Phorbol ester induces intracellular translocation of phospholipid/Ca^{2+}-dependent protein kinase and stimulates amylase secretion in isolated pancreatic acini

Marie W. Wooten and Robert W. Wrenn

Department of Anatomy, Schools of Medicine and Biomedical Graduate Studies, Medical College of Georgia, Augusta, GA 30912, USA

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Treatment of intact rat pancreatic acini with phorbol ester (12-*O*-tetradecanoyl-phorbol-13-acetate, TPA) resulted in a time- and concentration-dependent translocation of phospholipid/Ca^{2+}-dependent protein kinase (PL/Ca-PK) from the soluble fraction. Redistribution of PL/Ca-PK was concurrent with stimulation of amylase secretion by TPA-treated acini. Polymyxin B, a potent and selective inhibitor of PL/Ca-PK completely inhibited TPA-induced amylase secretion. These findings are consistent with a role for PL/Ca-PK in the regulation of pancreatic exocrine secretion.

Phorbol ester *Protein kinase* *Secretion* *Pancreatic acini*

1. INTRODUCTION

Tumor-promoting phorbol esters (such as 12-*O*-tetradecanoyl-phorbol-13-acetate, TPA) have been shown to exert a wide variety of effects on cells, including morphological transformation [1], as well as alterations in phospholipid metabolism [2], and protein synthesis [3]. Recent work has implicated the phospholipid/Ca^{2+}-dependent protein kinase (PL/Ca-PK; kinase C) as having a central role in mediating the biological actions of TPA. Studies have shown that TPA initially interacts with the plasma membrane by binding to a high affinity receptor [4]. Authors in [5,6] reported that treatment of cultured parietal yolk sac or EL4 thymoma cells with TPA resulted in a rapid translocation of PL/Ca-PK activity from the cytosol to the particulate fraction. Authors in [7] showed direct activation of purified PL/Ca-PK by TPA, and proposed that the TPA receptor may be identical to, or closely associated with this kinase. Further support for this idea has come from studies demonstrating that the phorbol ester receptor and PL/Ca^{2+}-PK copurify through several chromatographic steps [8].

To date, however, the relationship between phorbol esters and PL/Ca-PK and the possible functional significance of that relationship has not been examined in a secretory cell type. The author in [9] earlier reported that exposure to TPA induced protein discharge by pancreatic acini but did not provide an insight into the actual mechanism of this event. We here examine the effect of TPA treatment upon subcellular distribution of PL/Ca-PK and amylase secretion in pancreatic acinar cells in an attempt to define a relationship between phorbol ester and PL/Ca-PK in acinar cells and to suggest a role for this protein kinase in exocrine secretory regulation.

2. EXPERIMENTAL

2.1. *Materials*

TPA, β-phorbol, phosphatidylserine (bovine brain), lysine-rich histone, trypsin inhibitor (type I-S), carbamylcholine, 2-amylase (type VIII-A), aprotinin, PMSF (phenylmethylsulfonyl fluoride) and benzamidine were from Sigma. Polymyxin B was purchased from United States Biochemicals. Purified collagenase (low clostripain activity) and

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chymotrypsin were obtained from Worthington Biochemicals; MEM amino acid supplement was from Gibco Laboratories. Fluphenazine was a gift from the Squibb Institute.

2.2. *Preparation of isolated pancreatic acini*

Adult male Wistar rats (200–250 g) were fasted overnight and killed by decapitation. Isolated pancreatic acini were prepared essentially as in [10]. Excised pancreas was incubated for 50 min at 37°C in 10 mM Hepes (pH 7.35), equilibrated with 95% O_2/5% CO_2, containing MEM amino acid supplement and 0.01% soybean trypsin inhibitor, collagenase (70–90 units/ml), chymotrypsin (10–15 μg/ml), and BSA (2 mg/ml). Acini were dissociated by passage through polypropylene pipets of decreasing orifice and filtered through nylon mesh. The resulting suspension was layered onto, and centrifuged through isolation medium lacking enzymes but containing 4% BSA and 2.0 mM EDTA. Thereafter acini were resuspended in 10 mM Hepes (pH 7.35) containing 1% BSA. Cells prepared by this method were >95% viable (trypan blue exclusion). Isolated acini were incubated and secretory activity determined as in [10].

2.3. *Determination of amylase and protein*

Amylase was measured as in [11]. Protein was determined as in [12].

2.4. *Protein kinase assay*

Pancreatic acini were washed in 20 mM Tris–Cl (pH 7.5), subsequently centrifuged, and resuspended in the same buffer, containing 100 mg/ml aprotinin, 1 mM benzamidine, 1 mM PMSF, 2 mM EDTA, and 50 mM 2-mercaptoethanol. The cells were sonicated and soluble PL/Ca^{2+}-PK activity was recovered by rapid centrifugation in an Eppendorf microfuge. PL/Ca-PK activity was measured as in [13,14], substituting Pipes (pH 6.5) for Tris–Cl (pH 7.5) as the assay buffer. The assay system (0.2 ml) contained Pipes, 20 mM; lysine-rich histone, 40 μg; $MgCl_2$, 10 mM; phosphatidylserine, 5 μg; EGTA, 25 μM; with or without $CaCl_2$, 0.5 mM; and 10–50 μg sample protein. The reaction was initiated by the addition of [λ-^{32}P]ATP, 1 nmol, containing 0.5–1.0 × 10^6 cpm, and carried out at 30°C for 5 min. Protein kinase activity stimulated by Ca^{2+} is reported or used for calculations.

3. RESULTS AND DISCUSSION

Pancreatic acini treated with 10^{-6} M TPA displayed a rapid translocation of soluble PL/Ca-PK. This translocation was marked over the initial 5 min of treatment, progressively increased over a 30 min period (fig.1), and was dose-dependent over a wide concentration range (fig.1, inset).

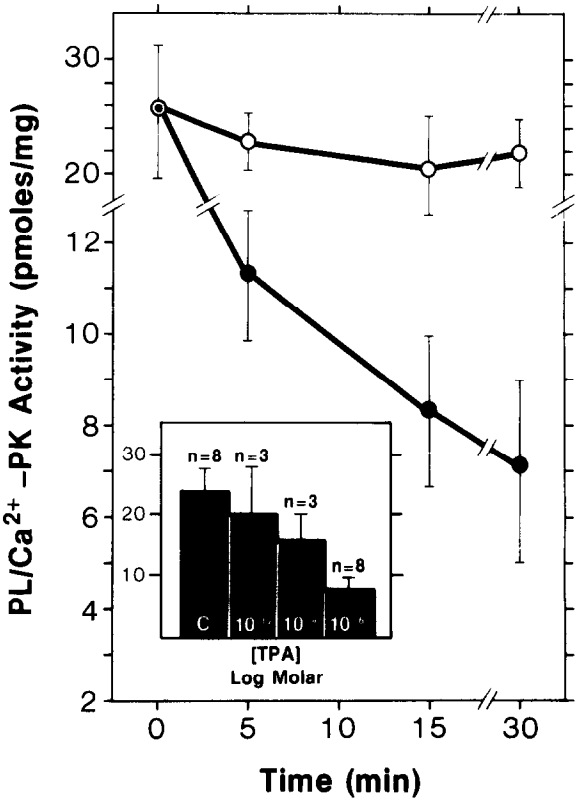

Fig.1. Effect of phorbol ester (TPA) on translocation of soluble PL/Ca-PK activity in pancreatic acini. Acini were treated with TPA (10^{-6} M) for various times, as indicated. Collection of the soluble fraction and assay for PL/Ca-PK activity was as described (section 2). Values given represent the means of triplicate incubations (± SE) and are typical of 5 experiments. Inset: effect of various concentrations of TPA (30 min exposure) on translocation of soluble PL/Ca-PK in pancreatic acini. Values given represent duplicate or triplicate incubations from several experiments as indicated. Vertical lines represent SE.

Amylase secretion was also stimulated by TPA over a concentration range identical to that effective in causing translocation of soluble PL/Ca-PK (fig.2). Induction of secretion was maximal at a TPA concentration of 10^{-6} M (fig.2) and was equivalent to that noted in response to an optimal concentration of carbamylcholine (fig.3). Similar to the kinetics of PL/Ca-PK redistribution, TPA treatment increased amylase discharge above that observed in control acini as early as 5 min (fig.3), and this increase was sustained up to 30 min. Neither non-esterified β-phorbol nor the vehicles dimethylsulfoxide (DMSO) or ethyl alcohol (ETOH) were effective in either stimulating secretion (fig.4) or effecting redistribution of PL/Ca-PK (not shown).

In further experiments, polymyxin B (PMB), an amphipathic peptide antibiotic which is a potent and selective inhibitor of PL/Ca-PK [15], completely inhibited TPA-induced amylase secretion (fig.4). The secretory response was also prevented in the presence of fluphenazine (FP, fig.4), an inhibitor of PL/Ca-PK which is equally potent as an inhibitor of calmodulin/Ca^{2+}-dependent reactions [16,17]. Both drugs are membrane-interacting and are thought to inhibit PL/Ca-PK by competing for

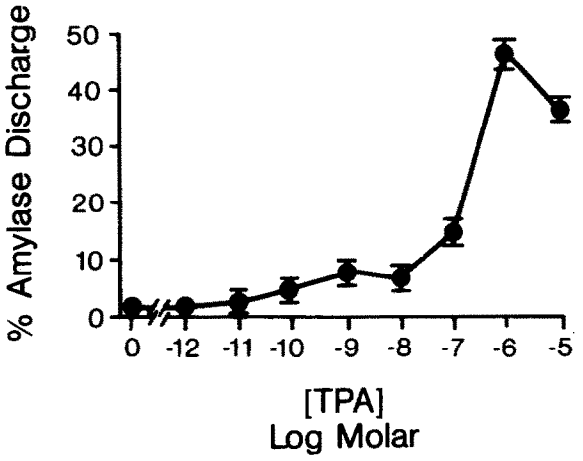

Fig.2. Effect of phorbol ester (TPA) on amylase discharge from pancreatic acini. Acini were incubated for 30 min in the presence of various concentrations of TPA, as indicated. Percent discharge was determined based on the total amylase content of acini at the beginning of the incubation period. Results given represent the means of triplicate incubations (± SE) and are typical of 7 experiments.

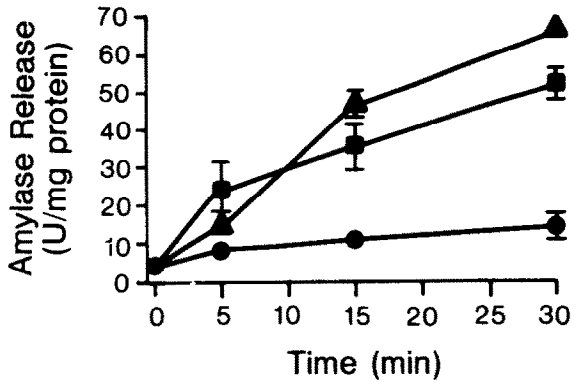

Fig.3. Kinetics of TPA-induced amylase secretion in isolated pancreatic acini. Acini were incubated in the presence of TPA (10^{-6} M; ■), carbamylcholine (10^{-6} M; ▲), or in their absence (●) for various times, as indicated. Aliquots of the incubation mixture were removed at the appropriate time, acini removed by rapid centrifugation and the medium amylase determined. Results presented are typical of 5 experiments and represent the means of duplicate determinations ± SE.

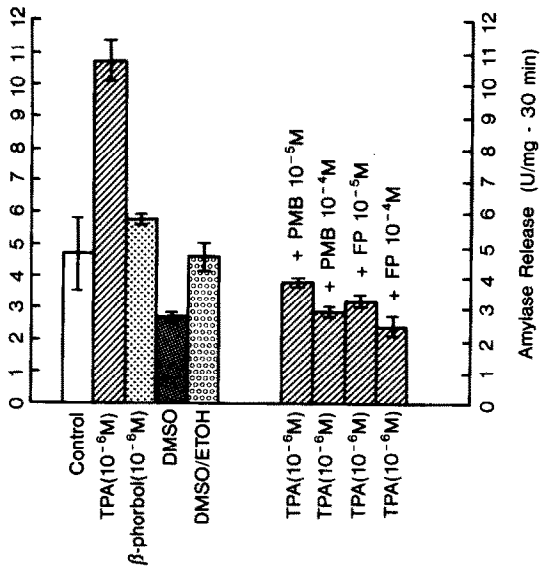

Fig.4. Effect of various agents on amylase secretion by pancreatic acini. The amount of amylase released was determined after 30 min exposure to various agents, alone or in combination, as indicated. Polymyxin B (PMB) or fluphenazine (FP), when present, were added simultaneously with TPA. The incubation media were recovered and amylase determined as described (fig.3; section 2). Values given are the means of triplicate determinations (± SE) and are representative of 6 experiments.

a hydrophobic region on the enzyme to which the phospholipid cofactor (diacylglycerol; phosphatidylserine) binds [17,18]. Since TPA is thought to activate PL/Ca-PK by directly substituting for diacylglycerol [7], it is at least conceivable that PMB is preventing TPA-induced secretion by blocking phorbol ester activation of membrane-associated kinase in situ.

The present studies are the first to associate phorbol ester treatment with intracellular redistribution of PL/Ca-PK in a secretory cell population. The similarities in kinetics and concentration-dependency of TPA-induced kinase translocation and amylase secretion, as well as prevention of TPA-stimulated secretion by inhibitors of PL/Ca-PK, are consistent with an active role for this protein kinase in the initiation of the secretory response in exocrine pancreas.

This would be in line with a recent hypothesis based on data obtained in two endocrine secretory systems [19,20]. In this concept, the overall regulatory action of Ca^{2+} resides in dual Ca^{2+}-activated pathways: the first expressed via PL/Ca-PK and responsible for the initiation of secretion, the second expressed via calmodulin and responsible for the maintenance of a sustained response. The present results suggest that this hypothesis, or at least that portion of it relative to PL/Ca-PK, may be extended to the control of pancreatic exocrine secretion as well. Other recent work in our laboratory has indicated a marked differential distribution of PL/Ca-PK, and endogenous substrate proteins for this enzyme, among various compartments within the acinar cell, including components of the secretory apparatus (unpublished). More detailed studies of the precise locations to which PL/Ca-PK and/or its substrates are redistributed within acinar cells following TPA treatment will further our understanding of Ca^{2+} as a modulator of exocrine pancreatic function.

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REFERENCES

[1] Driedger, P.E. and Blumberg, P.M. (1977) Cancer Res. 37, 3257–3265.
[2] Wertz, P.W. and Mueller, G.C. (1978) Cancer Res. 38, 2900–2904.
[3] Cabral, F., Gottesmann, M.M. and Yuspa, S.H. (1981) Cancer Res. 41, 2025–2028.
[4] Driedger, P.E. and Blumberg, P.M. (1980) Proc. Natl. Acad. Sci. USA 77, 567–571.
[5] Kraft, A.S., Anderson, W.B., Cooper, H.L. and Sando, J.J. (1982) J. Biol. Chem. 257, 13193–13196.
[6] Kraft, A.S. and Anderson, W.B. (1983) Nature 301, 621–623.
[7] Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847–7851.
[8] Neidel, J.E., Kuhn, L.J. and Vandenbark, G.R. (1983) Proc. Natl. Acad. Sci. USA 80, 36–40.
[9] Gunther, G.R. (1981) J. Biol. Chem. 256, 12040–12045.
[10] Williams, J.W., Korc, M. and Goldfine, R.L. (1978) Am. J. Physiol. 235,E517–E524.
[11] Jung, D.H. (1980) Clin. Chem. Acta 100, 819–827.
[12] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[13] Kuo, J.F., Andersson, R.G.G., Wise, B.C., Mackerlova, L., Salomonsson, I., Brackett, N.L., Katoh, N., Shoji, M. and Wrenn, R.W. (1980) Proc. Natl. Acad. Sci. USA 77, 7040–7043.
[14] Wrenn, R.W., Katoh, N., Wise, B.C. and Kuo, J.F. (1980) J. Biol. Chem. 255, 12042–12046.
[15] Kuo, J.F., Raynor, R.L., Mazzei, G.J., Schatzman, R.C., Turner, R.S. and Kem, W.R. (1983) FEBS Lett. 153, 183–186.
[16] Wrenn, R.W., Katoh, N., Schatzman, R.C. and Kuo, J.F. (1981) Life Sci. 29, 725–733.
[17] Schatzman, R.C., Wise, B.C. and Kuo, J.F. (1981) Biochem. Biophys. Res. Commun. 98, 669–676.
[18] Mazzei, G.J., Katoh, N. and Kuo, J.F. (1982) Biochem. Biophys. Res. Commun. 109, 1129–1133.
[19] Kojima, I., Lippes, H., Kojima, K. and Rasmussen, H. (1983) Biochem. Biophys. Res. Commun. 116, 555–562.
[20] Zawalich, W., Brown, C. and Rasmussen, H. (1983) Biochem. Biophys. Res. Commun. 117, 448–455.