Phorbol ester stimulates amylase secretion from rat parotid cells

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Phorbol myristate acetate (PMA), a potent activator of Ca^{2+}- and phospholipid-dependent protein kinase (protein kinase C), evoked amylase release from rat parotid cells. In dose-response studies, PMA stimulated amylase release independently of db-cAMP, but potentiated the effect of carbachol. PMA and A23187, a Ca^{2+} ionophore, synergistically increased amylase release. The maximum effect of carbachol was further enhanced by PMA but not by A23187, suggesting that protein kinase C is not fully activated by the muscarinic-cholinergic agonist under the condition where calcium is fully utilized for amylase secretion.

Phorbol ester *Protein kinase C* *cyclic AMP* *Ca^{2+} ionophore* *Amylase secretion* *(Parotid gland)*

1. INTRODUCTION

The exocytosis of amylase from the parotid gland is stimulated by both cAMP- and Ca^{2+}-modulating agonists. In rat parotid gland, β-adrenergic agonists and cAMP derivatives have a stronger effect than Ca^{2+}-modulating agonists, including muscarinic-cholinergic, α-adrenergic and peptidergic agonists [1]. Although its precise role is not fully understood, protein phosphorylation mediated by cAMP-dependent protein kinase is suggested to be involved as an obligatory step in amylase release by cAMP-modulating agonists [2–6]. On the other hand, the secretory mechanism stimulated by Ca^{2+}-modulating agonists is much less understood, since Ca^{2+} ionophores elicit only a limited response [1].

Recently, Ca^{2+}- and phospholipid-dependent protein kinase (protein kinase C) has been reported to be involved in a wide variety of exocytotic systems [7–9]. In the pancreas, where Ca^{2+}-modulating agonists play the main role in exocytosis, amylase secretion was synergistically stimulated by phorbol esters, direct activators of protein kinase C, and Ca^{2+} ionophores [10–15]. Phorbol esters also evoked protein secretion from parotid slices [16] and mucin secretion from submandibular cells [17] of the rat. Here, we further studied the effect of phorbol ester (PMA) on amylase release from rat parotid cells, and the results indicate that phorbol ester stimulates amylase release independently of cAMP and that it enhances the effect of carbachol.

Abbreviations: PMA, phorbol 12-myristate 13-acetate; db-cAMP, N^6,$O^{2'}$-dibutyryl adenosine 3',5'-monophosphate; DMSO, dimethyl sulfoxide

2. MATERIALS AND METHODS

2.1. *Materials*

PMA and A23187 were obtained from Calbiochem. Carbachol, db-cAMP and bovine testicular hyaluronidase (type 1-S) were from Sigma. Collagenase (CLS II) and Hank's balanced salt solution were from Cooper Biomedical, and Gibco, respectively.

2.2. *Preparation of parotid cell aggregates*

Rat parotid cell aggregates were prepared as

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described in [18] with minor modifications. Parotid glands were minced finely and incubated for 60 min at 37°C in Hank's balanced salt solution buffered with 20 mM Hepes-NaOH (pH 7.4, HBSS-H) containing collagenase (130 units/ml) and hyaluronidase (0.25 mg/ml) under 100% O_2 in a metabolic shaker. Minces were gassed and gently pipetted at 20-min intervals; after digestion, they were filtered through two layers of cheesecloth, washed 4 times with HBSS-H containing 0.1% bovine serum albumin, and suspended in the same medium.

2.3. *Assay of amylase release*

For amylase secretion, 1 ml of cell suspension prepared as above was transferred into a glass tube containing 10 μl of either secretagogue or vehicle and incubated for 15 min at 37°C. After incubation, the medium was collected by filtration through glass fiber paper mounted in a Toyo multiple filter holder (Toyo, Japan). For the measurement of total amylase activity, the cells were homogenized with the incubation medium in a Polytron homogenizer. The released amylase activity was expressed as a percentage of the total activity. Amylase activity was measured as described by Bernfeld [19].

3. RESULTS

As shown in fig.1, PMA evoked amylase release from rat parotid cells dose-dependently with a half-maximal dose of around 50 nM. The stimulatory effect of PMA was as strong as that of carbachol, but less than that of isoproterenol or db-cAMP. When the cells were stimulated concurrently with PMA and other agonists, additive or more than additive (PMA + A23187) effects on amylase release were observed (table 1).

To determine whether PMA potentiated the effects of other agonists, we studied the effect of PMA on the dose-response curves of db-cAMP and carbachol. As seen in fig.2, PMA additively increased amylase release at each dose of db-cAMP, suggesting that PMA and db-cAMP stimulate amylase release independently. On the other hand, PMA slightly potentiated the effect of carbachol, since 10^{-6} M carbachol was the half-

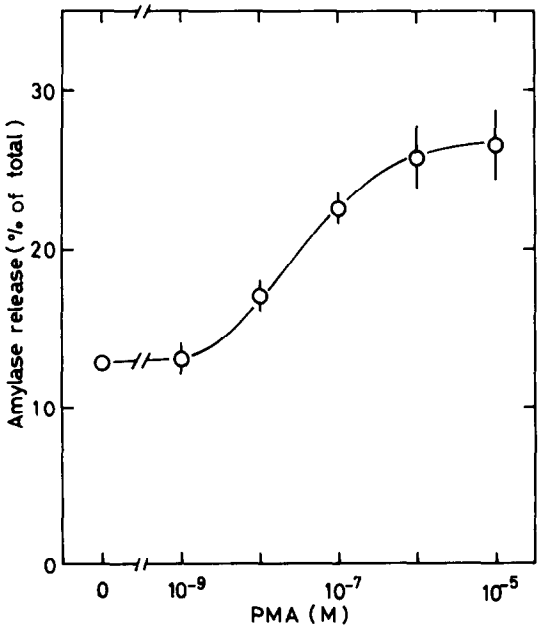

Fig.1. Effect of phorbol ester (PMA) on amylase release from rat parotid cells. Cells were incubated with various doses of PMA at 37°C for 15 min. Data shown are means ± SE (n = 6).

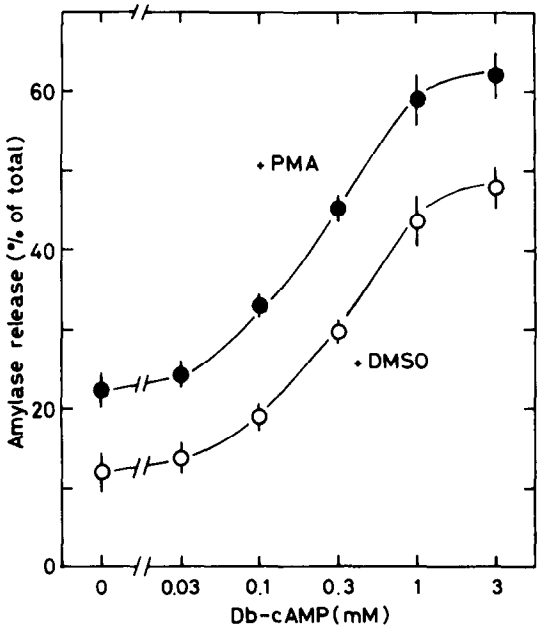

Fig.2. Effect of PMA on amylase release by db-cAMP. Rat parotid cells were incubated with various concentrations of db-cAMP and either 1 μM PMA or 1% DMSO at 37°C for 15 min. Data are means ± SE (n = 6).

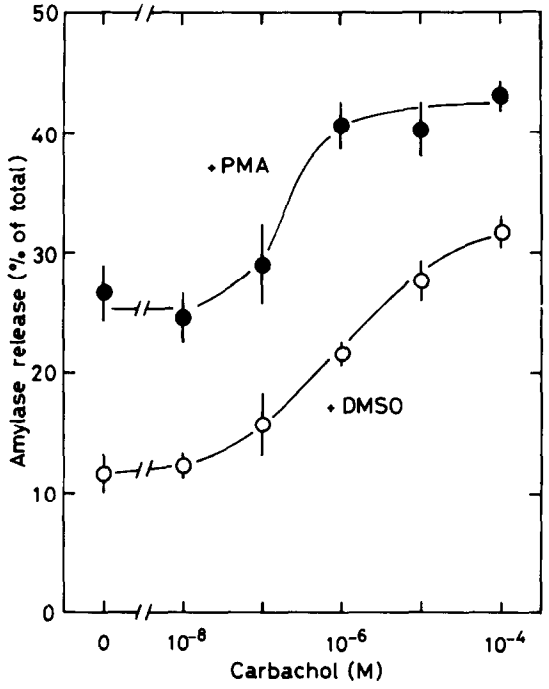

Fig.3. Effect of PMA on amylase release by carbachol. Parotid cells were incubated with various concentrations of carbachol and either 1 μM PMA or 1% DMSO for 15 min. Data are means ± SE (n = 6).

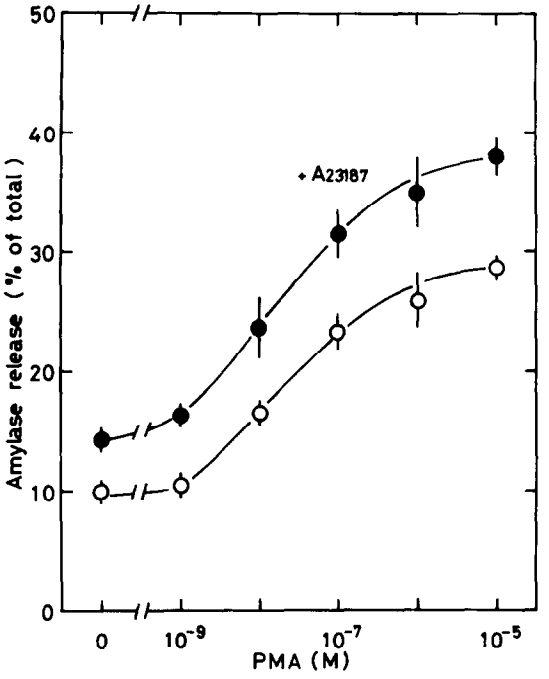

Fig.4. Effect of A23187 on amylase release by PMA. Parotid cells were incubated with various doses of PMA in the presence or absence of 1 μM A23187 for 15 min. Data shown are means ± SE (n = 6).

maximal dose in the absence of PMA but was almost the maximal dose in the presence of PMA (fig.3).

Weak synergism was also observed between PMA and A23187. Namely, A23187 alone stimulated amylase release less than 5% above the control level, but in the presence of PMA evoked amylase release around 10% above the level of PMA alone (table 1 and fig.4).

To evaluate the interrelationship between Ca^{2+} and protein kinase C in carbachol action, we investigated the effects of carbachol, A23187, and PMA in various combinations on amylase release. As shown in table 2, although PMA further in-

Table 1

Effect of PMA on amylase release from rat parotid cells stimulated by db-cAMP, carbachol, or A23187

Treatment	Amylase release (% of total/15 min)	
	+ DMSO	+ PMA (1 μM)
None	11.3 ± 0.6	25.9 ± 1.3
db-cAMP (1 mM)	42.1 ± 2.5	57.9 ± 2.8
Carbachol (10 μM)	27.0 ± 1.4	38.5 ± 1.8
A23187 (1 μM)	13.5 ± 1.1	38.6 ± 2.3

Data shown are means ± SE (n = 6)

Table 2

Effect of PMA and A23187 on amylase release from rat parotid cells stimulated by the maximum dose of carbachol

Treatment	Amylase release (% of total/15 min)
Carbachol (100 μM)	30.8 ± 1.1
+ A23187 (1 μM)	26.3 ± 1.3
+ PMA (1 μM)	39.8 ± 1.6
+ PMA and A23187	35.7 ± 1.3

Data shown are means ± SE (n = 6)

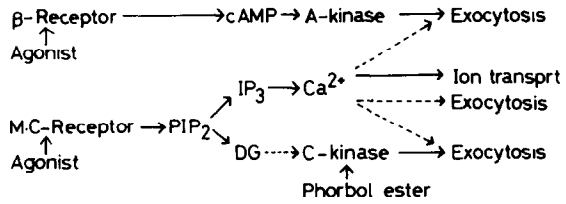

Fig.5. Secretory mechanisms by cAMP- and Ca^{2+}-modulating agonists in rat parotid gland. M·C-Receptor, muscarinic-cholinergic receptor; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; DG, diacylglycerol; A-kinase, cAMP-dependent protein kinase; C-kinase, Ca^{2+}- and phospholipid-dependent protein kinase.

creased amylase release stimulated by the maximum dose of carbachol (100 μM), A23187 did not increase, but rather decreased, the amylase release stimulated by carbachol alone or by carbachol plus PMA.

4. DISCUSSION

This study clearly demonstrates that the phorbol ester PMA evokes amylase release from rat parotid cells and that the effect is completely independent of that of db-cAMP. These results indicate that protein kinase C activated by phorbol esters and cAMP-dependent protein kinase are separately involved in amylase release from rat parotid gland.

On the other hand, the possible involvement of Ca^{2+} and protein kinase C in amylase release by carbachol was suggested by the following findings: (i) PMA potentiated the effect of carbachol, and (ii) PMA and A23187 synergistically stimulated amylase release to the extent stimulated by carbachol plus PMA. In these experiments, the maximum effect of carbachol was further enhanced by PMA but not by A23187 (table 2), suggesting that protein kinase C was not fully activated by diacylglycerol, a physiological activator liberated from phosphoinositide breakdown.

In the rat parotid gland, Ca^{2+} ionophore A23187 by itself has a weak ability to evoke amylase release, but has a potent effect on ion transport [20]. As reported by Putney et al. [16], phorbol ester did not stimulate ^{86}Rb efflux from rat parotid slices, implying that protein kinase C is not involved in ion transport. From these findings, it may be concluded that Ca^{2+} plays its main role in ion and fluid transport in the rat parotid gland, although it facilitates amylase release promoted via protein kinase C or cAMP-dependent protein kinase. Thus, excess Ca^{2+} introduced by A23187 decreased amylase release probably due to ATP consumption required for ion and fluid transport (table 2).

From these studies we have proposed a secretory mechanism for rat parotid gland, which is consistent with the above findings (fig.5).

REFERENCES

[1] Butcher, F.R. and Putney, J.W. (1980) Adv. Cyclic Nucleotide Res. 13, 215–249.
[2] Kanamori, T. and Hayakawa, T. (1980) Biochem. Int. 1, 359–402.
[3] Baum, B.J., Freiberg, J.M., Ito, H., Roth, S.G. and Filburn, C.R. (1981) J. Biol. Chem. 256, 9731–9736.
[4] Jahn, R. and Söling, H.D. (1981) Proc. Natl. Acad. Sci. USA 78, 6903–6906.
[5] Sperman, T.N., Hurley, K.P., Olivas, R., Ulrich, R.G. and Butcher, F.R. (1984) J. Cell Biol. 99, 1354–1363.
[6] Quissell, D.O., Deisher, L.M. and Barzen, K.A. (1985) Proc. Natl. Acad. Sci. USA 82, 3237–3241.
[7] Nishizuka, Y. (1984) Nature 308, 693–698.
[8] Berridge, M.J. (1984) Biochem. J. 220, 345–360.
[9] Rasmussen, H. and Barrett, P.Q. (1984) Physiol. Rev. 64, 938–984.
[10] Gunther, G.R. (1981) J. Biol. Chem. 256, 12040–12045.
[11] De Pont, J.J.H.H.M. and Fleuren-Jakobs (1984) FEBS Lett. 170, 64–68.
[12] Wooten, M.W. and Wrenn, R.W. (1984) FEBS Lett. 171, 183–186.
[13] Singh, J. (1985) FEBS Lett. 180, 191–195.
[14] Pandol, S.J., Schoeffield, M.S., Sachs, G. and Muallem, S. (1985) J. Biol. Chem. 260, 10081–10086.
[15] Merritt, J.E. and Rubin, R.P. (1985) Biochem. J. 230, 151–159.
[16] Putney, J.W., McKinney, J.S., Aub, D.L. and Leslie, B.A. (1984) Mol. Pharmacol. 26, 261–266.
[17] Fleming, N., Bilan, P.T. and Sliwinski-Lis, E. (1986) Pflügers Arch. 406, 6–11.
[18] Takuma, T., Kuyatt, B.L. and Baum, B.J. (1984) Am. J. Physiol. 247, G284–G289.
[19] Bernfeld, P. (1955) Methods Enzymol. 1, 149–158.
[20] Putney, J.W. (1983) Cell Calcium 4, 439–449.