Ornithine Decarboxylase and *S*-Adenosyl Methionine Decarboxylase in Skin Fibroblasts of Normal and Cystic Fibrosis Patients

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Summary

The key enzymes in the synthesis of the naturally occurring polyamines, ornithine decarboxylase (ODC) and *S*-adenosyl methionine (SAM) decarboxylase, were investigated during cell growth and aging in fibroblast cultures from normal patients and patients with cystic fibrosis. A linear correlation between increased *S*-adenosyl methionine activity and putrescine concentration was apparent in all cell lines. A putrescine concentration of 0.8 mM was optimal for enhancement of SAM decarboxylase activity.

The passage number of the cell line correlated inversely with maximal putrescine-stimulated SAM decarboxylase activity, earlier passage numbers having the highest specific activity (Fig. 1). No significant differences in basal or putrescine-stimulated SAM decarboxylase activity were noted between normal fibroblast cultures and cells from patients with cystic fibrosis (Fig. 2). SAM decarboxylase activity increased as the cell lines approached confluence. Activity was lowest during exponential growth (Fig. 3). ODC activity was increased during early exponential growth and fell as cells reached confluence (Fig. 4). No differences in ODC activity and putrescine inhibition between the normal and cystic fibrosis cell cultures at equivalent points of exponential growth were noted.

Speculation

Although there are definite alterations of polyamine levels in patients with cystic fibrosis, no abnormalities in ornithine decarboxylase or *S*-adenosyl methionine decarboxylase were found in cystic fibroblasts (Fig. 5). Differences in polyamine biosynthetic activity appeared to be a function of cell age. Therefore, it is postulated that polyamine abnormalities in cystic fibrosis are caused by enzymes other than SAM or ornithine decarboxylase. The polyamine abnormalities may relate to abnormalities in the metabolism of polyamines (*e.g.*, acetylation or catabolism) or be a consequence of increased *in vivo* synthesis secondary to humoral factors which have not yet been identified.

Table 1. Stimulation of *S*-adenosyl methionine decarboxylase activity by 2.5 mM putrescine

Cell line	Passage no.	Activity relative to basal level, -fold
Normal	6	38, 45
	28	39, 17, 22
Cystic fibrosis	8	30, 40
	11	20, 21

Several investigators (1, 9, 12) have indicated that polyamine levels in sera and urine closely reflect abnormal disease states in humans. In neoplastic diseases, polyamine levels reflect the rapid turnover rate of cells (13) and the potential indices of therapeutic efficacy (10, 13). In cystic fibrosis patients, abnormal blood concentrations of spermidine have been found (7), and these levels may be useful in diagnosis.

Previous *in vitro* studies of polyamine biosynthetic pathways (see Fig. 5) have utilized rat prostate and tumor cells (3, 4), but no extensive studies in human fibroblast cultures have been

Fig. 1. The potential *S*-adenosyl methionine (SAM) decarboxylase activity in the presence of putrescine decreased with increasing numbers of culture passages. The concentration of putrescine was 2.5 mM for all cultures studied. Each point represents the average of two separate experiments except passage 28 which is the mean of four experiments. Passages 8 and 11 are cystic fibrosis fibroblasts whereas passages 6 and 28 are normal fibroblasts.

Fig. 2. Basal *S*-adenosyl methionine (*SAM*) decarboxylase specific activity in cystic fibrosis and normal fibroblasts. Each value is the average of two to six experiments per culture. The number of experiments is given as (*N*) in bar graph. From left to right the passage number of the bar graphs for the control cultures are 28 and 6, and for the cystic fibrosis cultures 11 and 8, respectively.

reported. In this report, the key enzymes in the synthesis of the naturally occurring polyamines, ODC and *SAM* decarboxylase, were investigated during cell growth and aging in fibroblast cultures from normal patients and patients with cystic fibrosis (CF).

MATERIALS AND METHODS

Cultured skin fibroblasts from two normal CF male adults, three normal male infants, and four children with (age 2–5 years) were grown in 75-cm² Falcon flasks at 37° in a 5% CO₂ atmosphere. The cells were cultured in 15 ml Eagle's minimum essential medium with 10% or 20% fetal calf serum, penicillin (100 µg/ml), streptomycin (100 µg/ml), fungizone (25 µg/ml) (1% antibiotic antimycotic mixture), 10 mM HEPES buffer, and 1% nonessential amino acids with 2 mM L-glutamine. All culture materials were purchased from Gibco. Confluent flasks were trypsinized and split 1:2 or 1:3 24 hr before assay. At the time of assay, 2 ml 0.1 M sodium phosphate buffer, pH 7.2, were added to each flask, and the cells were removed by scraping with a rubber policeman. Cells from 20 flasks were harvested and centrifuged at 900 × *g* for 10 min at 4°. The resulting pellet was resuspended in 5.5 ml 0.1 M sodium phosphate buffer, pH 7.2, with 5 mM dithiothreitol and 0.05 mM pyridoxal phosphate. The cell suspension was freeze-thawed twice with liquid N₂. After freeze-thawing, the mixture was spun at 25,000 × *g* for 15 min at 4° in a Beckman LD3-40 ultracentrifuge. The supernatant and pellet were separated and the final volume of supernatant brought to a 6-ml volume with 1 mM EDTA (0.5 ml added). All assays were performed with this preparation. The assays were performed in triplicate on at least three different occasions and gave reproducible results with a variation of 10% or less.

Fig. 3. Putrescine stimulated *S*-adenosyl methionine (*SAM*) decarboxylase activity is a function of DNA content. Since DNA content/per cell is constant, the transition of cultured fibroblasts from the exponential growth phase to the stationary phase is expressed as a function of the DNA concentration per culture flask. Increasing DNA concentration is synonymous with time since passage of the inoculum or with doubling time. *SAM* decarboxylase specific activity increases as both cystic and normal cells transit from exponential growth to confluence (nonproliferative state).

Fig. 4. The transition of cultured fibroblasts from exponential growth towards confluence is characterized by increasing DNA concentration per culture vessel. Both ornithine carboxylase (ODC) and ornithine-ketoacid transaminase (OKT) specific activity decrease as cellular proliferation decreases.

SAM decarboxylase assay was accomplished with 0.5 ml enzyme supernatant incubated with 5 μ l *S*-adenosyl-[carboxy- 14 C]methionine, 14 C-labeled SAM, 7.5 μ Ci/mmol (New England Nuclear Corporation), 5.0 mM to 0.01 mM putrescine and 0.1 M sodium phosphate buffer, pH 7.2, in a final volume of 1 ml and a final concentration of 0.25 μ g of [14 C]SAM. Duplicate blanks contained boiled enzyme. The mixture was incubated at 37° in a 20-ml scintillation vial. A no. 1 Whatman filter paper disk was cut to fit into the cap and saturated with 0.1 ml Hyamine to trap CO_2 . The 37° water bath was kept in a 37° environmental chamber to prevent condensation of liquid at the rim of the vial. After 30 min, the reaction was stopped by addition of 1 ml 40% trichloroacetic acid, and the tightly capped vial was gently shaken for 60 min at room temperature to release all CO_2 . The Hyamine-impregnated disk was removed and placed in 10 ml of Aquasol scintillation counting fluid from New England Nuclear Corporation. Background counts of Hyamine-saturated filter paper and Aquasol were 34 cpm. Scintillation counting was done in a Hewlett Packard Tri-Carb scintillation spectrometer, model 3320.

For the assay of ODC, the preparation was incubated at 37° using the above outlined Hyamine-saturated filter paper disk method. Enzyme supernatant (0.5 ml) was incubated with 10 μ l [carboxy- 14 C]ornithine, specific activity = 54 μ Ci/mmol. The assay was incubated for 60 min and stopped by addition of 0.5 ml trichloroacetic acid and the vial gently shaken for 60 min to release all labeled CO_2 . Blanks contained boiled enzyme.

The activity of the enzyme, ornithine- α -ketoaminotransferase (OKT), which is not directly involved in polyamine synthesis, was assayed by adding 0.2 ml supernatant to 0.6 ml sodium, 1 mM, phosphate buffer with 5 mM dithiothreitol, 0.5 mM pyridoxal phosphate, and 1.5 mM EDTA (pH 7.2) in a conical centrifuge tube. Ornithine (0.15 ml) and α -ketoglutarate (0.05 ml) were added, mixed, and the tubes incubated at 37° for 2 hr. The reaction was stopped by addition of 0.6 ml 10% trichloroacetic acid and 0.6 ml 1% 2-aminobenzaldehyde. The optical density was determined at a wavelength of 443 nm in a Bausch and Lomb Spectronic 600.

DNA concentration was determined on 0.1 ml of the freeze-thawed suspension by the method of Richards (10) and protein content of the enzyme preparations was determined using the method of Lowry *et al.* (6). Since the amount of DNA in each cell is constant, an increase in measured total DNA per culture flask reflects increased cell number or density. Microscopic inspection of several culture flasks established confluency at a cell density of 3×10^7 cells/75-cm 2 flask. The DNA measured in 20 confluent 75-cm 2 culture flasks was approximately 2.5 mg.

RESULTS

Basal SAM decarboxylase activity without addition of putrescine was reproducibly detectable at 15–50 pmol/mg protein/hr. The addition of putrescine to the reaction mixture increased enzyme activity 17–45 times in both normal and cystic fibrosis

Table 2. Ornithine decarboxylase (ODC) activity in normal and cystic fibrosis cell lines¹

Cell line	Passage no.	ODC activity, pmol/mg protein·hr	n ¹
Normal	6	27	3
	8	40	3
	17	25	1
	28	55	6
Cystic fibrosis	8	38	3
	11	65	3

¹ Number of experiments.

Table 3. Inhibition of ornithine decarboxylase (ODC) by putrescine, 2.5 mM

Cell line	Passage no.	% of basal activity, $\bar{x}$ ¹
Normal		34
BOR	14	32
NFS	32	48
McC	15	24
Det	17	46
DeB	18	21
Cystic fibrosis		48
HeA	12	48
	14	58
	16	39

¹ $\bar{x}$ Mean.

Fig. 5. Biosynthetic pathway of polyamines. SAM: S-adenosyl methionine.

cell lines (Table 1). A linear correlation between increased SAM decarboxylase activity and linear correlation between increased SAM decarboxylase activity and putrescine concentration was apparent. A putrescine concentration of 0.8 mM was optimal for enhancement of SAM decarboxylase activity.

The passage number of the cell line correlated inversely with maximal putrescine-stimulated SAM decarboxylase activity, earlier passage numbers having the highest specific activity (Fig. 1). No significant differences in basal SAM decarboxylase activity were noted between normal fibroblast cultures and cells from patients with CF (Fig. 2).

The mean basal SAM decarboxylase for eight control cultures and five age-matched and passage number-matched cystic fibrosis cultured fibroblasts assayed as released radiolabeled CO₂ from S-adenosyl methionine showed no difference between CF and control cultures. SAM decarboxylase activity increased as the cell lines approached confluence. Activity was lowest during exponential growth (Fig. 3). ODC activity was increased during early exponential growth and fell as cells reached confluence (Fig. 4). No differences in ODC activity between the normal and

cystic fibrosis cell cultures at equivalent points of exponential growth were noted (Table 2).

As noted in rat prostate (5), the addition of putrescine to human fibroblast enzyme supernatant inhibited the ODC activity; we found 75–100% inhibition of basal activity at 5 mM and 50–80% inhibition at 2.5 mM concentrations of putrescine. In a preliminary study no difference in the effect of putrescine was found between the different fibroblast cultures if the growth phase was equivalent (Table 3). Ornithine α -ketoaminotransferase, an enzyme which uses ornithine as a substrate but is not directly involved in the polyamine pathways, was found to have equivalent activity in cystic fibroblasts and normal fibroblasts.

Ornithine-ketoacid transaminase (OKT) activity was monitored because it is an enzyme uninvolved in polyamine biosynthesis that is easily determined in cultured human fibroblasts derived from skin (8). OKT activity is proportional to the number of cells present but is not known to be influenced by the intermediates of polyamine biosynthesis. The negative slope, $Y_{OKT} = 19.1X + 118.7$, found by linear regression (Fig. 4) of OKT activity plotted against DNA, indicates that the activity level of this enzyme also decreases as the DNA amount reaches maximum levels, i.e., at cell confluence.

DISCUSSION

As human fibroblast cell cultures approach confluence, detectable differences in the activity of key enzymes in the production of polyamines are noted. During early exponential growth of human fibroblast cultures, there is a measurable increase in the activity of ODC. This results in increased production of putrescine, the substrate for spermine and spermidine synthesis (Fig. 5). With increased putrescine production, the rate-limiting step for spermine and spermidine synthesis becomes the decarboxylation of SAM. SAM decarboxylase activity is lowest during early exponential cell growth, increasing as cells achieve confluence. As demonstrated in this report, increasing putrescine concentration also decreases *in vitro* activity of ODC in human fibroblasts (2).

As demonstrated in Figure 1, the age of a cell line as reflected by passage number correlates with the maximal putrescine-stimulated SAM decarboxylase activity better than the age of the patient or whether the patient has cystic fibrosis. The observations that basal ODC and SAM decarboxylase activity are similar for normal and cystic fibrosis cultures indicate that abnormalities in spermidine concentration noted in cystic fibrosis patients are probably not due to an inherent abnormality in this component of the biosynthetic pathway.

CONCLUSION

The key polyamine biosynthetic enzymes, S-adenosyl methionine decarboxylase and ornithine decarboxylase, were investigated in normal and CF fibroblasts. No differences were observed in basal SAM decarboxylase and ornithine decarboxylase activity. Inhibition of ODC by putrescine and stimulation of SAM decarboxylase activity by putrescine showed parallel effects for normal and CF fibroblast enzyme preparations.

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A Review: Newer, Noninvasive Cardiac Diagnostic Methods

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This review article will focus on the application of two noninvasive techniques that have become increasingly more useful in the diagnosis and management of infants and children with heart disease. In a general sense, noninvasive diagnostic techniques provide two types of information; first, one seeks anatomic information in order to more clearly describe the architectural details of the heart and circulation, and, second, one hopes to derive data allowing analysis of functional characteristics of the circulation. The ability to obtain important structure-function correlations by the use of both scintillation scanning and cardiac ultrasound forms the basis for their widespread applicability in noninvasive diagnosis. We will describe selected aspects of current applications of scintillation scanning of the lungs and heart and of echocardiography, and will attempt to indicate new and interesting directions that may be anticipated by continuing developments in these fields. We await, with interest, additions to the armamentarium of the diagnostician that may be expected in the future from electrocardiographic-gated, computerized axial tomograms of the heart and from flow velocity data provided by range-gated, pulsed Doppler technology.

It should be understood that noninvasive methods infrequently offer a substitute for invasive hemodynamic and angiographic studies performed in the cardiac catheterization laboratory. Rather, the former most often provide valuable ancillary data that assist the clinician appreciably in decisions concerning patient management.

SCINTILLATION SCANNING

Isotopes are employed to quantify the distribution of pulmonary blood flow in patients with a wide variety of acquired and

congenital heart disease in order to determine indirectly the levels of pulmonary venous and arterial pressures (12, 13) and to determine the patency of systemic arterial and systemic venous-pulmonary arterial anastomosis (9, 12). In addition to these lung scanning methods which employ radionuclide labeled macroaggregated human serum albumin, radionuclide angiography is utilized to view alterations in the anatomy of the heart and great vessels, and to detect and quantify left to right, intra-, or extra-cardiac shunts (2-4, 22, 33, 35). The latter studies are performed after intravenous infusion of ^{99m} technetium with a scintillation camera coupled to a computer tape storage system.

ALTERATIONS IN REGIONAL PULMONARY BLOOD FLOW

VERTICAL DISTRIBUTION OF FLOW

The distribution of pulmonary blood flow vertically or to either lung may be determined in an accurate and reproducible manner by scintillation scanning of the lungs (12). The rationale for evaluating the vertical distribution of pulmonary blood flow resides in the importance of distinguishing patients with both pulmonary arterial and venous hypertension from those with pulmonary arterial hypertension alone.

In this regard, more precise selection may be anticipated of patients requiring an accurate hemodynamic assessment of pulmonary flows and resistance. It is recognized that because of the influence of gravity on the low pressure vascular bed, normal erect subjects have greater blood flow to the lung bases than to the apices. In patients with pulmonary venous hypertension, there is a relative increase in blood flow to the apices and a decrease in perfusion to the lung bases. A linear relationship