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Angiotensin Converting Enzyme in Cultured Fibroblasts in Gaucher and Niemann–Pick Diseases[1] (41427)

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Abstract. Similar low levels of angiotensin converting enzyme activity were found in cultured fibroblasts from types 2 and 3 Gaucher disease, type A Niemann–Pick disease, and normal subjects. The highly elevated levels of angiotensin converting enzyme in serum and spleen in Gaucher disease is not due to a marked general cellular elevation of the enzyme, but may be due to elevated synthesis of ACE secondarily stimulated in Gaucher cells.

Gaucher disease is an autosomal, recessively inherited disorder characterized by generalized deficient glucocerebroside β-glucosidase and deposition of glucocerebroside in reticuloendothelial cells (1). Angiotensin converting enzyme (ACE, EC 3.4.15.1, peptidyl-dipeptide hydrolase, angiotensin I→II) is strikingly elevated in serum and spleen in Gaucher disease (2–4). To test the possibility that the genetic alteration which diminishes glucocerebrosidase activity in all cells investigated also results in increased ACE in the same cells, presumably by derepression of the ACE gene, ACE was assayed in skin fibroblasts from Gaucher disease and compared with those from Niemann–Pick disease, another inherited disorder of lipid metabolism, and normal subjects.

Materials and Methods. Fibroblasts (Table I) were cultured in McCoy's 5A medium (GIBCO) with 20% fetal calf serum in 5% CO_2 at 37° for 4–8 days. The cells were washed with Dulbecco's calcium and magnesium-deficient buffered saline (PBSD) or Puck's saline/0.02% EDTA and harvested in 1 ml PBSD/0.25% trypsin/0.05% EDTA or 3 ml Puck's saline/0.04% trypsin/0.02% EDTA. Trypsinization was terminated by the addition of 3–5 ml McCoy's 5A/20% fetal calf serum. Cells were collected by centrifugation at 500*g* for 10 min, washed four times with a total of 38 ml PBSD, and suspended in 0.2 ml of 50 m*M* K phosphate, pH 8.3, and stored at −80° until sonicated and assayed in duplicate for ACE activity as previously described (5). In order to overcome any possible residual EDTA which inhibits ACE activity, the assay was also done with cobalt activation (1.25 m*M* $CoCl_2$ for 30 min at 37° prior to initiation of reaction with hippuryl-His-Leu; 1 m*M* $CoCl_2$ final concentration). Protein was assayed (6) with a bovine serum albumin standard.

Three of the four normal control fibroblast specimens were derived from foreskin cultures (Table I). Foreskin was washed in medium (McCoy's 5A or RPMI 1640), cut into small pieces, and at least four pieces allowed to adhere to the culture plates (10 × 60 or 10 × 35 mm) for 10 min. Culture medium was then added and cultures were incubated. When the cells become confluent, skin pieces were removed and cells harvested with 0.4 ml (small plates) or 1.5 ml (large plates) PBSD/0.25% trypsin/0.05% EDTA and subcultured into three plates, harvested after confluency was reached, and prepared for assay as aforementioned.

Cerebroside albumin was prepared by slowly mixing a solution of 18.45 mg of bovine brain cerebrosides (predominantly galactocerebroside; Serdary Research Laboratories, London, Ontario, Canada) dissolved in 4 ml of hot methanol into a solution of fat-free bovine serum albumin

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TABLE I. ANGIOTENSIN CONVERTING ENZYME IN SKIN FIBROBLASTS FROM GAUCHER AND NIEMANN–PICK TYPE A DISEASES AND NORMAL SUBJECTS

Fibroblasts		ACE specific activity (nmole/min·mg protein)	
No.[a]	Type	No cobalt	Cobalt, 1 m*M*
GM 877	Gaucher disease, Type 2, infantile	1.5 ± 0.5[b]	2.1 ± 0
GM 1260	Gaucher disease, Type 2, infantile	1.0 ± 0	2.2 ± 0.2
GM 1607	Gaucher disease, Type 3, juvenile and adult, cerebral	0.88 ± 0.12	1.8 ± 0.1
GM 112	Niemann-Pick disease, Type A	1.1 ± 0.3	1.5 ± 0
Normal control (n = 4)[c]		0.90 ± 0.36 $P < 0.5$*	1.5 ± 0.7 $P < 0.3$*

[a] The Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, N.J.
[b] Standard deviation.
[c] One specimen was GM 41; three specimens were newborn foreskin fibroblast cultures.
* Statistical significance (Student's t test) with respect to all Gaucher disease samples.

(Sigma) (133 mg/4 ml of 20 m*M* sodium acetate, pH 4.0). The mixture was cooled to 0° on ice, dialyzed against distilled water overnight at 5°, and then lyophilized.

Human monocytes were cultured essentially as previously described (7). The monocytes were plated in RPMI 1640 medium containing 10% autologous serum which had been heated for 40 min at 60° to inactivate ACE. After one hour the monocytes were washed four times with phosphate-buffered saline, removing nonadherent cells, and then cultured in 1 ml of RPMI 1640:medium 199 in 1:1 proportion containing 10% autologous serum. After 5 days 0.1 mg of cerebroside albumin in 0.1 ml medium was added (control cultures received 0.1 ml medium only) and the cells were harvested after an additional 2 days incubation and assayed for ACE (5).

Results and Discussion. ACE activities were similar in Gaucher, Niemann–Pick, and normal fibroblasts (Table I), in marked contrast to a greater than 10-fold increase in ACE in Gaucher spleen (3). The slight differences tended to be minimized under conditions of cobalt activation, suggesting that some variable residual EDTA inhibition may have been present. There was no statistically significant difference between the ACE activities of four normal control fibroblast cultures and three Gaucher disease fibroblast cultures. The present results indicate that, unlike the generalized diminution of glucocerebroside β-glucosidase, the genetic defect in Gaucher disease is not simultaneously manifested by a generalized massive increase in ACE as tested in fibroblasts under the conditions described, although a very slight increase cannot be ruled out. The genetic alteration in Gaucher disease may not be intimately related to the increased ACE, which may be a secondary phenomenon. The detection of ACE in Gaucher cells (8), the inducibility of mononuclear phagocytes in culture for ACE (5, 7), and modulation of the induction by lymphocytes and soluble factors (9) suggests that increased ACE in Gaucher disease may be due to induction of ACE in Gaucher cells stimulated by accumulated metabolites and by cellular influences.

Initial experiments to test the possibility that accumulation of cerebrosides may stimulate ACE synthesis in Gaucher cells have not revealed any enhancement of ACE activity. Human monocytes exposed to 0.1 mg/ml cerebroside for the final 2 days of a 7-day incubation contained ACE which was similar to control (experimental, 4.2 ± 0.8 (SD) nmol/min·mg protein, n = 2; control, 4.3 ± 0.1, n = 2).

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