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PHORBOL ESTER STIMULATES CALCITONIN SECRETION SYNERGISTICALLY WITH A23187, AND ADDITIVELY WITH DIBUTYRYL CYCLIC AMP IN A RAT C-CELL LINE

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Summary: The mechanism of action of 12-O-tetradecanoyl phorbol-13-acetate (TPA) on calcitonin secretion was studied in a rat C-cell line, rMTC 6-23. TPA stimulated calcitonin secretion at the concentration of 16nM. This effect was synergistically enhanced with calcium ionophore, A23187. Synthetic diacylglycerol, 1-oleoyl-2-acetyl-glycerol (OAG), also showed a synergism with A23187 on calcitonin secretion. When dibutyryl cyclic AMP was added with TPA, an additive effect was obtained. These data suggest that C-kinase might be a possible regulator of calcitonin secretion in addition to the cyclic AMP-mediated pathway.

There is a substantial evidence that Ca^{2+}activated, phospholipid-dependent protein kinase (C-kinase), widely distributed in various tissues and cells, plays a crucial role in signal transduction of many kinds of cellular functions (1). C-kinase might also be involved in the release of hormones, such as aldosterone (2), insulin (3), catecholamine (4), cortisol (5), prolactin (6, 7), and growth hormone (8).

Phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA), known to be a potent tumor promoter, has pleiotropic effects (9). One of its effect is to activate C-kinase as a substitute for diacylglycerol which is produced in the process of phosphoinositide turnover (10). C-kinase itself is thought to be a receptor for TPA (11).

Kaibuchi et al. showed that the effect of TPA is enhanced synergistically by the low concentration of calcium ionophore, A23187, in serotonin release from platelets, the former acting on C-kinase, the latter on Ca^{2+} mobilization

Abbreviations: CT, calcitonin; TPA, 12-O-tetradecanoyl phorbol-13-acetate; cAMP, adenosine 3',5'-monophosphate; DBcAMP, dibutyryl cyclic AMP; C-kinase, Ca^{2+} activated, phospholipid-dependent protein kinase; A-kinase, cyclic AMP-dependent protein kinase; OAG, 1-oleoyl-2-acetyl-glycerol;

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(12). Such a synergistic effect of these agents has been found in the secretion of hormones (2, 3, 4, 5, 7) as well as histamine release from mast cells (13) and lysosomal enzyme release from neutrophils (14).

Recently, deBustros et al. reported that TPA increases calcitonin (CT) secretion and its production at the transcriptional level from human medullary thyroid carcinoma cells (15). However, it is not yet determined whether the effect of TPA on CT secretion is mediated via C-kinase activation. Furthermore, the interaction of C-kinase with Ca^{2+} mobilization or with cAMP-dependent protein kinase (A-kinase) should be clarified.

In the present study, the effect of phorbol esters or synthetic diacylglycerol, 1-oleoyl-2-acetyl-glycerol (OAG), in combination with A23187 or dibutyryl cyclic AMP (DBcAMP), was investigated in rMTC 6-23 cells, originated from rat transplantable medullary thyroid carcinoma (16, 17).

Materials and Methods

Materials; TPA, 4α-and 4β-phorbol 12,13-didecanoate, and A23187 were purchased from Sigma Chemicals Co. (St. Louis, MO). OAG from Avanti Polar-Lipids, INC. (Birmingham, AL). DBcAMP from Toyo Jozo Co. (Choshi, Japan).

Cell culture; rMTC 6-23 cells (American Type Culture Collection) were grown as monolayers in Dulbecco's modified essential medium (Flow) supplemented with 15% horse serum (Gibco) in a humidified atmosphere with 5% CO_2 95% air.

Secretion experiment; After preincubation with serum-free Ham's F12 medium (Flow) for 15 min, confluent cells on replicate dishes were incubated with the medium containing test agents or vehicle alone for 60 min except for the time course study, as described previously (18). Then, the medium was collected, centrifuged, and kept at -20C until assayed. CT was measured by radioimmunoassay using human CT antibody (19), which completely cross-reacted with rat CT. CT concentration was corrected by cell protein measured by the method of Lowry et al.(20).

The results were expressed as the mean $\pm$ SEM. Statistical significance was assessed by Student's t-test.

Results

The time course effect of TPA and/or A23187 on CT secretion is shown in Fig.1. The stimulatory effect of TPA was sustained as compared to that of A23187. The effect of TPA was markedly enhanced at 60 and 90 min, when combined with A23187. At 60 min CT release induced by 250 nM A23187 was 130% of control, and that induced by 32 nM TPA was 160% of control, whereas the value

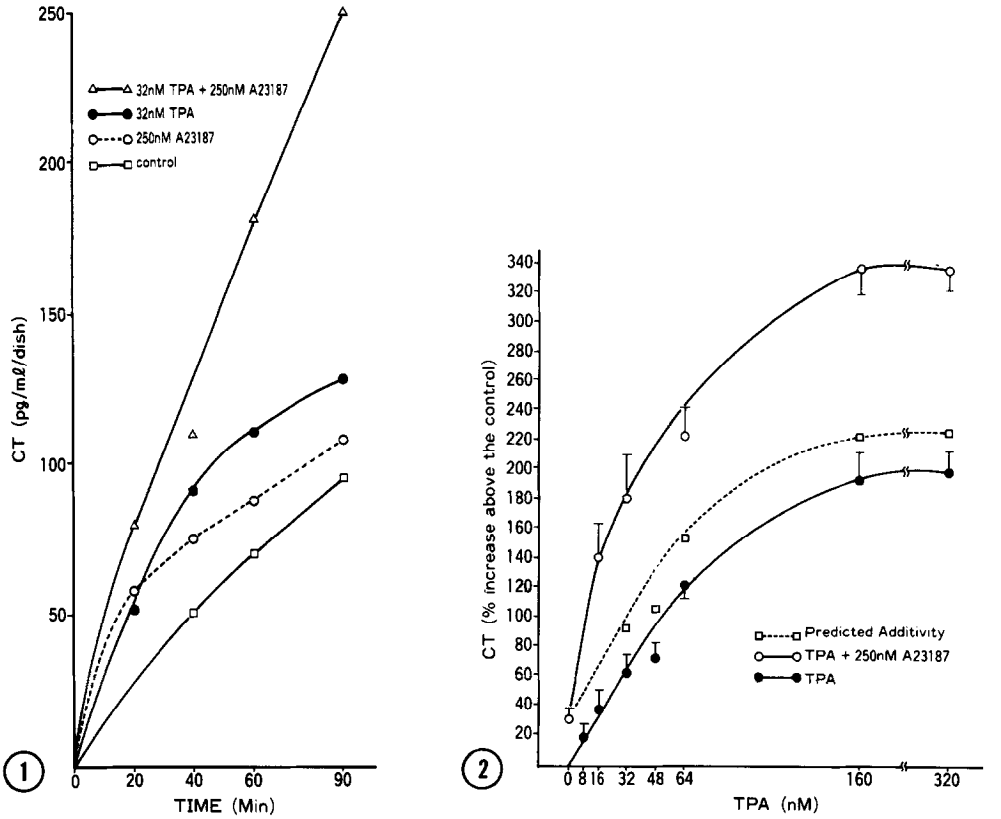

Fig. 1. The time course effect of TPA and/or A23187 on CT release. The effect of TPA was sustained as compared to that of A23187. At 60 and 90 min, the effect of TPA was synergistically enhanced by 250 nM A23187.

Fig. 2. The dose-dependent effect of TPA with or without A23187 (250 nM). TPA induced the increase of CT secretion in a dose-dependent manner. When combined with A23187, TPA caused a synergistic, more than additive, effect. The predicted additivety with TPA and A23187 is shown in (□-----□).

in combination of both was 260% of control, higher than the additive of each agent.

The dose-dependent effect of TPA with or without 250 nM A23187 is shown in Fig.2. TPA at the concentration of 16 nM caused a significant increase of CT release (33% increase above the control, $p < 0.05$, n=6), reaching the maximum at 160 nM (190% increase above the control, $p<0.001$, n=6). A synergism with A23187 was observed at each concentration of TPA.

To explore the role of phorbol ester on CT release, the effect of 4β-phorbol 12,13-didecanoate, an activator of C-kinase, and that of 4α-phorbol 12,13-didecanoate, non-activator of C-kinase, was compared. 4α-phorbol 12,13-

didecanoate failed to stimulate CT release even at the concentration of 150 nM, whereas 4β-phorbol 12,13-didecanoate stimulated CT release significantly at 30 nM and 150 nM (130%, 150% of control, respectively)

The effect of synthetic diacylglycerol, OAG, with or without A23187 was examined (Fig.3)。 OAG at the concentration of 25μg/ml induced 41% increase above the control. A simultaneous addition of 250 nM A23187 caused a synergistic enhancement of CT release (104% increase above the control).

In order to estimate the interaction between C-kinase and A-kinase, DBcAMP was added into the medium with or without TPA. Fig.4 shows the effect of DBcAMP on CT release。 DBcAMP induced a significant increase at the concentration of 200 μM and 500 μM (41%, 54% increase above the control, respectively). When combined with TPA, each concentration of DBcAMP caused an additive, but not synergistic, effect on CT release. This effect was exhibited with TPA at 160 nM, the maximal effective dose。

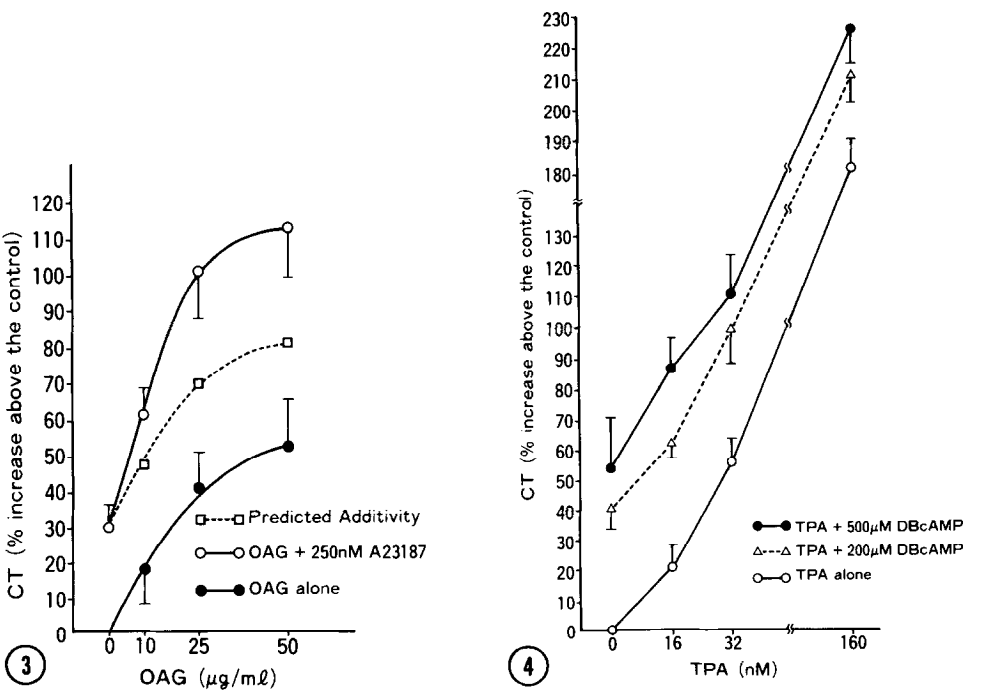

Fig. 3. The effect of OAG on CT release with or without A23187. At the concentration of 25μg/ml, OAG significantly stimulated CT release, which was synergistically enhanced in combination with A23187.

Fig. 4. The effect of DBcAMP on TPA-induced CT release. An additive effect of DBcAMP was demonstrated.

Discussion

It seems evident that a synergism exists between C-kinase and Ca^{2+} mobilization in several cell types. We have demonstrated that TPA stimulated CT secretion from a rat C-cell line, and that its effect was synergistically enhanced by the low concentration of A23187. Furthermore, we have shown that OAG which can penetrate the cell membrane and mimics the action of diacylglycerol (12) had a similar effect on CT release. Although the effect of OAG was less potent than TPA, this might be due to the fact that it is easily metabolized in situ (1). These data strongly suggest that TPA acted to stimulate CT secretion via C-kinase-mediated pathway. This is also supported by the data that 4α-phorbol 12,13-didecanoate, but not 4α-phorbol 12,13-didecanoate, stimulated CT release.

While a rise in extracellular Ca^{2+} (21) as well as cytosolic Ca^{2+}(18) serves as a stimulator of CT secretion, A-kinase system also seems to be involved (22). When added together, DBcAMP and TPA caused an additive effect, indicating that TPA, or C-kinase, acts independently from cAMP-mediated pathway. It has been reported that cAMP counteracts to the action of C-kinase in platelets (23) and erythrocytes (24). On the other hand, the secretion of prolactin (6) and growth hormone (8) is additively stimulated by DBcAMP and TPA, similar to that of CT, shown in the present paper. The cause of the difference of the interaction of cAMP and C-kinase between the cell types still remains to be solved.

In summary, the present data suggest that C-kinase might be involved in the regulation of CT secretion as well as cAMP-mediated pathway. However, further explorations would be required to define whether C-kinase plays a physiological role in CT secretion.

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