Synergistic effect of A23187 and a phorbol ester on amylase secretion from rabbit pancreatic acini

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The combination of the ionophore A23187 and the phorbol ester 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) stimulates amylase secretion from rabbit pancreatic acini up to a level equal to, or slightly higher than when carbachol is used as stimulant. Each of the two compounds alone gives only a minor stimulation. This synergistic effect of A23187 and TPA supports a role of protein kinase C in pancreatic enzyme secretion.

Secretion *Amylase* *Pancreas* *Phorbol ester* *A23187* *Protein kinase C*

1. INTRODUCTION

The secretion of enzymes from the pancreatic acinar cell can be stimulated by various hormones and neurotransmitters [1–4]. For the stimulants acetylcholine and cholecystokinin-pancreozymin an increase in cytosolic calcium appears to play an important role in the stimulus–secretion coupling process [2–4]. One of the arguments in favour of such a role is the fact that the enzyme secretion can be stimulated by the ionophore A23187 in the presence of extracellular calcium [5–7]. However, in many studies the rate of enzyme secretion obtained with A23187 is lower than that obtained with acetylcholine or cholecystokinin-pancreozymin [6–9].

It has gradually become clear that phosphoinositide breakdown often occurs in hormone-stimulated processes involving an increase in cytosolic calcium [10], such as pancreatic enzyme secretion [11–13]. One of the breakdown products is 1,2 diacylglycerol, which is an activator of the calcium- and phosphatidylserine-dependent protein kinase C [14,15]. Recently, it has been suggested that both an increase in cytosolic calcium and an activation of protein kinase C are involved in the stimulus–response coupling of physiological stimuli [16–18]. The known stimulating effect of phorbol esters, like TPA on protein kinase C [17–20] offers a tool to study the role of this enzyme system.

This has led us to examine the effects of A23187 and TPA, alone and in combination, on amylase secretion from rabbit pancreatic acini and to compare these effects with that of carbachol.

Abbreviation: TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate

2. MATERIALS AND METHODS

2.1. *Materials*

The following chemicals have been obtained from the indicated firms: A23187 (Calbiochem, San Diego, CA), TPA (Sigma, St. Louis, MO), carbachol (ACF Chemiefarma, Maarsen, The Netherlands) and Phadebas test kit (Pharmacia, Uppsala).

2.2. *Pancreatic acini*

Rabbit pancreatic acini are prepared essentially as described for acinar cells [21], except that the divalent cation chelating step is omitted. The method is based on two successive incubations with collagenase and hyaluronidase in a medium containing 0.1 mM Ca^{2+}. After this digestion the

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tissue is incubated in a medium containing 1.2 mM Ca^{2+} and is dispersed by pipetting. The suspension is filtered through nylon gauze and purified by centrifugation through a 4% albumin layer. The intactness of the isolated acini has been demonstrated by Trypan blue exclusion.

For secretion studies a Krebs-Ringer bicarbonate (KRB) medium (pH 7.4) containing 119 mM NaCl; 3.5 mM KCl; 1.2 mM KH_2PO_4; 25 mM $NaHCO_3$; 2.5 mM $CaCl_2$; 1.2 mM $MgCl_2$; 5.8 mM glucose; 1% bovine serum albumin and an amino acid mixture as in [22] is used. The isolated acini are first washed for 5 min in the above medium without $CaCl_2$ but with 0.1 mM EGTA. They are then briefly washed 3 times in KRB medium without $CaCl_2$ and incubated in this medium at 37°C. The stimulants are added after 30 min, and after 60 min the Ca^{2+} concentration is brought to 2.5 mM. Every 15 min samples of the incubation mixture are removed for determination of the amylase activity. At the end of the experiment the acini are homogenized in the incubation medium. Amylase activity is measured in the samples after rapid sedimentation of the acini and in the acinar homogenate by means of the Phadebas test. The ionophore A23187 is added as an ethanolic solution and TPA in dimethylsulfoxide. The solvents alone have no effect on amylase secretion.

3. RESULTS

Acini from rabbit pancreas are incubated in a calcium-free medium after a washing step in which 0.1 mM EGTA is present. After 30 min various stimulants are added and again 30 min later calcium is added to a final concentration of 2.5 mM. In the absence of calcium none of the stimuli gives a significant increase in the rate of amylase secretion (fig.1). This is probably due to the washing procedure, since without prior washing carbachol does stimulate amylase secretion in the absence of extracellular calcium (not shown).

After addition of 2.5 mM calcium all stimulants

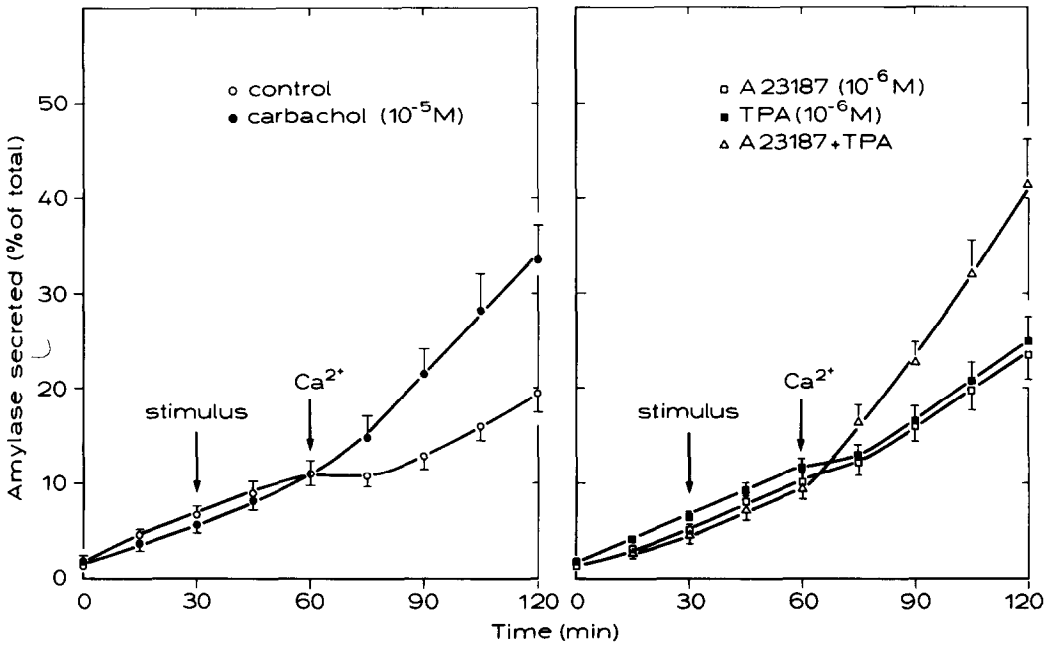

Fig.1. Time dependence of amylase secretion from rabbit pancreatic acini. Acini are incubated in a calcium-free medium and after 30 min no stimulant (—○—), 10^{-5} M carbachol (—●—), 10^{-6} M A23187 (—□—), 10^{-6} M TPA (—■—) or 10^{-6} M A23187 + 10^{-6} M TPA (—△—) is added. After 60 min the calcium concentration is brought to 2.5 mM. Amylase is measured in samples of the incubation mixture and in the acinar homogenate at the end of the experiment. Amylase secretion is expressed as a percentage of total amylase present in the acini. Results are given as means with SE for 5 experiments.

cause an increase in amylase secretion (fig.1). This increase is linear with time, although with A23187 a slight delay seems to occur. The effects of A23187 and TPA alone, both at 10^{-6} M, are small but significant (table 1). Higher concentrations of A23187 have not been used because of reported cytotoxic effects [23]. TPA stimulates maximally at 10^{-6} M (fig.2).

Combination of 10^{-6} M A23187 and 10^{-6} M TPA markedly potentiates the effect of each of the stimuli alone (fig.1) up to a rate which is even slightly higher than that with 10^{-5} M carbachol (table 1). The potentiating effect of TPA on the A23187-stimulated amylase secretion already occurs at a 10^{-8} M concentration and reaches a maximal effect at 10^{-7} M TPA (fig.2).

The stimulating effect of a combination of A23187 and TPA is not further enhanced by addition of 10^{-5} M carbachol. Conversely, the stimulating effect of carbachol is not increased by addition of A23187. With the combination of carbachol and TPA the same level of stimulation is reached as that obtained with the combination of A23187 and TPA (table 1).

Table 1

Effects of TPA on amylase secretion by rabbit pancreatic acini

Compounds added	Amylase secretion (% of carbachol-stimulated secretion)	
	− TPA	+ TPA (10^{-6} M)
Control	29 ± 2.9 (4)	52 ± 4.1 (5)
A23187 (10^{-6} M)	45 ± 3.3 (3)	129 ± 5.8 (6)
Carbachol (10^{-5} M)	≡100	117 ± 3.6 (3)
A23187 + carbachol	97 ± 5.9 (3)	125 ± 9.6 (2)

Acini are incubated in a Ca-free medium. After 30 min the various compounds are added and after 60 min the Ca^{2+} concentration of the medium is brought to 2.5 mM. The amylase secretion in the subsequent 45-min period (60–105 min) is expressed as a percentage of the secretion with 10^{-5} M carbachol. Carbachol induces in this period a secretion of 18.7 ± 3.0% of the total amount of amylase present. Averages with SE, and in parentheses the number of experiments

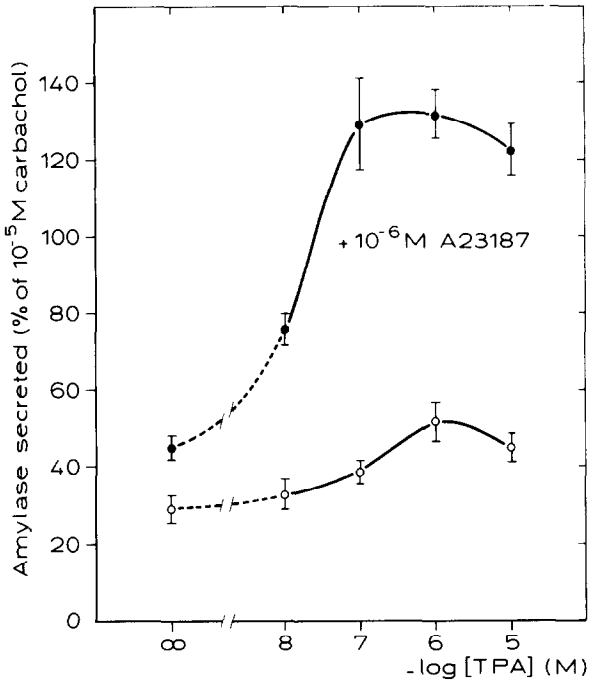

Fig.2. Dose-dependence of the effect of TPA on amylase secretion from rabbit pancreatic acini with and without 10^{-6} M A23187. Acini are incubated in a calcium-free medium. After 30 min TPA is added in the indicated concentration with (—●—) or without (—○—) 10^{-6} M A23187. After 60 min the calcium concentration is brought to 2.5 mM. The amount of amylase secreted in the period 60–105 min is expressed as the percentage of the secretion induced by 10^{-5} M carbachol. Carbachol induced in this period a secretion of 18.7% (SE 3.0) of the total amount of amylase present. Results are given as means with SE for 4–5 experiments.

4. DISCUSSION

The main finding of this study is that the ionophore A23187 and the phorbol ester TPA in combination stimulate amylase secretion from rabbit pancreatic acini much more than each of them alone. The rate of secretion reached with the combination is equal to, or slightly higher than that obtained with carbachol. This suggests that each of these stimulants affects an intracellular process involved in stimulus–secretion coupling. The ionophore in the presence of external calcium is known to lead to an increase in the cytosolic calcium concentration. Phorbol esters have recent-

ly been shown to stimulate protein kinase C [17–20], a Ca^{2+}- and phosphatidylserine-dependent protein kinase, which can also be stimulated by 1,2-diacylglycerols [14,15].

Diacylglycerol is formed from the breakdown of phosphoinositides, which process in the pancreas is stimulated by acetylcholine and cholecystokinin-pancreozymin [11–13]. Hence, our findings are in agreement with the assumption that stimulus–secretion coupling in the pancreas would involve an increase in cytosolic calcium and in diacylglycerol. Both effects would stimulate protein kinase C, which enzyme would then play a key role in stimulus–secretion coupling. The role of phosphoinositides in this process is supported by recent findings in [24] that inositol 1,4,5-triphosphate, a product formed from phosphatidylinositol 4,5-bisphosphate, stimulates the release of calcium from an intracellular pool in permeabilized rat acini.

A central role for protein kinase C in intracellular processes involving calcium is suggested in [16] on the basis of experiments with thrombin-stimulated platelets. Authors in [25] have shown that TPA can stimulate secretion and aggregation in platelets without elevating intracellular calcium above a basal level of 0.1 μM as tested with the intracellular indicator quin2. TPA and calcium also stimulate catecholamine secretion in bovine adrenal medullary cells [26], aldosterone secretion in porcine adrenal glomerulosa cells [27] and insulin secretion in rat islets [28] in a synergistic way.

Stimulating effects of TPA on ^{3}H-labeled protein secretion from guinea pig pancreatic acini have been reported in [29,30]. At that time the relationship between phorbol esters and protein kinase C had not yet been described. In contrast to our findings, they found the same degree of stimulation with TPA as with the cholecystokinin-pancreozymin analogue caerulein [30]. It might be that the cytosolic calcium concentration in acini of the guinea pig is already sufficiently high to obtain a maximally stimulating effect of TPA. An interesting point in [30] is that TPA did not increase the ^{45}Ca efflux, which is in agreement with a mechanism involving a protein kinase.

In summary, the findings reported here extend the hypothesis of a role of both calcium and 1,2-diacylglycerol in stimulus–response coupling to the enzyme secretion process in the pancreas.

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