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SYNERGISTIC INDUCTION OF ORNITHINE DECARBOXYLASE BY DIACYLGLYCEROL, A23187, AND CHOLERA TOXIN IN GUINEA PIG LYMPHOCYTES

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SUMMARY: When guinea pig lymphocytes were cultured with 1-oleoyl-2-acetylglycerol (OAG), A23187, and cholera toxin, ornithine decarboxylase activity was induced synergistically, peaking at 6 h. Addition of 12-O-tetradecanoylphorbol 13-acetate (TPA), A23187, and dibutyryl cAMP caused the same kind of induction. Cholera toxin potentiated the ability of A23187 to induce ornithine decarboxylase, but not that of OAG. Dibutyryl cAMP augmented the induction caused by A23187 but not by TPA. These results suggest that both the activation of Ca^{++}-sensitive, phospholipid-dependent protein kinase (protein kinase C) and the increase in intracellular levels of Ca^{++} and cAMP are necessary for this induction. cAMP may potentiate the induction by modulating a Ca^{++} messenger system other than that for protein kinase C activation.

Many stimuli increase the activity of ornithine decarboxylase (EC 4.1.1.17), and the resultant increases in polyamine levels have been implicated in different biological actions of cells responding to such stimuli (1-3). In eukaryotic cells cAMP (4, 5), Ca^{++}(6-12), glutamine, and asparagine (13) are inducers of the activity of this enzyme. Treating lymphocytes with Clostridium phospholipase C induces ornithine decarboxylase activity (14), and the activity of this enzyme increases in proportion to the amount of phosphatidic acid and diacylglycerol formed (15). Further, treatment of cells with both phospholipase C and the Ca^{++} ionophore A23187 caused the synergistic induction of the ornithine decarboxylase of guinea pig lymphocytes (16). It seems that both the activation of protein kinase C

ABBREVIATIONS: OAG, 1-oleoyl-2-acetylglycerol; TPA, 12-O-tetradecanoylphorbol 13-acetate; protein kinase C, Ca^{++}-sensitive, phospholipid-dependent protein kinase.

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caused by diacylglycerol and an increase in the intracellular Ca^{++} level are necessary for this induction. To check this, we tested the effect of 1-oleoyl-2-acetylglycerol (OAG), an activator of protein kinase C (17), on ornithine decarboxylase induction.

MATERIALS AND METHODS

Materials: DL-[5-^{14}C]Ornithine was purchased from Amersham International, Amersham, Buckinghamshire, K. 12-O-Tetradecanoylphorbol 13-acetate (TPA) was from the LC Service Corp., Woburn, MA. A23187 was from Calbiochem Behring, La Jolla, CA. OAG was synthesized by the method of Buchnea (18). Horse serum was the product of Commonwealth Serum Laboratories, Victoria, Australia. Dibutyryl cAMP was purchased from Sigma Chemical Co., St. Louis, MO. Cholera toxin was from the Chemo-sero-therapeutic Research Institute, Kumamoto.

Lymphocyte culture: Lymphocytes were prepared from guinea pig lymph nodes as described previously (6) and cultivated in Eagle's minimum essential medium supplemented with 5% horse serum and 2 mM glutamine in 5% CO_2 in air. The medium was exchanged for fresh medium after 18 h and agents were then added. OAG was dissolved in dimethyl sulfoxide and A23187 in ethanol. The final concentration of dimethyl sulfoxide was 0.2% and that of ethanol was 0.5%.

Assay of ornithine decarboxylase activity: After treatment of cells with OAG, A23187, and cholera toxin, cells were harvested by centrifugation. Preparation of the enzyme extract and the measurement of ornithine decarboxylase activity by estimating the amount of putrescine formed from [5-^{14}C]ornithine were described elsewhere (15).

RESULTS

Figure 1 shows the course of increases in ornithine decarboxylase activity. OAG or A23187 alone did not cause an increase. When added together with cholera toxin, A23187 increased ornithine decarboxylase activity, but OAG did not. OAG, A23187, and cholera toxin together induced this enzyme synergistically. Cholera toxin alone did not increas the activity (data not shown). The increase in activity caused by the combination of OAG, A23187, and cholera toxin was inhibited by actinomycin D or cycloheximide (data not shown), suggesting that de novo synthesis of RNA and protein was necessary for the increase.

Figure 2 shows the effect of OAG concentration on the activity of ornithine decarboxylase. OAG alone at the concentrations we tried did not increase the enzyme activity. For cells treated with a high concentration of OAG and with A23187, this activity was slightly higher than for cells

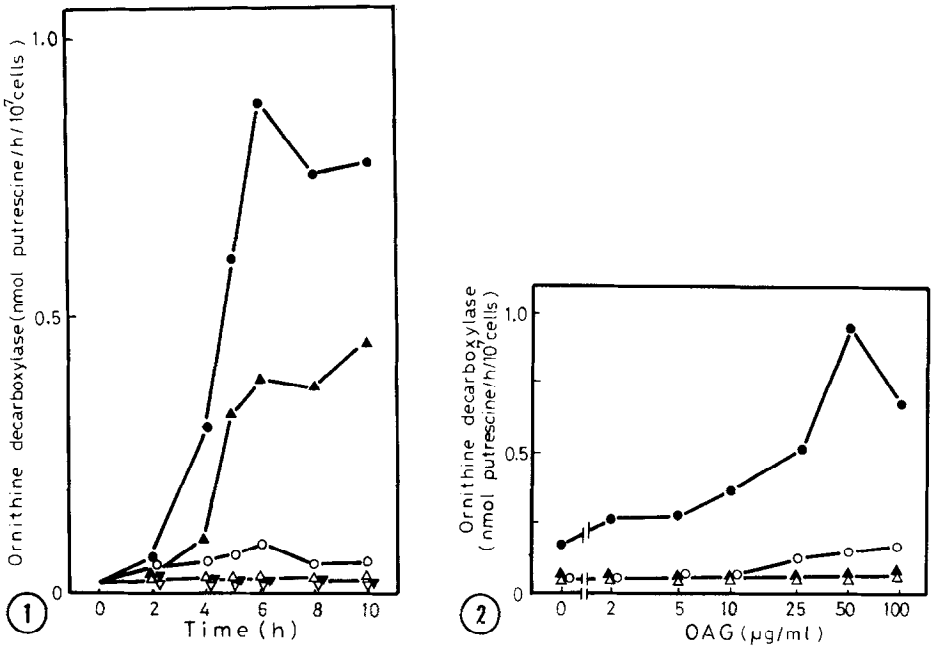

Fig. 1. Course of ornithine decarboxylase induction by OAG, A23187, and cholera toxin. OAG (50 μg/ml), A23187 (0.125 μg/ml) and cholera toxin (0.2 μg/ml) were added at time 0 of cell culture. Cells were harvested at the times indicated and ornithine decarboxylase activity was measured. Each point is the mean of duplicate experiments. ●, OAG, A3187, and cholera toxin; ▲, A23187 and cholera toxin; ○, OAG and A23187; △, A23187; ▽, OAG; ▼, OAG and cholera toxin.

Fig. 2. Effects of OAG on ornithine decarboxylase induction. Cells were cultivated with various concentrations of OAG, A23187 (0.125 μg/ml), and cholera toxin (0.2 μg/ml) for 6 h. Then ornithine decarboxylase activity was measured. Each point is the mean of duplicate experiments. ●, OAG, A23187, and cholera toxin; ○, OAG and A23187; △, OAG; ▲, OAG and cholera toxin.

treated with either OAG or A23187. However, OAG, A23187, and cholera toxin together caused synergistic induction of the enzyme activity. The optimum concentration of OAG for ornithine decarboxylase induction was 50 μg/ml. Since the tumor promoter TPA activates protein kinase C, as does OAG, we designed experiments to see if TPA can replace OAG in the induction of enzyme activity. In fact, results were similar when OAG was replaced by TPA (Fig. 3). When cells were treated with TPA, A23187, and dibutyryl cAMP, synergistic induction of ornithine decarboxylase occurred. TPA was the more potent inducer. TPA alone at high concentrations could induce the enzyme activity, while OAG could not. The induction by TPA of ornithine decarboxylase activity was greater than that by OAG when A23187 was also

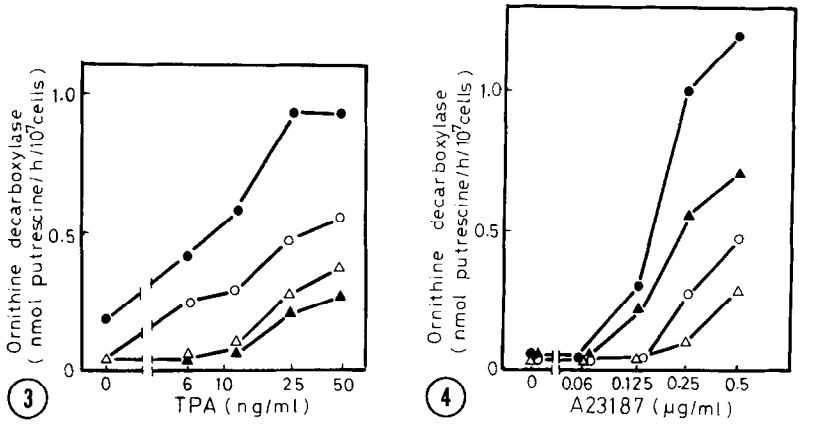

Fig. 3. Effects of TPA on ornithine decarboxylase induction. Cells were cultivated with various concentrations of TPA, A23187 (0.125 μg/ml), and dibutyryl cAMP (4 mM) for 6 h. Then ornithine decarboxylase activity was measured. Each point is the mean of duplicate experiments. ●, TPA, A23187, and dibutyryl cAMP; ○, TPA and A23187; △, TPA; ▲, TPA and dibutyryl cAMP.

Fig. 4. Effects of A23187 on ornithine decarboxylase with a combination of OAG and cholera toxin. Cells were cultivated with various concentrations of A23187, OAG (50 μg/ml), and cholera toxin (0.2 μg/ml) for 6 h. Then ornithine decarboxylase activity was measured. Each point is the mean of duplicate experiments. ●, A23187, OAG, and cholera toxin; ▲, A23187 and cholera toxin; ○, A23187 and OAG; △, A23187.

present. These results suggest that TPA influences ornithine decarboxylase induction in some way besides by its protein kinase C activation.

In Fig. 4 is shown the effect of the concentration of A23187. A23187 alone at concentrations of 0.25 μg/ml or more induced ornithine decarboxylase activity. Addition of cholera toxin enhanced this effect. Addition of OAG as well further augmented this effect. In the same way, TPA potentiated the ornithine decarboxylase induction caused by A23187 and dibutyryl cAMP (Fig. 5). The stimulation by dibutyryl cAMP of ornithine decarboxylase induction caused by A23187 alone or in combination with TPA was less at high concentration of A23187: the reason is unknown.

DISCUSSION

We found that addition of synthetic diacylglycerol OAG, the Ca^{++} ionophore A23187, and cholera toxin together, stimulated ornithine decarboxylase activity synergistically. Results were similar when TPA replaced OAG. Since OAG and TPA both activate protein kinase C (17, 19), these results

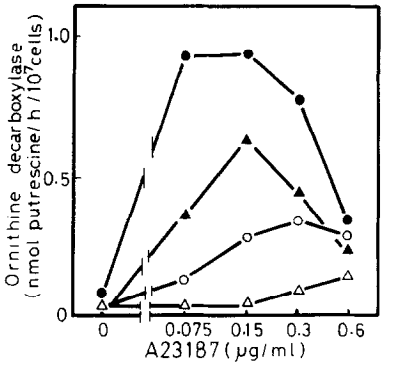

Fig. 5. Effects of A23187 on ornithine decarboxylase induction with a combination of TPA and dibutyryl cAMP. Cells were incubated with various concentrations of A23187, TPA (6 ng/ml), and dibutyryl cAMP (4 mM) for 6 h. Then ornithine decarboxylase activity was measured. Each point is the mean of duplicate experiments. ●, A23187, TPA, and dibutyryl cAMP; ▲, A23187 and dibutyryl cAMP; ○, A23187 and TPA; △, A23187.

suggest that both activation of protein kinase C and increases in the cytosolic Ca^{++} and cAMP levels are necessary for such induction. Our results agree with earlier findings that TPA and calcium ionophore are both comitogens of lymphocytes (20, 21) and that OAG and A23187 augment the mitogenic effect of suboptimal amounts of phytohemagglutinin (22). Lymphocyte mitogens stimulate phosphatidylinositol turnover (23, 24), formation of diacylglycerol (15, 25), and Ca^{++} influx (26), as well as ornithine decarboxylase activity and DNA synthesis, which is in agreement with our results. However, we found that cAMP is needed for ornithine decarboxylase induction, in addition to diacylglycerol and Ca^{++}. cAMP acts as a signal (positive or negative) for the proliferation of various eukaryotic cells (27). Agents that elevate the cAMP level almost all inhibit lymphocyte proliferation when added at the same time as a mitogen (28-30), which suggests that cAMP is a negative regulator of lymphocyte proliferation. However, sometimes cAMP is a positive regulator. Phytohemagglutinin activates adenyl cyclase (31), concanavalin A at the optimum concentration for DNA synthesis activates cAMP-dependent protein kinase I in human peripheral lymphocytes (32), and cAMP surge is observed in the G_1 phase of human blood lymphocytes stimulated with phytohemagglutinin (33) and in that of spleen lymphocytes activated by concanavalin A (34). Therefore, it is

possible that cAMP modulates protein kinase C activity or some other Ca^{++} -dependent process, resulting in stimulation of ornithine decarboxylase. cAMP modulates the cytosolic Ca^{++} level and many Ca^{++}-dependent biological activities (35). It also potentiates TPA. Treatment of CHO cells or epidermal cells with both TPA and cholera toxin results in a more than an additive increase in ornithine decarboxylase activity (36, 37). TPA-induced prolactin release from GH_4C_1 cells, cells of an established pituitary tumor cell strain, is enhanced by forskolin (38). These results suggest that cAMP modulates protein kinase C activity. However, we found that dibutyryl cAMP or cholera toxin augmented A23187-induced ornithine decarboxylase activity but not TPA-induced activity (Fig. 3,4,5). The combination of OAG and cholera toxin did not increase in the activity of this enzyme (Fig. 2). Thus, cAMP potentiates Ca^{++}-dependent processes other than protein kinase C, leading to the potentiation of ornithine decarboxylase induction.

Our results here show that both protein kinase C activation and increases in the levels of cytosolic Ca^{++} and cAMP are necessary for ornithine decarboxylase induction in guinea pig lymphocytes. A cAMP-dependent pathway may be involved in potentiating a Ca^{++}-dependent process, which consequently augments ornithine decarboxylase induction. We have no evidence concerning the mechanism of the potentiation.

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