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European Journal of Pharmacology 342 (1998) 311–317

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α_1-Adrenoceptor subtype activation increases proto-oncogene mRNA levels. Role of protein kinase C

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Received 16 June 1997; revised 20 October 1997; accepted 24 October 1997

Abstract

Noradrenaline increased the mRNA levels of c-*fos* and c-*jun* in rat-1 fibroblast lines stably expressing the cloned α_1-adrenoceptor subtypes. The efficacy to induce the expression of c-*fos* mRNA was similar for the three cell lines ($\alpha_{1d} = \alpha_{1b} = \alpha_{1a}$) but different for c-*jun* ($\alpha_{1a} \geq \alpha_{1b} > \alpha_{1d}$). The EC_{50} values were also different: $\approx$ 5 nM (c-*fos*) and $\approx$ 300 nM (c-*jun*) for cells transfected with the α_{1a} subtype, $\approx$ 30 nM (c-*fos*) and $\approx$ 300 nM (c-*jun*) for cells transfected with the α_{1b} subtype and $\approx$ 300 nM (c-*fos* and c-*jun*) for those transfected with the α_{1d} subtype. Staurosporine and protein kinase C down-regulation blocked such effects, indicating a role of this protein kinase. Endothelin-1 (10 nM) also increased the levels of c-*fos* and c-*jun* mRNAs. These actions of endothelin-1 were unaffected by staurosporine and protein kinase C down-regulation. It is concluded that activation of any of the three cloned subtypes can increase the levels of c-*fos* and c-*jun* mRNAs and that protein kinase C plays a major role in mediating such effects. © 1998 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor; Proto-oncogene expression; Protein kinase C; c-*fos*; c-*jun*

1. Introduction

α_1-Adrenoceptors mediate some of the physiological actions of the sympathetic nervous system and of the circulating natural catecholamines, adrenaline and noradrenaline (Hoffman and Lefkowitz, 1996). These receptors are members of the G-protein coupled family of receptors, and constitute a heterogeneous subfamily. Three α_1-adrenoceptor subtypes have been cloned (for reviews see García-Sáinz, 1993; Minneman and Esbenshade, 1994; Graham et al., 1996). They are referred to as α_{1a}-, α_{1b}- and α_{1d}-adrenoceptors, as recommended by the International Union of Pharmacology Committee for Receptor Nomenclature and Drug Classification (Hieble et al., 1995).

α_1-Adrenoceptor activation results in immediate responses such as synaptic transmission, arteriolar smooth muscle constriction, cardiac contraction or hepatic glycogenolysis (Hoffman and Lefkowitz, 1996). In addition to these immediate responses, α_1-adrenoceptors play important roles in longer term actions, such as modulation of cellular phenotype and cell growth and proliferation (Simpson, 1983; Knowlton et al., 1993; Okazaki et al., 1994; Chen et al., 1995; Hu et al., 1996; Rokosh et al., 1996). The long term actions of catecholamines are physiologically important and could be implicated in the pathogenesis of diseases such as benign prostatic hyperplasia (Hoffman and Lefkowitz, 1996). α_1-Adrenoceptors modulate growth and proliferation in smooth muscle cells (Okazaki et al., 1994; Chen et al., 1995; Hu et al., 1996) and mediate hypertrophy of myocardial cells (Simpson, 1983; Knowlton et al., 1993; Rokosh et al., 1996). In addition, cells expressing constitutively active α_1-adrenoceptor mutants exhibit enhanced mitogenesis and tumorgenicity (Allen et al., 1991). The myocardial expression of these active α_1-adrenoceptors induces cardiac hypertrophy in transgenic mice (Milano et al., 1994).

An initial action, usually observed in response to agents that modulate cell growth and proliferation (e.g., hormones, neurotransmitters and growth factors), is the expression of the so-called, immediate early genes (Curran and Morgan, 1987; Ransone and Verma, 1990; Herschman, 1991; Treisman, 1992). Among these early genes are c-*fos* and c-*jun*, whose products, Fos and Jun, consti-

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tute AP-1. AP-1 is a transcription factor that has been regarded as a nuclear third messenger that couples short-term stimulations to long-term actions by regulating the expression of target genes (Curran and Morgan, 1987; Ransone and Verma, 1990; Herschman, 1991; Treisman, 1992).

There is evidence that α_1-adrenoceptor activation induces c-*fos* expression in a variety of cells including cardiac myocytes (Iwaki et al., 1990), vascular smooth muscle cells (Okazaki et al., 1994), neuroblastoma cells (Schilling et al., 1991), brown adipocytes (Thonberg et al., 1994) or hepatocytes (González-Espinosa and García-Sáinz, 1992; García-Sáinz and Alcántara-Hernández, 1996). Different α_1-adrenoceptor subtypes seem to be involved in modulating c-*fos* expression in these cells, and in some cases more than one subtype participates. Comparison of these effects is complicated by the fact that different cells, containing different amounts and different subtypes of α_1-adrenoceptors were employed. The purpose of this study was to investigate the potential role of the different α_1-adrenoceptor subtypes in the expression of the mRNAs for c-*fos* and c-*jun* and the possible participation of protein kinase C in such effects. We took advantage of the availability of rat-1 fibroblasts transfected with the different cloned α_1-adrenoceptor subtypes, that stably express similar densities of such receptors (Vázquez-Prado and García-Sáinz, 1996).

2. Materials and methods

2.1. Materials

L-Noradrenaline, DL-propranolol, endothelin, phorbol 12-myristate 13-acetate (PMA), staurosporine, bovine serum albumin (fraction V), Triton X-100, 2-mercaptoethanol and soybean trypsin inhibitor were obtained from Sigma Chemical. Specific polyclonal antibodies against protein kinase C isozyme peptides (anti-protein kinase C α, δ, ϵ and ζ), (Dulbecco's modified Eagle's medium) DMEM, fetal bovine serum, trypsin, antibiotics and other reagents used for cell culture were from GIBCO BRL. Nylon membranes, nick translation kits, [^{32}P]dCTP (6000 Ci/mmol), biotinylated anti-rabbit Ig, streptavidin–horseradish peroxidase conjugate, ECL™ Western blotting detection reagents and the c-*fos* Amprobe were from Amersham. The c-*jun* probe (JAC.1 clone, 63026 plasmid) was from the American Type Culture Collection. Restriction enzymes were obtained from New England Biolabs.

2.2. Cell culture

Rat-1 fibroblasts were kindly given to us by Dr. Fernando López; clones stably expressing the different cloned α_1-adrenoceptors: α_{1a}, cloned from bovine brain (Schwinn et al., 1990), α_{1b}, cloned from DDT$_1$ MF-2 cells (Cotecchia et al., 1988) and α_{1d}, cloned from rat brain (Lomasney et al., 1991) were kindly provided by Dr. L. Allen, Dr. R.J. Lefkowitz and Dr. M.G. Caron (Duke University, Durham, NC). Cells were grown as described (Vázquez-Prado and García-Sáinz, 1996) in glutamine-containing high-glucose DMEM supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin, 100 units/ml penicillin and 0.25 μg/ml amphotericin B at 37°C under a 5% CO_2 atmosphere. For selection, cells were grown in the presence of the neomycin analogue G-418 sulfate (300 μg/ml).

2.3. Proto-oncogene mRNA expression

Total RNA was obtained according to the method of Chomczynski and Sacchi (1987); integrity of total RNA was verified in agarose-formaldehyde gels stained with ethidium bromide. RNA was quantified by OD A_{260} and equal amounts were spotted onto Nylon membranes. RNA was cross-linked, and hybridized under conditions of high stringency with nick translated probes, as described (González-Espinosa and García-Sáinz, 1992, 1995, 1996). Hybridization probes were as follows: human c-*fos* probe was the 615 bp *Eco*RI–*Pst*I fragment of Amprobe c-*fos*; mouse c-*jun* was the 2.6 kb *Eco*RI–*Eco*RI fragment of JAC.1 clone (63026 plasmid) and, as a control, a cDNA clone of GAPDH (Fort et al., 1985) was used. The fragments are highly conserved among species and gave very clear signals in Northern and dot blot analysis. Filters were exposed on screens for 5–7 days at −70°C. The films were scanned and analyzed using a DHU Beckman densitometer. Levels of mRNA expression were always normalized to its basal value, i.e., expression in the absence of stimulus.

2.4. Protein kinase C Western blots

Confluent rat-1 fibroblasts cultured in 10 cm Petri dishes were scraped with a rubber policeman and homogenized in buffer (Robles-Flores et al., 1991) containing: 20 mM Tris (pH 7.5), 10 mM EGTA, 2 mM EDTA, 0.1% Triton X-100, 50 mM 2-mercaptoethanol and 0.1 mg/ml trypsin inhibitor. The cells were homogenized with an Ultraturrax for 20 s and the homogenate was kept at 0–5°C for 30 min before being centrifuged at $28\,000 \times g$ for 20 min. The pellets were discarded and protein in the supernatants was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard. Samples (40 μg/lane) were separated by 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis followed by electrophoretic transfer to nitrocellulose membranes. Immunoblotting was performed using specific polyclonal antibodies against protein kinase C isozyme peptides, biotinylated anti-rabbit Ig, streptavidin–horseradish peroxidase conjugate and ECL™ Western blotting detection reagents as indicated by the suppliers.

Statistical analysis was performed with an analysis of variance with Bonferroni's correction for multiple comparisons, using a computer package (Instat, Graphpad).

3. Results

Rat-1 fibroblasts transfected with the different α_1-adrenoceptor subtypes had the following affinities for [^{3}H]prazosin and plasma membrane receptor densities: (a) cells expressing the α_{1a}-subtype: K_D 0.29 ± 0.04 nM, B_{max} 920 ± 120 fmol/mg protein; (b) cells expressing the α_{1b}-subtype: K_D 0.38 ± 0.03 nM, B_{max} 2425 ± 335 fmol/mg protein and (c) cells expressing the α_{1d}-subtype: K_D 0.37 ± 0.04 nM, B_{max} 583 ± 71 fmol/mg protein (data are the means $\pm$ S.E.M. of 3 determinations in each case, using different membrane preparations). These values are similar to those previously reported (Vázquez-Prado and García-Sáinz, 1996).

Noradrenaline (10 μM in the presence of 10 μM propranolol to block β-adrenergic effects) induced rapidly and transiently the mRNA expression of proto-oncogenes c-*fos* and c-*jun* in cells transfected with any of the three cloned subtypes (Fig. 1). No such effects were observed in untransfected rat-1 fibroblasts (data not shown). The adrenoceptor agonist induced a marked increase in the level of c-*fos* mRNA which reached its maximum at 30 min and then rapidly returned towards its basal level of expression. The time courses and the magnitude of the effects were very similar in fibroblasts that expressed the different α_1-adrenoceptor subtypes (Fig. 1, upper panel). Noradrenaline (plus propranolol) also rapidly induced c-*jun* mRNA expression in cells transfected with any of the three

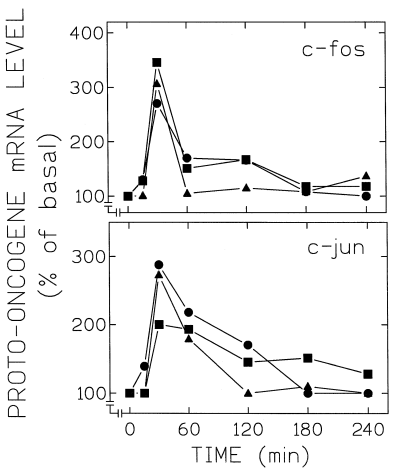

Fig. 1. Time course of the α_1-adrenoceptor stimulation of proto-oncogene mRNA level in rat-1 fibroblasts. Cells stably transfected with the cloned α_1-adrenoceptor subtypes (α_{1a}, circles; α_{1b}, triangles and α_{1d}, squares) were incubated with 10 μM noradrenaline (plus 10 μM propranolol) for the times indicated and the expression of *c-fos* (upper panels) and *c-jun* (lower panels) was determined. Data are presented as percentages of basal (absence of agonist) mRNA level for each cell line and condition. The average of 3 experiments using different cell cultures is presented (standard error bars were omitted for clarity).

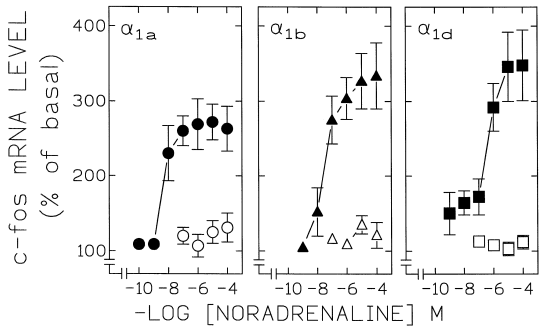

Fig. 2. Effect of noradrenaline on *c-fos* mRNA level in rat-1 fibroblasts and role of protein kinase C down-regulation. Cells stably transfected with the cloned α_1-adrenoceptor subtypes (α_{1a}, circles; α_{1b}, triangles and α_{1d}, squares) were preincubated overnight in the absence (closed symbols) or presence (open symbols) of 100 nM PMA and further incubated in the presence of different concentrations of noradrenaline (plus 10 μM propranolol) for 30 min. Data are presented as percentages of basal mRNA level for each cell line and condition. Plot shows the means and vertical lines represent the S.E.M. of 7–10 determinations using different cell cultures (where no error bar is presented the value is within the symbol).

cloned α_1-adrenoceptor subtypes which also reached its maximum at 30 min. However, the return towards basal levels of expression was slower for the mRNA for this proto-oncogene than for c-*fos* and the effect observed with cells transfected with the α_{1d} subtype was consistently smaller than the effect in cells transfected with the other subtypes (Fig. 1, lower panel). The noradrenaline-induced expression of these mRNA proto-oncogenes was abolished in the presence of 1 μM prazosin, confirming that such effects were mediated through α_1-adrenoceptors (data not shown).

The dose–response curves for noradrenaline (in the presence of 10 μM propranolol) for the expression of the mRNA for c-*fos* and c-*jun* are presented in Figs. 2 and 3,

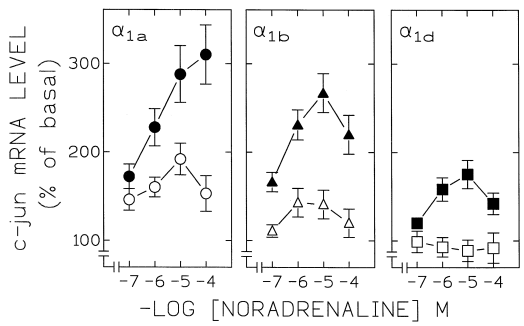

Fig. 3. Effect of noradrenaline on *c-jun* mRNA level in rat-1 fibroblasts and role of protein kinase C down regulation. Cells stably transfected with the cloned α_1-adrenoceptor subtypes (α_{1a}, circles; α_{1b}, triangles and α_{1d}, squares) were preincubated overnight in the absence (closed symbols) or presence (open symbols) of 100 nM PMA and further incubated in the presence of different concentrations of noradrenaline (plus 10 μM propranolol) for 30 min. Data are presented as percentages of basal mRNA level for each cell line and condition. Plot shows the means and vertical lines represent the S.E.M. of 7–10 determinations using different cell cultures (where no error bar is presented the value is within the symbol).

respectively. It can be observed that noradrenaline induced dose-dependent increases of c-*fos* mRNA in the three cell lines. The magnitude of the effects was very similar with the order of efficacy: $\alpha_{1d} = \alpha_{1b} = \alpha_{1a}$. Interestingly, the potency of noradrenaline to induce c-*fos* was significantly different for these cell lines. The EC_{50} values were: 5 ± 1 nM for cells transfected with the α_{1a} subtype, 30 ± 5 nM for cells transfected with the α_{1b} subtype and 300 ± 65 nM for those transfected with the α_{1d} subtype (data are the means $\pm$ S.E.M. of 7–10 determinations using different cell cultures; α_{1a} vs. α_{1d}, $P < 0.001$; α_{1b} vs. α_{1d}, $p < 0.001$; α_{1a} vs. α_{1b}, not significant) (Fig. 2).

Noradrenaline increased in a dose-dependent fashion the level of c-*jun* mRNA in the three lines (Fig. 3). No adrenergic effect was observed at concentrations below 100 nM, on the level of c-*jun* mRNA, in any of the cell lines (data nor shown). Interestingly, the potencies were very similar with EC_{50} values of ≈ 300 nM (Fig. 3), but the efficacies were clearly different, i.e., noradrenaline induced a slightly greater effect in cells transfected with the α_{1a} subtype than in those transfected with the α_{1b} subtype, but in cells transfected with the α_{1d} receptor the effect was much smaller (α_{1a} vs. α_{1d}, $P < 0.05$; α_{1b} vs. α_{1d}, $p < 0.05$; α_{1a} vs. α_{1b}, not significant). Similar effects were observed in cells incubated in the absence of propranolol (data not shown). The mRNA level of GAPDH was not modified by any of the agents employed (data not shown). Representative dot blot autoradiographs are presented in Fig. 4.

It is well known that activation of protein kinase C leads to expression of c-*fos* and c-*jun* in many cells (Curran and Morgan, 1987; Ransone and Verma, 1990; Herschman, 1991; Treisman, 1992). PMA (1 μM) increased $\approx$ 2-fold c-*fos* and c-*jun* mRNA levels of expression in the three cell lines (Fig. 5). Staurosporine (10 μM) an inhibitor of protein kinase C, did not affect basal mRNA expression of these proto-oncogenes (data not shown), but abolished the actions of PMA (Fig. 5). It is also known that sustained activation of protein kinase C with active phorbol esters induces down-regulation of the enzyme and cell refractoriness to PMA (Rodriguez-Peña and Rozengurt, 1984). Pretreatment (18 h) of rat-1 fibroblasts with PMA depletes the cells of measurable protein kinase C activity (Muldoon et al., 1990). In our studies, cells preincubated overnight ($\approx$ 18 h) with 100 nM PMA showed an increase ($\approx$ 30–50%) in the basal level of c-*fos* and c-*jun* mRNAs when compared to the results of parallel incubations in the absence of PMA (data not shown). In cells preincubated overnight with PMA the acute addition of 1 μM of this phorbol ester did not further increase proto-oncogene mRNA expression (Fig. 5).

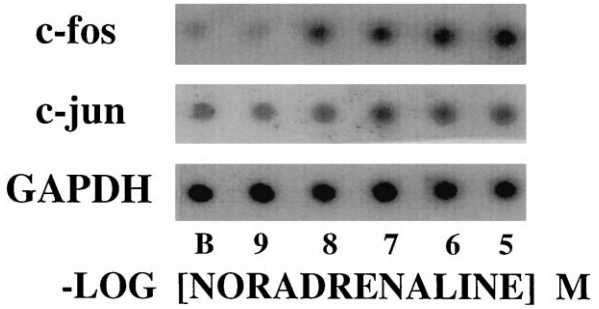

Fig. 4. Representative dot blot autoradiographs of the effect of noradrenaline on *c-fos*, *c-jun* and GAPDH mRNA levels in rat-1 fibroblasts stably expressing α_{1a}-adrenoceptors B, basal.

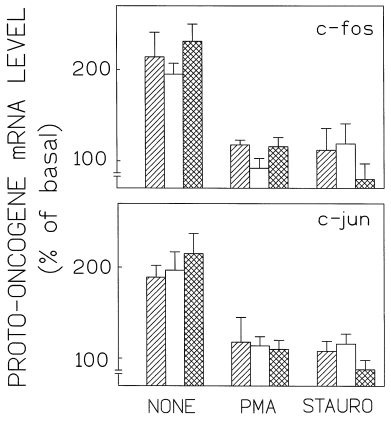

Fig. 5. Effect of protein kinase C on proto-oncogene mRNA levels in rat-1 fibroblasts. Cells stably transfected with the cloned α_1-adrenoceptor subtypes (α_{1a}, hatched bars; α_{1b}, open bars and α_{1d}, crisscrossed bars) were preincubated overnight in the absence (NONE and STAURO) or presence (PMA) of 100 nM PMA and further incubated in the presence of 1 μM PMA (NONE and PMA) or 1 μM PMA plus 10 μM staurosporine (STAURO) for 30 min. Data are presented as percentage of basal mRNA level for each cell line and condition. Plot shows the means and vertical lines represent the S.E.M. of 7–10 determinations, using different cell cultures.

In all these experiments, filters containing identical samples were hybridized with the GAPDH gene probe; no significant change in response to the adrenoceptor agonists or active phorbol esters was observed (not shown, see Fig. 4), indicating that such actions were not general effects on gene transcription but rather specific effects on the expression of early genes.

In order to explore the role of protein kinase C in the α_1-adrenoceptor-stimulated proto-oncogene mRNA expression the effects of staurosporine and overnight pretreatment with PMA were studied. It was observed that overnight pretreatment with PMA markedly inhibited the effect of noradrenaline on the level of c-*fos* and c-*jun* mRNAs (Figs. 2, 3 and 6) in the cell lines transfected with any of the cloned receptor subtypes. Similarly, staurosporine inhibited the effect of noradrenaline on proto-oncogene mRNA expression in the three cell lines (Fig. 6).

Endothelin-1 is a potent activator of proto-oncogene expression in rat-1 fibroblasts (Muldoon et al., 1990; Pribnow et al., 1992; Daub et al., 1996). In the cell lines studied here, we observed that this peptide induces 2 to 3-fold increases in the levels of c-*fos* and c-*jun* mRNAs. The magnitude of the stimulations was similar for the three

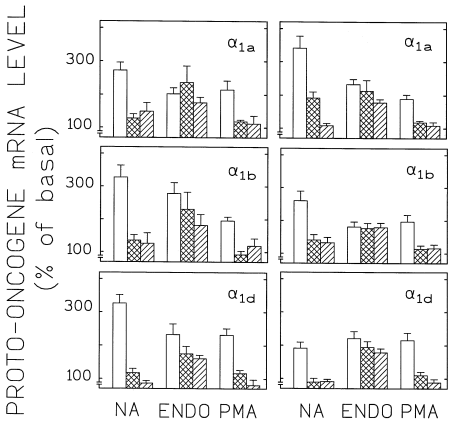

Fig. 6. Effects of noradrenaline, endothelin-1 and PMA on proto-oncogene mRNA level in rat-1 fibroblasts. Cells stably transfected with the cloned α_1-adrenoceptor subtypes (α_{1a}, upper panels; α_{1b}, middle panels and α_{1d}, lower panels). Right panels, *c-fos*; left panels, *c-jun*. Open bars represent cells preincubated without any agent and incubated 30 min with the agent indicated; cross-hatched bars represent cells preincubated overnight with 100 nM PMA and incubated 30 min with the agent indicated; hatched bars represent cells preincubated without any agent and incubated 30 min with the agent indicated plus 10 μM staurosporine. NA, noradrenaline 10 μM plus 10 μM propranolol, ENDO, 10 nM endothelin-1. Data are presented as percentages of basal mRNA level for each cell line and condition. Plot shows the means and vertical lines represent the S.E.M. of 5–7 determinations, using different cell cultures.

cell lines and, interestingly, the effect of the peptide was not blocked by either staurosporine or overnight pretreatment with PMA (Fig. 6).

In order to gain a further insight into the role of the different protein kinase C isoforms in the induction of proto-oncogene mRNA expression, the effect of overnight treatment with PMA on the relative amounts of different protein kinase C isoforms was evaluated by Western blot analysis. It is known that rat-1 fibroblasts express the protein kinase C α, δ, ϵ and ζ isoforms (Berti et al., 1994). It can be observed in Fig. 7 that sustained activation of protein kinase C with this phorbol ester markedly decreased the immunologically detectable α ($80 \pm 5\%$ decrease; mean $\pm$ S.E.M., $n = 3$), δ ($75 \pm 5\%$ decrease; mean $\pm$ S.E.M., $n = 3$), and ϵ ($66 \pm 5\%$ decrease; mean $\pm$ S.E.M., $n = 3$) isoforms with little change in the detectable amount of the ζ isoform ($3 \pm 2\%$ decrease; mean $\pm$ S.E.M., $n = 3$) (Fig. 7).

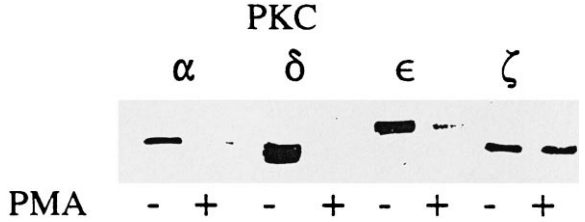

Fig. 7. Effect of overnight treatment with PMA on the immunologically detectable amounts of protein kinase C (PKC) isoforms. A representative immunoblot is presented, from 6 performed with identical results.

4. Discussion

Our present results clearly indicate that activation of any of the three cloned α_1-adrenoceptor subtypes results in an increased level of early gene mRNAs such as those for c-*fos* or c-*jun* and that protein kinase C plays a key role in mediating such effects. However, it is also clear that some differences exist. The density of α_1-adrenoceptors was similar in the three cell lines (Vázquez-Prado and García-Sáinz, 1996 and confirmed in the present work). There is no direct correlation between receptor densities and the magnitude of the adrenergic responses. We have observed that the order of efficacy of these receptors to increase [^{3}H]IP$_3$ (inositol 1, 4, 5 trisphosphate) production and [Ca^{2+}]$_I$ (intracellular calcium concentration) was: $\alpha_{1a} > \alpha_{1b} > \alpha_{1d}$ (Vázquez-Prado and García-Sáinz, 1996). Recently, an identical order of coupling efficiency ([^{3}H]IP$_3$ production and [Ca^{2+}]$_I$) was reported for the human α_1-adrenoceptor subtypes (Theroux et al., 1996). The same order of efficacy was observed in the present study for c-*jun* mRNA expression (Fig. 3), but the results obtained for c-*fos* mRNA level ($\alpha_{1d} = \alpha_{1b} = \alpha_{1a}$) differed markedly (Fig. 2). The EC_{50} values determined in the studies on signal transduction ([^{3}H]IP$_3$ production and [Ca^{2+}]$_I$, $\approx$ 200–500 nM (Vázquez-Prado and García-Sáinz, 1996)) were very similar to those for c-*jun* mRNA expression ($\approx$ 300 nM) but again, they were markedly different from those observed for c-*fos* mRNA (5, 30 and 300 nM for cells transfected with α_{1a}-, α_{1b}- or α_{1d}-adrenoceptors, respectively). Interestingly, there was a good correlation between the sensitivity to noradrenaline for c-*fos* mRNA level ($\alpha_{1a} > \alpha_{1b} > \alpha_{1d}$) and the order of efficacy for signal transduction ([^{3}H]IP$_3$ production and [Ca^{2+}]$_I$ (Vázquez-Prado and García-Sáinz, 1996)).

It is clear that very different levels of receptor occupation were required to increase the level of c-*fos* and c-*jun* mRNAs through α_{1a}-adrenoceptors (60-fold difference in EC_{50} values), and that, for the other receptor subtypes, either the difference was smaller (α_{1b}-adrenoceptor, 10-fold difference in EC_{50} values) or there was no difference (α_{1d}). These data are puzzling and their interpretation challenging. It is possible that one of the pathways for proto-oncogene (c-*fos*) mRNA expression could be more sensitive to the amplification of the signaling cascade than the other (c-*jun*), and/or that additional signaling devices could participate and magnify the responses. On the one hand, α_1-adrenoceptors are known to activate the phosphoinositide turnover/calcium mobilization signal transduction system, but there is evidence that other signaling pathways may also be activated by these receptors and that they can participate in some of the actions (García-Sáinz, 1993; Minneman and Esbenshade, 1994; Graham et al., 1996). On the other hand, although both c-*fos* and c-*jun* are rapidly and transiently expressed in many cells, this is not always co-regulated (Herschman, 1991). The amount of mRNA is the result of the balance between gene

transcription and mRNA degradation. It is clear that expression of early genes is due to both increased transcription (Curran and Morgan, 1987; Ransone and Verma, 1990; Herschman, 1991; Treisman, 1992) and regulation of mRNA stability (Schiavi et al., 1992). The signaling pathways employed to modulate transcription of these proto-oncogenes seem to differ; i.e., the signal transduction pathways for c-*fos* induction appear to converge at the Serum response element of the c-*fos* promoter whereas induction of c-*jun* expression seems to occur at a AP-1 site (Herschman, 1991).

Protein kinase C seems to play a cardinal role in mediating the α_1-adrenoceptor stimulation of proto-oncogene mRNA expression, as evidenced by the blockade by both staurosporine and overnight treatment with PMA. The down regulation observed for the α, δ and ϵ isoforms of this kinase suggests that they may be involved in such effects. It is interesting to mention that a similar treatment abolished the blockade of the α_1-adrenoceptor actions induced by protein kinase C activation (Vázquez-Prado and García-Sáinz, 1996). This suggests that such isoforms of protein kinase C may be also involved in the functional modulation of these receptors. It is clear that the role of each of the different isoforms in these actions needs to be addressed directly.

The action of endothelin-1 was an important control in these experiments. It is clear that down regulation of protein kinase C or inhibition of this family of enzymes did not block the mRNA expression of proto-oncogenes induced by the peptide. These data are consistent with the previous demonstration that the ability of endothelin-1 to induce second messenger production and transcription of c-*fos* and c-*jun* is largely independent of protein kinase C activity in rat-1 fibroblasts (Muldoon et al., 1990). Calcium (Pribnow et al., 1992) and transactivation of the epidermal growth factor (EGF) receptor (Daub et al., 1996) seem to play prominent roles in the action of this potent endothelial peptide on proto-oncogene mRNA expression.

In summary, our data clearly indicate that activation of any of the three cloned adrenoceptor subtypes can increase the amount of c-*fos* and c-*jun* mRNAs and that protein kinase C plays a major role in mediating such effects. Differences in the magnitude of the responses and in the sensitivity to noradrenaline were evidenced. These actions are likely involved in the long-term actions induced by α_1-adrenoceptor activation.

Acknowledgements

This research was partially supported by Grants from DGAPA (IN200596) and CONACyT (3170P-N9608). The authors also express their gratitude to Dr. Claudia González-Espinosa for her help and advice. The authors express their gratitude to Dr. F. López, Dr. L. Allen, Dr. M.G. Caron and Dr. R.J. Lefkowitz for the donation of the rat-1 fibroblast cell lines.

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