Enhancement of Ca^{2+}-induced catecholamine release by the phorbol ester TPA in digitonin-permeabilized cultured bovine adrenal chromaffin cells

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The phorbol ester, 4β-phorbol 12-myristate acetate (TPA), increased the extent of catecholamine release induced by Ca^{2+}, without affecting the basal release response in digitonin-permeabilized chromaffin cells. This finding is consistent with the hypothesis that protein kinase C has a role to play in stimulus-secretion coupling in the bovine adrenal medullary chromaffin cell.

Phorbol ester *Cathecholamine* *Protein kinase C* *Chromaffin* *Secretion* *Ca^{2+}-induced release*

1. INTRODUCTION

The seemingly central importance of Ca^{2+} as a second messenger in secretagogue-induced catecholamine release in the adrenal medulla [1] has led to the search for Ca^{2+}-activated enzymes which may be involved in the mediation of this process. One such enzyme may be protein kinase C, a Ca^{2+}-activated phospholipid-dependent protein kinase [2].

Protein kinase C has been shown to be present in a soluble fraction of a homogenate of bovine adrenal medulla prepared in the presence of EGTA and this activity has been partially characterized [3]. The adrenal medullary enzyme shows similar properties to those reported for the enzyme isolated from other tissues, including a reduced dependency for free Ca^{2+} in the presence of either diacylglycerol or the phorbol ester, TPA.

Although TPA has been reported to have no effect on catecholamine secretion from acutely isolated bovine adrenal chromaffin cells [4,5], it was found to induce catecholamine secretion from cultured cells and this effect was enhanced synergistically by the Ca^{2+} ionophore, A23187 [3]. This latter observation suggested a possible role for protein kinase C in the secretory mechanism of these cells. TPA has also been shown to increase the affinity of exocytosis for Ca^{2+} in acutely isolated bovine adrenal chromaffin cells which had been rendered permeable by exposure to an intense electric field [4]. We would now like to extend this latter observation by reporting the effect of TPA on cultured bovine adrenal chromaffin cells which have been permeabilized with the detergent, digitonin.

Abbreviations: TPA, 4β-phorbol 12-myristate acetate; Pipes, 1,4-piperazinediethanesulfonic acid

2. EXPERIMENTAL

Bovine adrenal chromaffin cells were isolated from medullae by collagenase digestion and maintained as primary monolayer cultures in 24-well plates for 3 days as previously described [3]. All experiments were performed at 37°C.

Culture medium was removed from the wells and the cells washed with 1 ml of balanced salt solution containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose and 25 mM Hepes/NaOH (pH 7.32). Cells were

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then permeabilized for 10 min by addition of 0.25 ml of medium containing 140 mM monosodium glutamate, 20 mM Pipes/NaOH (pH 6.8), 5 mM glucose, 5 mM $MgSO_4$, 5 mM ATP, 0.5 mM ascorbic acid, 10 μM digitonin and 5 mM EGTA [6,7]. This medium was then removed and the cells incubated for the designated time interval with 0.25 ml of the same medium without digitonin but with the addition of $CaCl_2$ or TPA as indicated in the figure legends. The incubation medium was then removed and centrifuged at 12000 × *g* for 2 min at 4°C and the supernatant assayed for catecholamines. To each well was added 0.25 ml of 10% (v/v) acetic acid, and after freeze-thawing, samples were assayed for catecholamines.

Catecholamines were assayed by the trihydroxyindole method performed on ice [8]. TPA was stored as a 1 mM stock solution in dimethyl sulfoxide, and the final concentration of the solvent presented to the cells was 1% which did not affect the rate of catecholamine release. Free Ca^{2+} concentrations were calculated as in [9].

3. RESULTS AND DISCUSSION

Permeabilization of cultured bovine adrenal chromaffin cells with digitonin rendered the process of release of catecholamines from these cells sensitive to Ca^{2+}, as has been reported [6,7]. The greatest effect of Ca^{2+} on catecholamine release was in the concentration range 10^{-7}–10^{-6} M with half-maximal release occurring at 330 nM (fig.1). TPA (100 nM) was found to increase the release of catecholamines at all Ca^{2+} concentrations shown to elicit release, but had no effect on basal release. In the presence of TPA, half-maximal release of catecholamines occurred at 230 nM free Ca^{2+}. The time course of catecholamine release evoked by 400 nM free Ca^{2+} and 100 nM TPA is shown in fig.2 and the effect of TPA concentration on the potentiation of release at 400 nM free Ca^{2+} shown in fig.3. The half-maximal effect of TPA was observed at approx. 50 nM.

These results are in general agreement with those reported for acutely isolated bovine adrenal chromaffin cells permeabilized by exposure to an intense electric field [4], although catecholamine release from the digitonin-permeabilized cells occurs at lower free Ca^{2+} concentrations. The most striking difference between the two sets of data is the concentration of TPA found to give half-maximal release of catecholamines, this being approx. 2 nM for the electrically permeabilized cells. This discrepancy could be due to a greater loss of the plasma membrane with digitonin treatment, the plasma membrane presumably being at least one of the sites of action of TPA, or possible blockage of TPA insertion sites on the membrane by digitonin.

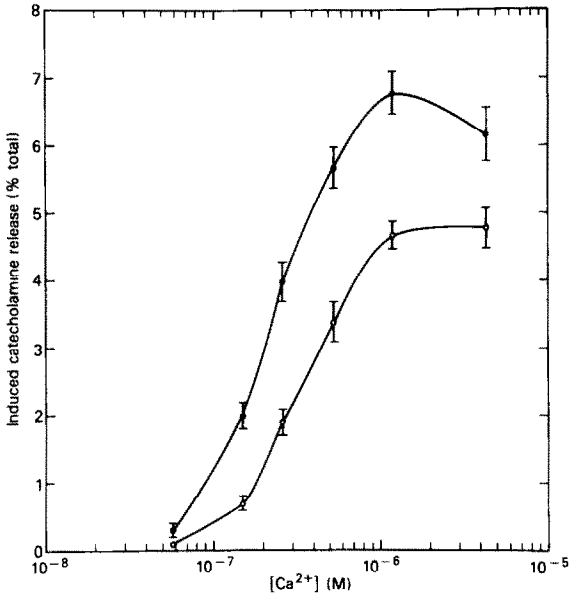

Fig.1. Effect of TPA on the free Ca^{2+} concentration dependency of catecholamine release from digitonin-permeabilized chromaffin cells. Cultured bovine adrenal chromaffin cells were permeabilized with digitonin medium for 10 min at 37°C and then incubated for 10 min in digitonin-free medium in the presence of 5 mM EGTA, various concentrations of $CaCl_2$ and either in the absence (○) or presence (●) of 100 nM TPA. Results were calculated as the percentage of total cellular catecholamine released from the cells into the medium and then corrected for basal release observed in the presence of 5 mM EGTA alone. Values shown are means ± SE (n = 14). Basal release was 5.6 ± 0.2% and was not significantly affected by 100 nM TPA when the release was 5.7 ± 0.1% of total catecholamines.

Using a cytosolic preparation from bovine adrenal medulla as a source of protein kinase C, TPA was shown to decrease the free Ca^{2+} concentration required for half-maximal activation of the enzyme in the presence of phosphatidylserine from 18 to 9 μM [3]. These free Ca^{2+} concentrations are

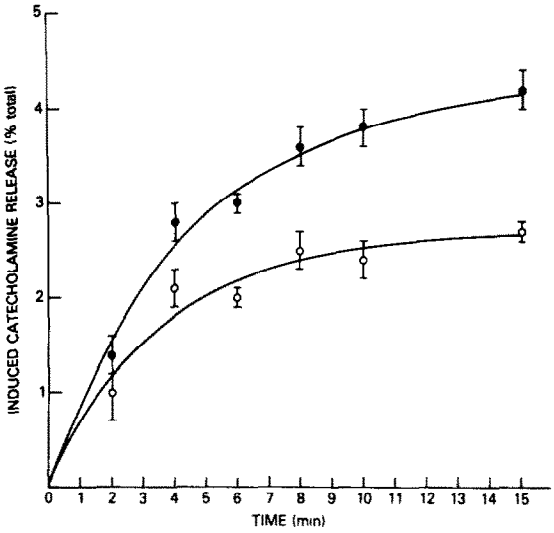

Fig.2. Time course of catecholamine release from digitonin-permeabilized chromaffin cells induced by Ca^{2+} and TPA. Cultured bovine adrenal chromaffin cells were permeabilized with digitonin-medium for 10 min at 37°C and then incubated for the designated time intervals in digitonin-free medium in the presence of 5 mM EGTA and 4 mM $CaCl_2$ (resulting in a free Ca^{2+} concentration of approx. 400 nM) either in the absence (○) or presence (●) of 100 nM TPA. Results were calculated as the percentage of total cellular catecholamine released into the medium and then corrected for basal release observed in the presence of 5 mM EGTA alone. Values shown are means ± SE (n = 8). Basal release did not increase after 2 min and was unaffected by TPA, being 4.8 ± 0.2% in the absence and 5.0 ± 0.2% of total catecholamines in the presence of 100 nM TPA.

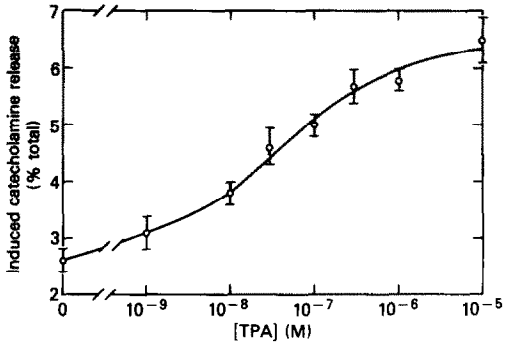

Fig.3. Effect of TPA concentration on catecholamine release from digitonin-permeabilized chromaffin cells. Cultured bovine adrenal chromaffin cells were permeabilized with digitonin medium for 10 min at 37°C and then incubated for 10 min in digitonin-free medium in the presence of 5 mM EGTA, 4 mM $CaCl_2$ (resulting in a free Ca^{2+} concentration of approx. 400 nM) and various concentrations of TPA. Results were calculated as the percentage of total cellular catecholamine released into the medium and then corrected for basal release observed in the presence of 5 mM EGTA alone. Values shown are means ± SE (n = 12). Basal release was 5.0 ± 0.1% of total catecholamines and was independent of TPA concentration.

almost two orders of magnitude higher than the corresponding values for the effect of TPA on the half-maximal free Ca^{2+} concentration that elicits release of catecholamines from digitonin-permeabilized cells. Given that protein kinase C appears to be the only receptor for TPA [10], the reason for this discrepancy is unclear but possibly reflects the presence of an inhibitor of protein kinase C in the cytosolic preparation or the greater ability of cellular membranes than phosphatidylserine to activate the enzyme.

These studies were undertaken primarily to attempt to reproduce in digitonin-permeabilized chromaffin cells the effect of TPA on catecholamine release reported for electrically permeabilized cells. Previously differences in the secretory properties of the two types of permeabilized cells have been noted [7] and it was important to characterize the effect of TPA on digitonin-treated chromaffin cells because of these differences and also as pointed out in [6] these cells may permit the introduction of antibodies into functioning cells whereas the size of the holes in the plasma membrane introduced by electrical permeabilization probably precludes this possibility. Thus the results are in agreement with the hypothesis that protein kinase C is involved in the secretory process in bovine adrenal chromaffin cells.

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