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Protein kinase C-mediated phosphorylation and desensitization of human α_{1b}-adrenoceptors

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Abstract

Human α_{1b}-adrenoceptors stably expressed ($B_{max} \sim 800$ fmol/mg membrane protein) in mouse fibroblasts were able to increase intracellular Ca^{2+} and inositol phosphate production in response to noradrenaline. Activation of protein kinase C desensitized the α_{1b}-adrenergic-mediated actions but did not block the ability of the cells to respond to lysophosphatidic acid. Inhibition or downregulation of protein kinase C also blocked the action of the tumor promoter on the adrenergic effects. Photolabeling experiments indicated that the receptor has an apparent molecular weight of ~ 80 kDa. The receptors were phosphorylated in the basal state and such phosphorylation was increased when the cells were incubated with phorbol myristate acetate or noradrenaline. Incubation of the cells with phorbol myristate acetate or noradrenaline blocked noradrenaline-promoted [^{35}S]GTP-γ-S binding to membranes, suggesting receptor-G protein uncoupling. The results indicate that activation of protein kinase C blocked/desensitized human α_{1b}-adrenoceptors and that such effect was associated to receptor phosphorylation.

Keywords: α_1-Adrenoceptor; Phosphorylation; Desensitization

1. Introduction

α_1-Adrenoceptors are a heterogeneous subgroup of receptors, members of the G protein-coupled family of receptors. Three α_1-adrenoceptor isoforms have been cloned and expressed, i.e., the α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors (uppercase is used when referring in general to a subtype or to receptors pharmacologically defined whereas lowercase is used when referring to cloned receptors) (Hieble et al., 1995). The hamster α_{1b} subtype was the first to be cloned (Cotecchia et al., 1988), is the best characterized and, therefore, it is considered prototypic of this subgroup of receptors. These receptors are structurally similar, containing seven putative transmembrane domains; they are mainly coupled to the phosphoinositide/Ca^{2+} mobilization signal transduction pathway, although other signaling processes can also be activated by them (Minneman and Esbenshade, 1994; Graham et al., 1996).

Attenuation of the receptor function (desensitization) is a characteristic of these serpentine receptors and can be elicited by activation of the same receptor (homologous desensitization) or of unrelated receptors (heterologous desensitization/transmodulation). Different cellular processes seem to be involved in such desensitizations (uncoupling from G proteins, internalization, degradation, regulation of receptor gene expression, etc.) with different time frames; a very initial event seems to be receptor phosphorylation (Premont et al., 1995; Ferguson et al., 1997). Three groups of protein kinases seem to be the major modulators of G protein-coupled receptor function. These groups are: (a) second messenger-activated kinases, such as protein kinase A and protein kinase C (Clark et al., 1988; García-Sáinz, 1991; Houslay, 1991), (b) members of the G protein receptor kinase family (Benovic et al., 1990; Premont et al., 1995) and (c) some receptors with tyrosine kinase activity (Hadcock et al., 1992; Karoor and Malbon, 1996; Karoor et al., 1995).

Using rat hepatocytes, it was first showed that activation of protein kinase C blocks α_{1B}-adrenergic-mediated action (Corvera and García-Sáinz, 1984; Corvera et al., 1986). Leeb-Lundberg et al. (1985) subsequently showed that α_{1B}-adrenoceptor phosphorylation underlies this blockade using DDT_1 MF2 cells. During the last 5 years, considerable progress has been gained in our understand-

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ing of the phosphorylation of this receptor. This includes the identification of the phosphorylation sites at the receptor carboxyl terminus (Lattion et al., 1994; Diviani et al., 1997); the effect of different G protein-coupled kinases (Diviani et al., 1996) and the cross-regulation/phosphorylation induced by activation of different receptors, such as the endothelin ET_A or the bradykinin B2 receptors (Vázquez-Prado et al., 1997; Medina et al., 1998). Interestingly, all the in vivo phosphorylation studies have been performed using the hamster α_{1b}-adrenoceptor, endogenously expressed in DDT_1 MF-2 cells or transfected into model cells. The human α_1-adrenoceptors have been cloned, expressed and characterized (Forray et al., 1994; Weinberg et al., 1994; Schwinn et al., 1995) but to the best of our knowledge, no data on desensitization/phosphorylation have been reported. The recent commercial availability of cell lines expressing these receptors prompted us to study the effect of protein kinase C activation on the function and phosphorylation of the human α_{1b}-adrenoceptor.

2. Materials and methods

2.1. Materials

(−)-Noradrenaline, phorbol 12-myristate 13-acetate (PMA), prazosin, guanylyl-imido-diphosphate, GDP, lysophosphatidic acid and protease inhibitors were obtained from Sigma. Phentolamine was a generous gift from Ciba-Geigy. 5-Methyl urapidil and 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione-dihydrochloride (BMY 7378) were from Research Biochemicals International. 3-[1-[3-(Amidinothio) propyl-1*H*-indol-3-yl]-3-(1-methyl-1*H*-indol-3-yl) maleimide methane sulfonate (Ro-31-8220) was from Calbiochem. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, trypsin, antibiotics, and other reagents used for cell culture were from Gibco BRL. [^{125}I-aryl]-azidoprazosin (2200 Ci/mmol), [^{3}H]prazosin (77.9 Ci/mmol), [^{35}S]GTP-γ-S (1250 Ci/mmol), [2,3-^{3}H]myo-inositol (22.9 Ci/mmol) and [^{32}P]Pi (8500–9120 Ci/mmol) were from New England Nuclear. Sepharose-coupled protein A was from Upstate Biotechnology. Indo-1/acetoxymethyl ester was from Molecular Probes. The carboxyl-terminal decapeptide of the α_{1b}-adrenoceptor was obtained from Multiple Peptide Systems.

2.2. Cell line and culture

Mouse fibroblasts stably transfected with the human α_{1b}-adrenoceptor were obtained form the American Type Culture Collection (ATCC number CRL-11139) and cultured in glutamine-containing high-glucose DMEM supplemented with 10% fetal bovine serum, 300 μg/ml of the neomycin analog, G-418 sulfate, 100 μg/ml streptomycin, 100 U/ml penicillin and 0.25 μg/ml amphotericin B at 37°C under a 95% air/5% CO_2 atmosphere.

2.3. Membrane preparation and radioligand binding

Overnight serum-deprived confluent cultures were washed and scraped with a rubber policeman in buffer containing: 20 mM HEPES, pH 7.5, 5 mM EDTA, 100 μM Na_3VO_4, 10 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 2 mM $MgCl_2$, and protease inhibitors (leupeptin 20 μg/ml, aprotinin 20 μg/ml, phenylmethylsulfonyl fluoride 100 μg/ml, bacitracin 500 μg/ml and soybean trypsin inhibitor 50 μg/ml). Membranes were prepared according to Mattingly et al. (1992) and resuspended in binding buffer (50 mM Tris, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.5). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. [^{3}H]Prazosin binding studies were performed by incubating the radioligand (0.05–5 nM in saturation experiments and 1 nM in binding competition studies) with the membranes (20 μg of protein) in a final volume of 0.25 ml of binding buffer for 60 min at 25°C in a water bath shaker. Incubation was terminated by addition of 5 ml of ice-cold buffer and filtration through GF/C filters using a Brandel harvester. Filters were washed twice, dried and radioactivity was measured in a liquid

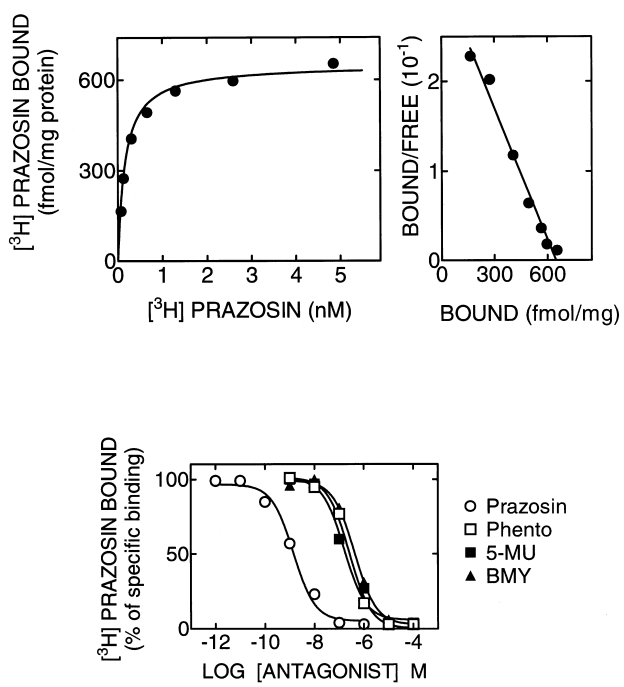

Fig. 1. [^{3}H]Prazosin binding to fibroblast membranes. Upper panels: saturation isotherm and Rosenthal transformation of the data. Lower panel: binding competition experiment using prazosin, phentolamine (Phento), 5-methyl urapidil (5-MU) and BMY 7378 (BMY). The data are representative of three to four experiments using different membrane preparations.

scintillation counter. Nonspecific binding was determined in the presence of 10 μM phentolamine; specific binding represented >90% of total binding at the K_D. Curves were analyzed by the EBDA program (Biosoft-Elsevier). K_i values were calculated according to Cheng and Prusoff (1973).

2.4. Northern analysis

Total RNA (40 μg), obtained by the method of Chomczynski and Sacchi (1987), was electrophoresed in 1.5% agarose gels containing 0.6 M formaldehyde and transferred to Hybond-N membranes (Amersham) according to standard procedures. Hybridization was done under high stringency conditions using nick translated human α_1-adrenoceptor cDNA probes (Weinberg et al., 1994).

2.5. Intracellular calcium ($[Ca^{2+}]_i$) determinations

Confluent fibroblasts were incubated overnight in DMEM without serum and antibiotics. Cells were loaded with 5 μM Indo-1/acetoxymethyl ester in Krebs–Ringer–HEPES containing 0.05% bovine serum albumin, pH 7.4 for 1 h at 37°C. Cells were detached by gentle trypsinization. Fluorescence measurements were carried as described (Vázquez-Prado et al., 1997) but the excitation monochromator was set at 340 nM with a chopper interval 0.5 s, and the emission monochromators were set at 410 and 490 nm. $[Ca^{2+}]_i$ was calculated using the software provided by AMINCO-Bowman; traces were directly exported to the graphs.

2.6. [^{3}H]inositol phosphate production

Cells approaching confluence were labeled with [^{3}H]inositol (5 μCi/ml) for 18–24 h in inositol-free DMEM containing 1% fetal bovine serum. On the day of the experiment, cells were washed twice with Krebs–Ringer–HEPES buffer containing 1.3 mM $CaCl_2$ and preincubated for 20 min in 2 ml of the same buffer containing 10 mM LiCl, at 37°C in a 5% CO_2 atmosphere. Incubations with noradrenaline and/or PMA were for 15 min and were

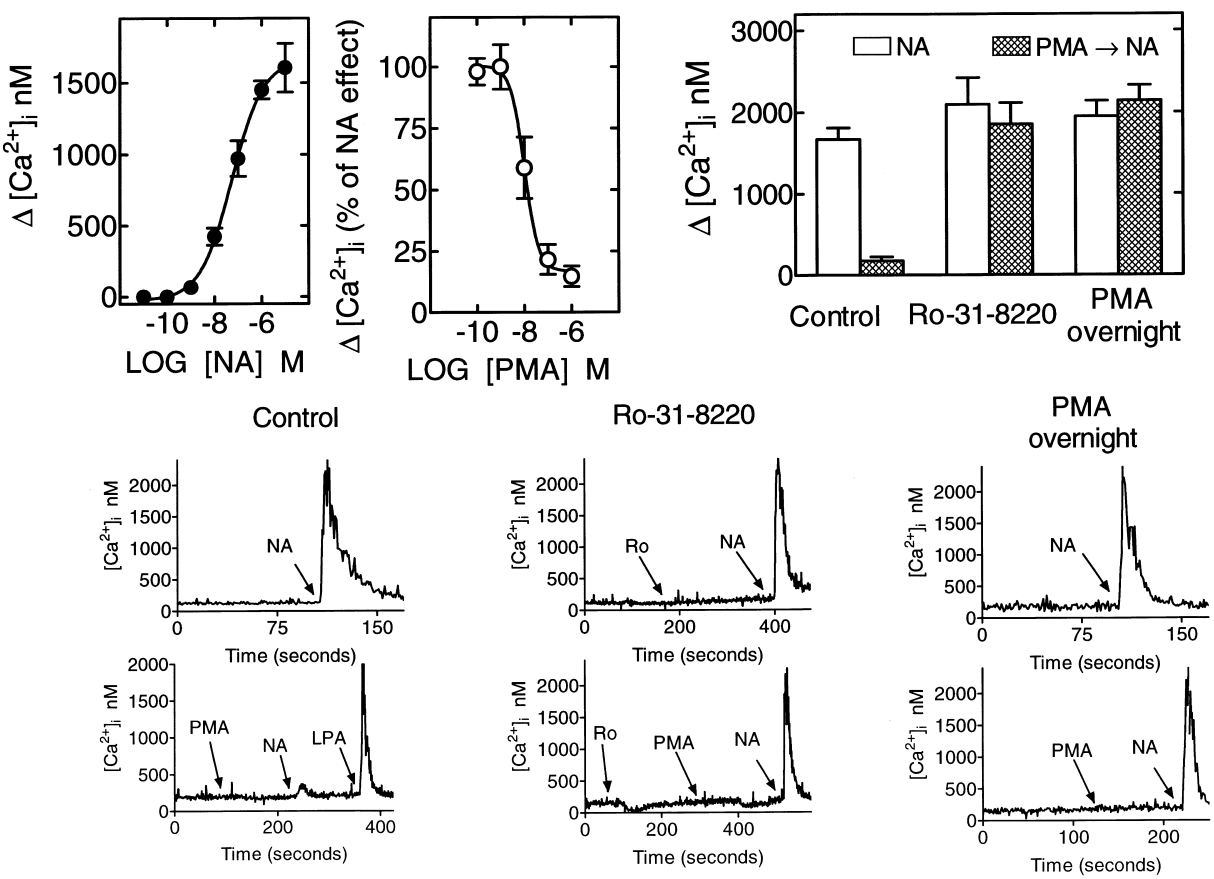

Fig. 2. Effects of PMA and noradrenaline (NA) on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Upper left panel: effect of different concentrations of noradrenaline on $[Ca^{2+}]_i$; upper middle panel: effect of different concentrations of PMA on noradrenaline-induced increase on $[Ca^{2+}]_i$; upper right panel: effect of 10 μM Ro-31-8220 and overnight incubation with 1 μM PMA on the effects of noradrenaline and PMA on $[Ca^{2+}]_i$. Plotted are the means and vertical lines represent the S.E.M. of six to seven experiments using different cell preparations. Lower panel: representative tracings of six to seven experiments are shown. NA, 10 μM noradrenaline; PMA, 1 μM PMA; LPA, 1 μM lysophosphatidic acid; Ro, 10 μM Ro-31-8220.

terminated by the addition of 0.4 ml of 30% ice-cold perchloric acid. Supernatants were neutralized and [^{3}H]inositol phosphates (IP_1, inositol monophosphate; IP_2, inositol bisphosphate; and IP_3, inositol trisphosphate) were separated by Dowex AG1-X8 chromatography (Berridge et al., 1983).

2.7. Photoaffinity labeling and phosphorylation

Membranes (25 μg protein), were incubated in the dark with 6 nM of [^{125}I-aryl]-azido-prazosin essentially as described by Lattion et al. (1994) and modified by us (Vázquez-Prado et al., 1997) for 1 h at room temperature; then 1 ml of 50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA containing protease inhibitors was added and the tubes were exposed to UV light for 3 min. After this treatment, membranes were centrifuged at $12700 \times g$ for 15 min, washed and electrophoresed in 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions.

Receptor phosphorylation studies were performed as described in detail (Vázquez-Prado et al., 1997). In short, cells were maintained in phosphate-free DMEM during 1 h and then incubated in 3 ml of the same medium containing [^{32}P]Pi (0.05 mCi/ml) for 3 h at 37°C. Labeled cells were stimulated as indicated, then they were washed twice with ice-cold phosphate buffered saline and solubilized with 1 ml of ice-cold solubilization buffer (Vázquez-Prado et al., 1997). The extracts were centrifuged and the supernatants transferred to tubes containing a rabbit antiserum generated against the carboxyl terminus decapeptide of the hamster α_{1b}-adrenoceptor and sepharose-coupled protein A and immunoprecipitated as described (Vázquez-Prado et al., 1997). The amount of phosphorylated receptor was determined by PhosphorImager analysis.

2.8. [^{35}S]GTP-γ-S binding

[^{35}S]GTP-γ-S binding was performed as described by Wieland and Jakobs (1994) with minor modifications (Vázquez-Prado et al., 1997). The binding reaction was carried out in a volume 250 μl for 30 min at 30°C in buffer containing 2 nM [^{35}S]GTP-γ-S. The reaction was initiated by the addition of membranes and terminated by the addition of 2 ml of ice-cold binding buffer without EDTA and filtration on Whatman GF/C filters using a Brandel harvester. Nonspecific binding was determined in the presence of 100 μM Gpp(NH)p and represented ~10% of total binding. Filters were washed three times, dried and radioactivity measured with a liquid scintillation counter.

2.9. Statistical analysis

Statistical analysis was performed using analysis of variance with Bonferroni post-test.

3. Results

The expression of α_{1b}-adrenoceptors was confirmed at the level of mRNA (Northern analysis, not shown) and protein (radioligand binding). [^{3}H]Prazosin bound to membranes in a rapid, reversible and saturable fashion. A representative saturation isotherm and the Rosenthal transformation of the data are presented in Fig. 1. It can be observed that the radioligand binds with high affinity (K_D 0.22 ± 0.05 nM; mean ± S.E.M., $n = 4$) and that a relatively high density of receptors were detected (B_{max} 807 ± 60 fmol/mg protein; mean ± S.E.M., $n = 4$). Binding competition experiments showed the following order of potency: prazosin (K_i 0.10 ± 0.03 nM) > phentolamine (K_i 127 ± 13 nM) = 5-methyl urapidil (K_i 134 ± 45 nM) ≥ BMY 7378 (K_i 214 ± 20 nM) (means ± S.E.M., $n = 3$) (in all cases, the slopes were ~1) which is consistent with what was expected for this receptor subtype (representative data are presented in Fig. 1).

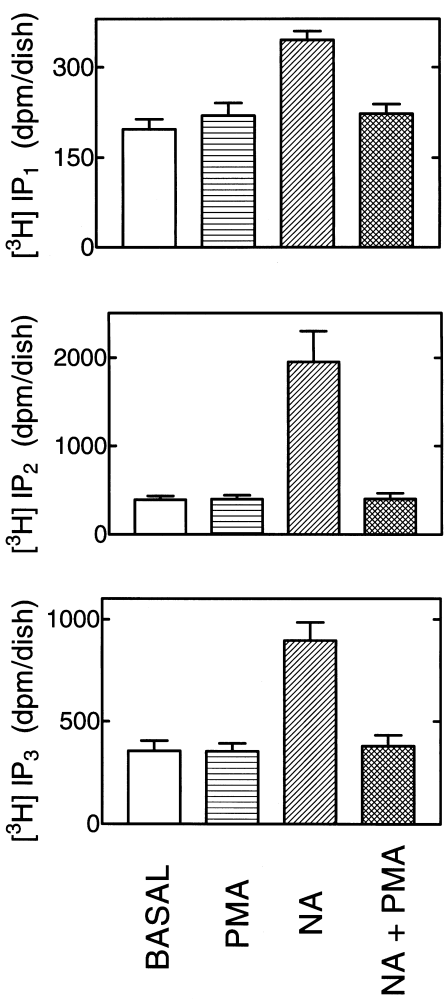

Fig. 3. Effects of PMA and noradrenaline (NA) on [^{3}H]inositol phosphate production. Plotted are the means and vertical lines represent the S.E.M. of six to eight determinations using four different cell preparations.

The ability of noradrenaline to increase $[Ca^{2+}]_i$ was next examined. As shown in Fig. 2, 10 μM noradrenaline induced an almost immediate increase in $[Ca^{2+}]_i$ from a basal of 100 ± 15 nM (mean ± S.E.M., $n = 7$) to ~2 μM. The effect of noradrenaline was concentration-dependent with an EC_{50} of 70 ± 30 nM (Fig. 2) and it was

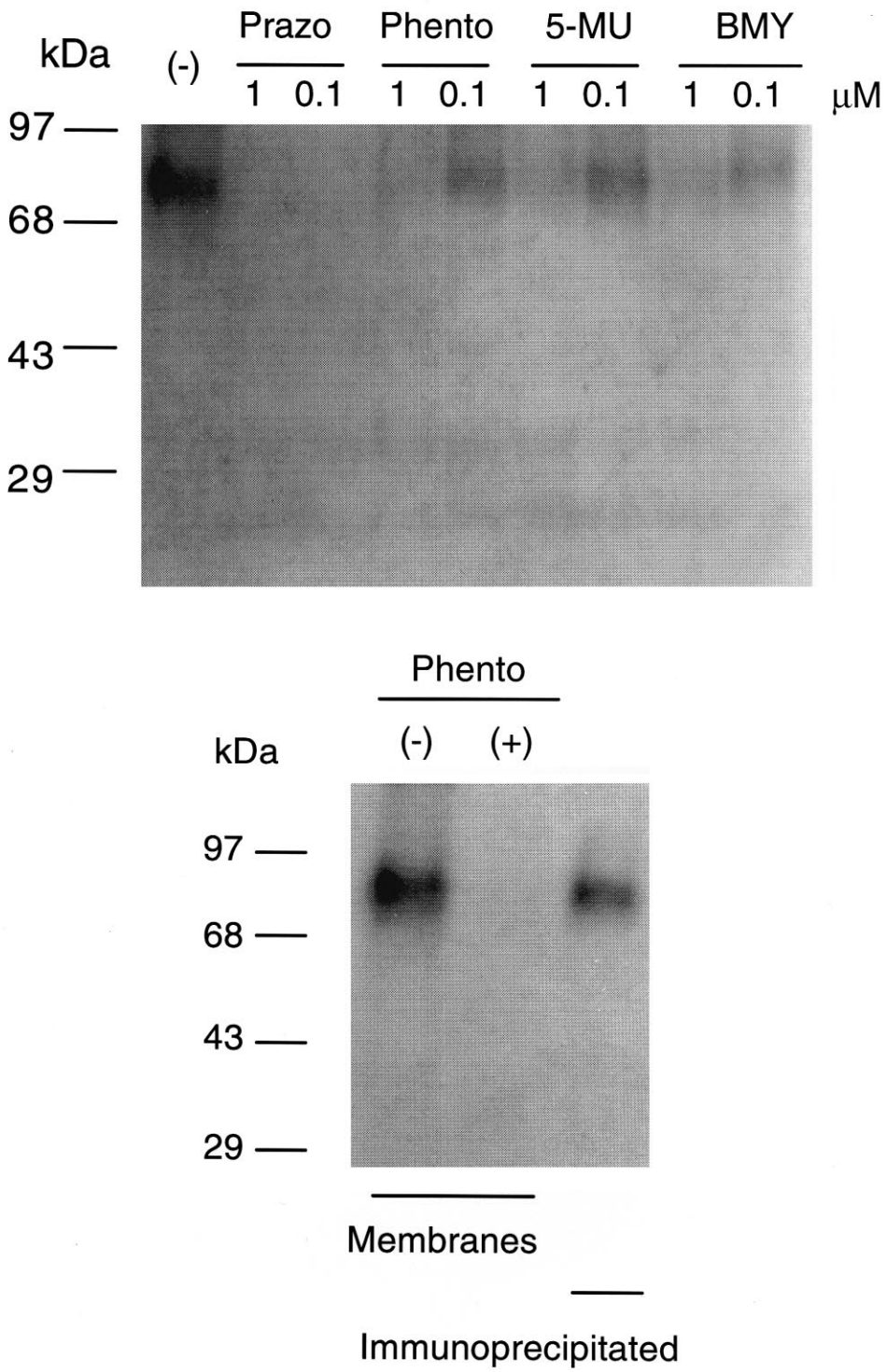

Fig. 4. Photoaffinity labeling and immunoprecipitation of the α_{1b}-adrenoceptor. Upper panel: photoaffinity labeling of the receptor with [^{125}I-aryl]-azidoprazosin in the absence of any agent (−) or presence of the indicated concentrations of prazosin (Prazo), phentolamine (Phento), 5-methyl urapidil (5-MU) and BMY 7378 (BMY). Lowert panel: photoaffinity labeled membranes were solubilized and the receptor immunoprecipitated. Autoradiographs are representative of three different experiments.

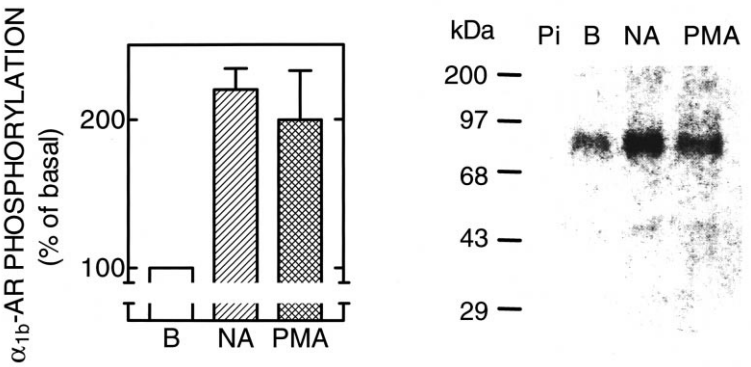

Fig. 5. Phosphorylation of the α_{1b}-adrenoceptor. Cells were incubated in the absence of any agent (B), with 10 μM noradrenaline (NA) or 1 μM PMA for 5 min. Plotted are the means and vertical lines represent the S.E.M. of three determinations using different cell preparations. A representative autoradiograph is shown; Pi, preimmune serum.

blocked by phentolamine (not shown). The active phorbol ester, PMA, also blocked in a concentration-dependent fashion the action of noradrenaline with an IC_{50} of 10 ± 1 nM (mean ± S.E.M., $n = 7$) (Fig. 2). Ro-31-8220, a selective protein kinase C inhibitor, did not alter the ability of noradrenaline to increase $[Ca^{2+}]_i$ but blocked the inhibitory action of PMA (Fig. 2). Similarly, overnight pre-incubation of the cells with PMA, to induce downregulation of protein kinase C (Rodriguez-Peña and Rozengurt, 1984; García-Sáinz et al., 1998), did not alter the effect of noradrenaline on $[Ca^{2+}]_i$ but under these conditions acute administration of PMA was without effect on the action of noradrenaline (Fig. 2).

These effects were further confirmed by studying the production of inositol phosphates. Noradrenaline (10 μM) markedly increased the production of [^{3}H]inositol phosphates (IP_1, IP_2 and IP_3; $p < 0.001$ vs. basal for each inositol phosphate) from [^{3}H]inositol labeled cells (Fig. 3). PMA (1 μM) did not alter the basal production of inositol phosphates but completely blocked the effect of noradrenaline (Fig. 3).

The α_{1b}-adrenoceptor was next studied by photoaffinity labeling and immunoprecipitation. As shown in Fig. 4, when membranes were incubated with [^{125}I-aryl]-azidoprazosin and subjected to UV irradiation a protein with an apparent molecular weight of ~80 kDa was labeled. The specificity of the labeling was evidenced in competition experiments in which it was observed that 100 nM prazosin completely abolished the labeling whereas the same concentration of phentolamine, 5-methyl urapidil or BMY 7378 were only partially effective. Higher concentrations of these antagonists completely blocked receptor labeling (Fig. 4).

The ability of rabbit antiserum generated against the carboxyl terminal decapeptide of the hamster α_{1b}-adrenoceptor to immunoprecipitate the human isoform was tested. It can be observed in Fig. 4. that the antiserum was clearly capable of immunoprecipitating the photolabeled receptor (~50%–60% of the total receptor labeled) (Fig. 4); preimmune sera was unable to do it (not shown).

Receptor phosphorylation was next examined. It was observed that the antibody immunoprecipitated a single band of the same molecular weight of the photolabeled receptor (Fig. 5). The band was labeled in the basal state, suggesting that the receptor is a phosphoprotein and such basal phosphorylation was markedly increased by stimulation by 10 μM noradrenaline and by 1 μM PMA (Fig. 5).

In order to gain further insight on the actions of the agonist and the phorbol ester, cells were incubated in the absence of any agent, with 10 μM noradrenaline or with 1 μM PMA. Membranes were prepared from these cells and the ability of the α_{1b}-adrenoceptor to interact with G proteins was evaluated by studying agonist-stimulated [^{35}S]GTP-γ-S binding. It can be observed in Fig. 6 that noradrenaline induced a small but consistent and significant ($p < 0.05$ vs. basal binding) increase in nucleotide binding in control membranes. Such effect was completely

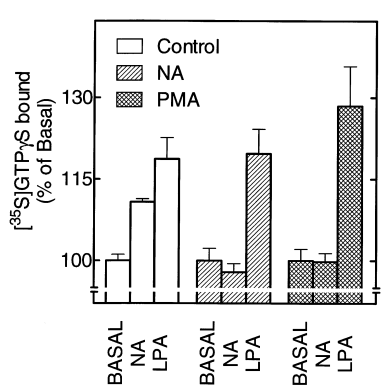

Fig. 6. Effect of noradrenaline and PMA on [^{35}S]GTP-γ-S binding. Membranes from cells incubated in the absence of any agent (Control, open colums), 10 μM noradrenaline (NA, dashed columns) or 1 μM phorbol 12-myristate 13-acetate (PMA, crisscrossed columns) were incubated with the labeled nucleotide in the absence of any agent (BASAL), with 10 μM noradrenaline (NA) or 1 μM lysophosphatidic acid (LPA). Data were normalized to the basal binding in each cell incubation condition (100%) which were: control 11,017 ± 895 dpm/25 μg protein; noradrenaline 8938 ± 814 dpm/25 μg protein and PMA 8073 ± 870 dpm/25 μg protein. Plotted are the means ± S.E.M. of 16–20 determinations using three different membrane preparations for each condition.

absent in membranes from cells treated with the agonist or with PMA. Under the same conditions, lysophosphatidic acid was able to increase [^{35}S]GTP-γ-S; the effect of lysophosphatidic acid was bigger than that of noradrenaline and was clearly observed in all the conditions studied ($p < 0.01$ in the three conditions) (Fig. 6).

4. Discussion

In the present work, we took advantage of the commercial availability of a cell line stably expressing the human α_{1b}-adrenoceptor. After confirming the expression of the receptor by Northern analysis and radioligand binding, we evaluated the functional response of the cells to noradrenaline. It is clear from our results that activation of these receptors resulted in the production of [^{3}H]inositol phosphates and marked increases in $[Ca^{2+}]_i$. The data clearly indicated that, as expected, these receptors are coupled to phosphoinositide turnover/Ca^{2+} signalling.

The main aim of the study was to define the sensitivity of the human α_{1b}-adrenoceptors to protein kinase C activation. As shown, an active phorbol ester drastically and essentially completely desensitized the cells to α_{1b}-adrenergic stimulation. This resembles what happens in rat hepatocytes (Corvera and García-Sáinz, 1984; Corvera et al., 1986) and in hamster DDT_1 MF-2 cells (Leeb-Lundberg et al., 1985) but differs to what take place in rat-1 fibroblasts stably expressing hamster α_{1b}-adrenoceptors. In those cells a partial (50%–70%) blockade of noradrenaline action was observed (Vázquez-Prado and García-Sáinz, 1996; Vázquez-Prado et al., 1997). It is clear, therefore, that PMA blocks the action of the human α_{1b}-adrenoceptors at relatively low concentrations. The ability of lysophosphatidic acid to increase $[Ca^{2+}]_i$, in cells treated with PMA clearly indicated that the action of this tumor promoter was not due to a general deterioration of the cells. The role of protein kinase C in the action of PMA was evidenced by use of a selective protein kinase C inhibitor, Ro-31-8220, and by the refractoriness to the acute action observed after prolonged stimulation. Such refractoriness is usually observed after overnight stimulation and results from protein kinase C downregulation (Rodriguez-Peña and Rozengurt, 1984; García-Sáinz et al., 1998).

The cloned human α_{1b}-adrenoceptor is a protein of 519 aminoacids with a MW of 56,778 Da (SWISS-PROT: P35368). Photoaffinity labeling experiments indicated that the receptor migrates as broad band of approximately 80–85 kDa, which is similar to what has been observed for the hamster (Lomasney et al., 1986) and rat (Sawutz et al., 1987) α_{1b}-adrenoceptor isoforms. These data suggests that human receptors are similarly glycosylated.

The antibody we generated against the carboxyl terminal of the hamster α_{1b}-adrenoceptor (Vázquez-Prado et al., 1997) was able to immunoprecipitate the human receptor. The carboxyl terminal decapeptide sequences of these receptor isoforms only differ in one aminoacid (SWISS-PROT: P35368 and P18841). Nevertheless, antisera, obtained from different rabbits, that were similarly effective in immunoprecipitating the hamster α_{1b}-adrenoceptor (70%–80% of the photoaffinity labeled receptor) varied widely in their ability to immunoprecipitate the human receptor (10%–50%).

The human α_{1b}-adrenoceptor is phosphorylated in the basal state and its phosphorylation state can be increased by receptor activation by agonists and by activation of protein kinase C with PMA. The action of agonists may probably involve G protein receptor kinases, as shown for the hamster α_{1b}-adrenoceptor (Diviani et al., 1996) and for many other G protein-coupled receptors (Benovic et al., 1990; Premont et al., 1995). It is also clear from our data that there is a correlation between phosphorylation and desensitization and that such effects are associated to an uncoupling of the receptor with G proteins as evidenced by the absence of agonist-stimulated [^{35}S]GTP-γ-S binding. This is consistent with what has been observed for the hamster α_{1b}-adrenoceptor (Vázquez-Prado et al., 1997).

Phosphorylation of the hamster α_{1b}-adrenoceptor seems to occur mainly on serine residues and to a much lesser extent in threonine residues (Diviani et al., 1997; Vázquez-Prado et al., 1997). The three intracellular loops of the human α_{1b}-adrenoceptors are 100% identical to those of the hamster isoform. The recent, very elegant, works of Lattion et al.(1994) and Diviani et al. (1997) have indicated that the main phosphorylation sites for protein kinase C and G protein receptor kinases are located in the carboxyl terminus. There are some differences in the carboxyl termini of these α_{1b}-adrenoceptors. The human isoform is four aminoacids bigger, i.e., aminoacids 341–519 in the human receptor as compared to aminoacids 341–515 in the hamster isoform. This includes two additional arginines in the poly-arginine region (aminoacids 370–379 in the human α_{1b}-adrenoceptor as compared to aminoacids 371–378 of the hamster isoform), an additional proline at position 449, additional alanine and proline residues at positions 457 and 458, respectively, and the absence of serine 369 of the hamster α_{1b}-adrenoceptor. The domain containing the phosphorylation sites involved in protein kinase C- and G protein receptor kinase-mediated desensitizations, identified by Diviani et al. (1997), is present in both of these α_{1b}-adrenoceptor isoforms. The overall identity of the caboxyl termini of these receptors is of 89.7% and a potential protein kinase C-phosphorylation site of the hamster receptor is not present in the human receptor.

The distribution of α_1-adrenoceptor subtypes has been extensively studied in rodent tissues both at the level of mRNA expression and protein. However, the information is largely incomplete for human tissues. In addition, the information available suggests that the distribution of α_1-adrenoceptors in human tissues is distinct from that ob-

served in other mammals (Price et al., 1994; García-Sáinz and Macías-Silva, 1995; García-Sáinz et al., 1995) which precludes extrapolation of findings. There is also evidence that suggests roles of different α_1-adrenoceptors in human diseases. Therefore, the physiological and pathophysiological roles of α_1-adrenoceptors make it important to define the functional properties of human receptors. In the present work, we show that activation of protein kinase C blocks/desensitizes α_{1b}-adrenoceptors and that such effect is associated to receptor phosphorylation and G protein uncoupling. In addition, evidence is presented which suggest that activation of these receptors by an agonist also induces receptor phosphorylation and G protein uncoupling.

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