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Effect of Phorbol Myristate Acetate on α_1-Adrenergic Action in Cells Expressing Recombinant α_1-Adrenoceptor Subtypes

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SUMMARY

We studied the ability of norepinephrine to stimulate [^{3}H]inositol trisphosphate production and calcium mobilization in rat-1 fibroblasts stably expressing the cloned α_1-adrenergic subtypes and their sensitivity to phorbol-12-myristate-13-acetate (PMA). It was observed that the three subtypes were able to activate this signal transduction process, but marked differences in efficacy were noted. The order of efficacy was $\alpha_{1a} > \alpha_{1b} > \alpha_{1d}$ in cells with similar receptor densities. The use of PMA blocked the α_{1d} responses at lower concentrations than those needed to block the α_{1b} action; little effect of PMA was observed on effects mediated through α_{1a}-adrenoceptors. These effects of PMA were not observed in cells preincubated overnight with the tumor promoter, suggesting that such actions were mediated through isoform or isoforms of protein kinase C subjected to down-regulation. The repetitive addition of norepinephrine induced desensitization of the α_1-adrenergic action in cells expressing any of the three subtypes, and this effect was not altered by overnight pretreatment with PMA. Our data indicate that there are differences in sensitivity to PMA among the α_1-adrenoceptor subtypes stably expressed in rat-1 fibroblasts.

Three distinct α_1-adrenoceptor subtypes have been cloned (1–4). As recommended by the International Union of Pharmacology Committee for Receptor Nomenclature and Drug Classification (5), they are referred to as α_{1a}- (1), α_{1b}- (2), and α_{1d}- (3, 4) adrenergic receptors. All of these subtypes seem to be primarily coupled to the hydrolysis of phosphoinositides (6–9) via G proteins of the G_q/G_{11} family (9), with subsequent stimulation of phospholipase Cβ activity. They can also couple to other signal transduction pathways (6, 8), but this varies among cell types (8). The existence of several receptor subtypes that modulate the same transduction pathways suggests the possibility that differences may exist in their regulation. Different regulatory properties have been described for members of the β- and α_2-adrenoceptor families (10, 11).

Activation of protein kinase C with active phorbol esters, such as PMA, blocks α_1-adrenergic actions (12–19), which is associated with receptor phosphorylation (14). This action of protein kinase C seems to be physiologically relevant in homologous and heterologous desensitization of the α_1-adrenergic action (15–17). However, other protein kinases also participate in these processes (18, 19).

Most of the studies on protein kinase C-mediated blockade and/or desensitization of the α_1-adrenergic action have been carried out in cells that either express naturally the α_{1B}-adrenergic receptor [e.g., rat hepatocytes (20) or DDT_1 MF-2 cells (2, 14, 17)] or have been transfected with this receptor subtype. This raises the possibility that different subtypes may have distinct sensitivities to the action of this protein kinase. We observed that hepatocytes obtained from different species express different α_1-adrenoceptor subtypes (20) and that such receptor subtypes vary in their sensitivity to phorbol esters (21). However, the observed differences in sensitivity could be due not only to the receptor subtype expressed by the cells but also to other differences in the molecular repertoires expressed in the distinct hepatocytes. Therefore, comparison of the receptor subtype sensitivity to PMA in the same cell type seems to be required to reveal intrinsic differences among these adrenoceptors. To the best of our knowledge, no such comparison has been reported. We characterized α_1-adrenergic responses (increase in $[Ca^{2+}]_i$ and [^{3}H]InsP_3 production) with the use of fibroblasts transfected with the different cloned α_1-adrenergic subtypes and their sensitivity to PMA. Our results suggest that distinct subtypes have different sensitivities to protein kinase C activation.

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Experimental Procedures

Materials. (−)-Norepinephrine, endothelin, PMA, and (±)-propranolol were obtained from Sigma Chemical Co (St. Louis, MO).

ABBREVIATIONS: $[Ca^{2+}]_i$, intracellular calcium concentration; PMA, phorbol-12-myristate-13-acetate; InsP, inositol phosphate; DMEM, Dulbecco's modified Eagle's medium; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Fura-2/AM was obtained from Molecular Probes (Eugene, OR). Phentolamine was a generous gift from Ciba-Geigy (Summit, NJ). DMEM, fetal bovine serum, trypsin, antibiotics, and other reagents used for cell culture were from GIBCO BRL (Gaithersburg, MD). [^{3}H]Prazosin (74.4 Ci/mmol) and *myo*-[2-^{3}H]inositol (22.9 Ci/mmol) were from New England Nuclear (Boston, MA).

Stably transfected cell lines. Rat-1 fibroblasts clones stably expressing one of the different cloned α_1-adrenoceptors [α_{1a}, cloned from bovine brain (1); α_{1b}, cloned from DDT$_1$ MF-2 cells (2); and α_{1d}, cloned from rat brain (3)] were kindly provided by Drs. L. Allen, R. J. Lefkowitz, and M. G. Caron (Duke University, Departments of Medicine and Cardiology, Durham, NC). Transfected rat-1 fibroblasts were grown 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° under a 5% CO_2 atmosphere. For selection, cells were cultured in the presence of the neomycin analogue G-418 sulfate (300 μg/ml).

Northern blot analysis. Expression of the mRNA for the transfected α_1-adrenoceptors was evaluated with Northern blot analysis. Total RNA (40 μg) obtained according to the method of Chomczynski and Sacchi (22) 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 with nick-translated probes (23). In brief, membrane filters were prehybridized for 4 hr at 55° in a solution containing 35% formamide, 10% dextran sulfate, 5× Denhardt's reagent (1× = 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone), 5× SSPE (1× = 0.15 M NaCl, 0.01 M NaH_2PO_4, 0.001 M EDTA, pH 7.4), 1% SDS, and 500 μg/ml denatured salmon sperm DNA. The probes used were an 812-bp *Bgl*II fragment of the bovine α_{1a}-adrenoceptor cDNA (1), an 803-bp *Eco*RI/*Bam*HI fragment of the hamster α_{1b}-adrenoceptor cDNA (2), and an 850-bp *Bam*HI fragment of the rat α_{1d}-adrenoceptor cDNA (3). Hybridization lasted 16–20 hr at 55°. The membranes were washed twice for 15 min in 0.2× standard saline citrate (1× = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0)/0.2% SDS at 55° and twice for 15 min in 0.1× standard saline citrate/0.1% SDS at 55°. The membranes were exposed to X-ray film at −70° with one intensifying screen.

Membrane preparation and [^{3}H]prazosin binding. Confluent transfected rat-1 fibroblasts cultured in 10-cm Petri dishes were scraped with a rubber policeman and pelleted through centrifugation. Membrane preparation was performed according to Mattingly *et al.* (24). Protein concentration was determined according to the method of Lowry *et al.* (25) with bovine serum albumin as standard. [^{3}H]Prazosin saturation isotherms were performed by incubating the radioligand with the membranes (25 μg of protein) in a final volume of 0.25 ml of buffer (50 mM Tris, 10 mM $MgCl_2$, pH 7.5) for 60 min at 25° in a shaking water bath. Incubation was terminated by the addition of 5 ml of ice-cold buffer and filtration through GF/C filters with a Brandel harvester. Filters were washed twice and dried, and radioactivity was measured with a liquid scintillation counter. Nonspecific binding was determined in the presence of 10 μM phentolamine. Saturation curves were analyzed by the EBDA program (Biosoft-Elsevier, Cambridge, UK).

[^{3}H]InsP$_3$ production. Cells approaching confluence on 35-mm plates were labeled with [^{3}H]inositol (6 μCi/ml) for 18–24 hr in 1 ml of inositol-free DMEM containing 1% fetal bovine serum. On the day of the experiment, cells were washed twice with Krebs-Ringer-HEPES buffer (1 × = 120 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.75 mM KCl, 10 mM glucose, 20 mM HEPES, and 0.05% bovine serum albumin, pH 7.4) containing 1.3 mM $CaCl_2$ and preincubated for 20 min in 2 ml of the same buffer containing 10 mM LiCl at 37° in a 5% CO_2 atmosphere. Incubations with norepinephrine and/or PMA were done for 15 min and were terminated by the addition of 0.4 ml of 30% ice-cold perchloric acid; plates were stored at −20° until the next day. Supernatants of thawed plates were neutralized, and [^{3}H]InsPs (InsP$_1$, InsP$_2$, and InsP$_3$) were separated through Dowex AG1-X8 chromatography (26). [^{3}H]InsP$_3$ production is presented.

[Ca^{2+}]$_i$ measurements. Confluent rat-1 fibroblasts expressing the adrenergic receptors were incubated overnight in G-418-free DMEM containing 1% serum; on the day of the experiment, cells were loaded with 5 μM Fura-2/AM in the same medium at 37° for 1 hr. After loading, the cells were washed with Krebs-Ringer-HEPES buffer containing 0.05% bovine serum albumin, pH 7.4; scraped from the plate with a rubber policeman; and washed again three times with the use of centrifugation to remove unincorporated dye (in some experiments, the cells were detached with trypsin; identical results were obtained). Cells were resuspended in the same buffer at a concentration of ~10^6 cells/ml. Fluorescence measurements were carried out with 2-ml cell suspensions (5 × 10^5 cells/cuvette) and constant stirring at 37° in an AMINCO-Bowman Series 2 luminescence spectrometer with excitation at 340 nm and emission at 510 nm. [Ca^{2+}]$_i$ was calculated according to the method of Grynkiewicz *et al.* (27) as described previously (21).

Results

The expression of the different α_1-adrenoceptors was studied in the three transfected lines, at both the mRNA and receptor levels. Binding saturation experiments indicate that, as expected, the cells expressed a relatively high level of receptors that was similar in the three cell lines (Fig. 1), although consistently the cell line expressing α_{1b}-adrenoceptors had higher values. The density of receptors and their affinities for [^{3}H]prazosin in membranes from these cell lines were as follows: (a) cells expressing α_{1a}-adrenoceptors, B_{max} = 1.04 ± 0.12 pmol/mg protein, K_D = 0.36 ± 0.04 nM; (b) cells expressing α_{1b}-adrenoceptors, B_{max} = 2.04 ± 0.47 pmol/mg protein, K_D = 0.30 ± 0.04 nM; and (c) cells expressing α_{1d}-adrenoceptors, B_{max} = 0.79 ± 0.11 pmol/mg protein, K_D = 0.29 ± 0.03 nM (results are mean ± standard error of six experiments in each case with different membrane preparations). A very strong signal was obtained in the Northern analysis (Fig. 2), which confirmed that each cell line expressed the expected receptor subtype.

Functional parameters were examined. Basal [Ca^{2+}]$_i$ and [^{3}H]InsP$_3$ productions were similar in the three cell lines (see

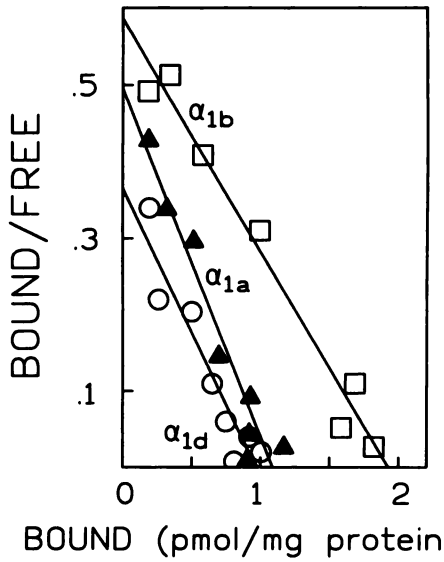

Fig. 1. Rosenthal analysis of saturation isotherms. [^{3}H]Prazosin binding to membranes from rat-1 fibroblasts stably expressing α_{1a}- (▲), α_{1b}- (□), or α_{1d}- (○) adrenoceptors was performed as indicated in Experimental Procedures. Data are representative of six experiments with different membrane preparations.

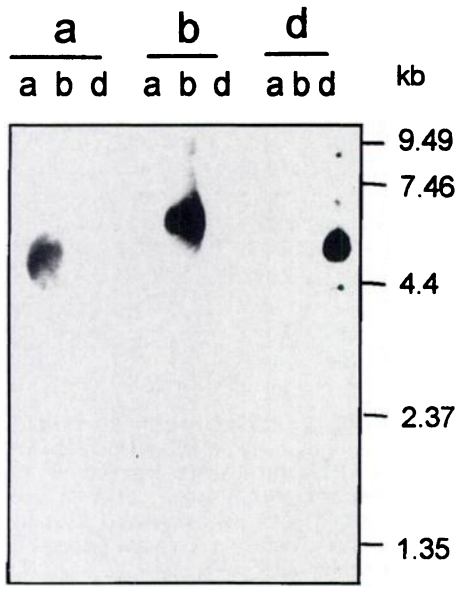

Fig. 2. Northern analysis of RNA obtained from rat-1 fibroblasts stably transfected with α_1-adrenoceptor subtypes. Total RNA obtained from cells transfected with the α_{1a}- (*lanes a*), α_{1b}- (*lanes b*), or α_{1d}- (*lanes d*) adrenoceptor genes was electrophoresed, transferred to membranes, and hybridized using labeled receptor probes (*top*). Autoradiograph is representative of eight experiments performed with identical results.

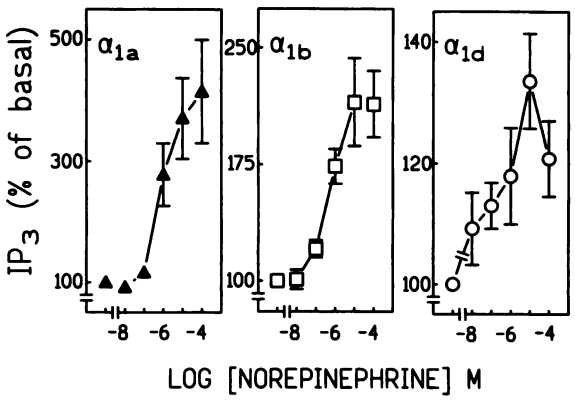

Fig. 4. Effect of norepinephrine on [^{3}H]InsP$_3$ (*IP$_3$*) production in rat-1 fibroblasts expressing α_{1a}- (▲), α_{1b}- (■), or α_{1d}- (○) adrenoceptors. Results are presented as percentage of basal [^{3}H]InsP$_3$ productions: (a) α_{1a} cells, 1343 ± 318 cpm/well; (b) α_{1b} cells, 1675 ± 340 cpm/well; and (c) α_{1d} cells, 1547 ± 330 cpm/well. Data are mean values. *Vertical lines*, standard error of five or six experiments in each case.

legends to Figs. 3 and 4). Norepinephrine induced an immediate increase in $[Ca^{2+}]_i$, followed by a slower decrease toward basal levels; the peak of the immediate $[Ca^{2+}]_i$ response was used to calculate the dose-response curves. For [^{3}H]InsP$_3$ production, maximal accumulation was observed 15 min after hormone addition, and this time was selected for these experiments. Cells expressing the α_{1a} subtype had a very strong response to norepinephrine in both $[Ca^{2+}]_i$ and [^{3}H]InsP$_3$ production (Figs. 3 and 4, respectively). In these cells, the agonist induced an ~8-fold increase in $[Ca^{2+}]_i$ and an ~4-fold increase in [^{3}H]InsP$_3$ production, with similar EC_{50} values (i.e., ~300 nM and ~500 nM, respectively). Cells expressing the α_{1b}-adrenoceptor increased $[Ca^{2+}]_i$ (~3-fold) and [^{3}H]InsP$_3$ (~2-fold) production in response to norepinephrine (Fig. 3), with an EC_{50} of ~200 nM for both processes. Interestingly, in the rat-1 fibroblasts that expressed α_{1d}-adrenoceptors, a very modest increase in [^{3}H]InsP$_3$ production was observed (~30% increase over basal levels, Fig. 4), and the maximal increase observed in the dose-response curve for $[Ca^{2+}]_i$ was ~150% over basal levels (Fig. 3). As expected, the functional responses to 1 μM norepinephrine were blocked by 10 μM phentolamine in the three cell lines studied (data not shown).

The acute effect of PMA on the α_1-adrenergic action was studied. Fibroblasts expressing the different adrenergic receptor subtypes were incubated in the presence of 10 μM norepinephrine in the absence or presence of different concentrations of PMA, and [^{3}H]InsP$_3$ production was assayed. PMA by itself did not alter basal [^{3}H]InsP$_3$ production. In cells expressing the α_{1d} subtype, PMA inhibited the α_1-adrenergic response at low concentrations (Fig. 5). In contrast, the adrenergic actions in cells expressing α_{1a}-adrenoceptors were essentially insensitive to the active phorbol ester (Fig. 5). Rat-1 fibroblasts that expressed α_{1b} -adrenoceptors had an intermediate response; i.e., PMA inhibited the effect of

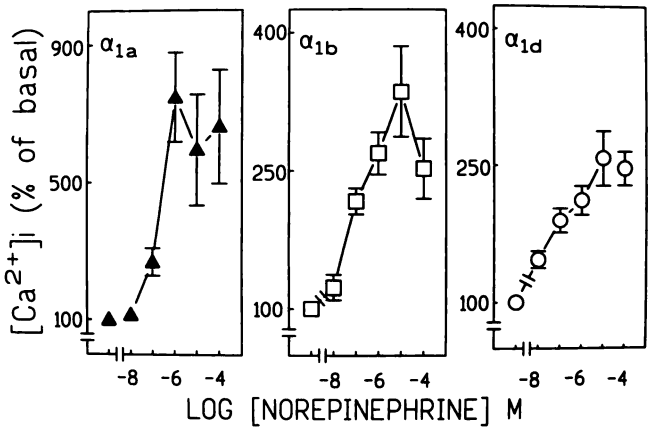

Fig. 3. Effect of norepinephrine on $[Ca^{2+}]_i$ in rat-1 fibroblasts expressing α_{1a}- (▲), α_{1b}- (□), or α_{1d}-(○) adrenoceptors. Results are presented as a percentage of basal $[Ca^{2+}]_i$ values: (a) α_{1a} cells, 211 ± 4 nM; (b) α_{1b} cells, 192 ± 3 nM; and (c) α_{1d} cells, 206 ± 8 nM. Data are mean values. *Vertical lines*, standard error of 5–12 experiments in each case.

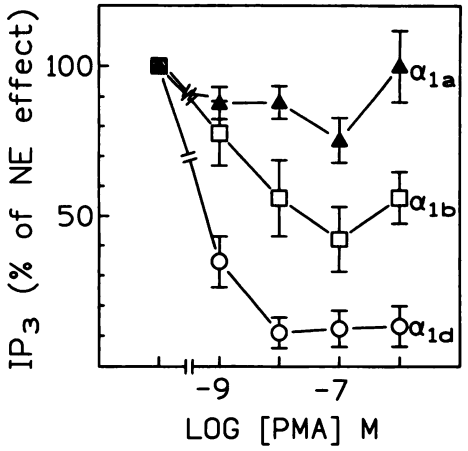

Fig. 5. Effect of PMA on the α_1-adrenergic-mediated increase in [^{3}H]InsP$_3$ (*IP$_3$*) production. Cells were incubated in the presence of 10 μM norepinephrine (*NE*) and different concentrations of PMA; results are presented as percentage of norepinephrine effect. Other indications are as in legend to Fig. 3.

norepinephrine, but the blockade was only partial (~50%), and relatively high concentrations were required (Fig. 5).

The effect of PMA was also tested on the $[Ca^{2+}]_i$ response. For this purpose, PMA was added to the cells 30 sec before the agonist. The tumor promoter did not alter basal $[Ca^{2+}]_i$. In these experiments, a similar pattern was observed. PMA dose-dependently inhibited the effect of 10 μM norepinephrine in cells expressing α_{1b}-adrenoceptors (Fig. 6). Fibroblasts that expressed the α_{1d} subtype were very sensitive to the action of PMA; even with a relatively high concentration of agonist (100 μM norepinephrine), the active phorbol ester blocked dose-dependently the response at relatively low concentrations (Fig. 6). In contrast, cells that expressed the α_{1a} subtype were very resistant to the action of PMA; even when a relatively low concentration of agonist (1 μM norepinephrine) was used, the active phorbol ester did not inhibit the $[Ca^{2+}]_i$ response (Fig. 6). The use of different concentrations of agonist involves the actions of different mechanisms. To test this point, we challenged the cells with different concentrations of norepinephrine in the absence or presence of 1 μM PMA. The results showed that cells expressing α_{1a}-adrenoceptors were very insensitive to PMA, whereas those expressing the other subtypes were much more sensitive, and this effect was not dependent on the dose of agonist (data not shown).

It is well known that sustained activation of protein kinase C with active phorbol esters induces down-regulation of the enzyme and refractoriness to PMA (28). We tested the effect of overnight treatment with PMA (100 nM) on the α_1-adrenergic-mediated $[Ca^{2+}]_i$ response. Pretreatment with PMA did not affect the effect of norepinephrine on this parameter but abolished the acute effect of 1 μM PMA in cells expressing α_{1d}- or α_{1b}-adrenoceptors (Fig. 7). Fibroblasts expressing the α_{1a} subtype were, as expected, insensitive to PMA under these conditions.

When cells were challenged with increasing concentrations of norepinephrine, the $[Ca^{2+}]_i$ response decreased sequentially, indicating that the cells became desensitized. This effect was not due to depletion of the calcium pools in cells expressing the α_{1b} or α_{1d} subtype, as demonstrated by the ability of 10 nM endothelin to increase $[Ca^{2+}]_i$ (Fig. 8). In cells expressing the α_{1a} subtype, 10 nM endothelin induced a large increase in $[Ca^{2+}]_i$ but this effect was no longer observed in cells previously challenged with norepinephrine (Fig. 8). Interestingly, norepinephrine induced desensitizations identical to those just described in cells preincubated overnight with PMA (data not shown).

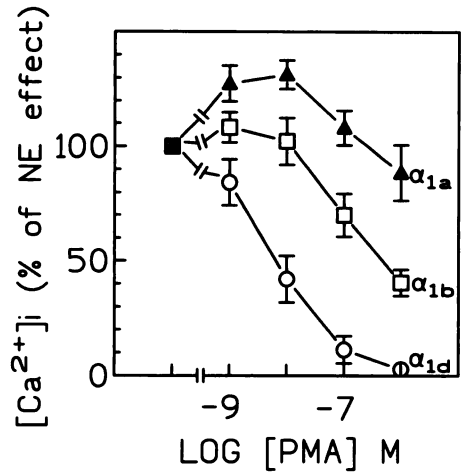

Fig. 6. Effect of PMA on the α_1-adrenergic-mediated increase in $[Ca^{2+}]_i$. Cells were incubated in the presence of norepinephrine (*NE*) (1 μM, α_{1a} cells; 10 μM, α_{1b} cells; and 100 μM, α_{1d} cells) and different concentrations of PMA. Results are presented as percentage of norepinephrine effects. Other indications are as in legend to Fig. 3.

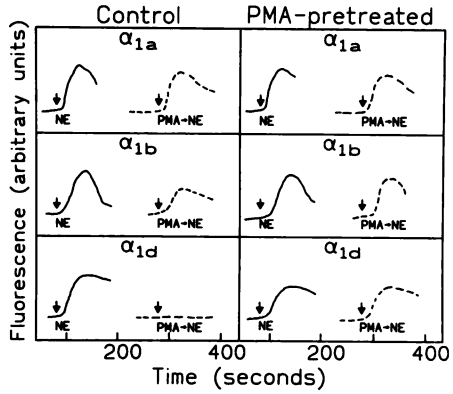

Fig. 7. Effect of overnight preincubation with 100 nM PMA on the acute effect of PMA on the α_1-adrenergic-mediated increase in $[Ca^{2+}]_i$. Cells were incubated overnight in the absence or presence of 100 nM PMA. The effect of norepinephrine (*NE*) (1 μM, α_{1a} cells; 10 μM α_{1b} cells; and 100 μM, α_{1d} cells) on $[Ca^{2+}]_i$ is presented. PMA (1 μM, *dotted lines*) was added 30 sec before norepinephrine. Data are representative of three separate experiments with each cell line.

Discussion

Our results indicate that the three cloned α_1-adrenoceptors expressed in rat-1 fibroblasts are coupled to the phosphoinositide/calcium signal transduction pathway. This is consistent with what has been observed by other authors using different cell lines (6–9, 29–32). It is particularly interesting that in rat-1 fibroblasts expressing similar receptor densities, the abilities of the α_1-adrenergic subtypes to activate this pathway differed. In our experiments, the α_{1a} subtype had considerable more efficacy to activate this signal transduction pathway than the other subtypes; the α_{1d} subtype had particularly low efficacy.

In some cell systems, receptor density seems to be associated with the ability of receptors to activate signaling pathways; Esbenshade *et al.* (31) observed a linear correlation between receptor density and maximum $[^3H]$InsP production

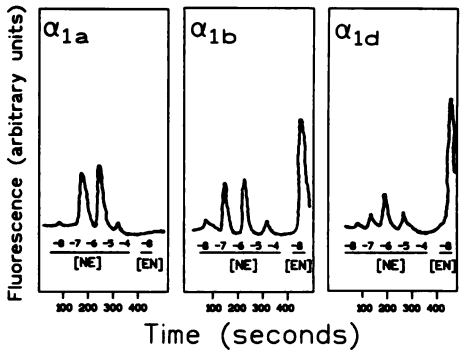

Fig. 8. Effect of sequential addition of norepinephrine (*NE*) on $[Ca^{2+}]_i$. Norepinephrine was added at the times and concentrations indicated. *EN*, endothelin. Data are representative of six or seven separate experiments with each cell line.

in DDT_1 MF-2 cells, and Horie and Tsujimoto (30) reported that the $[Ca^{2+}]_i$ response increased disproportionately in response to the α_{1b}-adrenoceptor density in CHO cells. In our experiments, the differences observed in receptor density do not seem to explain the distinct efficacy of the receptor subtypes; i.e., the receptor density of cells that expressed the α_{1a} subtype was smaller than that of the cells transfected with the α_{1b} subtype, but the maximal response of the former cells was considerable larger. Similarly, the response of the cells transfected with α_{1d} receptors was considerable smaller than that of the cells that expressed the α_{1a} subtype, despite the fact that the receptor densities of these two cell clones were very similar. However, we cannot ignore potential differences due to clonal variation. Wise *et al.* (32) also studied α_1-adrenergic actions in rat-1 fibroblasts transfected to express the α_1-adrenoceptor subtypes; these authors observed distinct receptor densities in their cells but did not detect any major variation in receptor efficacy. They reported that receptor occupation accelerated degradation of $G_{11\alpha}/G_{q\alpha}$; the agonist-induced degradation of these G proteins was greater in the cells expressing α_{1a}-adrenoceptors, but this was attributed to the higher observed receptor density (32).

The most interesting finding in our study was the difference observed in sensitivity to PMA. The order of sensitivity was $\alpha_{1d} > \alpha_{1b} \gg \alpha_{1a}$. Again, this difference does not seem to be explained by disparate receptor densities. However, a correlation exists with the ability of the subtypes to activate the signaling pathway. Comparison of the putative intracellular sequences of the three cloned subtypes revealed main differences in the intracellular loops and the carboxyl-terminal tail. These domains seem to be important both for G protein coupling and as target sites for phosphorylations. The putative protein kinase C phosphorylation sites [consensus sequence X-(Arg/Lys_{1-3},X_{0-2})-(**Ser/Thr**)-(X_{0-2},Arg/Lys_{1-3})-X] present in these receptors are (a) for the α_{1a} subtype, Thr224 (third intracellular loop) and Ser351 and Ser407 (carboxyl terminus) (none of these sites are conserved in the other receptor subtypes); (b) for the α_{1b} subtype, Thr233 (corresponding to Thr278 of the α_{1d} receptor), Thr252 (third intracellular loop), and Ser369 and Ser440 (carboxyl terminus); and (c) for the α_{1d} subtype, Ser195 (second intracellular loop), Thr278 and Thr322 (third intracellular loop), and Ser424, Ser448, and Ser480 (carboxyl terminus). There are other putative phosphorylation sites in these receptors. At this point, the role of these sites is unknown. However, it is worth mentioning that truncation of the α_{1b}-adrenoceptor carboxyl terminus impairs phosphorylation and desensitization (19).

Differences have been observed in the α_1-adrenergic sensitivity to PMA in hepatocytes isolated from diverse species and that express distinct α_1-adrenoceptors (20, 21); liver cells that express the α_{1B} subtype were more sensitive than those that express the α_{1A} receptor. Thus, the data obtained with liver cells that naturally express the receptor subtypes are consistent with those that were obtained with the transfected clones. The data obtained with cells pretreated overnight with PMA strongly suggest that this effect is mediated through isoform or isoforms of protein kinase C that are subjected to down-regulation. The isoform or isoforms of protein kinase C that mediate this effect have not been defined.

An additional interesting finding was the observation that repeated α_1-adrenergic stimulation induces desensitization of the response and that such desensitization is observed even in cells that have been pretreated overnight with PMA. These data indicate that processes in addition to protein kinase C activation are involved in α_1-adrenoceptor desensitization. Lattion *et al.* (19) presented evidence that receptor phosphorylation by protein kinases other than protein kinase C plays a role in α_{1b}-receptor desensitization. Our results are consistent with these findings. The type of desensitization induced by repeated stimulation with norepinephrine in cells that express the α_{1b}- and α_{1d}-adrenoceptors was homologous, as demonstrated by the ability of endothelin to elevate $[Ca^{2+}]_i$. In cells that express the α_{1a}-adrenoceptor, no $[Ca^{2+}]_i$ response to endothelin was observed after repeated stimulation with norepinephrine, which suggests that the adrenergic stimulation may have induced depletion of the hormone-sensitive calcium pools. This is consistent with the finding that this adrenoceptor subtype had greater efficacy than the other subtypes. However, the possibility that other processes could be involved cannot be ruled out.

PMA not only induces the blockade/desensitization of α_1-adrenoceptors but also alters the subcellular distribution of these receptors. Fonseca *et al.* (33) elegantly showed that PMA induced internalization to endosomes of α_{1b}-adrenoceptors stably expressed in human embryonic kidney 293 cells. This effect is also induced by agonists, and, interestingly, it is blocked by staurosporine, an inhibitor of protein kinase C, which suggests a physiological role of protein kinase C in agonist-induced receptor internalization (33).

The distribution of mRNA for the different α_1-adrenoceptor subtypes in human tissues has shown that in most tissues there is coexpression of several subtypes, although one of these subtypes may predominate (34). Signal transduction is the language through which cells communicate and transmodulate their responsiveness. Cells that receive a message may alter their responses differentially through receptor subtypes with diverse sensitivity to such modulation. In this physiological context, the observation that α_1-adrenergic subtypes have different sensitivities to protein kinase action may be of importance.

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