INDUCTION OF ORNITHINE DECARBOXYLASE IN GLIOMA AND NEUROBLASTOMA CELLS

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1. Introduction

Ornithine decarboxylase (ODC, L-ornithine carboxylyase; EC 4.1.17), catalyzes the conversion of ornithine to the diamine, putrescine. This reaction is the rate-limiting step in the synthesis of putrescine and the naturally occurring polyamines, spermidine and spermine which have been linked to many processes crucial for cell growth, division and differentiation [1,2]. Recent studies indicated the presence of polyamines in neural tissues [3,4] and in cultures of mouse neuroblastoma cells [5,6].

This paper deals with the induction of ODC in C6-BU-1 glioma and N115 neuroblastoma cells by fresh medium added to confluent cultures. A similar induction of ODC by fresh medium has been reported for other cell lines [7–12].

2. Materials and methods

Rat glioma clone C6-BU-1 [13] and mouse C1300 neuroblastoma clone N115 subcultures were grown in Falcon 100-mm plastic dishes in Dulbecco's modified Eagle's minimum medium (DMEM, GIBCO, Cat. No. H-21), supplemented with 10% fetal-calf serum, in atmosphere of 90% air–10% CO_2.

Ornithine decarboxylase activity was determined as described elsewhere [6]: Cells were washed with ice-cold PBS (0.8% NaCl; 0.02% KCl; 0.115% Na_2HPO_4; 0.02% KH_2PO_4; pH 7.2), and suspended in assay buffer (50 μM ethylenediamine-tetraacetic acid; 25 μM pyridoxal phosphate; 2.5 mM dithiothreitol in 25 mM Tris–HCl buffer, pH 7.1), 1.6 ml per plate. Cells were frozen and thawed three times and centrifuged at 4500 × g for 10 min. Ornithine decarboxylase activity was determined by incubating 0.5-ml quantities of the supernatant fluid with 100-μl quantities of [1-^{14}C] ornithine (0.4 μCi, 1.25 nmol, New England Nuclear) in 16 × 125-mm plastic tubes equipped with a rubber stopper supporting a polyethylene well. After incubating at 37°C for 45 min, 0.2 ml of hydroxide of hyamine (Packard Instrument Co.) was injected into each center well. Tubes were incubated at 37°C for another 15 min, followed by the injection of 0.2 ml of 6% perchloric acid, to stop the reaction. To release bound CO_2, tubes were agitated for 15 min. Center wells were then removed and their radioactivities determined. Proteins were assayed by a colorimetric method [14].

Polyamines were assayed by thin-layer chromatography of their dansyl derivatives [15]. Aliquots of the fluorescent derivatives were spotted on silica gel plates (Uniplate, 250 μ thick, Analtech, 75 Blue Hen Dr., Newark Del. 19711) and developed in benzene/cyclohexane/methanol (85:15:1.5) as solvent. Fluorescent spots were extracted with 0.5-ml quantities of dioxane and fluorescence determined in an Aminco-Bowman spectrophotofluorometer (activation wavelength 365 nm; emission wavelength 520 nm).

3. Results

Ornithine decarboxylase activity of C6-BU-1 glioma and N115 neuroblastoma cells fluctuated during their growth cycle. Table 1 shows that enzyme activity was high one or two days after seeding when cells pro-

Table 1
Ornithine decarboxylase activity of glioma and neuroblastoma cells

Cell line		Age of culture (days)										
		1	2	4	4	4	5	5	5	7	7	7
Glioma C6-BU-1	Time after feeding (h)	24	48	0	2	4	0	2	4	0	2	4
	Ornithine decarboxylase activity (pmoles/mg/h)	340	354	2.4	41	230	0.2	17	189	0.03	5	143
	Protein (mg/plate)	2.0	5.0	6.0			7.5			8.2		
Neuroblastoma N115	Time after feeding (h)		48	0	2	4	0	2	4	0	2	4
	Ornithine decarboxylase activity (pmoles/mg/h)		305	6.6	20	408	0.5	21	209	0.02	5.5	145
	Protein (mg/plate)		2.8	5.0			5.8			6.8		

Cells were fed 4,5 and 7 days after seeding and assayed for ornithine decarboxylase activity at times indicated. The protein content of the cultures was also determined.

liferated rapidly and their protein content doubled. Thereafter, the activity declined and rose again after adding fresh medium.

It may be seen (table 1) that old cultures responded less readily to feeding than young ones. The maximal activity of ODC in a 7-day-old culture was approximately one third that of a young one. It is also apparent that the addition of fresh medium to 4- to 7-day-old cultures, led to the increase in ODC only after a definite lag period.

A more detailed analysis (fig.1) showed that the activity was maximal 5 to 8 h after feeding, being 800–1000-fold higher than the basal values. Thereafter, the activity declined gradually and reached basal levels within 15–20 h after feeding (fig.1).

The addition of fresh medium to confluent C6-BU-1 glioma cells, not only caused the induction of ODC, but also brought about a significant elevation in cellular putrescine. Figure 1 shows that 6 h after adding fresh medium to glioma cultures, cellular putrescine doubled, and at 10 h, was seven times that of the initial level. It should be noted that the kinetics of putrescine accumulation in C6-BU-1 cells, paralleled that of ODC, with a lag period of 2 h. In analogy to ODC, putrescine levels returned to basal values, approximately 20 h after feeding.

The effect of fresh medium on ODC induction appears to be specific; the decarboxylation rate of

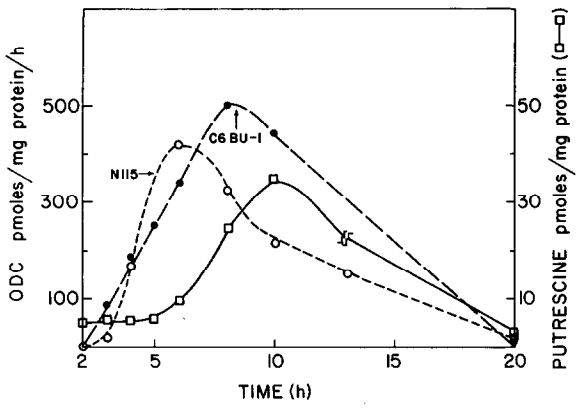

Fig.1. Induction of ornithine decarboxylase and putrescine content of cultured neural cells. Cells were fed on the first and third day after feeding. Experiment was started on day 5. ODC activities at zero times were 0.8 and 0.5 pmol/mg/h for N115 neuroblastoma and C6-BU-1 glioma cells, respectively. Putrescine content of C6-BU-1 cells (□—□).

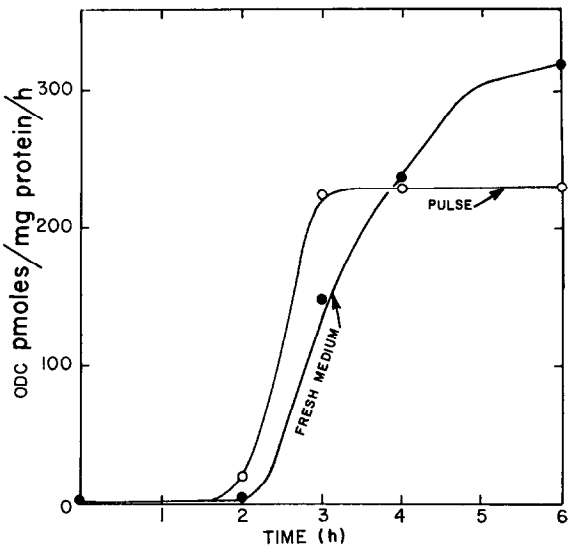

Fig.2. Induction of ornithine decarboxylase in N115 neuroblastoma cells. Experimental conditions were those given in the legend to fig.2 except that one culture was incubated with fresh DMEM medium (supplemented with 10% fetal-calf serum) for 30 min followed by incubation with a serum-free medium (pulse, ○—○). The other culture was incubated with fresh DMEM-serum medium throughout the experiment (fresh medium, ●—●).

other amino acids, such as lysine, was not altered markedly when confluent C6-BU-1 glioma cells were fed with fresh medium.

A short exposure (30 min) of N115 neuroblastoma cells to fresh medium, was sufficient for ODC induction. It may be seen (fig.2) that when N115 neuroblastoma cells were incubated with fresh DMEM medium (supplemented with 10% fetal-calf serum) for 30 min and then washed and further incubated in medium A (Dulbecco's modified Eagle's medium with 25 mM Hepes [N-2-hydroxy-ethylpiperazine-*N'*-ethanesulfonic acid, pH 7.4, instead of $NaHCO_3$, adjusted to 340 mosmol/liter with 1.1 g of NaCl/liter], the activity of ODC was not reduced significantly). Medium A, which does not contain serum, had only a slight effect on ODC activity.

The increase in ODC activity can be explained either by synthesis of a new enzyme or by interference with its inactivation.

The latter possibility was ruled out by an experiment in which the half-life of ornithine decarboxylase

was determined. Cycloheximide (20 μg/ml) was added to C6-BU-1 cells, 6 h after feeding, and the activity of the enzyme was determined at various time intervals. This experiment showed that the half-life of ODC, was 15 min, when assayed 6 h after feeding. This value is very close to that obtained with cells before the addition of fresh medium. It thus appears that the increased activity of ODC is due to new synthesis, which can be blocked by actinomycin D [6].

4. Discussion

Data presented in this paper clearly show the presence of ODC in C6-BU-1 glioma and in N115 neuroblastoma cells. This enzyme is probably responsible for the synthesis of putrescine and polyamines which have been detected in some neural cells [3,4]. The induction of ODC by fresh medium is very dramatic and the degree of activation (up to 1000-fold) is one of the most spectacular inductions ever described in a eukaryotic system. The induction of ODC appears to be specific, since the activity of related enzymes, such as lysine decarboxylase was not elevated when C6-BU-1 glioma cells were fed with fresh medium. It is also of interest that a relatively short pulse with fresh medium permitted the induction of ornithine decarboxylase.

Ornithine decarboxylase activity varies considerably during the development of rat [16] and human [17] brain. Activities are high during perinatal development and low after birth [16,17]. In the rat brain, greatest enzyme activities are found during cell proliferation at the various regions of the brain [16]. A similar correlation between ornithine decarboxylase and proliferation rate has also been reported in the developing chick embryo retina [18]. Our data also suggest a correlation between growth rate and ornithine decarboxylase activity in cultured glioma and neuroblastoma cells.

Figure 1 shows that putrescine accumulated in C6-BU-1 glioma cells after the induction of ODC. It may be seen (fig.1) that cellular putrescine levels were maximal 10 h after feeding, whereas ODC activity was maximal 2 h earlier. The decline in ornithine decarboxylase activity upon putrescine accumulation could be interpreted in terms of feed-back inhibition of ODC induction by the end-product, putrescine. Preliminary experiments indicated that this is indeed the case, and the induction of ODC by fresh medium was significantly repressed when 10^{-5} M putrescine was added to N115 neuroblastoma cells in fresh medium. A similar feed-back inhibition by polyamines of the induction of ODC in rat hepatoma cells has recently been reported [19].

The high activity of ODC in tumor cells of the nervous system, may be related to their ability to proliferate. This system may be useful to elucidate control mechanisms and gene expression in neural cells. As ODC may be induced effectively in glioma and neuroblastoma cells, cultures of these cells may also be employed for the characterization and purification of ODC.

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