FORMATION OF PUTRESCINE AND ACETYLSPERMIDINE FROM SPERMIDINE BY CULTURED HUMAN LYMPHOCYTES

Joseph FABER, Moshe MENASHE, Uriel BACHRACH+ and Hans DESSER*

Shaare Zedek Medical Center, Jerusalem, +Department of Molecular Biology, Hebrew University-Hadassah Medical School, Jerusalem, Israel and **Ludwig Boltzman Institute for Leukemia Research and Hematology, A-1140, Vienna, Austria*

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1. Introduction

Phytohemagglutinins induce the proliferation of lymphocytes in vitro [1,2]. This process is accompanied by the activation of ornithine decarboxylase (EC 4.1.1.17), the rate limiting enzyme in the biosynthesis of the naturally occurring polyamines [3]. Another enzyme which is also activated during the transformation process is *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50), which is involved in the synthesis of spermidine [4].

The activation of the biosynthetic decarboxylases during the transformation process, results in the accumulation of polyamines in the stimulated lymphocytes [5]. In general, the concentration of spermidine and spermine, is maximal when DNA synthesis reaches its peak. After prolonged incubation, when the rate of DNA synthesis declines, a decrease in cellular polyamines is observed [5]. The concentrations of polyamines in cultured lymphocytes may be regulated by controlling the rate of their biosynthesis, excretion or degradation. Some information is available concerning the control of polyamine biosynthesis in cultured lymphocytes. The induction of ornithine decarboxylase during lymphocyte mitogenesis may be regulated by cyclic AMP-dependent protein kinase [6]. However, the metabolism of polyamines and the regulation of their intracellular levels in cultured lymphocytes remain a mystery.

This study deals with the metabolism of polyamines by cultured lymphocytes. We show that in these cells, radioactive spermidine is converted into putrescine and *N*-acetylspermidine, and that these products are also excreted into the growth medium.

+ To whom reprint requests should be addressed

2. Materials and methods

Heparinized blood was drawn from healthy human volunteers. After separation of plasma, cells were mixed with RPMI-1640 medium (Bio-Lab, Jerusalem) and mononuclear cells were separated by using an Isolymph (Gallard Schlesinger Chemicals, New York) gradient as in [7]. For autoradiography studies, lymphocytes were grown in microwells at 37°C in a humidified atmosphere of 5% CO_2. The final composition of a single well was: (a) 0.18 ml RPMI-1640, supplemented with 2 mM L-glutamine and combined antibiotics (per ml: 200 μg penicillin, 200 μg streptomycin, 10 μg neomycin and 10 μg kanamycin); (b) 0.02 ml autologous plasma; (c) 0.4 μg purified phytohemagglutinin (PHAP, Wellcome, Beckenham); (d) 80 000 lymphocytes; (e) 0.08 μCi [^{14}C]spermidine, spec. act. 120 mCi/mmol, (New England Nuclear, Boston, MA). Controls without purified phytohemagglutinin were cultured simultanously under the same conditions. At specified times the culture medium was separated by centrifugation at 2000 rev./min for 10 min and cells were harvested by means of an automated cell harvester.

Radioactive polyamines were extracted with 3% (v/v) perchloric acid, from the cells or growth medium and dansylated as in [8]. Dansyl derivatives were separated on silica-gel plates (Kodak 13179, Eastman chromatogram sheets, 100 μm thick) using benzene/methanol (19:1) as a solvent [9]. The plates were exposed to films (Kodak, Noscreen) for several days. Spots identified by this radioautography were scraped off the plates, placed in scintillation vials and counted. *N*-Acetylspermidine, which served as a marker, was kindly provided by Dr N. Seiler (Centre de Recherche Merrell International, Strasbourg).

Polyamine content: Cells were cultured in 25 cm^2 tissue culture flasks (Falcon, Oxnard, CA) as above, but the final composition of a single culture was: (a) 9 ml RPMI 1640 medium; (b) 1 ml autologous plasma; (c) 10 μg purified phytohemagglutinin; (d) 4 000 000 lymphocytes. At specified times cells and medium were separated by centrifugation and lyophylized. Polyamines were then extracted with 3% (v/v) perchloric acid and analyzed by means of an automatic amino acid analyzer as in [10].

3. Results

Fig.1 represents a typical experiment, illustrating a 10-fold increase in cellular polyamine concentrations during lymphocyte stimulation. All these experiments were done in triplicate and the variations were <10%. It may be seen that putrescine levels, which were elevated during transformation, were consistently lower than those of spermidine or spermine. All the polyamines reached maximal levels 72 h after stimulation and then declined (fig.1).

The increase in cellular polyamines in cultured lymphocytes, is easily explained by the activation of the biosynthetic decarboxylases. On the other hand, the reason for their decline, several days after lectin stimulation, has not yet been clarified. To study the metabolic activity of the lymphocytes, radioactive spermidine was added to the various cultures.

It may be seen (fig.2) that the concentration of

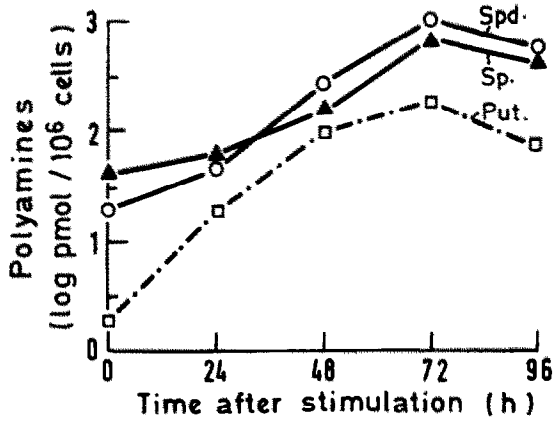

Fig.1. Intracellular concentrations of polyamines in cultured lymphocytes. Cells were transformed by purified phytohemagglutinins. Spd., spermidine; Sp., spermine; Put., putrescine.

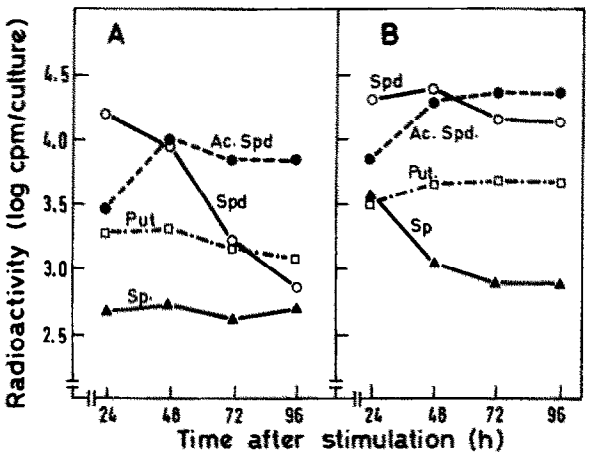

Fig.2. Accumulation of radioactive metabolites of [^{14}C]spermidine in the growth medium of cultured human lymphocytes. (A) Lymphocytes were transformed by purified phytohemagglutinins. (B) Unstimulated controls. Spd., spermidine; Sp., spermine; Put., putrescine; AcSpd., *N*-acetylspermidine.

[^{14}C]spermidine, gradually decreased in the growth medium throughout the 96 h incubation. It is also obvious that this process was more pronounced in the medium of PHAP-stimulated lymphocytes (fig.2A), than in the unstimulated controls (fig.2B). Concomitant with the disappearance of [^{14}C]spermidine from the culture medium, the formation of [^{14}C]acetylspermidine was noticed. The concentrations of this derivative were higher in the medium after the growth of unstimulated lymphocytes (fig.2B) than their concentrations in the medium of the PHAP-stimulated cells (fig.2A). After 48 h incubation the concentrations of acetylspermidine in the growth medium increased by a factor of 5 (fig.2). In addition, [^{14}C]-putrescine was also detected in the growth medium. The concentrations of this radioactive diamine paralleled those of [^{14}C]acetylspermidine and were higher in the medium in which unstimulated lymphocytes were grown (fig.2B), than in the medium of the PHAP-stimulated cells (fig.2A). The concentrations of [^{14}C]spermine, formed from spermidine, were considerably lower than those of [^{14}C]putrescine, in the medium after growing stimulated and unstimulated lymphocytes (fig.2AB).

[^{14}C]Spermidine was also taken up by cultured lymphocytes. This process was more active in PHAP-stimulated lymphocytes (fig.3A) than in the unstimulated controls (fig.3B). As in the culture medium, both radioactive putrescine and acetylspermidine were

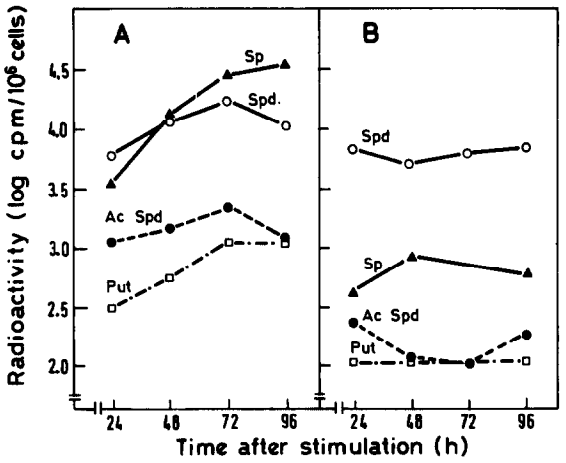

Fig.3. Accumulation of radioactive metabolites of [^{14}C]spermidine in cultured human lymphocytes. (A) Lymphocytes were transformed by purified phytohemogglutinins. (B) Unstimulated controls. Spd., spermidine; Sp., spermine; Put., putrescine; AcSpd., *N*-acetylspermidine.

found in the cultured cells. Again, the levels of radioactive putrescine corresponded well with those of acetylspermidine. It should, however, be noted that these two compounds accumulated mainly in the stimulated lymphocytes (fig.3A) and not in the unstimulated controls (fig.3B), while the reverse relationship was found when the growth medium was analyzed (fig.2A,B).

Stimulated lymphocytes also differed from the unstimulated controls by their capacity to convert [^{14}C]spermidine into spermine. The stimulated cells accumulated spermine at relatively high concentrations, even after 96 h (fig.3A).

4. Discussion

These data confirm the findings that transformation of lymphocytes leads to changes in their cellular polyamines [5]. It may be seen (fig.1) that polyamine levels rise when lymphocytes are stimulated by PHAP and that cellular putrescine concentrations are lower than those of spermidine and spermine.

We also show that transformed lymphocytes are more active, metabolically, than the unstimulated controls. Thus, transformed cells incorporate more [^{14}C]spermidine than the unstimulated lymphocytes (fig.3). In addition, the conversion of [^{14}C]spermidine into radioactive spermine or putrescine is more pronounced in transformed lymphocytes than in the untreated controls (fig.3). This increase in metabolic activity may explain the rapid disappearance of radioactive spermidine from the growth medium after lymphocyte stimulation (fig.2).

It is evident from fig.3, that cultured human lymphocytes, are capable of converting radioactive spermidine into putrescine. Radioactive putrescine, derived from spermidine, is detectable in the medium during the growth of transformed and unstimulated lymphocytes (fig.2). It is most likely that this conversion is catalyzed by an enzyme, similar to that described in [11]. This enzyme, which catalyzes the oxidation of spermidine to putrescine, and to aminopropionaldehyde, had been purified from rat liver [11]. This reaction, thus leads to the 'recycling' of putrescine. A similar process may also occur in mouse brain [12]. The interconversion of spermidine into putrescine has also been described for mouse parotid glands [13].

We also show that the formation of [^{14}C]putrescine from spermidine, parallels the accumulation of *N*-acetylspermidine in the growth medium (fig.2) and in the cultured lymphocytes (fig.3). This derivative has been identified as N^1-acetylspermidine [NH_2-$(CH_2)_4NH(CH_2)_3NHCO$-CH_3] [9]. This acetyl derivative of spermidine, has been implicated in the oxidation of spermidine to putrescine by the enzyme in [14,15]. Our findings (fig.2,3) that the formation of putrescine from spermidine parallels the acetylation of spermidine, strongly suggests that acetylspermidine is an intermediate in the oxidation of spermidine to putrescine. Recent studies, dealing with the acetylation of spermidine in rat liver extracts, after carbon tetrachloride treatment, also led to the conclusion that acetylation of spermidine is required for its conversion into putrescine [16]. It thus appears that N^1-acetylspermidine is an intermediate in the 'recycling' of putrescine and its formation from spermidine.

In addition, the acetylation process alters the cationic nature of the polyamines by blocking primary amino groups. This reversible process may facilitate the excretion of polyamines and their transport through the cellular membrane. It is of special interest that acetylspermidine accumulated in the medium during the growth of transformed and unstimulated lymphocytes. Acetylation of polyamines may thus serve as an additional control mechanism which regulates intracellular levels of the polycations. Such regulatory mechanisms involving acetylation and deacytalation of polyamines have been proposed [15,17].

This study thus demonstrates the 'recycling' of spermidine and its conversion into putrescine in cultured lymphocytes. This process and the acetylation of polyamines may play an important role in regulating polyamine levels in lymphocytes during mitogenesis.

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