation is shown in Fig. 2. Maximal desensitization of all activities was observed at 0.1–1 μM PMA with a half-maximal effect at 1–2 nM. In contrast to PMA, incubation of the cells with 4α-phorbol 12,13-didecanoate (4α-PDD), which is devoid of tumour-promoting activity and which does not activate protein kinase C, failed to promote desensitization in these cells (Fig. 2). PMA (0.1 nM–1 μM) added directly to control membranes had no effect on adenylate cyclase activity (not shown).

Table 1 summarizes the maximal effects of PMA (1 μM, 30 min incubation at 37°) on various adenylate cyclase responses and beta-adrenergic receptor characteristics. In accordance with the results presented in Figs 1 and 2, the desensitization of adenylate cyclase was not restricted to the catecholamine-induced activity, but also implied significantly reduced activities in response to other agonists (histamine, prostaglandin $E_1$), guanine nucleotides (GTP, GppNHp), and NaF. All these activities and basal activity were similarly reduced by approximately 50%, which strongly suggests that PMA exerts its effect mainly at site distal to the hormone receptors. However, the adenylate cyclase response

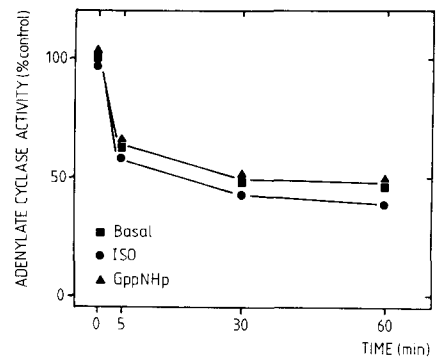


Fig. 1. Time course of PMA-induced desensitization of adenylate cyclase. MNL were incubated at 37° in HEPES-buffered RPMI-1640 medium in the absence (control cells) or in the presence of 1 μM PMA for the times indicated. After incubation, the cells were rapidly chilled by the addition of an excess of ice-cold medium and centrifuged immediately. The cells were washed twice, crude membranes were prepared, and adenylate cyclase activities were determined in the presence of buffer (■; basal), 100 μM isoproterenol + 100 μM GTP (●), or 100 μM GppNHp (▲) as described under Methods. Adenylate cyclase activities in membranes of the PMA-treated cells are expressed as percentage of the control activities found in membranes of the non-treated cells. These control values were 134.6 ± 2.0, 195 ± 17.9 and 238.5 ± 15.9 pmol cAMP/mg protein/min for basal, isoproterenol-stimulated, and GppNHp-stimulated adenylate cyclase activity, respectively. The results are derived from duplicate determinations in each membrane preparation and are representative of two separate experiments.

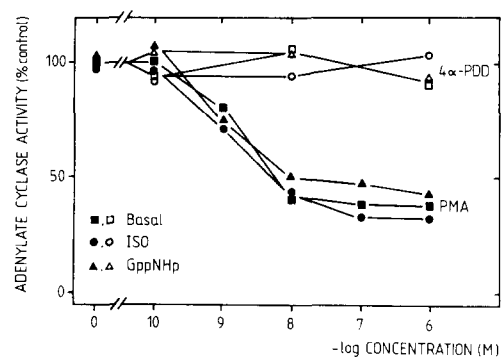


Fig. 2. Dose dependence of PMA-induced desensitization of adenylate cyclase. MNL were incubated for 30 min at 37° in HEPES-buffered RPMI-1640 medium in the absence (control cells) or in the presence of various concentrations of PMA (closed symbols) or 4α-PDD (open symbols) as indicated. After incubation, the cells were washed twice, crude membranes were prepared, and adenylate cyclase activities were determined in the presence of buffer (■, □; basal), 100 μM isoproterenol + 100 μM GTP (●, ○), or 100 μM GppNHp (▲, △) as described under Materials and Methods. The data shown are expressed as percentage of the control activities found in the membranes of the non-treated cells. The control values were 97.2 ± 11.2, 185.2 ± 12.5, and 182.6 ± 23.0 pmol cAMP/mg protein/min for basal, isoproterenol-stimulated, and GppNHp-stimulated adenylate cyclase activity, respectively. The results are derived from duplicate determinations in each membrane preparation and are representative of two separate experiments.

Table 1. Adenylate cyclase activities and beta-adrenergic receptor characteristics in membranes of human mononuclear leukocytes after 30 min incubation of the cells with buffer (Controls) or 1 μM PMA

| | Incubation | | | |
|---|---|---|---|---|
| | Controls | PMA | | N |
| Adenylate cyclase (pmol/mg/min) | | | | |
| Basal | 105.9 ± 9.1* | 48.9 ± 7.6‖ | (46.2)† | 13 |
| GTP (100 μM) | 120.0 ± 10.0 | 56.2 ± 8.4‖ | (46.8) | 13 |
| ISO (100 μM)/GTP (100 μM) | 165.4 ± 13.3 | 75.2 ± 9.8‖ | (45.5) | 13 |
| HIS (100 μM)/GTP (100 μM) | 143.7 ± 35.2 | 84.3 ± 24.2‡ | (58.7) | 4 |
| $PGE_1$ (10 μM)/GTP (100 μM) | 180.8 ± 34.0 | 112.1 ± 22.7‡ | (62.0) | 4 |
| GppNHp (100 μM) | 168.8 ± 15.0 | 87.9 ± 9.9‖ | (52.1) | 13 |
| NaF (10 mM) | 161.1 ± 20.9 | 86.5 ± 12.0§ | (53.0) | 6 |
| Forskolin (100 μM) | 178.5 ± 21.2 | 175.1 ± 19.3 | (98.1) | 3 |
| Beta-adrenergic receptors | | | | |
| number/cell | 866 ± 92 | 744 ± 56 | (89.4) | 3 |
| $K_d$ $^3$H-DHA (nM) | 0.6 ± 0.1 | 0.8 ± 0.1 | (133.3) | 3 |

* Mean ± S.E.M.
† Values in parentheses represent the percentage of adenylate cyclase activity in PMA-treated preparations relative to control membranes.
‡ $P < 0.05$.
§ $P < 0.005$.
‖ $P < 0.001$ vs buffer incubation.

to forskolin was unchanged after incubation of the cells with the phorbol ester (Table 1).

PMA had no significant effect on the beta-adrenergic receptor number and on the affinity of this receptor for $^3$H-DHA (Table 1). Since the isoproterenol-induced adenylate cyclase activity was significantly reduced by about 55% ($P < 0.001$), this observation implied uncoupling of the beta-adrenergic receptor from adenylate cyclase. Figure 3 demonstrates that the uncoupling was associated with a reduced affinity of the receptor for the agonist, since the isoproterenol competition curve for $^3$H-DHA binding to membranes of PMA-treated cells was shifted to the right of that obtained in the control membranes. Computer analysis of the shallow competition curves from the control membranes revealed a two-affinity state model for the agonist–receptor interaction, with 42 ± 5% of the receptors having a high-affinity dissociation constant ($R_H$; $K_H$ = 20 ± 6 nM) and 58 ± 5% of the receptors having a low-affinity dissociation constant ($R_L$; $K_L$ = 632 ± 148 nM; see Table 2). Competition curves obtained from membranes of PMA-treated cells were steeper and showed a significant shift from high- to low-affinity states ($\%R_H = 12 \pm 12\%$; $P < 0.05$). In the presence of 100 μM GppNHp, the competition curves from both membrane preparations were essentially indistinguishable and best described by a one-affinity state model with a low-affinity dissociation constant similar to the $K_L$-values found in the absence of this guanine nucleotide analogue, indicating a complete shift from high- to low-affinity sites (Fig. 3, Table 2).

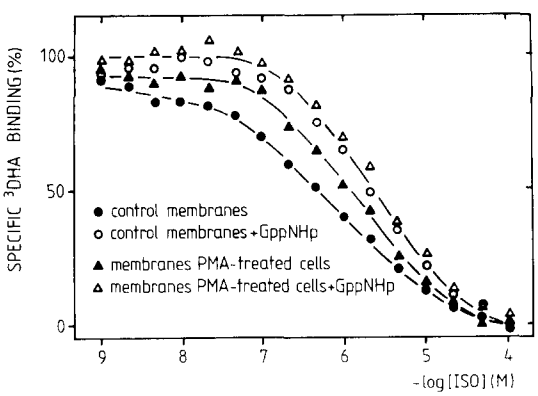


Fig. 3. Isoproterenol competition curves for $^3$H-DHA binding to membranes of MNL incubated with buffer (30 min, 37°; circles) and MNL incubated with 1 μM PMA (30 min, 37°; triangles). The membranes were incubated for 15 min at 37° with 2.9 ± 0.2 nM $^3$H-DHA in the presence of the indicated concentrations of isoproterenol, with (open symbols) or without (closed symbols) 100 μM GppNHp. $^3$H-DHA binding is expressed as the percentage of specific binding, defined as total $^3$H-DHA bound in the absence of competing isoproterenol minus $^3$H-DHA bound in the presence of 100 μM isoproterenol. Hundred percent binding was 44.6 ± 1.4 fmol/mg protein for control membranes and 41.6 ± 4.5 fmol/mg protein for membranes of PMA-treated cells. The results shown represent the mean of single (in the presence of GppNHp) or duplicate (in the absence of GppNHp) determinations from three (in the presence of GppNHp) or four (in the absence of GppNHp) separate experiments with a standard error of generally less than 10%.

## DISCUSSION

The present findings indicate that the tumour-promoting phorbol ester PMA may induce a time- and dose-dependent desensitization of isoproterenol-stimulated adenylate cyclase activity in human MNL, to a maximal degree of about 55%. In contrast to beta-agonist-induced desensitization of MNL [23], PMA-induced beta-adrenergic subsensitivity was not associated with down-regulation of the beta-adre-

Table 2. Binding parameters for isoproterenol competition curves in membranes of control cells (Controls) and PMA-treated cells (PMA), determined in the absence and in the presence of 100 $\mu$M GppNHp

| | Controls (N = 4) | PMA (N = 4) | Controls + GppNHp (N = 3) | PMA + GppNHp (N = 3) |
|---|---|---|---|---|
| $R_H$ (%) | 42 ± 5* | 12 ± 12‡ | 0 | 0 |
| $R_L$ (%) | 58 ± 5 | 88 ± 12‡ | 100 | 100 |
| $K_H$ (nM) | 20 ± 6 | 67 ± 32† | — | — |
| $K_L$ (nM) | 632 ± 148 | 564 ± 217 | 445 ± 30 | 546 ± 47 |

* Results are the mean ± S.E.M.

† Value (±SD) obtained from one experiment; no high affinity binding was observed in three other experiments.

‡ $P < 0.05$ vs controls.

nergic receptor, indicating that the receptor was uncoupled from adenylate cyclase. Similar findings have also been reported for mouse epidermis cells [16, 17] and avian erythrocytes [19, 20]. In the latter cell type it was further established that PMA-induced desensitization was associated with increased phosphorylation of the beta-receptor, which could represent an important mechanism for the observed uncoupling and consequently for the reduced hormonal stimulation of adenylate cyclase. Although our results do not exclude such a phosphorylation reaction, it appears that other imortant mechanisms must be involved in the PMA-induced desensitization of MNL adenylate cyclase. This assumption is based on the observation that in addition to the beta-adrenergic response, adenylate cyclase responses to other agonists such as histamine and $PGE_1$, and to activators of $N_s$ such as GppNHp and NaF, were also reduced by approximately the same degree. This non-specific desensitization suggests that alterations distal to the receptor must play a prevalent role in the action of PMA. By contrast, PMA-induced desensitization in the avian erythrocytes was largely specific for the beta-adrenergic response, although there was a small but significant change in fluoride-stimulated adenylate cyclase activity in these cells [20]. The cause of this apparent difference is not clear. However, it has been established that agonist-induced non-specific or heterologous desensitization can be associated with modifications in the adenylate cyclase system at both the level of the receptors and the level of $N_s$-protein, which contributions may differ in extent in different cell types [34]. This could possibly also apply to PMA-induced desensitization. Thus, our results are more in line with the recent observation of Mukhopadyay and Schumacher [12], who found that PMA-induced non-specific desensitization of hCG-responsive adenylate cyclase in purified mouse Leydig cells is mainly determined by a change in the $N_s$-protein. Several indications suggest that $N_s$ is also the main site of PMA-induced alteration in human MNL. First, the reduced adenylate cyclase responses to GppNHp and NaF indicated a reduced activity of $N_s$-stimulated C. This reduced stimulation appeared not to be due to a change at the catalytic site since the adenylate cyclase response to forskolin was unaltered. Particularly in membrane preparations, forskolin is thought to act mainly via direct stimulation of C [35, 36]. Secondly, isoproterenol competition curves indicated a reduced ability of the agonist to promote the guanine nucleotide-sensitive high affinity state of the beta-adrenergic receptor. This high affinity state has been identified as a ternary complex of agonist, receptor, and $N_s$ [37], which is an essential intermediate in adenylate cyclase activation [38]. Thus, a reduced ability to form this complex indicates a reduced coupling between the receptor and $N_s$. Although other explanations, such as receptor phosphorylation, could be advanced to account for this reduced coupling, our data are most consistent with the hypothesis that a change in $N_s$ contributes to both the impaired receptor–$N_s$ and the impaired $N_s$–C interaction.

The mechanism of the PMA-induced change in the adenylate cyclase complex of MNL is still unclear; however, it appears to differ from the specific, homologous desensitization induced by agonists such as isoproterenol and histamine (manuscript in preparation). Several lines of evidence indicate that the observed effects of PMA are probably mediated by an action of the phorbol ester on protein kinase C. First, treatment of the intact cells with the inactive phorbol ester, 4$\alpha$-PDD, did not have any effect on MNL adenylate cyclase activation. Secondly, the $EC_{50}$-value for PMA-induced desensitization (1–2 nM) was in the range of the $K_d$- and $K_{act}$-values of active phorbol esters for binding to and activation of protein kinase C, respectively [3, 6, 8]. Finally, PMA had no effect on adenylate cyclase activity when added directly to the membrane preparation of untreated cells (data not shown), indicating that PMA was not in some way interacting with (one of) the components of the adenylate cyclase system, either specifically or non-specifically.

The physiological significance of the PMA-induced adenylate cyclase desensitization remains to be established. However, it is tempting to speculate that the phorbol ester effect represents a mechanism of adenylate cyclase regulation which is involved in the activation of MNL, and which is physiologically mediated by hormone-induced diacylglycerol production and activation of protein kinase C.

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