ORNITHINE DECARBOXYLASE IN PHYTOHAEMAGGLUTININ STIMULATED LYMPHOCYTES: CONTROL OF DEGRADATION RATE BY AMINO ACIDS

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Received 31 January 1972

1. Introduction

Ornithine decarboxylase (ODC) catalyses the first step in the biosynthesis of polyamines. Its activity is rapidly and markedly increased in many different systems when growth stimuli are applied to non-growing or slowly growing cells [1–5], and it also has an unusually short half life [6]. These properties suggest that it may have an important role in the growth stimulation of animal cells, and it has been shown that its activity correlates with the rate of ribosomal RNA synthesis [7, 8, 4].

We have shown previously that ODC activity increases greatly in lymphocytes during growth stimulation by phytohaemagglutinin (PHA) [4]. We now report that ODC activity in PHA-stimulated lymphocytes is also regulated by the amino acid levels in the culture medium. This regulation appears to operate through control of the rate of degradation of the enzyme, and seems independent of the rate of RNA and protein synthesis.

2. Materials and methods

Lymphocytes were purified from human blood [9] and cultured at 2×10^6/ml in Eagle's minimal essential medium containing 10% autologous plasma. PHA (batches Q1 and PM1, Wellcome Research Laboratories Beckenham, Kent) was added at a final concentration of 2 μg/ml 20–25 hr before harvesting. The amino acid mixture used in these experiments contained the 7 non-essential amino acids omitted from Eagle's minimal essential medium. They were added such that the final concentration of each in the culture medium was 1 mM.

Incorporation of ^{3}H uridine into RNA or ^{14}C phenylalanine into protein was determined after incubation of 10^6 lymphocytes with 10 μCi 5-^{3}H-uridine for 1 hr or 1 μCi U-^{14}C-L-phenylalanine for 2 hr [10]. ODC activity in 5000 g or 10,000 g supernatant fractions of lymphocytes disrupted by freezing and thawing, and RNA polymerase activity of lymphocyte nuclei incubated in the presence or absence of 1 μg/ml α-amanitin were determined as described elsewhere [4, 11, 12]. The percentage of ribosomes active in protein synthesis was estimated by determination of the ability of ribosomes to resist dissociation to subunits in the presence of 0.5 M NaCl and 50 mM $MgCl_2$ [13]. Under the conditions used only those lymphocyte ribosomes attached to messenger RNA and carrying a nascent polypeptide chain resist dissociation [14].

3. Results

Lymphocytes incubated with PHA for 20–25 hr have markedly greater rates of RNA and protein synthesis than unstimulated lymphocytes, but have not yet initiated DNA synthesis [15]. ODC activity has risen from the very low levels found in resting lymphocytes to a peak activity which is maintained for the following 24 hr [4].

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Table 1
Effect of 3 hr incubation with non-essential amino acids on ODC activity, RNA synthesis and protein synthesis of lymphocytes incubated with PHA for 20–25 hr.

	Control	With amino acids	Amino acids / control
Ornithine decarboxylase[a]	32	158	4.9
^{14}C phenylalanine incorporation[b]	4,922	5,110	1.04
Percentage ribosomes active	69	68	0.99
^{3}H uridine incorporation[b]	75,851	77,694	1.02
Amanitin-resistant RNA polymerase[c]	1.9	1.8	0.97
Amanitin-sensitive RNA polymerase[c]	3.5	3.3	0.93

a. pMoles ornithine decarboxylated/10^6 lymphocytes/hr.
b. Counts/min incorporated/10^6 lymphocytes.
c. pMoles ^{3}H-UMP incorporated/10^6 nuclei.

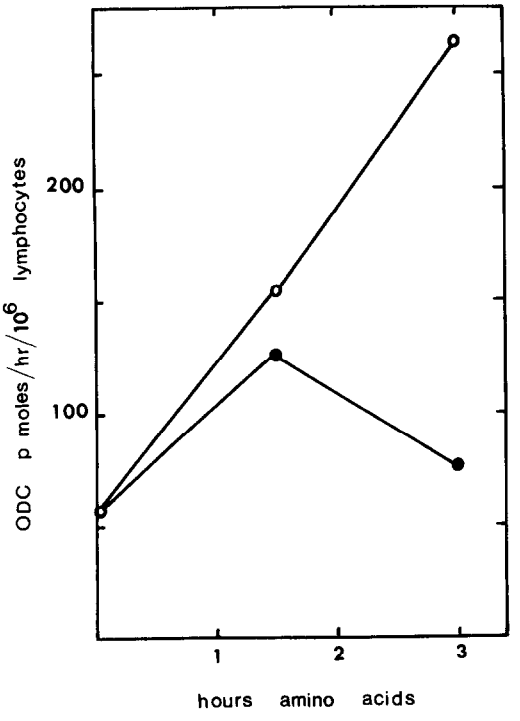

Fig. 1. Effect of actinomycin on the stimulation of ODC activity by amino acids. (○) Amino acids only. (●) 5 μg/ml actinomycin added 5 min before the amino acids.

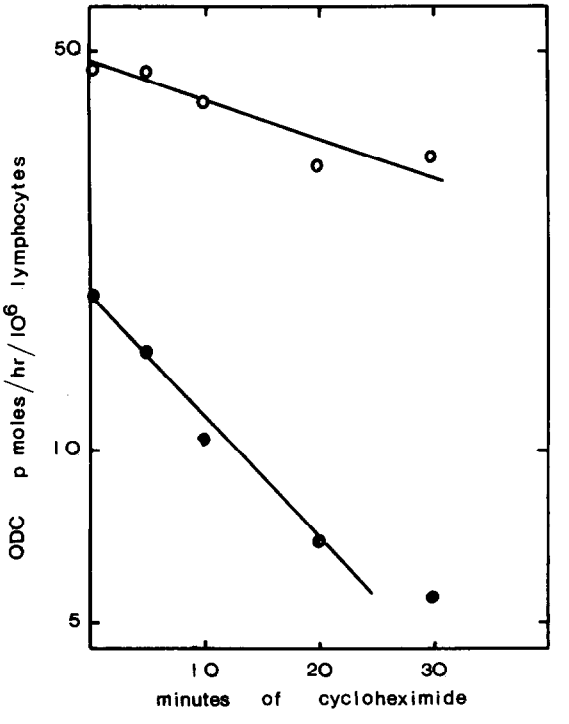

Fig. 2. Effect of amino acids on the stability of ODC. Lymphocytes were incubated with PHA for 22 hr and then with 100 μg/ml cycloheximide as indicated. (●) Control. (○) Amino acids added 1 hr prior to the addition of cycloheximide.

Table 1 and fig. 1 show that the level of ODC activity can be further increased at this time by the addition of high concentrations of non-essential amino acids to the culture medium. When the amino acids were added 3 hr before harvesting the mean increase was by a factor of 4.8, and varied from 2.8 to 7.8 over 10 experiments. In contrast, such treatment with amino acids did not increase the rate of incorporation of ^{14}C-phenylalanine into protein, the percentage of ribosomes active in protein synthesis, the rate of ^{3}H-uridine incorporation into RNA or the amanitin-sensitive or amanitin-resistant RNA polymerase activity of isolated nuclei (table 1). Addition of the amino acids together with the PHA usually gave a significant stimulation of ODC activity 20–25 hr later, although less than when the amino acids were added 3 hr before harvest. Again, no effects on protein or RNA synthesis were observed (data not shown).

Fig. 1 shows that at least part of the increase in ODC activity caused by amino acids is resistant to actinomycin. Actinomycin alone has no effect on ODC activity for 1.5 hr, but thereafter causes a precipitate decline [4].

Since ODC in rat liver has a very short half-life [6], the amino acid dependent increase in activity in lymphocytes could be caused by an increase in the stability of the enzyme as easily as by an increase in the rate of synthesis or activation. We therefore determined the stability of the enzyme in the presence of cycloheximide, both in the presence and absence of amino acids. Fig. 2 shows that the half life in stimulated lymphocytes was about 14 min, close to that found in rat liver. 1 hr after the addition of amino acids the half life had increased considerably, to approx. 40 min.

4. Discussion

In many systems the ODC activity correlates with the cell growth rate [1–5] and, in particular, with the rate of ribosomal RNA synthesis [7, 8, 4]. The amino acid dependent rise in ODC activity reported here differs from these systems in several respects.

Firstly, the amino acids do not seem to impart any detectable growth stimulus to the cells. They did not increase the rate of ^{14}C-phenylalanine incorporation into protein or the percentage of ribosomes active in protein synthesis, leading to the conclusion that they did not affect the rate of protein synthesis. Nor did they affect the rate of incorporation of ^{3}H-uridine into RNA or the RNA polymerase activities of isolated nuclei, indicating that they did not affect RNA or nucleotide metabolism [16, 17]. When starvation of mammalian cells for an amino acid is reversed most of these parameters are rapidly affected [18–20]. We conclude that while the amino acids added were not present in the Eagle's medium used, enough were available from the serum or from endogenous production to ensure that they did not limit the rate of RNA or protein synthesis. Control studies, not shown here, established that only 3 of the amino acids in the mixture used, glycine, serine and asparagine, were responsible for the stimulation. In addition, several of the essential amino acids, normally present in the Eagle's medium, could also cause an increase in ODC activity if added at high concentrations.

Secondly, the stimulation of ODC activity is mediated primarily through the control of the degradation or inactivation of the enzyme. While an effect on the rates of synthesis or activation has not been ruled out, the change in the half life is of the order required to account for the stimulation observed, assuming that the decay of enzyme activity in the presence of cycloheximide is an accurate indication of the normal stability of the enzyme. The increase in ODC during liver regeneration occurs without any major change in the stability of the enzyme [6] but this possibility has not been tested in other situations where ODC activity increases as a feature of growth stimulation.

Thirdly, the increase in ODC activity due to amino acids is at least partially insensitive to actinomycin. Stimulation of ODC activity during liver regeneration [6], growth hormone stimulation of liver [21] and lymphocyte stimulation by PHA [4] is abolished by actinomycin. However, the increase in rat liver ODC caused by injections of histidine [22] and in hepatoma cell ODC on dilution of cells growing at high densities [5] are also insensitive to actinomycin. This does not necessarily imply that the increase in ODC in the latter 2 cases is due to a change in the stability of the enzyme, but it is of interest that both involve an increase in amino acid availability.

Starvation of mammalian cells for amino acids is known to increase their rate of protein degradation [23]. It may be that the control of ODC degradation reported here is just a specific case of a general feature

of protein metabolism. However, 2 main conclusions follow from the results presented here. Firstly, that while the rise in ODC activity may be necessary for the increase in ribosomal RNA synthesis with which it is frequently associated, it does not, of itself, control it. Secondly, a rise in the activity of ODC does not necessarily indicate an increase in the growth rate of the cell.

Acknowledgements

We thank Dr. Brigid Hogan for many helpful discussions and for criticising the manuscript, Alan Betteridge for technical assistance, the Medical Research Council for financial support and the Science Research Council for a postdoctoral fellowship for A.C.

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