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Okadaic acid increases the phosphorylation state of α_{1A}-adrenoceptors and induces receptor desensitization

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Abstract

Okadaic acid, a protein phosphatase inhibitor, and phorbol myristate acetate, an activator of protein kinase C, increased the phosphorylation state of α_{1A}-adrenergic receptors. The effects of these agents were of similar magnitude but that of okadaic acid developed more slowly. Wortmannin (inhibitor of phosphoinositide 3-kinase), but not staurosporine (inhibitor of protein kinase C), abolished the effect of okadaic acid on the α_{1A}-adrenoceptor phosphorylation state. The effect of phorbol myristate acetate on this parameter was blocked by staurosporine and only partially inhibited by wortmannin. Okadaic acid markedly increased the co-immunoprecipitation of both the catalytic and regulatory subunits of phosphatidylinositol 3-kinase and of Akt/protein kinase B with the adrenoceptor and only marginally increases receptor association with protein kinase Cε. Okadaic acid induced desensitization of α_{1A}-adrenoceptors as evidenced by a decreased ability of noradrenaline to increase intracellular calcium. Such desensitization was fully reverted by wortmannin. Our data indicate that inhibition of serine/threonine protein phosphatases increases the phosphorylation state of α_{1A}-adrenergic receptor and alters the adrenoceptor function.

Keywords: α_1-adrenoceptor; Receptor phosphorylation; Protein kinase C; Protein phosphatase; Desensitization; Phosphatidylinositol 3-kinase

1. Introduction

Modulation of receptor function is a key event in the adaptation of cells to the changes in the internal milieu and to the overall homeostasis, finely tuning their sensitivity to different stimuli (desensitization–resensitization). Many molecular and cellular processes are involved in such modulation (receptor uncoupling to G proteins, internalization, receptor degradation and recycling and changes in the receptor gene expression, among others); receptor phosphorylation seems to be a very initial key event (Lefkowitz, 1998).

The phosphorylation state of a receptor results from the balance of the activities of two groups of enzymes: protein kinases and protein phosphatases. Three groups of protein kinases are the main modulators of G protein-coupled receptors: a) the G protein-coupled receptor kinases, b) second messenger activated protein kinases such as protein kinase A and protein kinase C, and c) receptors with tyrosine protein kinase activity (Vázquez-Prado et al., 2003). The identity of the specific phosphatases involved is largely unknown.

α_1-Adrenoceptors are a heterogeneous subfamily of G protein-coupled receptors comprised by three isoforms, the α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (Hieble et al., 1995). There is evidence that the three α_1-adrenoceptor subtypes are subjected to phosphorylation and the roles of G protein-coupled receptor kinases, protein kinase C and other kinases has been studied in some detail (García-Sáinz et al., 2000; Vázquez-Prado et al., 2003). In contrast, the role(s) of protein phosphatases is very little known. Previously, we reported that okadaic acid, tautomycin and calyculin A, protein phosphatase 2A and 1 selective inhibitors, and cypermethrin, an specific protein phosphatase 2B inhibitor, are able to increase the α_{1B}-adrenoceptor phosphorylation state in living cells (Alcántara-Hernández et al., 2000); such effect seems to involve protein kinase C as evidenced by the ability of staurosporine and Ro 31-8220 to inhibit the effect okadaic acid (Alcántara-Hernández et al., 2000). Interestingly, in spite of inducing a marked increase in the receptor phosphorylation state, okadaic acid alters α_{1B}-adrenoceptor function only marginally, which is in marked contrast with the effect of direct activation of protein kinase C

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with phorbol myristate acetate that induces α_{1B}-adrenoceptor phosphorylation and an almost total desensitization (Alcántara-Hernández et al., 2000).

α_{1A}-Adrenoceptors mediate important actions of adrenaline and noradrenaline such as vasoconstriction, cardiac inotropy, genitourinary smooth muscle contraction and modulation of liver metabolism in some species (García-Sáinz et al., 1992; García-Sáinz et al., 1995); these receptors also participate in the development of prostatic hypertrophy (Piascik and Perez, 2001; Schulman et al., 1996). It is particularly in this latter action in which selective α_{1A}-adrenoceptor antagonists have shown important therapeutic value, since long-term therapy seems to be safe and well-tolerated, improving urinary flow and decreasing symptoms (Schulman et al., 1996). In spite of the clear physiological and pathophysiological importance of these receptors little is know on their regulation and even less on the role that protein kinases and phosphatases might play. We have previously shown that agonists and activation of protein kinase C by phorbol myristate acetate induce α_{1A}-adrenoceptor phosphorylation (Vázquez-Prado et al., 2000). Interestingly, the phosphorylation of α_{1A}-adrenoceptors and the desensitization observed were of much lesser magnitude than those observed with the α_{1B} subtype (Vázquez-Prado et al., 2000). It has been reported that okadaic acid markedly attenuated α_1-adrenoceptor (putatively α_{1A})-mediated actions in glia and that such effect is blocked by inhibitors of protein kinase C (Assari et al., 2003). In the present work we studied the effect of okadaic acid in rat-1 cells expressing α_{1A}-adrenoceptors, our results show that the protein phosphatase inhibitor is capable of increasing the receptor phosphorylation state associated to receptor desensitization and that this effect is associated to recruitment of protein kinase C, phosphoinositide 3-kinase and Akt/protein kinase B. Differences were observed with the actions of phorbol myristate acetate. Our results indicate that the actions of okadaic acid are more complex than previously anticipated and that differences in the sensitivity to this phosphatase inhibitor exist among α_1-adrenoceptor subtypes.

2. Materials and methods

2.1. Materials

(−)-Noradrenaline, phorbol myristate acetate, staurosporine, wortmannin, and protease inhibitors were obtained from Sigma. Dulbecco´s modified Eagle´s medium, fetal bovine serum, trypsin, antibiotics, and other reagents used for cell culture were from Life Technologies. [^{32}P]P_i (8500–9120 Ci/mmol) was from NEN Life Science Products. Sepharose-coupled protein A was from Upstate Biotechnology. Fura-2/AM was from Molecular Probes. Nitrocellulose membranes were from Bio-Rad and the chemiluminescence's kits were obtained form Pierce. Antibodies against protein kinase C-selective isoforms, p85αPI3K and p110αPI3K were from Santa Cruz Biotechnology, anti-Akt and anti-*phospho*-Akt antibodies were from BD Pharmigen, and secondary antibodies were from Zymed.

2.2. Cell line and culture

Rat-1 fibroblasts stably expressing the bovine α_{1A}-adrenoceptor, generously provided to us by Drs. R. J. Lefkowitz, M. G. Caron, and L. Allen (Duke University), were cultured in glutamine-containing high-glucose DMEM supplemented with 10% fetal bovine serum, 300 μg/ml neomycin analog G-418 sulfate, 100 μg/ml streptomycin, 100 units/ml penicillin, and 0.25 μg/ml amphotericin B at 37 °C under a 95% air, 5% CO_2 atmosphere as described previously (Vázquez-Prado and García-Sáinz, 1996; Vázquez-Prado et al., 2000).

2.3. Receptor phosphorylation

Rat-1 fibroblasts, expressing the bovine α_{1A}-adrenoceptors were cultured in culture dishes (10-cm diameter). Cells reaching confluence were incubated in phosphate-free DMEM over night in 3 ml that containing [^{32}P]P_i (50 μCi/ml) at 37 °C. Labeled cells were stimulated with as indicated, and then they were washed with ice-cold phosphate-buffered saline and solubilized with 1.0 ml of ice-cold buffer containing 1% Triton X-100, 0.05% sodium dodecyl sulfate, 50 mM NaF, 1 mM Na_3VO_4, 10 mM β-glycerophosphate, 10 mM sodium pyrophosphate, 1 mM *p*-serine, 1 mM *p*-threonine, and 1 mM *p*-tyrosine. The plates were maintained on ice for 1 h. Then the extracts were centrifuged at 12,700 ×*g* for 15 min at 4 °C, and the supernatants were immunoprecipitated with a rabbit antiserum against GST-α_{1A}-adrenoceptor fusion protein (Vázquez-Prado and García-Sáinz, 1996; Vázquez-Prado et al., 2000). At least three independent experiments were performed for each treatment. Receptor phosphorylation was detected with a Molecular Dynamics PhosphorImager and quantified with ImageQuant software. Data were within the linear range of detection of the apparatus and were plotted using Prism 3.01, GraphPad software.

2.4. Intracellular calcium concentration

Confluent fibroblasts were incubated for 2 h in DMEM without serum and antibiotics. Cells were loaded with 5 μM Fura-2/AM in Krebs-Ringer Hepes containing 0.05% bovine serum albumin, pH 7.4, for 1 h at 37 °C as described (Vázquez-Prado and García-Sáinz, 1996; Vázquez-Prado et al., 2000). Cells were detached by gentle trypsinization. Experiments were performed with about 10^6 cells suspended in 3 ml of the above-mentioned buffer supplemented with 1.2 mM $CaCl_2$. Fluorescence measurements were carried out with an Aminco-Bowman Series 2 spectrometer with the excitation monochromator set at 340 and 380 nm, chopper interval of 0.5 s, and the emission monochromator set at 510 nm. $[Ca^{2+}]_i$ was calculated (Grynkiewicz et al., 1985) using the software provided by Aminco-Bowman; traces were directly exported to the graphs. When protein kinase inhibitors, okadaic acid or phorbol myristate acetate were used, the cells were in contact with each these agents for 15 min.

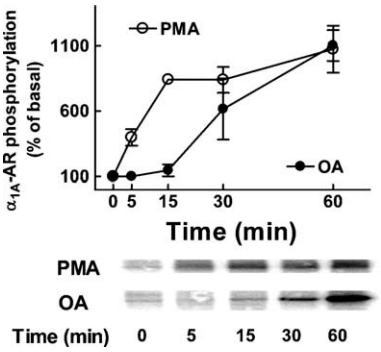

Fig. 1. Effects of okadaic acid and phorbol myristate acetate on α_{1A}-adrenoceptor phosphorylation state. Cells were incubated with 1 μM okadaic acid (OA, solid circles) or 1 μM phorbol myristate acetate (PMA, open circles) for the times indicated. Data are presented as percentage of basal receptor phosphorylation and plotted are the means±S.E.M. of four experiments using different cell preparations. Representative autoradiographs are shown.

2.5. Co-immunoprecipitation of α_{1A}-adrenoceptors

Co-immunoprecipitation studies were performed as described (Alcántara-Hernández et al., 2001, 2004). The rabbit antiserum against the GST-α_{1A}-adrenoceptor fusion protein has been described in detail (Vázquez-Prado et al., 2000). Membranes were solubilized in 1 ml of buffer (50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 5 mM EGTA, 1% Triton X-100, and protease inhibitors (Alcántara-Hernández et al., 2001, 2004). After 1 h at 4 °C, the extracts were centrifuged at 12,700 ×*g* for 15 min at 4 °C, and the supernatants were transferred to new tubes containing 15 μl of immune serum. Tubes were incubated overnight at 4 °C with the antiserum. Beads were washed two times (1 ml/each) with 50 mM Hepes, 50 mM NaH_2PO_4, 100 mM NaCl, pH 7.2, containing 0.1% Nonidet P40 and 100 mM NaF. Samples were incubated for 10 min in a boiling bath and subjected to electrophoresis in 10% sodium dodecyl sulfate-polyacrylamide mini-gels containing 7 M urea and electrotrasferred to nitrocellulose membranes. Incubation with the antibodies against the protein kinase C isoforms, p85αPI3K, p100αPI3K, Akt and phospho-Akt was for 12 h at 4 °C and the incubation with secondary antibodies was for 1 h at room temperature. Super signal enhanced chemiluminescence's kits form Pierce were used. Western blots were analyzed by densitometry.

2.6. Statistical analysis

Statistical analysis between comparable groups was performed using analysis of variance with Bonferroni´s Multiple Analysis subtest using the software included in the GraphPad Prism program.

3. Results

In agreement with previous work (Vázquez-Prado et al., 2000), 1 μM phorbol myristate acetate induced a 10-fold increase in the phosphorylation state of α_{1A}-adrenoceptors (Fig. 1); the effect was significant as early as 5 min and reached its maximum at 15 min. It is also shown in Fig. 1 that 1 μM okadaic acid induced a clear increase (similarly of ≈10-fold) in the phosphorylation state of α_{1A}-adrenoceptors. The effect of the protein phosphatase inhibitor took place in a slower fashion., being clear at 15 min and further increasing at 30 and 60 min of incubation (Fig. 1). No further increase was observed at longer times (data not shown).

To get some insight on the protein kinases involved in the effect of okadaic acid effect on the α_{1A}-adrenoceptor phosphorylation state, Rat-1 fibroblasts were treated with inhibitors. The effect of phorbol myristate acetate was also studied for comparison. As anticipated, the α_{1A}-adrenoceptor phosphorylation induced by phorbol myristate acetate was completely blocked by staurosporine, an inhibitor of serine/threonine protein kinases, with some selectivity for protein kinase C. Interestingly and to our surprise, the increase in the α_{1A}-adrenoceptor phosphorylation state induced by 1 μM okadaic acid, was only marginally inhibited by staurosporine (Fig. 2). We have previously observed that phosphoinositide 3-kinase is a key intermediary enzyme in the signaling events that lead to an increase in the phosphorylation state of α_{1B}-adrenoceptors (Casas-González et al., 2003, 2000; García-Sáinz et al., 2004, 2000; Medina et al., 2000; Romero-Ávila et al., 2002; Vázquez-Prado et al., 2003). Therefore, we tested the effect of low concentrations of wortmannin, a selective inhibitor of this phospholipid kinase. Wortmannin (100 nM) was able to completely inhibit okadaic acid-induced increase in the α_{1A}-adrenoceptor phosphorylation state and only partially that induced by phorbol myristate acetate (Fig. 2).

To further confirm that protein kinase C and phosphoinositide 3-kinase were involved in the increases in the α_{1A}-adrenoceptor phosphorylation state induced by phorbol myristate acetate and okadaic acid we performed co-immunoprecipitation assays. It can be observed in Fig. 3 that phorbol myristate acetate clearly increases the co-immunoprecipitation of α_{1A}-adrenoceptors and some protein kinase C isoforms, i.e., protein kinase Cα (183%), protein kinase Cδ (283%) and

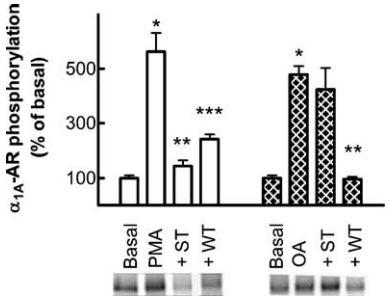

Fig. 2. Effects of protein kinase inhibitors on okadaic acid- and phorbol myristate acetate-induced increase in α_{1A}-adrenoceptor phosphorylation state. Cells were incubated in the absence of any agent (BASAL), in presence of 1 μM okadaic acid (OA) or 1 μM phorbol myristate acetate (PMA) for 30 min. Where indicated, cells were incubated with inhibitors (100 nM staurosporine (ST) or 100 nM wortmannin (WT)) 15 min before the addition of okadaic acid or phorbol myristate acetate. Data are presented as percentage of basal receptor labelling and plotted are the means±S.E.M. of four experiments using different cell preparations. Representative autoradiographs are shown. $^{*}P<0.001$ vs. basal; $^{**}P<0.001$ vs. phorbol myristate acetate alone or okadadic acid alone; $^{***}P<0.05$ vs. phorbol myristate acetate alone.

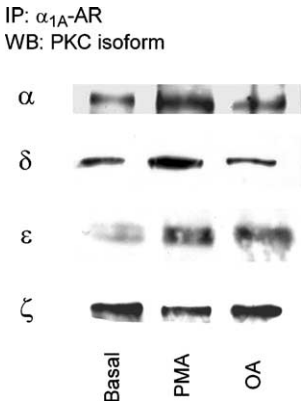

Fig. 3. Effects of phorbol myristate acetate (PMA) and okadaic acid (OA) on the co-immunoprecipitation of α_{1A}-adrenoceptors and protein kinases C isoforms. Cells were incubated for 30 min in the absence (Basal) or in presence of 1 μM phorbol myristate acetate or 1 μM okadaic acid. Blots are representative of three experiments using different cell preparations. IP, immunoprecipitation; WB, Western blot.

protein kinase Cε (163%). Basal association of protein kinase Cζ with α_{1A}-adrenoceptors was observed but no effect of phorbol myristate acetate was noticed. These data show similarity with what we have observed with α_{1B}-adrenoceptors under phorbol myristate acetate-stimulated conditions (Alcántara-Hernández et al., 2001). It should be kept in mind that Western blotting is not quantitative and only provides a semi-quantitative profile. Okadaic acid only induced a small increase (136%) in the co-immunoprecipitation of protein kinase Cε with the adrenoceptor. The co-immunoprecipitation of α_{1A}-adrenoceptors with phosphoinositide 3-kinase and its substrate Akt/protein kinase B was next examined (Fig. 4). Phorbol myristate acetate induced small increases in the co-immunoprecipitation of the adrenoceptor with the regulatory and

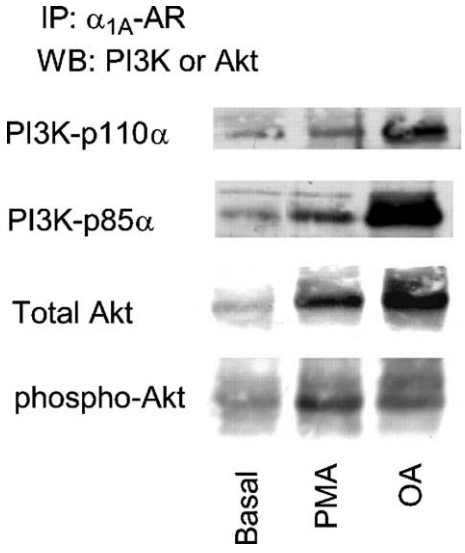

Fig. 4. Effects of phorbol myristate acetate (PMA) and okadaic acid (OA) on the co-immunoprecipitation of α_{1A}-adrenoceptors and phosphatidylinositol 3-kinase (PI3K) subunits, total Akt or phospho-Akt. Cells were incubated for 30 min in the absence (Basal) or in presence of 1 μM phorbol myristate acetate or 1 μM okadaic acid. Blots are representative of three experiments using different cell preparations. IP, immunoprecipitation; WB, Western blot.

catalytic subunits of phosphoinositide 3-kinase (p85 and p110) (120% and 185%, respectively) and slightly bigger effects on the co-immunoprecipitation with total Akt/protein kinase B (250% and 210%, respectively). In contrast, marked increases in the association of phosphoinositide 3-kinase with α_{1A}-adrenoceptors were observed following treatment with okadaic acid: p110α (345%), p85α (380%), total Akt/protein kinase B (438%) and phospho-Akt/protein kinase B (290%).

The functional repercussion of the increase in α_{1A}-adrenoceptor phosphorylation state induced by these agents was next studied. In Fig. 5 (upper left panel) it can be observed that 1 μM phorbol myristate acetate induced only a

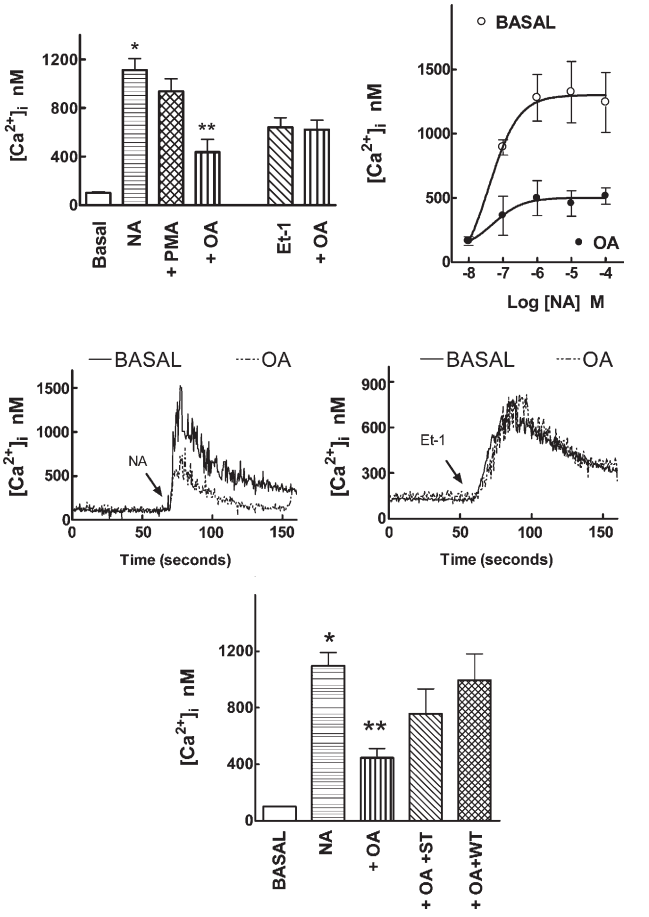

Fig. 5. Functional consequences of the effects of okadaic acid on α_{1A}-adrenoceptor action. Upper left panel; cells were preincubated in the absence of any agent (BASAL) or 1 μM phorbol myristate acetate (+PMA) or 1 μM okadaic acid (+OA) for 15 min and challenged with 10 μM noradrenaline (NA) or 100 nM endothelin 1 (Et-1). Upper right panel: cells were incubated for 15 min in the absence of any agent (BASAL, open circles) or 1 μM okadaic acid (OA, solid circles) and then challenged with different concentrations of noradrenaline. Middle panels: representative traces of the intracellular calcium response to 10 μM noradrenaline (NA) or 100 nM endothelin 1 (Et-1) of cells incubated for 15 min in the absence (BASAL, solid traces) or presence of 1 μM okadaic acid (OA, dotted traces) Lower panel: indicated is the basal intracellular calcium (BASAL) or the effect of 10 μM noradrenaline (NA) in cells preincubated with 1 μM okadaic acid (+OA) alone or with 100 nM staurosporine (+OA+ST) or 100 nM wortmannin (+OA+WT). In the upper and lower panels plotted are the means and vertical lines represent the S.E.M. of 8–10 determinations using different cell preparations.$^{*}P<0.001$ vs. basal; $^{**}P<0.001$ vs. NA.

marginal decrease of the effect of 10 μM noradrenaline on $[Ca^{2+}]_i$ which is consistent with previous observations (Vázquez-Prado et al., 2000). In marked contrast, pretreatment of the cells with 1 μM okadaic acid for 15 min produced a clear desensitization of noradrenaline-induced increase in $[Ca^{2+}]_i$ (similar effect were observed at longer times of incubation, data not shown). Concentration–response curves to noradrenaline in cells pretreated with and without okadaic acid further confirmed the finding (Fig. 5, upper right panel). It can be observed that maximal response to noradrenaline was markedly decreased by okadaic acid whereas the EC_{50} values were similar (30–60 nM). The effect of okadaic acid on this parameter was not due to a nonspecific perturbation of the cells since the effect of endothelin was not altered (see Fig. 5, upper panel and middle traces). Finally, we observed that pretreatment with 100 nM wortmannin blocked the inhibition of noradrenaline action on $[Ca^{2+}]_i$ induced by okadaic acid; staurosporine also blocked this action of okadaic acid but to a lesser extent (Fig. 5, lower panel).

4. Discussion

The present results indicate that inhibition of serine/threonine protein phosphatases increases the phosphorylation state of α_{1A}-adrenoceptors. Previously, we reported that that protein phosphatase 2A, 1 and 2B inhibitors, are able to increase the phosphorylation state of α_{1B}-adrenoceptors in Rat-1 fibroblasts (Alcántara-Hernández et al., 2000). Such data indicated that protein phosphatases play a role maintaining these adrenoceptors in a non-phosphorylated state. In the present study it was evident that the increase in the phosphorylation state of α_{1A}-adrenoceptors in response to by okadaic acid was very slow, which is consistent with the observation that these adrenoceptors are phosphorylated to a much lesser extent than α_{1B}-adrenoceptors (Vázquez-Prado et al., 2000); the data also suggest participation of a complex signaling pathway linking inhibition of phosphatases and activation of the protein kinase(s) that determine the phosphorylation state of the receptor. Additionally, differences in regulation of α_{1B} and α_{1A}-adrenoceptors are evidenced. Even without considering the functional consequences (see below), the data clearly indicate that although the final result is the same (i.e., an increased phosphorylation state of these receptors), the processes that lead to such result, differ.

In the case of the α_{1A}-adrenoceptor phosphorylation induced by phorbol myristate acetate, the data are consistent with a key participation of protein kinase C. Staurosporine was able to completely block the effect and the co-immunoprecipitation studies clearly suggest the interaction of the adrenoceptor with protein kinase C isoforms. Data are also consistent with our observation that α_{1B}-adrenoceptors form dynamic complexes with protein kinase C isoforms of the three classes: classical, novel and atypical (Alcántara-Hernández et al., 2001), which suggest that it is not just a particular observation but a more general effect. In contrast, the role of protein kinase C in the action of okadaic acid is far more complex. Staurosporine only marginally decreased the increase in the phosphorylation state of α_{1A}-adrenoceptors induced by okadaic acid and the co-immunoprecipitation studies indicate that only protein kinase Cε associates with the receptor. Nevertheless, a role of this group of kinases cannot be excluded. On the contrary, the ability of staurosporine to partially block the effect of the phosphatase inhibitor on noradrenaline-induced increase in intracellular calcium strongly indicated that protein kinase C plays a role in this effect.

One of the major finding of this study is the participation of other kinases, such as phosphoinositide 3-kinase and Akt/protein kinase B in the effect of okadaic acid and likely also in that of phorbol myristate acetate. The ability of wortmannin to block the effect of okadaic acid and the strong increase in co-immunoprecipitation of α_{1A}-adrenoceptors and the catalytic (p110α) and regulatory (p85α) subunits of phosphoinositide 3-kinase, together with total and activated Akt (phospho-Akt), clearly suggest so. At this point it is not possible to define how these kinases participate in the effect. The formation of complexes that co-immunoprecipitate do not necessarily indicate a direct receptor-kinase interaction, it could take place through other anchoring of scaffolding elements. Nevertheless, α_{1A}-adrenoceptor contains tyrosine residues, putative targets of phosphorylation to which the p85 regulatory subunit of phosphoinositide 3-kinase could potentially bind. Besides, p110 subunits interact with the β_2-adrenergic receptor kinase through the phosphoinositide kinase domain (Naga Prasad et al., 2002). Phosphoinositide 3-kinase recruits PH domain-containing proteins, such as Akt to the plasma membrane and has also the ability to activate protein kinase C isoforms (Cantley, 2002; Wymann and Pirola, 1998). Analysis of the primary sequence of α_{1A}-adrenoceptors indicates that these receptors contain putative phosphorylation sites by protein kinase C isoforms and Akt/protein kinase B. Akt/protein kinase B participate in the control a variety of regulatory responses in mammalian cells, including inhibition of apoptosis, cellular proliferation, metabolism and hypertrophy, among many others. A role of this kinase has been observed in the modulation of β_2-adrenoceptors by insulin. β_2-adrenoceptors are substrates of Akt/protein kinase B, and its activity has a key role in machinery of β_2-adrenoceptors endocytosis in response to insulin (Doronin et al., 2002).

The ability of wortmannin to partially block phorbol myristate acetate-induced α_{1A}-adrenoceptor phosphorylation is somehow surprising but nor completely unexpected. Phosphoinositide 3-kinase plays a key role as a connecting intermediate enzyme in α_{1B}-adrenoceptor phosphorylation and desensitization (Casas-González et al., 2003, 2000; García-Sáinz et al., 2004; Medina et al., 2000; Romero-Ávila et al., 2002; Vázquez-Prado et al., 2003). Very recent evidence indicates that phosphoinositide 3-kinase, is directly involved through its protein kinase activity in β_2-adrenoceptor endocytosis (Naga Prasad et al., 2005).

It should be mentioned that the present findings illustrate again the fact that the phosphorylation state of G protein-coupled receptors does not correlate linearly with sensitivity. The receptor phosphorylation state observed with phorbol myristate acetate was bigger (at shorter times) or comparable (at

longer times) to that induced by okadaic acid. In frank contrast is the fact that okadaic acid markedly desensitized the receptor whereas phorbol myristate acetate induced a very small functional effect.

It is now accepted that G protein-COUPLED receptor can act as scaffolds binding a variety of proteins and this might promote the activation of novel G protein-independent signaling pathways (Cotecchia et al., 2004; Vázquez-Prado et al., 2003). There is evidence indicating that multiproteic complexes are necessary for signaling, and a number of proteins have been found to interact with G protein-coupled receptors including G protein coupled receptor kinases, β-arrestins, subunits of heterotrimeric G proteins, calmodulin, A-kinase anchoring proteins (AKAPS), receptor-associated modulating proteins, tubulin, SH3-domain containing adaptor molecules like Grb2 and Src, (Cotecchia et al., 2004; Vázquez-Prado et al., 2003). A recent study using the yeast two-hybrid screening for interacting proteins, with the carboxyl-terminus of α_{1A}-adrenoceptors as bait, has suggested the possibility that Abr (Bcr-related protein), bone morphogenetic protein-1 (BMP-1), a metalloproteinase, and filamin-C might interact with α_{1A}-adrenoceptor (Zhang et al., 2004).

It is likely that all these protein interactions participate in modulating the function of α_{1A}-adrenoceptors and that fine mechanisms would be required to coordinate such complicated systems. Further studies will be required to define the precise roles and molecular mechanisms. The physiological roles of these receptors and the already shown therapeutic value of selective antagonists warrant these studies.

Acknowledgements

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