INDUCTION OF *S*-ADENOSYL-L-METHIONINE DECARBOXYLASE IN GLIOMA AND NEUROBLASTOMA CELLS

Uriel BACHRACH

Department of Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

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1. Introduction

The polyamines, spermine and spermidine, and the diamine, putrescine, have received considerable attention in recent years as they have been implicated in various growth processes and in cellular differentiation [1–4]. The biosynthetic pathway of these polyamines involves the decarboxylation of L-ornithine and *S*-adenosyl-L-methionine by ornithine decarboxylase (ODC, L-ornithine-carboxylyase, EC 4.1.17) and *S*-adenosyl-L-methionine decarboxylase (SAM decarboxy-lyase, EC 4.1.1.50), respectively [5].

Recent studies indicated the presence of polyamines in neural tissues [6–8] and in cultures of mouse neuroblastoma [9–11] and rat glioma cells [10–11]. We have also described the induction of ODC in cultured glioma and neuroblastoma cells and showed that it is mediated by adenosine 3′:5′ cyclic monophosphate (cyclic AMP) [10]. Since both ODC and SAM decarboxylase are involved in growth processes, it is reasonable to assume that the induction of both enzymes is mediated by similar agents.

This paper deals with the induction of SAM decarboxylase in C6-BU-1 glioma and N115 neuroblastoma cells. It will be shown that the enzyme can be induced by adding fresh medium, or various hormones, to confluent cultures.

2. Materials and methods

Rat glioma clone C6-BU-1 [12] and mouse C1300 neuroblastoma clone N115 subcultures were grown in 94 mm plastic dishes (Greiner und Söhne, Kunststoffwerke D-7440 Nürtingen, FRG) in Dulbecco's modified Eagle's minimum medium (DMEM, GIBCO, Cat. No.H-21) supplemented with 10% calf serum at 37°C, in an atmosphere of 90% air–10% CO_2.

SAM decarboxylase activity was determined as follows: 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-tetra-acetic acid, 25 μM pyridoxal phosphate, 2.5 mM dithiothreitol in 25 mM Tris–HCl buffer, pH 7.1) 1.6 ml/plate. Cells were frozen and thawed three-times and centrifuged at 4500 × *g* for 10 min. SAM decarboxylase activity was determined (in duplicate) by incubating 0.25 ml quantities of the supernatant fluid with 50 μl quantities of *S*-[1-^{14}C]adenosyl-L-methionine (0.2 μCi 2.67 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. ODC activity was determined as described elsewhere [10], by incubating 0.5 ml quantities of cellular extracts with 100 μl quantities of [1-^{14}C]ornithine (0.4 μCi – 1.25 nmol, New England Nuclear). Proteins were determined by a colorimetric method [13]. Experiments were carried out in duplicate. The values found in different experiments were within 10–15% of the values shown.

The effect of the various agents on enzyme activity was studies by adding 100 μl quantities of aqueous solutions of either 1-norepinephrine, 3-isobutyl-1-methylxantine (IBMX), isoproterenol (Sigma, Chemical Co.), or prostaglandin E_1 (PGE_1, kindly provided by Dr John Pike, Upjohn Co.) in ethanol, to confluent cultures in 100 mm plastic dishes containing 10 ml of medium. In some experiments, a complete medium, consisting of DMEM medium supplemented with 10% calf serum was used, whereas the synthetic medium [Medium A-DMEM with 25 mM HEPES (*N*-2-hydroxyethyl piperazine-N-ethanesulfonic acid) pH 7.4, instead of $NaHCO_3$ (adjusted to 340 mosmol/liter with 1.1 g of NaCl/liter) was employed in others.

3. Results

SAM decarboxylase activity of C6-BU-1 glioma and N115 neuroblastoma cells declined with the age of the culture. Enzyme activities in high stationary cultures (5–7 mg protein/100 mm plate) were barely above background values. The addition of fresh medium to 5-day-old C6-BU-1 glioma cells, resulted in a dramatic increase in SAM decarboxylase activity. Maximal activity of the enzyme was attained 11 h after the addition of the fresh complete medium (fig.1). When the complete medium (containing 10% calf serum) was substituted by the synthetic medium A, a moderate increase in SAM decarboxylase activity was noticed (fig.1).

Ornithine decarboxylase activity also increased after adding fresh medium to confluent cultures of C6-BU-1 glioma cells (fig.1). These results are in line with published data [11]. It should be noted that after feeding the cells with complete medium, the activities of ODC and SAM decarboxylase had a different time course and were maximal after 8 h and 11 h, respectively. It is also obvious that the specific activity of ODC was significantly higher than that of SAM decarboxylase (fig.1).

Numerous studies demonstrated that ODC activity can be increased by hormones that affect growth [14] and that cyclic nucleotides play an important role in this process. Clonal lines derived from tumors of the nervous system may be very useful in elucidating the mechanism of the activation of SAM decarboxylase and establishing the role of cyclic nucleotides in

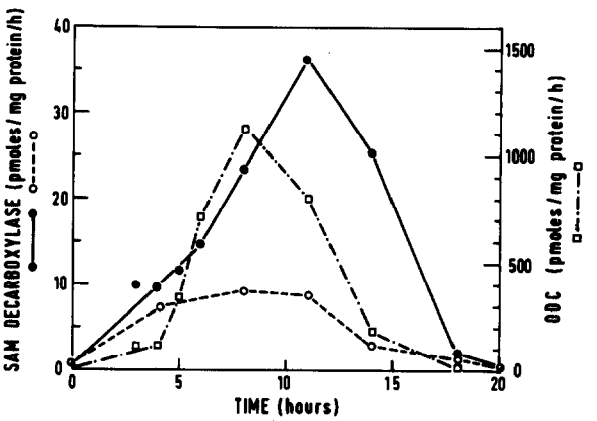

Fig.1. Induction of decarboxylases in C6-BU-1 glioma cells. Glioma cells (equivalent to 0.5 mg protein) were seeded on 94 mm culture dishes as described under Materials and methods. Cells were fed on the first and third day after seeding. Experiment was started on day 5 (when the protein content was 7.0–7.5 mg/plate) by adding complete medium containing 10% calf serum. ODC activity (□-·-□) and SAM decarboxylase activities (●—●) were determined at various times. SAM decarboxylase activity was also determined after the addition of the synthetic medium A (○- - -○).

enzyme induction. It is well known that neurons respond differently to environmental stimuli, which include hormones. Thus, neuroblastoma cells increase intracellular levels of cyclic AMP in the presence of prostaglandin E_1 (PGE_1) [15,16] and adenosine [17,18], while rat glioma cells do so when exposed to catecholamines [19]. It has also been demonstrated that guanosine 3′:5′ monophosphate (cyclic GMP) levels of neuroblastoma N115 cells increase upon activation of muscarinic acetylcholine receptors, by agents like the cholinergic agonist carbamylcholine [20].

To elucidate the possible role of cyclic nucleotides in the activation of SAM decarboxylase, confluent cultures of the respective tumors were incubated with hormones added to the synthetic medium A. Table 1 shows that the addition of the phosphodiesterase inhibitor IBMX to confluent glioma C6-BU-1 cultures resulted in a significant increase in SAM decarboxylase activity, indicating the involvement of cyclic nucleotides in this process. Catecholamines, such as isoproterenal or norepinephrine caused a similar increase in activity. Higher activities of SAM decarboxylase were observed when these

Table 1
SAM decarboxylase activity in glioma and neuroblastoma cells

Cell line	Treatment	Enzyme activity (pmoles/mg/h)
Glioma C6-BU-1	No addition	< 1
	Medium A	5.4
	Medium A + IBMX, 0.5 mM	11.5
	Medium A + isoproterenol, 0.1 μM	10.5
	Medium A + norepinephrine, 1 μM	8.8
	Medium A + IBMX + isoproterenol	14.6
	Medium A + IBMX + norepinephrine	22.3
	DMEM + calf serum, 10%	17.8
Neuroblastoma N115	No addition	< 1
	Medium A	7.1
	Medium A + IBMX, 0.5 mM	19.3
	Medium A + IBMX + PGE_1, 10^{-6} M	42.1
	Medium A + IBMX + adenosine, 0.5 mM	19.4
	Medium A + IBMX + carbamylcholine, 1.0 mM	21.0
	Medium A + IBMX + carbamylcholine + adenosine	30.3
	DMEM + calf serum, 10%	10.6

Enzyme activity was determined in extracts of 5-day-old cells, prepared 6 h after the onset respective treatment.

catecholamines were added to the glioma cells along with IBMX. The addition of complete medium (containing 10% calf serum) to confluent cells led to a similar increase in SAM decarboxylase activity (table 1). As expected, IBMX also caused the increase of SAM decarboxylase activity in neuroblastoma N115 cells. On the other hand, adenosine hardly affected the activity of the enzyme, even in the presence of the phosphodiesterase inhibitor IBMX. Table 1 also shows that PGE_1 or a mixture of carbamylcholine and adenosine led to a pronounced increase in SAM decarboxylase activity.

The increase in enzyme activity can be explained either by net synthesis or alternatively by stabilization of the labile enzymes. To distinguish between the two possibilities, the half-life of ODC and SAM decarboxylase was determined as follows: Glioma C6-BU-1 cells were fed with fresh complete medium, 4 days after seeding. After 6 h, cycloheximide (20 μg/ml) was added to the cells and the activity of the decarboxylases was determined at various times. It is evident from fig.2 that ODC had a half-life of 17 min and SAM decarboxylase of approximately 80 min. These values are in line with reported data [21].

4. Discussion

Even though considerable information is now available on the induction of ODC by various hormones, the effect of similar agents on the activity of SAM decarboxylase has not been studied as exten-

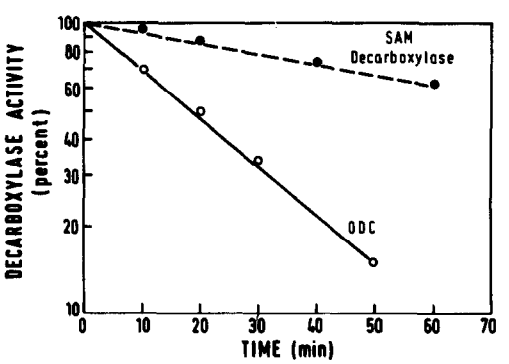

Fig.2. Half-life of ornithine and SAM decarboxylases. C6-BU-1 cells were fed on the first and third day after seeding. Complete medium was added to 4-day-old cells. After 6 h, cycloheximide (20 μg/ml) was added to the respective cultures and enzyme activities determined at various times. SAM decarboxylase (•- - -•), ornithine decarboxylase (○—○).

sively. It has recently been reported [22] that glucocorticoid hormones together with insulin stimulate the activity of SAM decarboxylase in mouse mammary epithelium in vitro. Russell and Taylor [21] showed that SAM decarboxylase activity is increased in the uterus of the rat after estradiol stimulation. In rat ovary the activity of this enzyme fluctuates during normal estrous cycle and rises during pregnancy [23]. Sturman has recently demonstrated the presence of SAM decarboxylase in the brain of rat or guinea pig [24]. The conversion of [^{14}C]putrescine to spermidine in cultured mouse neuroblastoma cells has also been documented [9].

Data presented in this paper clearly show the presence of SAM decarboxylase in both neuroblastoma and glioma cultures. Moreover, it appears that the activity of the enzyme can be increased by adding a serum-containing medium to confluent cultures. Phosphodiesterase inhibitors also increased SAM decarboxylase activity, suggesting the involvement of cyclic nucleotides in the induction process.

The effect of carbamylcholine on SAM decarboxylase is most interesting. This compound, which causes the accumulation of cyclic GMP in N115 neuroblastoma cells [20], prevents the induction of ODC by adenosine or PGE_1 [10], but stimulates the activity of SAM decarboxylase, mainly in the presence of adenosine (table 1).

These preliminary findings suggest that ODC may be induced in glioma cells either by a complete medium (which contains DMEM and 10% calf serum) or by compounds which increase the cellular level of cyclic AMP. It appears that the induction of SAM decarboxylase is mediated by a complete medium and by cyclic GMP. Obviously more studies have to be carried out to establish this assumption.

It should be noted that in horse peripheral blood lymphocytes, cyclic GMP and cholinergic agents stimulate the incorporation of phosphate into specific nuclear acidic proteins [25]. This phosphorylation appears to be related to lymphocyte proliferation [25] and to the synthesis of spermidine, which is essential for cell division [26].

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