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Effects of phorbol ester on catecholamine secretion and protein phosphorylation in adrenal medullary cell cultures

(exocytosis/phorbol 12-myristate 13-acetate/calcium)

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ABSTRACT **The effects of phorbol 12-myristate 13-acetate (PMA) on catecholamine secretion and protein phosphorylation from intact and digitonin-treated chromaffin cells were investigated. PMA (10–300 nM), an activator of protein kinase C, caused a slow Ca^{2+}-dependent release of catecholamine from intact chromaffin cells that was potentiated by the Ca^{2+} ionophore ionomycin. PMA also enhanced secretion induced by Ba^{2+}. In cells with plasma membranes rendered permeable by digitonin to Ca^{2+}, ATP, and protein, PMA (100 nM) enhanced Ca^{2+}-dependent secretion ≈70% at 0.5 μM Ca^{2+} and 30% at 10 μM Ca^{2+}. PMA enhanced the maximal response to Ca^{2+} ≈25% and decreased the Ca^{2+} concentration required for half-maximal secretion ≈30%. The effects of PMA on chromaffin cells were associated with a 2- to 3-fold increase in the phosphorylation of a 56-kDa protein that may be tyrosine hydroxylase. Other proteins were phosphorylated to a lesser extent. The experiments suggest that PMA increases protein kinase activity and secretion in chromaffin cells and raise the possibility that protein kinase C modulates catecholamine secretion in chromaffin cells.**

The exocytotic release of catecholamines from bovine adrenal chromaffin cells is normally triggered by the influx of extracellular Ca^{2+} and the rise in cytosolic Ca^{2+} concentration that occur on stimulation of nicotinic receptors (1–4). Membrane depolarization in the presence of Ca^{2+} or the substitution of Ba^{2+} for Ca^{2+} also stimulates exocytosis. Although the mechanism of exocytosis was first described using biochemical techniques in the adrenal medulla, the underlying mechanisms triggered by Ca^{2+} are not understood.

One reaction that could be involved in exocytosis is protein phosphorylation. Previous studies concerning the role of protein phosphorylation in secretion from adrenal chromaffin cells revealed that two proteins, one of 56–60 kDa and one of 100 kDa, were phosphorylated when secretion was stimulated by secretagogues (5, 6). The 56–60-kDa protein was identified as tyrosine hydroxylase (7), the rate-limiting enzyme in catecholamine biosynthesis, and is probably not directly involved in exocytosis. The 100-kDa protein has not been identified but its phosphorylation is not well correlated with secretion.

Protein kinase C is Ca^{2+}-dependent and requires acidic phospholipids for activity (8, 9). Diglyceride in the presence of phosphatidylserine increases the Ca^{2+} sensitivity of the enzyme to micromolar or perhaps submicromolar concentrations (10). The ester phorbol 12-myristate 13-acetate (PMA) can substitute for diglyceride *in vitro* (11) and similarly increases the Ca^{2+} sensitivity of the enzyme. Most importantly, PMA activates protein kinase C and protein phosphorylation in intact platelets and enhances serotonin secretion (11, 12).

In the present study, we have investigated the effects of PMA on catecholamine secretion and protein phosphorylation in bovine adrenal chromaffin cells in monolayer culture. We have used both intact chromaffin cells and cells treated with a low concentration of digitonin to render the plasma membrane permeable to Ca^{2+}, ATP, and proteins (13, 14). Digitonin-treated cells secrete catecholamine directly in response to micromolar Ca^{2+} in the medium by a mechanism that is similar, if not identical, to exocytosis and can be used to investigate the intracellular events associated with exocytosis directly.

MATERIALS AND METHODS

Primary dissociated cells from bovine adrenal medulla were prepared and maintained as monolayer cultures as described (4). In some experiments, suspended chromaffin cells were purified by differential plating (15). Cells were usually cultured as monolayers in 6.4-mm-diameter plastic culture wells (Costar, Cambridge, MA) at a density of 500,000 cells per cm^2. Cellular catecholamine stores were labeled by incubation of intact cells with [^{3}H]norepinephrine and secretion was determined by measuring the percentage of total cellular radioactivity released into the medium (13). Experiments with intact cells were performed in physiological salt solution (145 mM NaCl/5.6 mM KCl/2.2 mM $CaCl_2$/0.5 mM $MgCl_2$/5.6 mM glucose/15 mM Hepes, pH 7.4/0.5 mM sodium ascorbate). Cells were rendered leaky with 20 μM digitonin in potassium glutamate solution [139 mM potassium glutamate/20 mM Pipes, pH 6.6/1 mM MgATP/5 mM EGTA containing various amounts of $CaCl_2$ to yield buffered Ca^{2+} concentrations of 0–20 μM (13)]. All experiments were performed at 25°C.

Intracellular phosphorylation in intact cells was determined by incubation of cells for 30 min in physiological salt solution containing $^{32}P_i$ (0.25–0.4 mCi/ml; 1 Ci = 37 GBq; carrier free), followed by incubation for 10 min in the same salt solution without $^{32}P_i$ and then by incubation in the salt solution containing various drugs. Experiments were usually terminated by replacing the medium with 0.15 ml of stop solution [3% $NaDodSO_4$/2% 2-mercaptoethanol/5% (vol/vol) glycerol/62 mM Tris·HCl, pH 6.7 /0.05% bromphenol blue]. Cellular proteins were denatured by incubation at 90°C for 3 min. Solution, 0.1 ml, was analyzed for phosphoprotein by $NaDodSO_4$/polyacrylamide (6.9% or 5–20% gradient) slab gel electrophoresis and subsequent autoradiography (16). For two-dimensional electrophoresis, incubations were terminated by addition of 0.03 ml of 9.5 M

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Abbreviation: PMA, phorbol 12-myristate 13-acetate.

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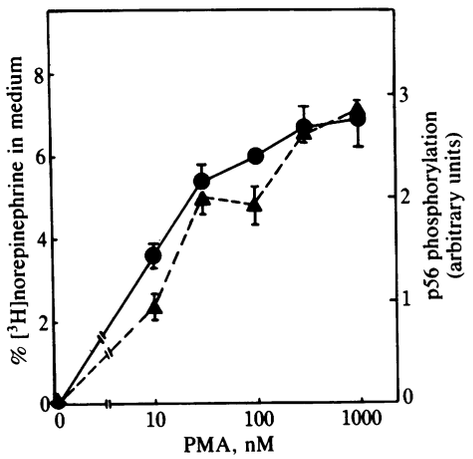

FIG. 1. Effect of PMA on catecholamine secretion and phosphorylation of p56 in intact chromaffin cells. Chromaffin cells were incubated in physiological salt solution containing various concentrations of PMA. Proteins were denatured by addition of trichloroacetic acid before addition of $NaDodSO_4$-containing solution. Proteins (12 μg) from each well were separated on one-dimensional $NaDodSO_4$/5–20% polyacrylamide gels. Percentage of the total catecholamine released into the medium (●) and amount of $^{32}P_i$ incorporation into p56 (▲) were determined after 60 and 30 min, respectively, in separate experiments. Background release of catecholamine and $^{32}P_i$ incorporation into p56 were 7% and 1.95, respectively. Each group contained four wells.

urea/2% Nonidet P-40/5% 2-mercaptoethanol/2% Ampholines (pH 3.5–10) to each 6.4-mm-diameter well. Aliquots (15 μl) were analyzed by isoelectric focusing followed by $NaDodSO_4$/5–20% gradient polyacrylamide gel electrophoresis according to O'Farrell (17).

The percentage of cellular lactate dehydrogenase activity released into the medium was determined according to Dunn and Holz (13) without desalting of the medium.

Data are expressed as mean ± SEM. Error bars smaller than the symbols or lines used in the figures have been

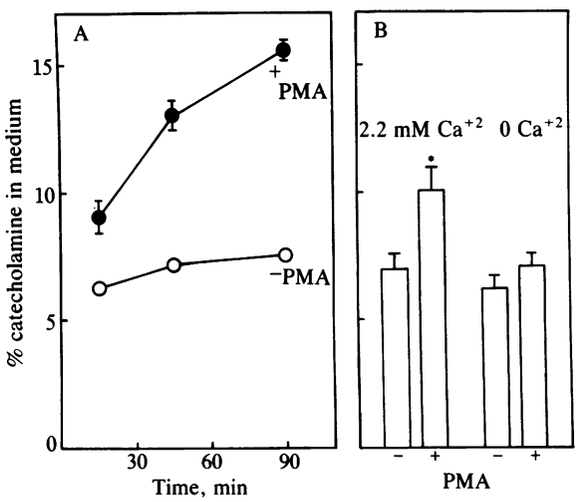

FIG. 2. Effect of PMA on catecholamine release from intact chromaffin cells. (*A*) Time course: intact cells were incubated in Ca^{2+}-containing physiological salt solution in the presence (●) or absence (○) of 160 nM PMA. At the indicated times, the percentage of endogenous catecholamine released into the medium was determined. (*B*) Ca^{2+} dependency: intact cells were incubated in physiological salt solution in the presence or absence of 2.2 mM Ca^{2+} with (+) or without (−) 100 nM PMA. After 30 min the percentage of endogenous catecholamine released into the medium was determined. In both experiments, each group contained four wells. *$P <$ 0.05 vs. 2.2 mM Ca^{2+}, 0 PMA.

Table 1. Effect of PMA on Ba^{2+}-induced secretion

	% [3H]norepinephrine in medium
Ca^{2+}, 0 PMA	6.2 ± 0.4
Ba^{2+}, 0 PMA	23.9 ± 0.6*
Ca^{2+}, 100 nM PMA	8.6 ± 0.5
Ba^{2+}, 100 nM PMA	32.7 ± 0.7*

Intact chromaffin cells prelabeled with [3H]norepinephrine were incubated in physiological salt solution containing 2.2 mM Ca^{2+} with or without 100 nM PMA. After 15 min, the solution was replaced with physiological salt solution containing either 2.2 mM Ca^{2+} or 2.2 mM Ba^{2+} in the continuing presence or absence of 100 nM PMA. After 5 min, the percentage of [3H]norepinephrine released into the medium was determined. Each group contained four wells.
*$P < 0.001$ vs. Ca^{2+}-containing solutions.

omitted. Differences between means of groups were tested for significance with Student's *t* test.

RESULTS

Effect of PMA on Catecholamine Release from Intact Chromaffin Cells. PMA caused a dose-dependent increase in catecholamine release from intact chromaffin cells with a half-maximal effect at ≈10 nM (Fig. 1). PMA-induced catecholamine secretion increased over 90 min (Fig. 2*A*) and was Ca^{2+}-dependent (Fig. 2*B*). The relatively slow catecholamine release induced by PMA contrasts with the rapid release induced by nicotinic agonists or by depolarization of 15–20% of the total catecholamine in 5–10 min (4).

Effect of PMA on Stimulated Catecholamine Secretion from Intact Cells. Secretion induced by substituting Ba^{2+} for Ca^{2+} was enhanced 30–40% by PMA (Table 1). Ionomycin, a Ca^{2+} ionophore, at a concentration that by itself caused minimal secretion, enhanced PMA-induced catecholamine release ≈2-fold (Table 2). Catecholamine release induced by PMA and ionomycin was not accompanied by lactate dehydrogenase release and, therefore, was not caused by cell lysis. Ionomycin at concentrations as low as 0.1 μM caused a similar potentiation of the effects of PMA. The release induced by the combination of ionomycin and PMA was reduced by >80% in Ca^{2+}-free medium (with 1 mM EGTA) and was, therefore, largely Ca^{2+}-dependent. The increased release in the presence of Ca^{2+} probably resulted from a potentiation of the effects of PMA by increased cytosolic Ca^{2+}.

Table 2. Effect of PMA and ionomycin on catecholamine secretion and lactate dehydrogenase release from intact chromaffin cells

Addition	% of total released into medium	
	[3H]NE	LDH
None	4.1 ± 0.3	5.2 ± 0.3
Ionomycin (1 μM)	5.0 ± 0.1	6.1 ± 0.6
PMA (100 nM)	7.0 ± 0.3*	6.6 ± 0.3
PMA (100 nM) + 1 μM ionomycin	11.5 ± 0.3*	6.3 ± 0.6

Chromaffin cells that had been prelabeled with [3H]norepinephrine were incubated in 16-mm-diameter wells for 15 min in physiological salt solution in the presence or absence of 100 nM PMA. Cells were then incubated with physiological salt solution containing bovine serum albumin at 5 mg/ml in the continuing presence or absence of 100 nM PMA and the presence or absence of 1 μM ionomycin. After 15 min, the percentage of total [3H]norepinephrine ([3H]NE) or lactate dehydrogenase (LDH) released into the medium was determined. The total amount of LDH was not altered by PMA. Each group contained four wells.
*$P < 0.001$ vs. no additions.

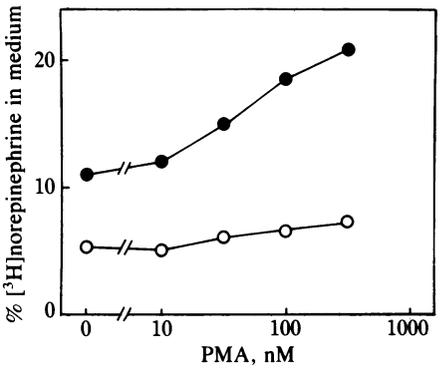

FIG. 3. Effect of PMA concentration on secretion from digitonin-treated cells. Chromaffin cells prelabeled with [^{3}H]norepinephrine were incubated in physiological salt solution with various concentrations of PMA. After 30 min, the solutions were replaced with potassium glutamate solution containing 20 μM digitonin, 1 mM MgATP, 0 (○) or 1 (●) μM Ca^{2+}, and the same concentration of PMA as in the initial incubation. The percentage of [^{3}H]norepinephrine released into the medium was determined after 11 min. Each group contained four wells.

PMA did not have reproducible effects on secretion induced by the nicotinic agonist DMPP or by elevated K^+. Prior incubation of the cells with 100 nM PMA for 15–30 min enhanced secretion 10–30% in only 5 of 10 experiments with 1,1-dimethyl-4-phenylpiperazinium ion and in one of three experiments with elevated K^+. The reason for this lack of reproducibility is unclear.

Dose Response and Ca^{2+} Dependency of Effects of PMA on Secretion from Digitonin-Treated Chromaffin Cells. To determine the effects of PMA on the secretory mechanism directly, the effects on secretion from digitonin-permeabilized cells were investigated. Chromaffin cells were incubated for 30 min in physiological salt solution containing various concentrations of PMA and were then stimulated to secrete

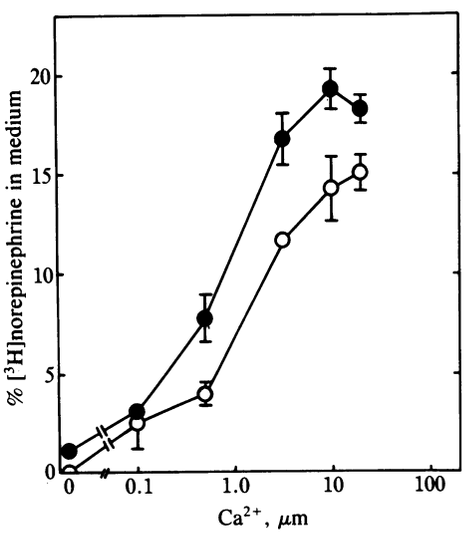

FIG. 4. Ca^{2+} dose response for secretion from digitonin-treated chromaffin cells in the presence and absence of PMA. Chromaffin cells prelabeled with [^{3}H]norepinephrine were incubated in physiological salt solution in the presence (●) or absence (○) of 100 nM PMA. After 15 min, the solutions were replaced with potassium glutamate solution containing various concentrations of free Ca^{2+} in the continuing presence or absence of 100 nM PMA. The percentage of [^{3}H]norepinephrine released into the medium was determined after 10 min. The percentage of [^{3}H]norepinephrine released in the absence of PMA and Ca^{2+} (8.3%) was subtracted from the data. Each group contained four wells.

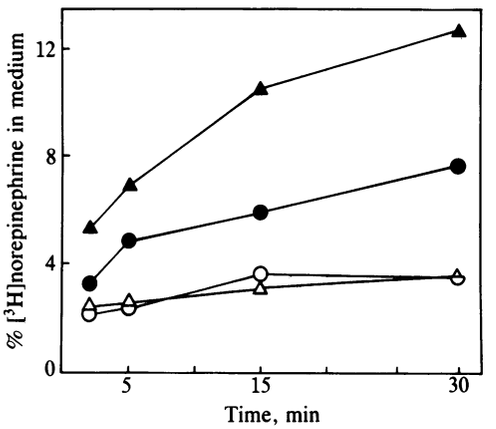

FIG. 5. Effect of PMA on the time course of secretion from digitonin-treated chromaffin cells. Chromaffin cells prelabeled with [^{3}H]norepinephrine were incubated in physiological salt solution in the presence (△, ▲) or absence (○, ●) of 100 nM PMA for 15 min. The solutions were then replaced with Ca^{2+}-free potassium glutamate solution containing 20 μM digitonin/1 mM MgATP in the presence or absence of 100 nM PMA for 5 min, after which cells were incubated in potassium glutamate solution without digitonin in the absence or presence of 100 nM PMA, with 1 mM MgATP, and 0 free Ca^{2+} (○, △) or 1 μM free Ca^{2+} (●, ▲). The percentage of [^{3}H]norepinephrine released into the medium was determined at various times. Each group contained four wells.

in potassium glutamate solution containing 20 μM digitonin, 1 mM MgATP, various concentrations of PMA, and either 0 or 1 μM Ca^{2+}. PMA caused a dose-dependent increase in Ca^{2+}-dependent [^{3}H]norepinephrine secretion between 30 and 300 nM (Fig. 3). The enhancement by PMA of Ca^{2+}-dependent secretion was observed between 0.5 and 20 μM Ca^{2+} (Fig. 4) and was relatively larger at the lower concentrations. For example, in Fig. 4, Ca^{2+}-dependent secretion was enhanced by PMA by 68% at 0.5 μM Ca^{2+} and by 27% at 10 μM Ca^{2+}. We reproducibly found that the secretion leveled off between 10 and 20 μM Ca^{2+} and that the maximal response was ≈25% (average of three experiments) greater in the presence than in the absence of PMA (100 nM). The concentration of Ca^{2+} that resulted in half-maximal Ca^{2+}-dependent secretion in the absence of PMA was ≈1 μM. PMA (100 nM) caused an ≈30% decrease in the Ca^{2+} concentration that gave half-maximal Ca^{2+}-dependent secretion. The enhancement by PMA of secretion induced by 1 μM Ca^{2+} was observed as early as 2 min (Fig. 5). PMA did not substantially alter the pattern of the time course of secretion.

In the experiments described above with digitonin-treated cells, PMA was first incubated with the cells for 15–30 min in physiological salt solution. Although in some experiments addition of PMA coincident with or after digitonin treatment was sufficient to enhance secretion in micromolar Ca^{2+}, prior incubation in physiological salt solution before digitonin treatment was necessary to obtain reproducible results.

Effects similar to those of PMA on intact and digitonin-treated cells were also observed with another phorbol ester activator of protein kinase C, 4β-phorbol 12,13-dibutyrate (10–300 nM; data not shown). 4β-Phorbol (100 nM), which does not activate protein kinase (11), did not increase secretion from intact or digitonin-treated cells.

Effects of PMA on Protein Phosphorylation in Chromaffin Cells. Incubation of chromaffin cells with PMA induced the phosphorylation of many proteins (Fig. 6). Two-dimensional electrophoresis reproducibly resolved >15 spots that were phosphorylated on incubation with PMA (100 μM) for 30 min. For example, PMA induced the phosphorylation of 3

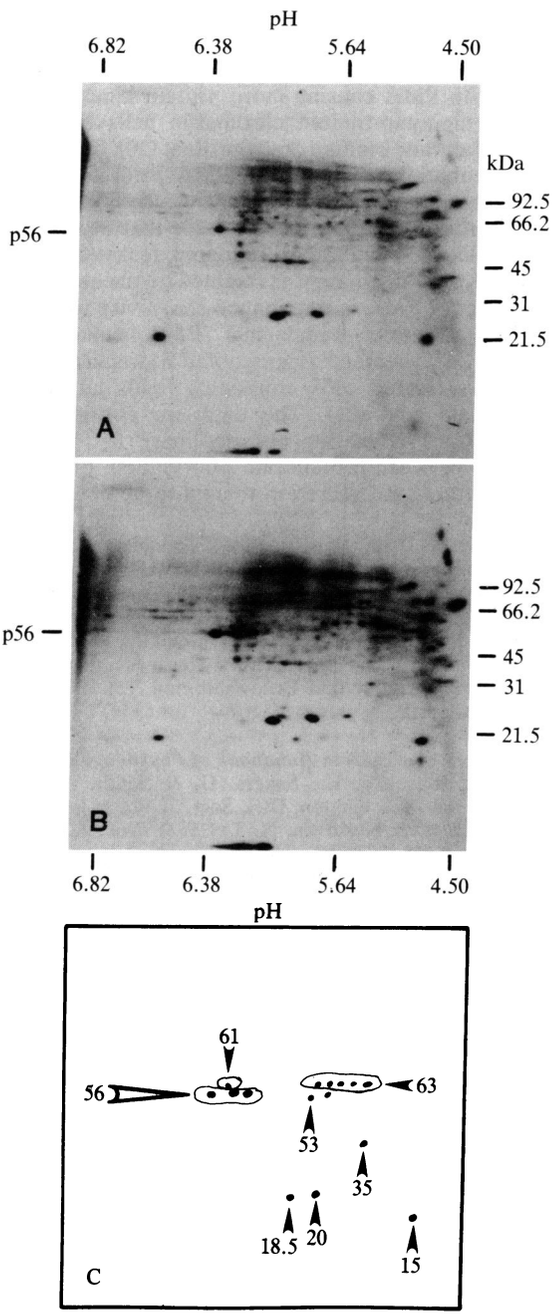

FIG. 6. Effect of PMA on protein phosphorylation in intact chromaffin cells. Purified chromaffin cells that had been labeled with $^{32}P_i$ for 30 min were incubated in physiological salt solution without (*A*) or with (*B*) PMA (100 nM) for 30 min. Phosphorylated cellular proteins were separated by two-dimensional polyacrylamide (5–20%) gel electrophoresis, applying 9 μg of protein to each gel, and were visualized by autoradiography. pH values were obtained by isoelectric focusing. Molecular mass markers are indicated on the right. The extrapolated position for 56 kDa is indicated on the left. (*C*) Tracing of *B* indicating some of the spots [with molecular masses ($\times 10^{-3}$)] phosphorylated by PMA. The open arrow marks the position of the three spots at 56 kDa.

spots at 56 kDa (pH values, 6.13, 6.26, and 6.34) as well as the phosphorylation of spots at 63 kDa (pH values, 5.20, 5.46, 6.56, 5.64, and 5.65), at 61 kDa (pH 6.30), at 53 kDa (pH 5.65), at 35 kDa (pH 5.20), at 20 kDa (pH 5.64), at 18 kDa (pH 5.80), and at 15 kDa (pH 4.77). In one-dimensional electrophoresis, the only band that could be reproducibly resolved that was phosphorylated in response to PMA was at

Table 3. Phosphorylation of p56 by PMA in intact chromaffin cells

Time, min	Phosphorylation, arbitrary units/well	
	0 PMA	100 nM PMA
3	0.87 ± 0.20	1.13 ± 0.15
30	0.50 ± 0.10	2.03 ± 0.03*

Chromaffin cells that had been preincubated with $^{32}P_i$ were incubated in the presence or absence of PMA for 3 or 30 min. The proteins were separated on $NaDodSO_4$/polyacrylamide (6.9%) gels and analyzed for phosphoproteins by autoradiography and densitometry. Each group contained three wells.
*$P < 0.001$ vs. no PMA.

56 kDa (p56, data not shown). This band corresponded in two dimensions to the set of 3 spots at 56 kDa. PMA also increased the background phosphorylation in one-dimensional electrophoresis, probably because of other proteins that were phosphorylated but were not resolved.

Because p56 could be detected readily in one dimension, its phosphorylation was quantitated by densitometry. Virtually identical relative stimulation of phosphorylation was calculated if phosphorylation was expressed as peak height (in arbitrary units as in Fig. 1 and Table 3) or as the ratio of p56 peak height to the entire span of the densitometric tracing (data not shown). PMA caused a barely detectable phosphorylation of p56 at 3 min but stimulated a 2- to 4-fold increase in phosphorylation of the band by 30 min (Table 3). In other experiments, PMA stimulated phosphorylation of p56 at least 2-fold in 15 min. PMA stimulated phosphorylation of p56 over a concentration range similar to that which stimulated catecholamine secretion (Fig. 1).

PMA-induced phosphorylation of p56 was not dependent on Ca^{2+}. For example PMA (100 nM) increased the phosphorylation (in arbitrary units) of p56 in 30 min from 1.20 ± 0.18 to 2.25 ± 0.17 in the presence of Ca^{2+} and from 1.30 ± 0.17 to 2.83 ± 0.24 in the absence of Ca^{2+}† (four wells per group, $P < 0.01$ for 100 nM PMA vs. 0 PMA). Thus, although PMA-induced secretion was dependent on medium Ca^{2+}, PMA-induced phosphorylation of p56 was independent of medium Ca^{2+}.

DISCUSSION

Catecholamine Secretion and Protein Phosphorylation. Experiments with the phorbol ester PMA, an activator of protein kinase C in other systems (11, 12), suggest that protein phosphorylation can modulate exocytosis from bovine adrenal chromaffin cells. PMA increased catecholamine secretion in intact and digitonin-treated chromaffin cells at concentrations comparable with that necessary to activate protein kinase C *in situ* in human platelets (12). In intact chromaffin cells, PMA caused a gradual Ca^{2+}-dependent catecholamine release that was potentiated by the Ca^{2+} ionophore ionomycin. PMA also enhanced Ba^{2+}-induced secretion. Secretion activated by micromolar calcium in digitonin-treated cells was enhanced if cells had been preincubated with PMA. Most importantly, PMA also increased phosphorylation of many proteins in intact chromaffin cells including a several-fold increase of phosphorylation of a band at 56 kDa (p56). Catecholamine release and p56 phosphorylation had similar PMA dose–response curves (10–300 nM) and were significantly affected by PMA over similar time periods (15 min or longer). The data suggest that protein kinase activity is activated by PMA in chromaffin cells and are consistent with a protein kinase, presumably protein

†0 Ca^{2+} solution did not contain EGTA.

kinase C, modulating catecholamine secretion from intact and digitonin-treated chromaffin cells. It is also possible, however, that the stimulation by PMA of protein phosphorylation results from decreased phosphatase activity or decreased protein turnover, although these effects of PMA have not to our knowledge been reported.

In platelets, activation of protein kinase C is necessary but not in itself sufficient to cause serotonin secretion. Activation of another Ca^{2+}-dependent pathway is also required (11, 12). PMA-stimulated secretion from chromaffin cells may also involve two pathways. PMA-induced catecholamine secretion but not PMA-induced increase in phosphorylation of p56 required Ca^{2+} in the medium. Medium Ca^{2+} may have been required for PMA-induced secretion to maintain the cytosolic Ca^{2+} concentration during the relatively long incubation required to cause significant catecholamine release. It is unknown whether protein kinase C is activated during nicotinic agonist-induced secretion.

Identity of p56. It is likely that p56 corresponds to subunits of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis. It had been previously demonstrated that a 56- to 60-kDa protein is phosphorylated upon nicotinic agonist-induced catecholamine secretion from chromaffin cells and that the protein is a subunit of tyrosine hydroxylase (5–7). We have recently confirmed the identity of the protein as tyrosine hydroxylase using one- and two-dimensional $NaDodSO_4$ slab gel electrophoresis and immunoblotting (unpublished work). p56 in PMA-treated chromaffin cells and tyrosine hydroxylase, which had been phosphorylated by incubation of chromaffin cells with nicotinic agonist, have identical molecular sizes (56 kDa) on one-dimensional $NaDodSO_4$/polyacrylamide slab gels and identical molecular sizes (56 kDa) and pI values ($\approx$6.1, 6.2, and 6.3) on two-dimensional gels (unpublished observations). Tyrosine hydroxylase from chromaffin cells has been reported to be a substrate for protein kinase C (18).

If p56 corresponds to subunits of tyrosine hydroxylase, then it is unlikely that the phosphorylation of this protein is directly involved in exocytosis. Nevertheless, its phosphorylation suggests that the effects of PMA on catecholamine secretion are correlated with the activation of protein kinase activity in the cells.

Comparison with Other Studies. In digitonin-treated cells, PMA both increased (25%) the maximal response and decreased (30%) the Ca^{2+} concentration necessary for half-maximal secretion. In a recent study of suspended chromaffin cells with plasma membranes rendered leaky by intense electric fields (19), the Ca^{2+} concentration necessary for half-maximal secretion was reduced by $\approx$50% by PMA with little shift in maximal response in the Ca^{2+}-activation curve for secretion. A PMA-induced increase in the Ca^{2+} sensitivity of the secretory process and a PMA-induced increase in the maximal secretory response are both consistent with the characteristics of protein kinase C and a possible role of the enzyme in modulating exocytosis in chromaffin cells. PMA *in vitro* increases the Ca^{2+} sensitivity of the rat brain enzyme (11); PMA *in situ* in cultured parietal yolk sac cells increases the fraction of the membrane-bound form of the enzyme (20), which is probably the active form.

The somewhat different effects of PMA on the Ca^{2+} dose–response curve for catecholamine secretion in our study and in a previous study (19) may reflect the different plasma membrane permeability characteristics of the two methods of permeabilizing the cells. Digitonin-treated cells are leakier than cells subjected to high electric fields and lose soluble cytosolic proteins much more rapidly. We have recently found that protein kinase C rapidly leaves digitonin-treated chromaffin cells (data not shown). Our data are consistent with PMA causing more protein kinase C to be retained by digitonin-treated chromaffin cells because of increased membrane binding of the protein (20). The requirement for incubation of cells with PMA prior to digitonin treatment for reproducible enhancement of secretion is also consistent with this notion. It is possible that in suspended cells with plasma membranes rendered leaky by intense electric fields protein kinase C is retained by the cells and the primary effect of PMA is to enhance sensitivity to Ca^{2+}.

We have recently found that PMA enhances the micromolar Ca^{2+}-induced release of [^{3}H]arachidonic acid from digitonin-treated cells containing lipids labeled with [^{3}H]arachidonic acid (21). The data are consistent with micromolar Ca^{2+} activating phospholipase A_2. The interrelationships between protein phosphorylation, phospholipase A_2 activation, and secretion remain to be determined.

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