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COMBINED EFFECT OF PHORBOL ESTER AND, A23187 OR DIBUTYRYL CYCLIC AMP ON PEPSINOGEN SECRETION FROM ISOLATED GASTRIC GLANDS

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Summary: In isolated guinea pig gastric glands, pepsinogen secretion was stimulated by the phorbol ester, 12-0-tetradecanoyl -phorbol-13-acetate (TPA) in a dose dependent manner. Calcium-deprivation from the medium resulted in the decrease in TPA-induced pepsinogen secretion. The combination of 0.4 μM Ca^{2+}ionophore A23187 and TPA stimulated pepsinogen secretion slightly higher than the caliculated additive value for each agent. This synergistic effect of the agents supports a role of calcium-activated, phospholipid-dependent protein Kinase (protein Kinase C) in gastric pepsinogen secretion. Furthermore, pepsinogen secretion was also stimulated by dibutyryl cyclic AMP (dbc AMP) and dbc AMP slightly enhanced TPA-induced pepsinogen secretion. Results suggest that gastric chief cells possess at least two different secretory pathways for pepsinogen which are probably dependent on protein kinase C and cyclic AMP, respectively.

Recent reports have suggested that CCK, gastrin and cholinergic agents, whose actions are probably mediated by intracellular Ca^{2+} mobilization, were effective stimuli for pepsinogen secretion in gastric glands (1). It has gradually become clear that phosphoinositide breakdown often occurs in hormone-stimulated processes involving an increase in cytosolic Ca^{2+}. Takai, Kaibuchi and co-workers have found that not only Ca^{2+} mobilization but also a particular protein Kinase (protein Kinase C) activated by diacylglycerol, a derivative of

Abbreviations used are: TPA, 12-0-tetradecanoyl-phorbol-13-acetate; protein Kinase C, Ca^{2+}-activated, phospholipid-dependent protein Kinase; dbcAMP, N^6-2'-0-dibutyryladenosine-3'5'-cyclic monophosphate; VIP, vasoactive intestinal polypeptide; and CCK, cholecystokinin.

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phosphoinositide breakdown in response to extracellular messenger, are essential to cause serotonin release from platelets (2). Following this observation, it has been demonstrated that Ca^{2+} mobilization and activation of protein Kinase C are synergistically effective for the physiological cellular response such as serotonin release from platelets, amylase secretion from pancreatic acini and insulin secretion from islets (3)(4)(5). The known stimulating effect of phorbol esters like TPA on protein Kinase C offers a tool to investigate the role of this enzyme in those studies (6).

With respect to the pepsinogen secretion, secretin, VIP and forskolin whose actions are probably mediated by cyclic AMP are also effective stimuli in isolated gastric glands (7)(8). Therefore, present study was conducted to see the effect of TPA, Ca^{2+}ionophore A23187 or dbcAMP, alone and in combination, on pepsinogen secretion from isolated guinea pig gastric glands.

MATERIALS AND METHODS

Gastric Glands Preparation

The guinea pig stomach was removed by blunt dissection. After stripping the smooth muscle coat, the fundus and corpus were minced and digested for 50 min at 37°C in a incubation solution containing 0.1% collagenase. At the end of incubation, the glands were filtered through nylon mesh to remove coarse fragments and rinsed. Finally, the glands were suspended in the medium consisting of 132 mM NaCl, 4.7 mM KCl, 1.1 mM $MgCl_2$, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 0.5% BSA, pH7.4 with or without 1.28 mM $CaCl_2$.

Pepsinogen Determination

Glands suspension was pre-incubated at 37°C for 30 min. Agents were then added and the suspension was incubated for an additional 30 min. An aliquot of the suspension was taken and centrifuged, and the supernatant separated for pepsinogen determination. Pepsinogen was measured by the method of Anson and Mirsky (9) using acid-denaturated hemoglobin as the substrate. The reaction was stopped by adding trichloroacetic acid solution. Supernatant samples, read at 280 nm, were caliculated using a standard curve for peptic activity of crystallized pepsinogen. Pepsinogen released into the medium was then corrected as the percentage of the total peptic activity present in the glands before incubation.

Chemicals

The phorbol ester, 12-0-tetradecanoylphorbol-13-acetate (TPA), Ca^{2+}ionophore A23187 dibutyryladenosine-3'-5'-cyclic monophosphate (dbcAMP), and type I collagenase were obtained

from Sigma chemical (St. Louis). Secretin was obtained from Protein Research Foundation (Osaka, Japan). Synthetic C-terminal octapeptide of cholecystokinin (CCK_8) was a gift from Squibb Institute for Medical Research (Princeton).

RESULTS

A comparison of the pepsinogen secretory responses to TPA, Ca^{2+}ionophore and dbcAMP is shown in Fig. 1. Pepsinogen was stimulated by each agent alone in a dose-dependent manner. Detectable stimulation of pepsinogen secretion occurred with 3 ng/ml TPA, $3x10^{-7}$M Ca^{2+}ionophore or 10^{-4}M dbcAMP.

In the next experiments, the effect of agents in combination on pepsinogen secretion was tested. To elucidate the influence of Ca^{2+} mobilization, gastric glands were first pre-incubated at 37°C for 30 min in a Ca^{2+}-free medium. Then, the effect of TPA in the absence of Ca^{2+} was compared to that in the presence of 1.28 mM Ca^{2+} plus 0.4 μM Ca^{2+}ionophore. Pepsinogen secretion was occurred by the TPA stimulation even in the absence of Ca^{2+}, though Ca^{2+}-deprivation resulted in the decrease in pepsinogen secretion. Moreover, as shown in Fig. 2, pepsinogen secreted by the stimulation of TPA plus Ca^{2+}ionophore was greater than the caliculated additive value for these agents.

The effect of a submaximal concentration of dbcAMP on TPA-induced pepsinogen secretion was also tested. Pepsinogen secretion caused by 10^{-4}M dbcAMP plus TPA was always a little greater than the caliculated additive value in 3 separate experiments. Results shown in Fig. 3 are representative of these experiments. In our systems, CCK_8 or secretin caused the pepsinogen secretion as shown in Fig. 4. When the effect of dbcAMP on CCK-induced pepsinogen secretion was examined, the responses to 10^{-4}M dbcAMP plus various doses of CCK_8 were almost equal to the caliculated additive values. Similarly, the

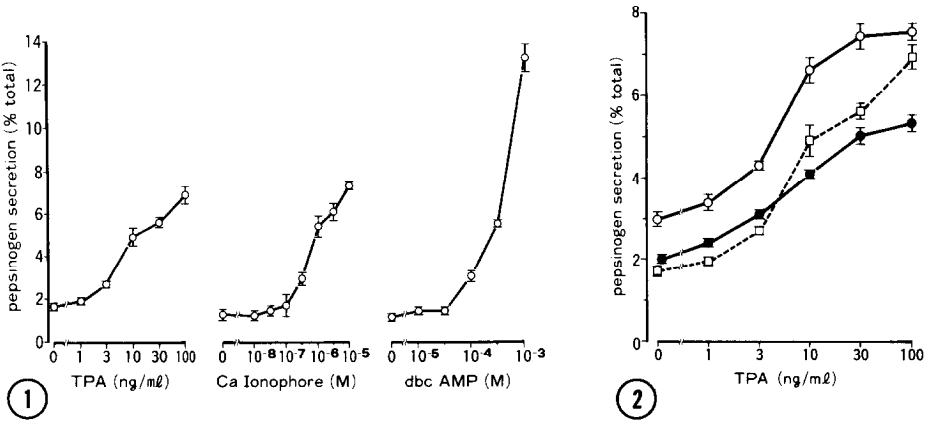

Fig. 1 Effect of TPA, Ca^{2+}ionophore A23187 and dbcAMP on pepsinogen secretion. Gastric glands were incubated in a standard medium containing 1.28 mM Ca^{2+}. TPA, Ca^{2+}ionophore dissolved in dimethylsulfoxide and dbcAMP were then added at the indicated concentrations. Values shown are the mean±SE from triplicate determinations and representative of 3 separate experiments.

Fig. 2 Synergistic effect of TPA and Ca^{2+}ionophore on pepsinogen secretion. Gastric glands were pre-incubated in a Ca^{2+}-free medium. After 30 min, TPA at the indicated concentrations and 0.4 μM Ca^{2+}ionophore were added in the presence of Ca^{2+} (o——o). For comparison, pepsinogen secretion stimulated by TPA in the absence of Ca^{2+} was shown (●——●). TPA-induced pepsinogen secretion from glands pre-incubated and incubated in the standard medium (the same data as Fig. 1) was shown at the dotted line (□---□).
Values shown are the mean±SE from triplicate determinations and representative of 3 separate experiments.

pepsinogen responses to 3 ng/ml TPA plus various doses of secretin were almost equal to the caliculated additive values.

DISCUSSION

Major findings of the present study is that pepsinogen secretion can be stimulated by TPA which can substitute for diacylglycerol and thus has been suggested to activate the protein Kinase C directly (10)(11). Pepsinogen secretion stimulated by TPA was partially abolished in the absence of extracellular Ca^{2+}. Results are good accordance with that protein Kinase C absolutely require Ca^{2+} for its activation, but it can be activated by TPA without detectable cellular Ca^{2+} mobilization (12). Thus, the known stimulating effect of TPA on

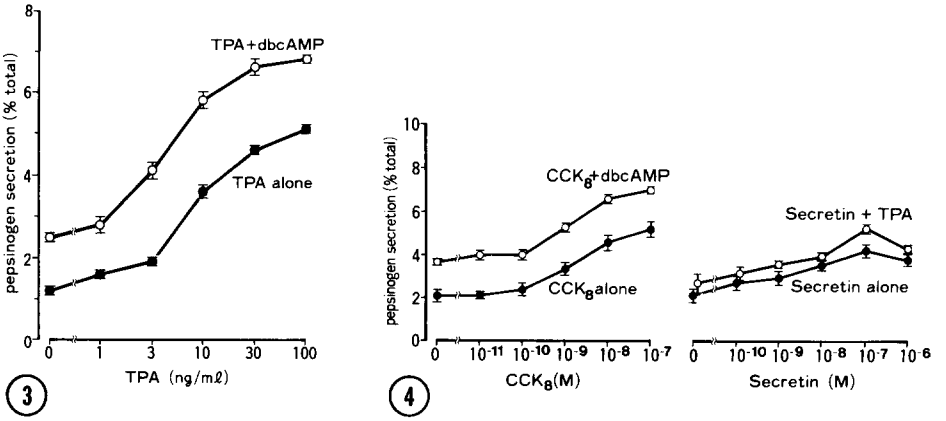

Fig. 3 The combined effect of TPA and dbcAMP on pepsinogen secretion. Gastric glands were pre-incubated and incubated in a Ca^{2+}-free medium. TPA was added at the indicated concentrations with (o——o) or without (●——●) 10^{-4}M dbcAMP.
Values shown are the mean±SE from triplicate determinations and representative of 3 separate experiments.

Fig. 4 Effect of dbcAMP or TPA on dose-response curves for peptides. Gastric glands were preincubated in a standard medium. After 30 min, 10^{-4}M dbcAMP or 3 ng/ml TPA was added to the glands suspended in a stadard medium containing peptides at the indicated concentrations.
Values are the mean±SE from triplicater determinations and representative 3 separate experiments.

protein Kinase C appears to support a role of this enzyme in gastric pepsinogen secretion. In our systems, 0.4 μM Ca^{2+}ionophore was also found to be synergistically effective for TPA-induced pepsinogen secretion. Results may indicate that Ca^{2+} mobilization, in addition to the activated protein Kinase C, is also essential for full biological response in the gastric chief cell.

It has been well known that CCK or cholinergic agents stimulate pancreatic enzyme secretion through Ca^{2+} dependent process (13). In addition, the breakdown of the phosphoinositide can be stimulated in the pancreas by those agents (14). Thus it is possible to speculate that the stimulatory effect of CCK or cholinergic agents on pepsinogen secretion may be due to the activated protein Kinase C in the chief cell. Furthermore, pepsinogen responses to dbcAMP were additive to those of CCK and

the responses to TPA were additive to those of secretin in the present study. Of interest is the observation that pepsinogen secretion stimulated by the combined addition of TPA and dbcAMP is equal to or greater than the additive value, suggesting that the chief cell possesses at least two different pathway for pepsinogen secretion. Our finding may give an explanation to the earlier observation that potentiation of pepsinogen secretion occurred with secretin or VIP plus carbachol or CCK (15). That is the speculation that cyclic AMP dependent protein Kinase and protein Kinase C may be synergistically effective for the physiological chief cell response such as pepsinogen secretion.

REFERENCES

1. Hersey, S.J., May, D. and Schyberg, D. (1983) Am. J. physiol. 244, G192-G197
2. Takai, Y., Kishimoto, A., Iwasa, Y., Kawahara, Y., Mori, T. and Nishizuka, Y. (1979) J. Biol. Chem. 254, 3692-3695
3. Kaibuchi, K., Takai, Y., Sawamura, M., Hoshijima, M., Fujikura T. and Nishizuka, Y. (1983) J. Biol. Chem. 258, 6701-6704
4. de Pont, J.J.H.H.M. and Fleuren-Jakobs, A.M.M. (1984) FEBS Lett. 170, 64-68
5. Zawalich, W., Brown, C. and Rasmussen, H. (1983) Biochem. Biophys. Res. Commun. 117, 448-455
6. Castagna, M., Takai Y., Kaibuchi, K., Sano, K., Kikkawa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7847-7851
7. Sanders, M.J., Amirian D.A., Ayalon, A. and Soll, A.H. (1983) Am. J. Physiol. 245, G641-G646
8. Hersey, S.J., Owirodu, A. and Miller, M. (1983) Biochim. Biophys. Acta 755, 293-299
9. Anson, M.L. and Mirsky, A.E. (1932) J. Gen. Physiol. 16, 59-63
10. Niedel, J.E., Kuhn, L.J. and Vandenbark, G.R. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 36-40
11. Kikkawa U., Takai, Y., Tanaka, Y., Miyake, R. and Nishizuka, Y. (1983) J. Boil. Chem. 258, 11442-11445
12. Yamanishi, J., Takai, Y., Kaibuchi, K., Sano, K., Castagna, M. and Nishizuka, Y. (1983) Biochem. Biophys. Res. Commun. 112, 778-786
13. Chandler, D.E. (1978) Life Sciences 23, 323-334
14. Tennes, K.A. and Roberts, M.L. (1982) Biochim. Biophys. Acta 719, 238-243
15. Raufman, J.P., Kasbekar, D.K., Jensen, R.T. and Gardner, J.D. (1983) Am. J. Physiol. 245, G525-G530