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Phorbol ester (TPA) potentiates noradrenaline and acetylcholine-evoked amylase secretion in the rat pancreas

Jaipaul Singh*

M.R.C. Secretory Control Research Group, Department of Physiology, University of Liverpool, PO Box 147, Liverpool, L69 3BX, England

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The effect of the phorbol ester 12-*O*-tetradeconyl-phorbol-13-acetate (TPA) on noradrenaline (NA) and acetylcholine (ACh)-evoked amylase secretion in isolated segments of rat pancreas was investigated. TPA alone evoked a relatively small increase in amylase output. However, when combined with either noradrenaline or ACh, the phorbol ester markedly enhanced the secretagogue-induced amylase secretion. These effects were dose related. TPA also enhanced the amylase secretion evoked by either the Ca^{2+} ionophore A23187 or dibutyryl cyclic AMP. This potentiation by TPA of noradrenaline, ACh, Ca^{2+} ionophore A23187 and dibutyryl cyclic AMP-evoked amylase output may suggest the existence of a third pathway controlling enzyme secretion in the pancreas.

Amylase secretion *Rat pancreas* *Phorbol ester* *Acetylcholine* *Noradrenaline* *Cyclic AMP*

1. INTRODUCTION

Enzyme secretion in the exocrine pancreas is regulated by secretagogues which act via two different stimulus-secretion coupling mechanisms. One pathway is activated by secretin, vasoactive intestinal polypeptide (VIP), cholera toxin and catecholamines resulting in an increase in endogenous adenosine 3′,5′-cyclic monophosphate (cyclic AMP). The other pathway is stimulated by ACh, cholecystokinin (CCK-like and bombesin-like peptides and involves changes in membrane potential, release of cellular Ca^{2+} and an elevation in intracellular guanosine 3′,5′-cyclic monophosphate (cyclic GMP) [1–5]. Two secretagogues (e.g., VIP and ACh) acting via the two different pathways can potentiate one another whereas two secretagoguges (e.g., ACh and CCK) acting through the same mechanism cannot [6].

Recently, several studies have demonstrated that TPA can markedly stimulate enzyme output in the pancreas without affecting either Ca^{2+} or cyclic nucleotide metabolism [7–9]. There are some suggestions that TPA may exert its effect on enzyme secretion through activation of protein kinase C [10–13]. Since TPA cannot stimulate either cellular Ca^{2+} release or cyclic AMP metabolism then it might act to potentiate enzyme secretion evoked either by hormones and neurotransmitters which are known to elevate cytoplasmic Ca^{2+} and cyclic AMP or by agents which can artificially elevate intracellular Ca^{2+} and cyclic AMP. It has been shown that TPA can potentiate the enzyme secretion induced by the Ca^{2+} ionophore A23187 [11], a substance which is known to increase cytoplasmic Ca^{2+} [14].

The present study was designed mainly to investigate whether TPA can also enhance the enzyme secretion evoked by agents which can elevate endogenous cyclic AMP. Furthermore, the effect of TPA on the acetylcholine and A23187-induced amylase secretion was examined for comparison.

* Present address: School of Applied Biology, Lancashire Polytechnic, Corporation Street, Preston, PR1 2TQ, England

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2. METHODS

All experiments were performed on isolated segments of rat pancreas. Adult animals were killed by a blow to the head and the pancreas was quickly removed and placed in a modified Krebs-Henseleit solution of the following composition (mM): NaCl 103, KCl 4.7, $CaCl_2$ 2.56, $MgCl_2$ 1.13, $NaHCO_3$ 25, NaH_2PO_4 1.15, D-glucose 2.8, Na pyruvate 4.9, Na fumarate 2.7 and Na glutamate 4.9. The solution was gassed with 95% O_2/5% CO_2 and maintained at 37°C.

The rat pancreas was cut into small segments (3–5 mg) and a total weight of about 300 mg was placed in a perspex flow chamber and superfused with Krebs-Henseleit solution at a flow rate of 1 ml · min^{-1}. The amylase concentration in the effluent from the chamber was measured using an on-line fluorometric assay by the methods described in [15,16]. Throughout an experiment the generation of fluorescence, which is a linear function of amylase concentration, was continuously monitored on a pen recorder. The secretagogue-evoked amylase release (i.e., that above basal output) was routinely expressed in terms of the output at the peak of the response in units · ml^{-1} (100 mg tissue)$^{-1}$. One unit of amylase is defined as the amount which will liberate 1.0 mg of maltose from starch in 3 min at pH 6.9 and 20°C. In this study of α-amylase (Sigma type IIA) was used as a standard for calibration. TPA, noradrenaline, ACh, Ca^{2+} ionophore A23187 and dibutyryl cyclic AMP (Sigma) were added directly to the superfusing solution in known concentrations.

3. RESULTS

The mean (± SE) basal output of amylase from superfused segments of rat pancreas was 2.93 ± 0.11 unit · ml^{-1} · (100 mg tissue)$^{-1}$, $n = 54$. Fig.1 shows an original chart recording of the response produced by superfusing a preparation with 10^{-5} M TPA. The phorbol ester evoked a relatively slow increase in amylase output reaching a maximum after 25–30 min and then gradually declining to a lower level. The peak amylase secretion above basal level was 1.45 ± 0.08 unit · ml^{-1} · (100 mg tissue)$^{-1}$, $n = 9$. Fig.1B shows the effect of varying the concentrations (10^{-8}–10^{-5} M) of TPA on amylase output. Concentrations between 10^{-8} and

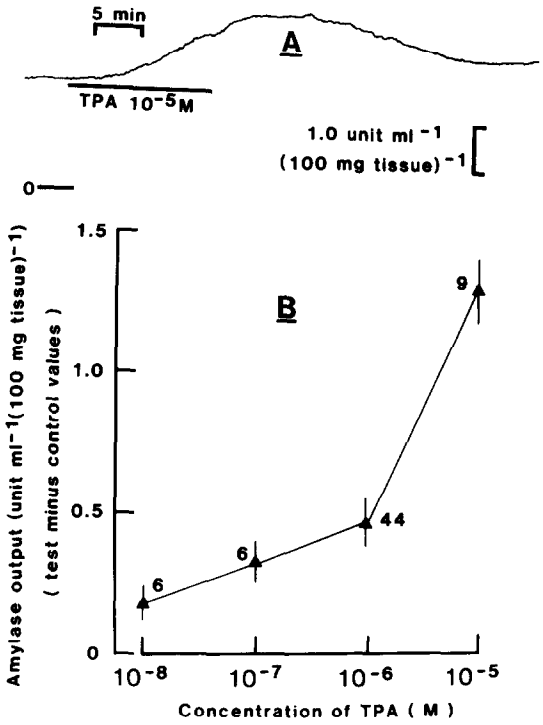

Fig.1. (A) Effects of 10^{-5} M TPA on amylase secretion from superfused rat pancreatic fragments. The horizontal bar indicates the duration of TPA stimulation. Vertical calibration: 1.0 unit · ml^{-1} · (100 mg tissue)$^{-1}$. In this and fig.2–4 the horizontal line labelled O represents the fluorescence reading without pancreatic tissue in the flow chamber. (B) Dose-response curve showing the effect of TPA (10^{-8} – 10^{-5} M) on amylase secretion. Each point is mean ± SE, n is indicated by the numbers beside each mean point.

10^{-6} M evoked relatively small increases (0.43 ± 0.02–0.18 ± 0.01 unit · ml^{-1} · (100 mg tissue)$^{-1}$) in amylase secretion.

Fig.2A shows an original chart recording of amylase output from an individual experiment following superfusion of rat pancreatic fragments with 10^{-7} M noradrenaline in the absence (a) and presence (b) of 10^{-6} M TPA. Exposure of pancreatic segments to 10^{-7} M noradrenaline resulted in a mean (± SE) peak amylase secretion of 0.62 ± 0.02 unit · ml^{-1} · (100 mg tissue)$^{-1}$, $n = 6$. In the presence of 10^{-6} M TPA the noradrenaline-induced amylase secretion increased to 2.10 ± 0.13 unit · ml^{-1} · (100 mg tissue)$^{-1}$, $n = 6$. These effects of noradrenaline were dose related. Fig.2B shows dose response curves for the noradrenaline-elicited

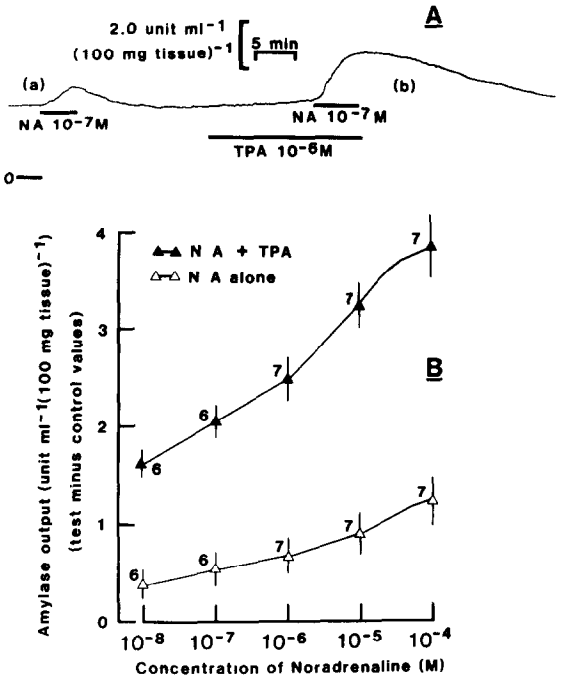

Fig.2. (A) Effects of 10^{-7} M noradrenaline (NA) on amylase output from superfused rat pancreatic segments in the absence (a) and presence (b) of 10^{-6} M TPA. The responses (a) and (b) are continuous fluorescence intensity from a single experiment. The horizontal bars indicate the duration of stimulation. Vertical calibration: 2.0 units $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$. (B) Dose-response curves showing the effect of differing concentrations of noradrenaline (10^{-8}–10^{-4} M) on peak amylase secretion above basal levels in the absence (open triangles) and presence (solid triangles) of 10^{-6} M TPA. Each point is mean ± SE, n is shown besides each point.

amylase output in the absence (open triangles) and presence (solid triangles) of 10^{-6} M TPA. The dose-dependent curve obtained in the presence of TPA was displaced to the left, indicating the marked enhancement by TPA of the noradrenaline-induced amylase secretion.

The effect of TPA on the ACh-evoked amylase secretion was also investigated. Fig.3A shows recordings of amylase output from a preparation superfused with 10^{-7} M ACh in the presence (a,c) and absence (b) of 10^{-6} M TPA. The mean (±SE) peak increase in amylase output above basal level evoked by 10^{-7} M ACh was 4.74 ± 0.34 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 11$. In the presence of 10^{-6} M TPA the ACh-induced amylase output was

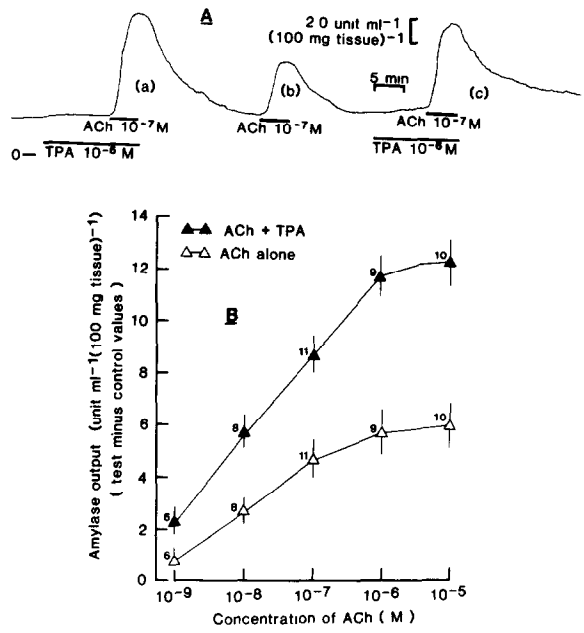

Fig.3. (A) Effects of 10^{-7} M ACh (acetylcholine) in the presence (a,c) and absence (b) of 10^{-6} M TPA on amylase release from an individual experiment. The horizontal bars indicate the duration of stimulation. Vertical calibration: 2.0 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$. (B) Dose-dependent curves showing the effects of ACh (10^{-9}–10^{-5} M) on amylase secretion in the absence (open triangles) and presence (solid triangles) of 10^{-6} M TPA. Each point is mean ± SE, n is shown besides each mean point.

8.66 ± 0.46 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 11$. Fig.3B shows the effect of varying the concentrations (10^{-9}–10^{-5} M) of ACh on amylase secretion in the absence (open triangles) and presence (solid triangles) of 10^{-6} M TPA. The results show that the phorbol ester markedly enhanced the ACh-elicited amylase output.

In another series of experiments the effects of the Ca^{2+} ionophore A23187 and dibutyryl cyclic AMP on enzyme secretion were investigated in the absence and presence of TPA. Fig.4A shows a chart recording of amylase output from one experiment following exposure of rat pancreatic fragments with 10^{-6} M Ca^{2+} ionophore A23187 in the absence (a) and presence (b) of 10^{-6} M TPA. In the absence of TPA the ionophore-evoked increase in amylase secretion was 0.61 ± 0.05 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 6$. In the presence of 10^{-6} M TPA the ionophore-induced amylase

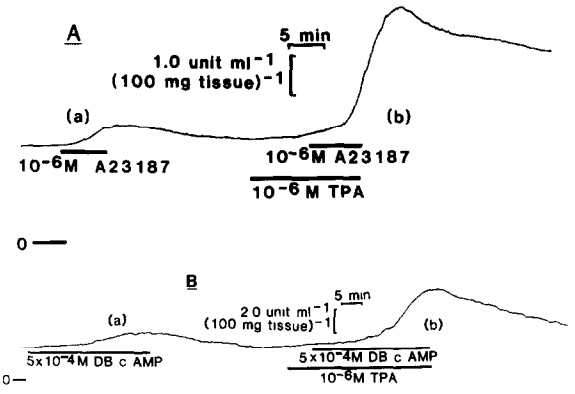

Fig.4. (A) Effects of 10^{-6} M Ca^{2+} ionophore A23187 on amylase secretion from one single experiment in the absence (a) and presence (b) of 10^{-6} M TPA. (B) Effect of 5×10^{-4} M dibutyryl (DB) cyclic AMP on amylase output from an individual experiment in the absence (a) and presence (b) of 10^{-6} M TPA. The horizontal bars indicate the duration of stimulation. Vertical calibration: 1.0 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$ in (A) and 2.0 units $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$ in (B).

output was 3.31 ± 0.26 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 6$. Fig.4B shows the effect of superfusing a preparation with 5×10^{-4} M dibutyryl cyclic AMP in the absence (a) and presence (b) of 10^{-6} M TPA. Exposure of rat pancreatic fragments with 5×10^{-4} M dibutyryl cyclic AMP resulted in a peak increase in amylase secretion of 1.01 ± 0.06 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 4$. In the presence of 10^{-6} M TPA the dibutyryl cyclic AMP elicited amylase output was 3.96 ± 0.08 unit $\cdot$ ml^{-1} $\cdot$ (100 mg tissue)$^{-1}$, $n = 4$. The data show that in the continuing presence of TPA both dibutyryl cyclic AMP and the Ca^{2+} ionophore A23187 evoked significant ($P < 0.001$) increases in amylase secretion as compared to these substances in the absence of TPA.

4. DISCUSSION

The present study has demonstrated that the phorbol ester TPA alone evokes a relatively small increase in amylase secretion but it can markedly potentiate the secretory responses induced by noradrenaline, ACh, dibutyryl cyclic AMP and the Ca^{2+} ionophore A23187 in the isolated rat pancreas. The question which now arises is: how does TPA act to augment the secretagogue-induced amylase secretion? TPA is known to stimulate enzyme secretion but has no effect on either Ca^{2+} or cyclic AMP metabolism [7–9]. The phorbol esters have been shown to exert their biological effects by stimulating a phospholipid and Ca^{2+}-dependent protein kinase (kinase C) [10,17–20]. Moreover, it has recently been demonstrated that the selective inhibitor (polymyxin B) of protein kinase C can completely block the TPA-induced amylase secretion in rat pancreatic acini [12]. These findings, taken together, would suggest that TPA is acting via a different pathway, involving protein kinase C, to stimulate enzyme secretion.

It has recently been shown that the noradrenaline-evoked amylase secretion is associated with an elevation in endogenous cyclic AMP [5] and it is also well known that the secretory action of ACh is associated with changes in cellular Ca^{2+} metabolism [21]. The combination of two secretagogues which are acting via two different pathways, involving both cyclic AMP and Ca^{2+}, can markedly potentiate one another [6]. The present results have shown that TPA can enhance the secretory action of both noradrenaline and ACh. Moreover, the phorbol ester can also potentiate the secretory responses of the Ca^{2+} ionophore A23187 and dibutyryl cyclic AMP, agents which are known to artificially elevate intracellular Ca^{2+} and cyclic AMP, respectively. The most straightforward interpretation of the effect of TPA on secretagogue-evoked amylase secretion is to postulate that there is a third pathway in the pancreas by which secretagogues can stimulate enzyme output. This pathway is believed to involve protein kinase C [10–13], which in turn can potentiate the actions of pancreatic secretagogues involving either Ca^{2+} or cyclic AMP metabolism.

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