Vol. 98, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1981 Pages 324-329

COLCHICINE BINDING ACTIVITY AND TYROSYL TUBULIN LIGASE ACTIVITY IN NORMAL AND CYSTIC FIBROSIS FIBROBLASTS

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Received December 1,1980

SUMMARY

Colchicine binding was measured in the cytosol and particulate fractions of normal human fibroblasts and in cystic fibrosis fibroblast cultures. Colchicine binding in the cytosol fraction of the cystic fibrosis cultures was 37%-42% lower than the binding in normal fibroblasts. Particulate colchicine binding was 1%-3% of the total binding in all cell cultures. The activity of tyrosyl tubulin ligase, an enzyme tightly associated with tubulin, was 31%-49% lower in the cystic fibrosis cell cultures.

INTRODUCTION

Cystic fibrosis (CF) acts as an autosomal recessive disease occurring with an incidence of 1/1500 live births. The effects of cystic fibrosis are manifested primarily in the exocrine glands and include pancreatic dysfunction, chronic pulmonary disease, and electrolytic imbalance (1). Although the major effects of CF are in the exocrine glands, the genetic nature of the disease requires that the defective gene be present in all cells. Indeed, many abnormalities in CF fibroblasts grown in tissue culture have been reported (1,2). The use of cultured fibroblasts allows one to study CF under more convenient and controlled conditions.

I have measured levels of colchicine binding and tyrosyl tubulin ligase in normal and CF cultures because these measurements involve microtubule interaction and many observations

0006-291X/81/010324-06$01.00/0

describing CF may be associated with microtubules. Several reports either imply or show that microtubule structure and function is important for cell secretion (3), for transport (4,5,6), and for the structural integrity of membrane glycoproteins (7). In addition, calcium and other ions play a role in the regulation of microtubule polymerization and depolymerization (8). All of the above processes have been reported to be abnormal in CF (1,2).

The following report shows that there is a difference in colchicine binding and tyrosyl tubulin ligase activity between normal human fibroblasts and a culture of CF fibroblasts.

MATERIALS & METHODS

Cell Culture

Cells were grown in N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) buffered McCoy's 5a modified medium supplemented with 10% fetal calf serum. Cultures were fed twice a week and subcultured every week with a one to three split. Two human skin fibroblast cultures were obtained from the Human Genetic Mutant Cell Repository in Camden, New Jersey. GM0142 was from a 14 year old male with severe cystic fibrosis. GM0316 was from a 12 year old normal male. HF1 is a human fibroblast culture derived from human foreskin.

Colchicine Binding

Cells were harvested by trypsinization, washed with Hank's balanced salt solution and resuspended in 0.5 ml of 0.01M phosphate buffer pH 6.8 containing 0.1M sodium glutamate. The cells were sonicated for 10 seconds and [^{3}H] colchicine (2×10^{-6}M final concentration) was added. The samples were incubated at 37°C for one hour and binding was determined by filtering through DE81 filter paper according to the procedure of Borisy (9).

Initial colchicine binding was determined by measuring the decay of the colchicine binding sites at 37° and by extrapolating to zero time using linear regression analysis (10). Five time points were used and colchicine was added at 30 minute intervals. Binding in the presence of colchicine was carried out at 37° for one hour. Blanks for each sample were run as described previously (10) but contained a 5000 fold excess of unlabeled colchicine.

Tyrosyl Tubulin Ligase

Tyrosyl tubulin ligase was assayed as described previously

(11). Cells were harvested by a brief trypsin treatment, washed and resuspended in ligase buffer (0.05M Tris, pH 7.4, 0.015M Mg acetate, 0.1M KCl, 1mM dithiothreitol, 1M glycerol, 200 µg/ml RNase). The cells were sonicated for 10 sec and the following reagents were added to give a final concentration of 4 mM ATP, $1x10^{-6}$M [^{14}C] tyrosine (513 mCi/mmole, New England Nuclear, Boston, Mass.) and 200 µg/ml of purified rat tubulin. The reaction mixture was incubated at 37°C for 2.5 hr and assayed for TCA insoluble tyrosine incorporation on GFC Whatman glass fiber filters.

Protein

Protein was determined by the Lowry procedure using bovine serum albumin as a standard (12).

RESULTS

The amount of colchicine binding in the cytosol of two normal human skin fibroblast cultures was compared to the amount of colchicine binding in the cytosol of a cystic fibrosis fibroblast culture. Since the colchicine binding sites decay with time (13), binding was assayed over a period of three hours and the initial binding was determined by extrapolating the decay curve back to zero time. Linear regression analysis was used to establish the best curve fit. Figure 1A shows the results of three experiments comparing colchicine binding in normal fibroblast (HF1 and GM316) and cystic fibrosis fibroblasts (GM142). In each experiment the amount of colchicine binding in cystic fibrosis fibroblasts was lower than in normal cells. Colchicine binding was 37% to 42% lower in the cystic fibrosis cells. Measurement of colchicine binding in the particulate fraction was 1%-3% of the total binding. Greater than 97% of the colchicine binding was in the cytosol fraction.

Tyrosyl tubulin ligase, an enzyme which is tightly associated with tubulin, was also assayed (14). This enzyme ligates tyrosine to the carboxyl terminal end of the α chain of tubulin. Its function in the cell is unknown. As shown

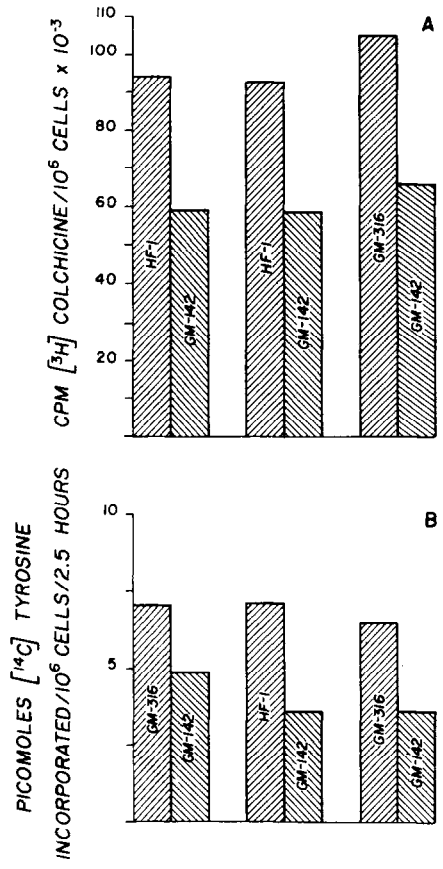

Figure 1. Colchicine binding and tyrosyl tubulin ligase activity in cystic fibrosis and normal human fibroblasts.

1A. Colchicine binding activity extrapolated back to zero time. GM316 and HF1, normal fibroblasts; GM142, cystic fibrosis fibroblasts.

1B. Tyrosyl tubulin ligase activity. GM316 and HF1, normal fibroblasts; GM142, cystic fibrosis fibroblasts.

in figure 1B, tyrosyl tubulin ligase is also lower in the cystic fibrosis fibroblasts compared to the normal fibroblasts. The results show that tyrosyl tubulin ligase was 31% to 49% lower in the cystic fibrosis cells. These results are approximately equal to the lower amount of colchicine binding in CF cells.

In order to rule out an overall lower protein content of the cystic fibrosis cells, total protein was determined for

the normal GM316 fibroblasts and for GM142 CF fibroblasts. The amount of protein in the CF cells was 10% greater than in the normal cells. Therefore a lower protein content of CF cells does not explain the results.

DISCUSSION

The results show that there is a definite difference in colchicine binding and tyrosyl tubulin ligase between normal human fibroblasts and a culture of cystic fibrosis fibroblasts. Colchicine binding was 37% to 42% lower and tyrosyl tubulin ligase was 31% to 49% lower in cystic fibrosis fibroblasts compared to normal fibroblasts. Since colchicine binding can be an accurate method of measuring levels of tubulin within cells (15), the lower binding activity implies that CF cells have lower amounts of tubulin. This result needs further verification in that there are many factors that can affect colchicine binding either by stabilizing, destabilizing, or competitively inhibiting binding (16). The level of some interfering factor may be altered in CF cells and thus account for the differences in colchicine binding.

Tyrosyl tubulin ligase is an enzyme tightly associated with tubulin (14). Ligase activity parallels tubulin levels and colchicine binding activity during chick brain development (17). The fact that tyrosyl tubulin ligase activity paralleled colchicine binding activity in CF cells supports the idea that tubulin levels are lower in CF cells.

It has also been reported that tyrosylation may control the partitioning of tubulin into cellular compartments (18). In neuroblastoma cells, tyrosine was incorporated almost exclusively into an insoluble fraction which might be membrane

bound (18). If these implications are correct, the pathological effects of cystic fibrosis on secretion, altered glycoproteins, and transport are consistent with alterations in microtubule structure and function. However, the question of whether the lower colchicine binding and tyrosyl tubulin ligase activity in CF cells reflects an alteration in microtubule function or whether this is a secondary effect of the disease needs to be proven.

ACKNOWLEDGEMENT

This work was made possible in part by support from the Norm Crosby Research Fund, by NIH Grant #1-S07-RR05841-01, a Biomedical Research Support Grant awarded to City of Hope Research Institute, and NIH grants AG00434 and GM26015.

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