Induction of ornithine decarboxylase by treatment of guinea pig lymphocytes with phospholipase C

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Treatment of guinea pig lymphocytes with *Clostridium perfringens* phospholipase C but not with *Naja naja* snake venom phospholipase A_2 increased ornithine decarboxylase activity. The increase in ornithine decarboxylase activity was suppressed by actinomycin D or cycloheximide, suggesting that de novo syntheses of RNA and protein are necessary for the increase in the enzyme activity. These results suggest that the activation of phospholipase C rather than that of phospholipase A_2 is responsible for induction of ornithine decarboxylase during lymphocyte transformation.

Ornithine decarboxylase *Phospholipase C* (Clostridium perfringens) *Phospholipase A_2* (Naja naja *snake venom*) *Lymphocytes*

1. INTRODUCTION

Physiological extracellular stimuli induce biochemical changes in membrane phospholipids of the responded cells and these changes have been considered to be involved in biological signal transmission [1,2]. Mitogens of lymphocytes, such as phytohemagglutinin and concanavalin A, bind to their membrane receptors and elicit stimulation of phosphatidyl inositol turnover [3,4] which is ascribed to the activation of phospholipase C [5]. Concanavalin A also increases methylation of membrane phospholipids, leading to the increase in Ca^{2+} influx and the activation of phospholipase A_2 [6]. However, it has been unclear whether one or both phospholipase activations are obligatory processes for lymphocyte transformation. ODC, a rate-limiting enzyme of polyamine biosynthesis, is markedly induced in lymphocytes within several hours after stimulation by mitogens [7–9] and the extent of the enzyme induction is proportional to that of the stimulation of [^{3}H]thymidine incorporation into DNA [9]. As an approach to determining if these activations of phospholipases are essential processes in the mechanism of initiation of lymphocyte transformation, we examined the effects of treatments of cells with various exogenously added phospholipase enzymes on ODC activity.

Abbreviation: ODC, ornithine decarboxylase [L-ornithine carboxylase, EC 4.1.1.17]

2. MATERIALS AND METHODS

2.1. *Materials*

D,L-[1-^{14}C]Ornithine (47.2 mCi/mmol) and [^{3}H]arachidonic acid (78.2 Ci/mmol) were purchased from New England Nuclear (Boston MA). *Clostridium perfringens* phospholipase C (type I and X), *Bacillus cereus* phospholipase C (type V) and *Naja naja* snake venom phospholipase A_2 were obtained from Sigma (St Louis MO). A purified *C. perfringens* phospholipase C giving a single band on polyacrylamide gel electrophoresis [10] was kindly supplied from Dr Ohsaka (National Institute of Health, Tokyo). Horse serum was from Commonwealth Serum Laboratories (Victoria).

2.2. *Lymphocyte cultures*

Guinea pig lymphocytes were prepared from

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lymph nodes as in [11] and were purified as in [12]. The lymphocytes were suspended in Eagle's minimum essential medium supplemented with 2 mM glutamine and 5% horse serum at 1×10^7 cells/ml, and cultivated in a CO_2-incubator with aeration by 5% CO_2 in air at 37°C.

2.3. *Assay of enzyme activities*

ODC activity was measured by estimation of release of $^{14}CO_2$ from D,L-[1-^{14}C]ornithine as in [13] with modifications as in [9]. Phospholipase A_2 activity was measured as in [14]. Phospholipase C activity was assayed by turbidimetric method as in [15]. Protein concentration was determined by ultraviolet absorption [16].

2.4. *Assay of release of [^{3}H]arachidonic acid from cellular phospholipids*

Release of [^{3}H]arachidonic acid from prelabelled cells was measured as in [6].

3. RESULTS

In order to know whether activation of phospholipase A_2 or C participates in ODC induction, lymphocytes were treated with various amounts of phospholipase A_2 or C and the activity of ODC of the treated cells was measured. The experiments presented in fig.1 showed that treatment of cells with *C. perfringens* phospholipase C, but not with phospholipase A_2, caused the increase in ODC activity. To ascertain whether phospholipase A_2 degraded cellular phospholipids, we examined the release of radioactivity from cells prelabelled with [^{3}H]arachidonic acid. The released radioactivities from control and cells treated with 0.5 and 1.0 unit/ml of phospholipase A_2 were 3354 ± 86, 6017 ± 48 and 7879 ± 179 cpm.h^{-1}.5 × 10^6 cells^{-1}, respectively, indicating that phospholipase A_2 degraded cellular phospholipids, but was incapable of increasing ODC activity. Treatment of cells with a large amount of *Bacillus cereus* phospholipase C elicited a small increase in ODC activity, but the increase was much lower than that caused by *Clostridium* enzyme. The low effectiveness of *Bacillus* enzyme consists with the finding that the activity of *Bacillus* enzyme is much lower than that of *Clostridium* enzyme when assayed in Eagle's minimum essential medium (not shown). Low activity of *Bacillus* enzyme in Eagle's minimum

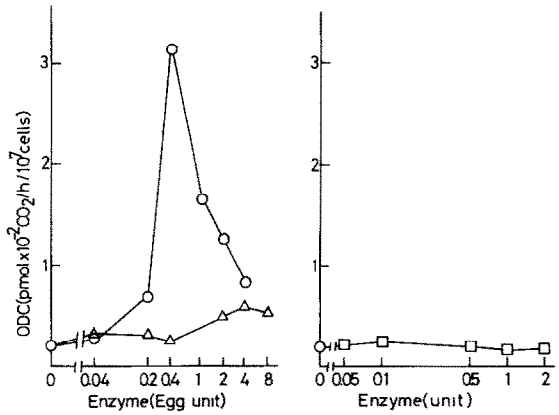

Fig.1. Effects of treatment of cells with phospholipase C or A_2 on ODC activity. Cells were incubated with various amounts of phospholipase C or A_2 for 6 h. ODC activity of the treated cells was measured as in section 2. Each point represents the mean of duplicate expts: (○) enzyme activity of cells treated with phospholipase C from *Clostridium perfringens* (Sigma type I); (△) enzyme activity of the cells treated with phospholipase C from *Bacillus cereus*; (□) enzyme activity of cells treated with phospholipase A_2 from *Naja naja* snake venom.

essential medium may be due to the fact that *Bacillus* enyme is inhibited by univalent anions [17]. We confirmed that the enzyme from *Bacillus*, but not that from *Clostridium*, was inhibited to 20% by 0.3 M NaCl.

To examine whether de novo syntheses of RNA and protein are necessary for the increase in ODC activity, we tested the effects of actinomycin D and cycloheximide on the increase in ODC activity caused by phospholipase C treatment. The experiments summarized in table 1 show that actinomycin D and cycloheximide inhibited the increase in ODC activity, indicating that treatment with phospholipase C caused expression of gene coding for ODC and de novo synthesis of the enzyme protein.

To ascertain that ODC induction is attributed to the activity of phospholipase C, we have compared the activity of phospholipase C and the effectiveness in inducing ODC of 3 independent preparations of phospholipase C. Fig.2 shows that phospholipase C activity required to induce ODC corresponded in the crude and the purified enzyme preparations. The increase in specific activity of phospholipase C is accompanied with the increase

Table 1

Effects of actinomycin D and cycloheximide on the increase in ODC activity by treatment with phospholipase C

Additions	ODC (pmol.h^{-1}. 10^7 cells^{-1})
None	21 ± 1
Phospholipase C	228 ± 2
Phospholipase C + actinomycin D (0.5 μg/ml)	95 ± 3
Phospholipase C + actinomycin D (2.0 μg/ml)	9 ± 2
Phospholipase C + cycloheximide (1.0 μg/ml)	9 ± 3
Phospholipase C + cycloheximide (5.0 μg/ml)	6 ± 1

Cells were incubated for 6 h with *Clostridium* phospholipase C (0.4 egg unit/ml) in the presence or absence of inhibitors. ODC activity was measured as described in section 2. Each value is the means ± SE of triplicate expts

in the effectiveness in inducing ODC, which suggests that ODC induction is ascribed to the activity of phospholipase C, but not to some factor contaminated in these enzyme preparations (table 2). This is also supported by the finding that phospholipase C from *B. cereus*, of which activity is very low in Eagle's minimal essential medium, is not effective to induce ODC activity.

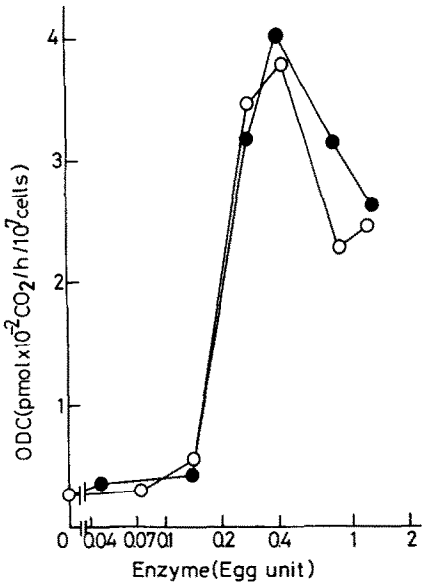

Fig.2. Effects of treatment of cells with crude or purified phospholipase C from *Clostridium perfringens*. Cells were incubated with various amounts of crude (Sigma type I) or purified *Clostridium* phospholipase C for 6 h. ODC activity of the treated cells was measured as described in section 2. Each point shows the mean of duplicate experiments: (●) crude enzyme; (○) purified enzyme.

4. DISCUSSION

ODC activity was stimulated by exogenously added *Clostridium* phospholipase C but not by phospholipase A_2, suggesting that the activation of

Table 2

Relationship between activity of phospholipase C and effectiveness in inducing ODC activity

Enzyme preparations	Specific activity of phospholipase C		Effectiveness in inducing ODC activity	
	Egg units/mg protein	(-fold)	nmol CO_2.10^7 cells^{-1}.h^{-1}.mg protein^{-1}	(-fold)
Crude enzyme				
Type I	29.4	(1.0)	27.9	(1.0)
Type X	367.5	(12.5)	340.0	(12.2)
Purified enzyme	3500.0	(119.0)	3235.3	(116.0)

Cells were incubated with the optimal amounts of phospholipase C for 6 h and then ODC activity of the treated cells was assayed. The activities of ODC and phospholipase C and the amounts of protein were measured as in section 2. The effectiveness in inducing ODC activity is shown as the ratio of ODC activity induced to mg protein of phospholipase C added in the cell culture

phospholipase C rather than phospholipase A_2 involved in induction of ODC. Furthermore, dibutyryl cyclic AMP inhibits not only phytohemagglutinin-induced stimulation of phosphatidyl inositol turnover [18], which is ascribed to the activation of phospholipase C, but also induction of ODC [19,20]. These results also support the possibility that phospholipase C activation is important to ODC induction in lymphocytes. In [21] treatment of platelets with *Clostridium* phospholipase C as well as with thrombin caused production of diacylglycerol, the activation of Ca^{2+}-acctivated, phospholipid-dependent protein kinase (protein kinase C) and phosphorylation of M_r 40000 protein which is proposed to be intimately related to release reaction [22–24]. Treatment of pancreas islets of Langerhans with phospholipase C also induced the activation of protein kinase C and secretion of insulin [25]. Therefore, it is possible that treatment of lymphocytes with phospholipase C produces diacylglycerol which activates protein kinase C, leading to ODC induction. However, since it was demonstrated [26] that phosphatidic acid stimulated Ca^{2+} influx and consequently cyclic GMP was synthesized in neuroblastoma cells, it is also possible that phosphatidic acid derived from diacylglycerol involved in ODC induction. Progressive studies are under way to determine whether ODC induction is attributable to the formation of diacylglycerol or phosphatidic acid.

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