Aspects of Biochemical Differentiation in the Central Nervous System

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Abstract—A demonstration of cell-specific patterns of development in the immature CNS is provided by examples of characteristic, cell-specific time-courses of enzyme development in different classes of brain cells isolated in highly purified form by bulk-separation from the cerebral and cerebellar cortex of the growing rat. The enzymatic analysis was carried out at the level of the nerve and glial cell lysosomes and mitochondria, two subcellular organelles crucial to the economy of all cells. The findings reveal rather similar developmental patterns for the lysosomal hydrolase N-acetyl-β-D-glucosaminidase in neurons and glial cells of the cerebral cortex as well as in two different cerebellar nerve cell types, the Purkinje and the granule cell. However, significant differences in the post-natal chronology of development of the mitochondrial enzyme α-glycerophosphate dehydrogenase were noted between cortical nerve and glial cells, the glial enzyme exhibiting 6-fold higher levels of activity than the neuronal one throughout the first month of postnatal life. The findings emphasize the feasibility as well as the necessity of studies aimed at the elucidation of the cell-specific aspects of the biochemistry of developing nerve and glial cells.

This paper presents results of experiments recently conducted in our laboratory which were designed to provide biochemical information on the fundamental biological issue of cellular specialization in the central nervous system. Being neurochemists, we examined the development of dynamic metabolic processes rather than the genesis of specialized structural characters or electrical signals. In our work, we have taken advantage of the recently acquired capability of bulk isolation of large amounts of neuronal cell bodies and glial cells and have thus attempted to analyze their biochemical development separately, sampling events at multiple time points during early post-natal development.

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Two processes were studied in the developing cerebral and cerebellar cortex of the rat: (1) the degradative reactions exhibited by the lysosomes of the neurons and the glial cells; and, (2) the differential ability of the neurons and glial cells to use cellular substrates for energy generation.

1. The Post-Natal Development of the Neuronal and Glial Lysosome

We present below the results of our recent findings on the post-natal development of a lysosomal enzyme, the glycosidase N-acetyl-β-D-glucosaminidase in nerve cell bodies and glial cells prepared by bulk-isolation methods, developed in our laboratory (Sellinger, et al. 1971) from the cerebral cortex of the rat, as well as using more recent methodologies (Sellinger, et al. 1974) in two different nerve cell types, the Purkinje and the granule cell, which we isolate from the immature rat cerebellar cortex. Our interest in this particular lysosomal glycosidase stems from its importance in the maintenance of the general well-being of nerve cells, and hence, of the total living organism; in fact, a partial or total deficiency of this enzyme activity characterizes the distinct forms of human cerebral gangliosidosis.

Materials and Methods

Chemicals. The two substrates of N-acetyl-β-D-glucosaminidase, p-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside and 4-umbelliferyl-N-acetyl-β-glucosaminide were purchased from Sigma Chemical Company, St. Louis, Missouri, or from Pierce Chemical Company, Rockford, Illinois. Bovine serum albumin (Fraction V) was obtained from Pentex Biochemicals, Kankakee, Illinois, and polyvinylpyrrolidone (Plasdone C) from GAF Corp., Calvert City, Kentucky. Sucrose and calcium chloride were of analytical reagent grade. Nylon bolting cloth was purchased from Tobler, Ernst and Traber, Elmsford, New York.

Animals

The rats were males of the Sprague-Dawley strain; they were generally sacrificed between 11 a.m. and 12 noon.

Methods

Analytical. RNA was determined by the procedure of Fleck and Begg (1965) and the activity of N-acetyl-β-D-glucosaminidase either according to Idoyaga-Vargas, et al. (1972) (colorimetrically)