DIFFERENCES IN POLYAMINE METABOLISM OF THE UNDIFFERENTIATED AND DIFFERENTIATED NEUROBLASTOMA CELLS

Metabolic labeling of an 18 000-M_r protein by [^{14}C]putrescine and the conversion of putrescine to GABA

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1. Introduction

In [1–3] we demonstrated differences in the regulation of ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) activity and putrescine transport of the undifferentiated and differentiated neuroblastoma cells, suggesting that alteration of polyamine metabolism may be involved in the differentiation of mouse neuroblastoma cells. Polyamines (putrescine, spermidine and spermine) are ubiquitous organic cations occurring abundantly in living organisms [4–8]. Although polyamines have been implicated in proliferative and cancerous growth, their precise function(s) in growth regulation remains to be elucidated. One approach to define the possible role(s) of polyamines in growth regulation is to identify and characterize the specific biochemical reactions involving polyamines.

[^{3}H]Putrescine metabolically labels several low M_r (rel. mol. wt) proteins in human lymphocytes [9]. We have observed [10] that [^{14}C]putrescine or [^{14}C]-spermidine metabolically labeled an 18 000-M_r protein in at least 4 different cultured cell lines [10]. This metabolic labeling reaction by polyamines appeared to be specific; under the experimental conditions used, only one protein band with an app. M_r of 18 000 was prominently labeled [10]. Putrescine can also be converted to γ-aminobutyric acid in the presence of diamine oxidase and aldehyde dehydrogenase. This reaction occurs in a number of biological systems [11,12] including C-1300 neuroblastoma cells maintained in fetal calf serum-containing medium [13].

The purpose of this study is to compare the metabolism of [^{14}C]putrescine in the NB and ND neuroblastoma cells. We report our findings on the differences in the labeling of the 18 000-M_r protein and the metabolic conversion of [^{14}C]putrescine into [^{14}C]GABA in the NB and ND cells. Our results indicated a 50–60% decrease of labeling of the 18 000-M_r protein of the ND cells when compared to that of the NB cells. In addition, there was an 8–10-fold increase in the conversion of [^{14}C]putrescine into amino [^{14}C]acids via [^{14}C]GABA.

Abbreviations: NB, undifferentiated neuroblastoma; ND, differentiated neuroblastoma; GABA, γ-aminobutyric acid; Bt_2cAMP, N^6,$O^{2'}$-dibutyryl cyclic AMP; IBMX, 3-isobutyl-1-methylxanthine; FCS, fetal calf serum

2. Materials and methods

2.1. *Chemicals*

The following compounds were purchased: [1,4-^{14}C]putrescine · 2 HCl (122 mCi/mmol) from Amersham (Arlington Heights IL); γ-amino [U-^{14}C]butyric acid (203 mCi/mmol) from New England Nuclear, (Boston MA); Dulbecco's modified Eagle medium and fetal calf serum from Gibco (Grand Island NY); N^6,$O^{2'}$-dibutyryl cyclic AMP, aminoguanidine, phenylmethyl sulfonyl fluoride from Sigma Chem. Co. (St Louis MO); 3-isobutyl-1-methylxanthine from Aldrich Chem. Co. (Milwaukee WI).

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2.2. *Cell culture and differentiation*

Mouse NB-15 neuroblastoma cells were grown as monolayer cultures as in [2]. The differentiation of neuroblastoma cells was induced by adding 1 mM Bt_2cAMP and 0.5 mM IBMX to the cell culture 15 h after subculture (seeding was at ~1 × 10^4 cells/cm^2). Unless otherwise specified, the NB and ND neuroblastoma cells at stationary phase of growth (~4–5 days after subculture) were used.

2.3. *Metabolic labeling with [^{14}C]putrescine of [^{14}C]GABA*

Both the NB and ND cells were washed twice and re-incubated in fresh Dulbecco's medium. [^{14}C]Putrescine or [^{14}C]GABA was added to the cell culture to a designated final concentration. FCS or other agents were then added to the appropriate concentrations. Cells were incubated at 37°C in a water-jacketed CO_2 incubator (95% air, 5% CO_2, 100% humidity) for 20 h. At the end of this incubation period, cells were washed 3 times with cold phosphate-buffered saline (pH 7.2), cells were scraped off the substratum in 0.7 ml Tris–HCl buffer (20 mM, pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride. The cell suspension was then homogenized by sonication at 4°C. The cell homogenate thus obtained was used for analysis of the incorporation of radioactivity into trichloroacetic acid-insoluble material and for sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoresis and fluorography. Protein was assayed by Lowry's method [14].

2.4. *SDS–Polyacrylamide gel electrophoresis*

Cell homogenate which contained 0.5–3.0 mg protein/ml was mixed with 1/5th of its volume of an SDS–stop solution containing 12% SDS, 0.5 M Tris–HCl (pH 9.0), 10% β-mercaptoethanol, 5 mM EDTA, 25% glycerol and 0.005% pyronin Y and heated at 100°C for 5 min. The samples were then subjected to SDS–polyacrylamide gradient slab-gel electrophoresis (7.5–15% acrylamide) as in [15].

Fluorograms were prepared according to [16]. Fluorograms were scanned with a Schoeffel SD-3000 spectro-microdensitometer and the peak areas of the optical tracings were used as a quantitative measurement of the amount of radioactivity incorporated.

3. Results

Fig.1 illustrates the amount of radioactivity incorporated into the trichloroacetic acid-insoluble material after incubation of the NB and ND cells with [^{14}C]-putrescine for 20 h. Our results demonstrated an increased incorporation of radioactivity into the ND cells both in the absence and presence of 10% fetal calf serum. The presence of FCS stimulated the amount of radioactivity incorporated into trichloroacetic acid-insoluble material of both the NB and ND cells. It should be noted that the increased incorporation of radioactivity into the ND cells was not due to an increase in the transport or specific activity of [^{14}C]putrescine in the ND cells; total cellular uptake of radioactivity in the NB cells was actually 50–80% higher than that of the ND cells.

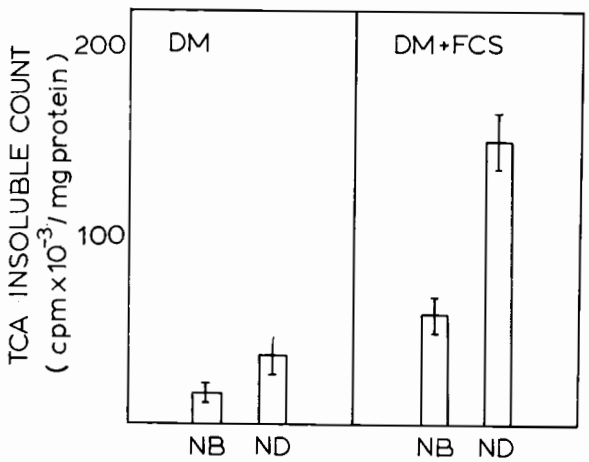

Fig.1. The incorporation of [^{14}C]putrescine into acid-insoluble material into the NB and ND cells. Cells were incubated with [^{14}C]putrescine (1 μCi/ml) in fresh Dulbecco's medium in the absence or in the presence of 10% FCS for 20 h at 37°C. Trichloroacetic acid-insoluble counts were determined by the filter-disc assay method in [20].

The pattern of incorporation of radioactivity into individual protein bands was analyzed by SDS–polyacrylamide gel electrophoresis and fluorography. The result of such an experiment is shown in fig.2. In the absence of FCS (fig.2B), an 18 000-M_r protein was identified as the major radioactive band of both the NB and ND cells. The absolute amount of radioactivity incorporated into the 18 000-M_r protein of the ND cells was only 60% of that of the NB cells as estimated from the densitometric tracings of the fluorogram. The presence of FCS during the 20 h incubation period with [^{14}C]putrescine increased the amount of radioactivity incorporated into the 18 000-M_r pro-

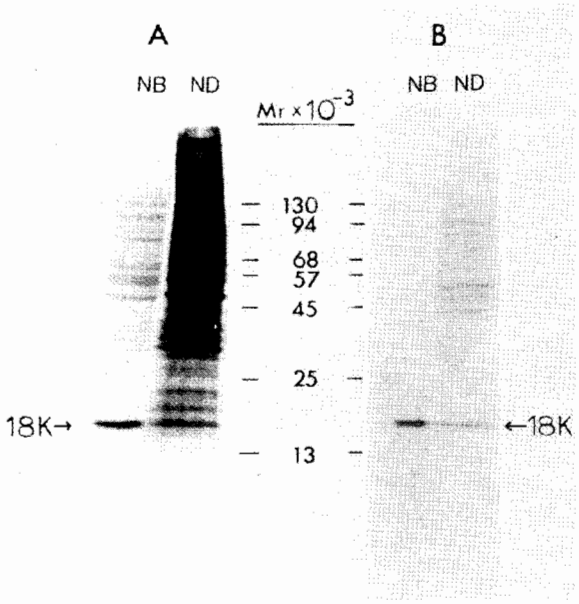

Fig.2. Fluorogram of the radioactively labeled proteins in the NB and ND cells exposed to [^{14}C]putrescine. Both NB and ND cells were incubated with [^{14}C]putrescine (1 μCi/ml) in fresh Dulbecco's medium in the presence (A) or the absence (B) of 10% FCS. At the end of 20 h incubation, cells were harvested and prepared for SDS–polyacrylamide gel electrophoresis and fluorography as in section 2. Each lane contained 50 μg protein.

tein while retaining the difference of the NB and ND cells (fig.2A). More importantly, however, was the marked stimulation by FCS of the incorporation of radioactivity into a variety of protein bands of the ND cells. FCS also caused the incorporation of radioactivity into various protein bands other than the 18 000-M_r band in the NB cells. However, the intensity of those bands was significantly less when compared to that of the ND cells (fig.2A).

In attempting to gain a better understanding of this FCS-dependent incorporation of radioactivity from [^{14}C]putrescine into cellular proteins, we noted, with the exception of the 18 000-M_r protein, the radioactive labeling patterns in fig.2A of both the NB and ND cells were qualitatively similar to the Coomassie blue-stained protein patterns (not shown). In view of this consideration, and the observation that putrescine can be converted to GABA [11–13] which can be further channeled to form amino acids via the GABA shunt and Krebs cycle, we reasoned that the labeling of the various cellular proteins (with the exception of the 18 000-M_r protein) in the presence of FCS may be attributable to radioactive amino acids derived from the [^{14}C]putrescine.

In this connection, we have carried out experiments to examine the effects of aminoguanidine on the incorporation of radioactivity, and to study the pattern of radioactivity using [^{14}C]GABA as precursor for metabolic labeling of NB cells. As shown in fig.3 the presence of aminoguanidine, a potent inhibitor of diamine oxidase [17], abolished the incorporation of radioactivity into all protein bands except the 18 000-M_r protein. Furthermore, with the exception of 18 000-M_r band the labeling pattern of the NB cells by [^{14}C]putrescine could be duplicated by using [^{14}C]GABA as the precursor (fig.3C). Similar results were also obtained with the ND cells (not shown).

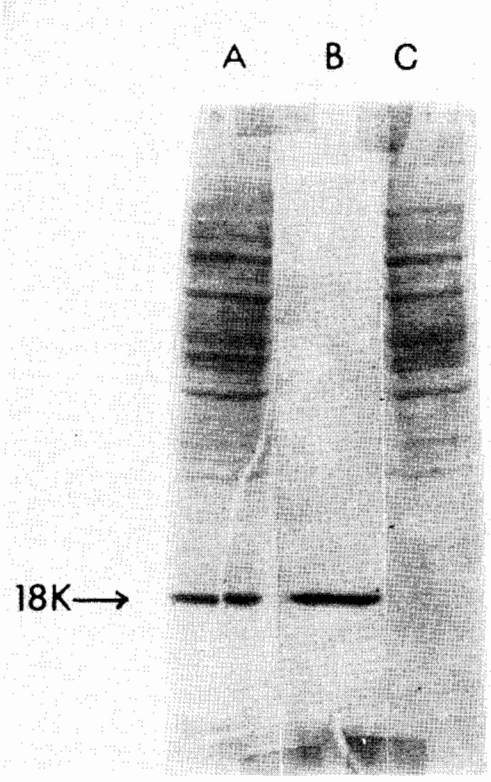

Fig.3. Effect of aminoguanidine on the [^{14}C]putrescine labeling pattern and the metabolic labeling pattern of 0 [^{14}C]GABA: (A,B) NB cells were incubated with [^{14}C]putrescine (1 μCi/ml) in fresh Dulbecco's medium plus 10% FCS in the absence (A) or in the presence (B) of 2×10^{-5} M aminoguanidine for 20 h; (C) cells were incubated with [^{14}C]GABA (0.5 μCi/ml) in fresh Dulbecco's medium plus 10% FCS for 20 h.

We thus concluded that:

(i) The 18 000-M_r protein represented a unique polyamine labeled protein;
(ii) There was enhanced conversion of putrescine to GABA and then to various amino acids in the ND cells;
(iii) The FCS-dependent labeling of various cellular proteins other than the 18 000-M_r band was attributable to the presence of radioactive amino acids derived from [^{14}C]putrescine.

4. Discussion

These data have identified two specific biochemical events associated with polyamine metabolism occurring in the NB and ND cells, namely:

(i) Specific labeling of the 18 000-M_r protein; and
(ii) Conversion of putrescine to amino acids via GABA.

Comparative studies of these biochemical events in the NB and ND cells showed that the labeling of the 18 000-M_r band was more prominent in the NB cells than in the ND cells whereas the conversion of putrescine to amino acids via GABA was more prominent in the ND cells than in the NB cells.

The metabolic labeling of the 18 000-M_r protein band in cultured cells exposed to [^{14}C]putrescine may represent a direct polyamine-conjugation reaction, presumably catalyzed by cellular transglutaminase [9,10]. Alternatively, it is possible that hypusine [N^{ϵ}-(4-amino-2-hydroxybutyl)-lysine], derived from spermidine may serve as the radioactive source for the specific labeling of this protein [18]. The identity of the radioactive moiety in the labeled 18 000-M_r protein of NB and ND cells is under investigation.

The physiological significance of the conversion of putrescine to amino acids via GABA is not clear. In view of the fact that the ND cells bear a closer resemblance to the mature neuron cells [19], and that GABA is an important neurotransmitter, our finding that the ND cells had enhanced capacity to convert putrescine to amino acids via GABA may represent another biochemical index of differentiation of mouse neuroblastoma cells.

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