ORNITHINE TRANSCARBAMYLASE (OTC) IN WHITE BLOOD CELLS AND JEJUNAL MUCOSA

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INTRODUCTION

The presence of OTC in white blood cells was demonstrated by Wolfe and Gatfield (1) and Snodgrass et al. (2), but was denied by Rabier et al. (3). OTC deficiency was diagnosed using white blood cells by Wolfe and Gatfield (1) and Krieger et al. (4). On the other hand, Snodgrass et al. (2) reported that OTC deficiency in the liver can not be inferred from the measurements of the enzyme's activity in peripheral white blood cells, because the latter parameter was normal in their patients. The presence of OTC in jejunal mucosa and reliability of this material for detecting OTC deficiency were demonstrated by Levin et al. (5) and Cathelineau et al. (6). However, the nature of OTC in intestinal mucosa was not clearly defined. We would like to describe some kinetic studies on OTC in white blood cells and jejunal mucosa.

MATERIAL AND METHOD

L-ornithine hydrochloride and carbamylphosphate (dilithium salt) used were purchased from Sigma Chemical Co, St. Louis. 14C-ornithine was purchased from New England Nuclear, Boston. All other chemicals were of reagent quality.

White blood cells: white blood cells were prepared by the method of Wolfe and Gatfield (1). Separation of mononuclear cells from the granulocytes was performed by differential filtration using a modified method of Boyum (7).

Establishment of lymphoid cell line: lymphoid cell lines were established from normal subject and OTC-deficient infant after incubation with Epstein-Barr (EB) virus (8). Liver and jejunum: post mortem specimens of the liver and jejunum were obtained from...
Materials were stored at -60°C for three months before being studied.

Measurement of OTC activity: the radiochemical method of Sinatra et al. (9) was used for OTC assay in white blood cells. White cell pellet was suspended in 0.1% cetylpyridium chloride, frozen and thawed three times, and centrifuged at 12,000 X g for 25 min. One hundred μl of the supernatant, 14C-carbamylphosphate (final concentration 0.87 mM; specific activity 1.3X10^3 cpm/μmol) and ornithine (final concentration 5 mM) were mixed and 0.05 M triethanolamine acetic acid buffer pH 8.5 was added. The mixture was incubated at 37°C for 60 min. The reaction was then terminated by adding 100 μl of 3N formic acid. After heating 5 min in boiling water; the excess 14CO₂ was removed by adding crushed dry ice. The supernatant, after centrifugation for 10 min at 2,000 X g, was transferred to a vial and counted by liquid scintillation spectrometer.

OTC in liver was measured by both radiochemical method and conventional colorimetric method (10) (11), and OTC in jejunal mucosa by colorimetric method only.

Autoradiography: logarithmically growing cells, each 5 x 10^5 in number were washed with arginine-free RPMI 1640 and incubated for 48 h at 37°C in RPMI 1460, which contained 14C-ornithine or 14C-citrulline instead of arginine. After the incubation, slides were dipped in Kodak NTB 3 emulsion, exposed for 5 days, developed in D-19 for 3 min, and stained with Giemsa.

Growth curve of lymphoid cells: lymphoid cells were cultured in two different media: RPMI 1640 with 1 mM arginine (ornithine-, arginine+); and RPMI 1640 with 1 or 5 mM ornithine (ornithine+, arginine-).

RESULT

OTC activity in white blood cells and cultured lymphoid cells: a positive linear relationship was found between incubation time (40 and 60 min) and conversion of 14C-carbamylphosphate. The relationship between the conversion rate of radioactive substrate and the amount of cell lysate, measured after 60 min incubation, was linear. The results obtained are summarized in Table 1. Apparent Km of OTC for ornithine and carbamyl phosphate are shown in Table 3.

Growth curves of cultured lymphoid cells: lymphoid cells from both the controls and the OTC-deficient infant showed normal logarithmic growths in the arginine (+), ornithine (-) medium, whereas the cells failed to grow in the arginine (-), ornithine (+) medium.

Autoradiography: when in the presence of 14C-ornithine, few grains were found on lymphoid cells from both the normal subjects and the OTC-deficient patient.