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Aspartate transcarbamylase
carbamyl phosphate synthetase
cerebellum
developmental biochemistry
nucleic acid synthesis
pyrimidine biosynthesis
uridine kinase

Pyrimidine Biosynthesis during Development of Rat Cerebellum

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Extract

The activities of the first two enzymes of the *de novo* pyrimidine biosynthetic pathway, carbamyl phosphate synthetase (CPS) and aspartate transcarbamylase (ATC) (EC.2.1.3.2), were closely correlated with cellular proliferation in rat cerebellum. Uridine kinase demonstrated a different developmental pattern which suggests an important role, in nondividing cells, for the pathway by which preformed pyrimidines are utilized for nucleic acid synthesis.

Speculation

De novo synthesis provides the major source of pyrimidine nucleotides required for nucleic acid synthesis in developing rat brain. Mature brain obtains its pyrimidine nucleotides for nucleic acid synthesis primarily from utilization of bases and nucleosides released from the breakdown of preexisting nucleic acid.

Introduction

The first two enzymes of the *de novo* pyrimidine biosynthetic pathway in mammals are glutamine-requiring CPS and ATC. These enzymes are both found in the soluble fraction of the cell and are associated as a multifunctional complex [14]. The activities of both enzymes have been shown to be correlated with the rate of cellular proliferation in tissues. For example, CPS was found to be elevated in Walker carcinosarcoma 256 [28] and ATC was very active in ascites tumor cells [6]. Activity of ATC was also high in tissues of chicken embryo [10], rat fetus [21], and in regenerating rat liver [5].

Pyrimidine nucleotides can also be formed by a mechanism in which preformed bases are phosphorylated by kinases. Uridine kinase (UK), a representative enzyme of this salvage pathway, has been shown to increase in activity towards the completion of organogenesis in several organs of the chick embryo [10], which suggests that the *de novo* pathway may be a primary source of pyrimidine nucleotides during early stages of development, whereas synthesis from preformed bases may be of greater importance at a later stage of development, when DNA synthesis has virtually ceased.

Since the major development of rat cerebellum occurs during the first three postnatal weeks [27], this organ is admirably suited for a study of the development of certain enzymatic activities important for synthesis of nucleic acid precursors. In the present investigation, we were concerned with obtaining developmental patterns for CPS and ATC from rat cerebellum and comparing them with patterns obtained for UK.

Materials and Methods

$Na_2{}^{14}CO_3$ was prepared from $Ba^{14}CO_3$ (61.1 mCi/mmole) [29]. (U-^{14}C) L-aspartate (10 mCi/mmole) [29] was purified to remove a small amount of radioactive impurity [14]. All other chemicals used in this study were of analytical reagent grade.

Sprague-Dawley rats were used in the study [30]; all pups were products of a second pregnancy. Dams were fed [31] and housed at room temperature.

To prepare enzyme extracts, rats were decapitated and then the cerebellum or cerebral cortex was rapidly dissected from surrounding brain tissue. Cerebral cortices were dissected by removal of olfactory lobes and separation of cerebral cortex from underlying white matter and brain stem. Tissue was homogenized [32] without delay in ice cold homogenizing medium consisting of sucrose (0.25 M), potassium phosphate, pH 7.4 (0.01 M), magnesium-ATP (0.015 $MgSO_4$/0.01 M ATP), dithiothreitol (1 mM), and glutamine (3 mM).

Preliminary experiments indicated that 20 up-and-down movements of the plunger of the homogenizer provided complete and optimum release of enzyme from the tissue. Homogenates were centrifuged at 35,000 × *g* at 5° for 15 min [33]. The developmental curves for CPS, ATC, and UK were determined with 1:10 (w/v) tissue homogenates.

Enzyme assays were performed on the supernatant solution derived from an individual cerebellum; four animals of each age were used. Cerebella from animals of 3- and 6-day post-gestational age were too small to homogenize singly so that 2 cerebella were pooled for these assays.

Enzyme activities were assayed as follows: CPS by the radiochemical method of Levine and Kretchmer [17], ATC by the radiochemical method of Porter *et al.* [23], UK by the Herbst *et al.* modification [12] of the method of Sköld [24]. Radioactivity was measured in a scintillation spectrometer [34].

Protein was determined on samples of supernatant solutions, using the Oyama and Eagle modification [22] of the method of Lowry *et al.* [18]. DNA was determined on samples of the homogenates using the modification by Giles and Myers [11] of the method of Burton [4].

Results

In Figure 1 the developmental patterns for CPS, ATC, and UK in cerebellum are compared with the total DNA content. Enzyme activities were calculated per milligram protein. Activities of the two enzymes of the *de novo* pathway were very similar, with maximal activity at approximately 9 days of age; activity decreased rapidly thereafter to 50% of maximum on *day 18*. The developmental pattern of UK was different, with a slow rise in activity between 3 and 5 days of age followed by a more rapid rise, with maximal activity

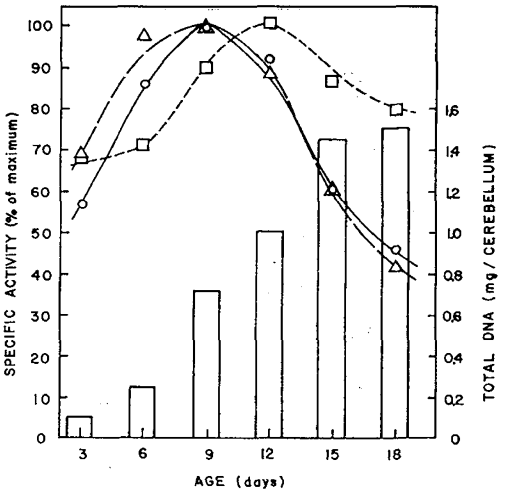

Fig. 1. Activity of pyrimidine nucleotide biosynthetic enzymes carbamyl phosphate synthetase (CPS) (○——○), aspartate transcarbamylase (ATC) (△——△), and uridine kinase (UK) (□---□) in cerebellum of the developing rat. The data are reported as a percentage of maximal activity and are compared with the total content of DNA in cerebellum (histograms). Enzymes were assayed as described in *Materials and Methods*. The maximal activities (100%), in mμmoles/min/mg protein were: CPS, 1.56 × 10^{-2}; ATC, 8.23; and UK, 0.34.

being attained at 12 days of age; afterwards, activity of UK was at 80% of maximum while DNA content became constant.

Figure 2 depicts the activities of CPS, ATC, and UK as calculated per milligram DNA. Since the content of DNA per cell is constant [3], these values represent enzymatic activity per cell, at different stages of development of the cerebellum. These activities are compared with the DNA synthesized in the time that elapsed (3 days) between the ages at which enzymatic activity was determined. This synthesis is expressed as a percentage of the total DNA present at that age (Δ DNA/mg DNA × 100). Activities of CPS and ATC were highest at the time of maximal DNA synthesis in the cerebellum. Activities fell abruptly between 6 and 9 days of age, just prior to the observed decrease in DNA synthesis in the cerebellum. Activity of UK was high initially and then decreased, but at the time when DNA synthesis dropped most sharply (between ages 9 and 12 days), UK activity remained constant.

Developmental patterns of ATC activity in homogenates of cerebellum and cerebral cortex are compared in Figure 3. Activity of ATC in cerebellum rose from 19-day gestational age so that by the time the pups were 4 to 10 days old, the activity had increased 40%. In contrast, the ATC activity of the cerebral

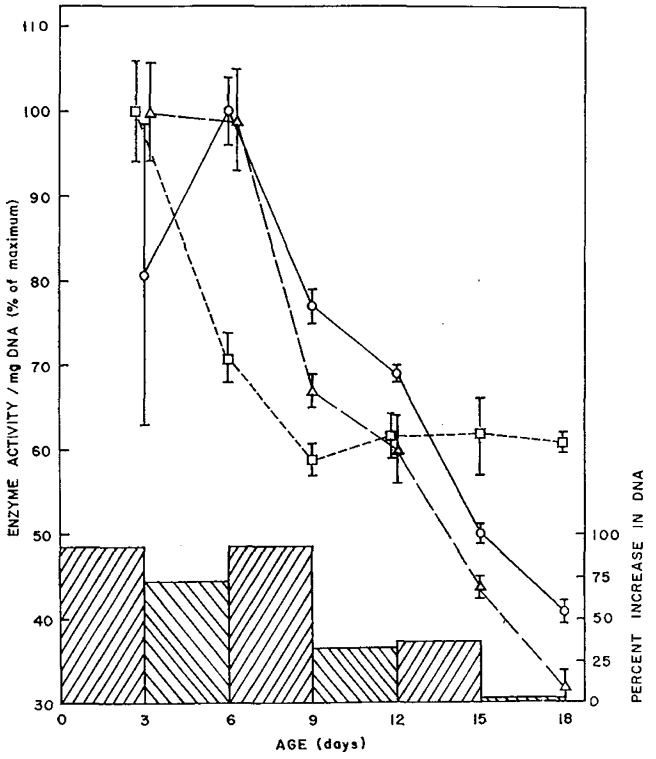

Fig. 2. Activities of carbamyl phosphate synthetase (CPS) (○——○), asparate transcarbamylase (ATC) (△——△), and uridine kinase (UK) (□---□) per mg DNA compared with the rate of DNA synthesis. Activities are expressed as a percentage of maximum. The bars represent ± one SEM [35]. Maximal activities (100%), in mμmoles/min/mg DNA, were: CPS, 8.3 × 10^{-2}, ATC, 50.9; and UK, 2.12. The height of the hatched bars represents the percentage increase in DNA during a 3-day period, as calculated by the formula:

$$\frac{\text{Increase in DNA during 3-day period}}{\text{Average DNA for 3-day period}} \times 100$$

Data are derived from the experiment depicted in Figure 1.

cortex was very high at 19-day gestational age and then fell gradually from a specific activity of 6.0 to one of 1.0 by 22 days after birth. Samples of homogenates from both cerebellum and cerebral cortex were tested for the presence of the CPS found in mitochondria and associated with the urea cycle in liver. Neither *N*-acetyl glutamate nor ammonia stimulated incorporation of $^{14}CO_2$ into carbamyl phosphate.

Discussion

These findings for rat cerebellum support the suggestion of Galofré and Kretchmer [10] that *de novo* synthesis is likely to be the most important source of pyrimidine nucleotides during times of rapid cellular proliferation while the pathway that utilizes preformed bases is probably involved in synthesis of nucleic acids required during the nonproliferative phase of the life cycle. Nevertheless, high activity of UK early in the development of the cerebellum suggests that formation of pyrimidine nucleotides from preformed bases may also be an important early source of precursors for DNA and RNA synthesis. Recently, Hogans *et al.* [13] found that, in adult rats, preformed pyrimidine nucleotides are far more important precursors for neural RNA synthesis than orotic acid, an intermediate in *de novo* synthesis of pyrimidine nucleotides.

The developmental curves for specific activity of CPS and ATC in the cerebellum were very similar (Fig. 1): both enzymes had their maximal activities at 9 days of age and both activities decreased after this age. The parallel behavior of these enzymes is consistent with, and may be accounted for, by the observation of Hoogenraad *et al.* [14] that the two activities can be copurified from hematopoietic mouse spleen. Similar copurification of activities of CPS and ATC from baker's yeast was reported by Lue and Kaplan [19, 20], and from *Neurospora* by Williams *et al.* [25]. Genetic evidence that the CPS and ATC of yeast and

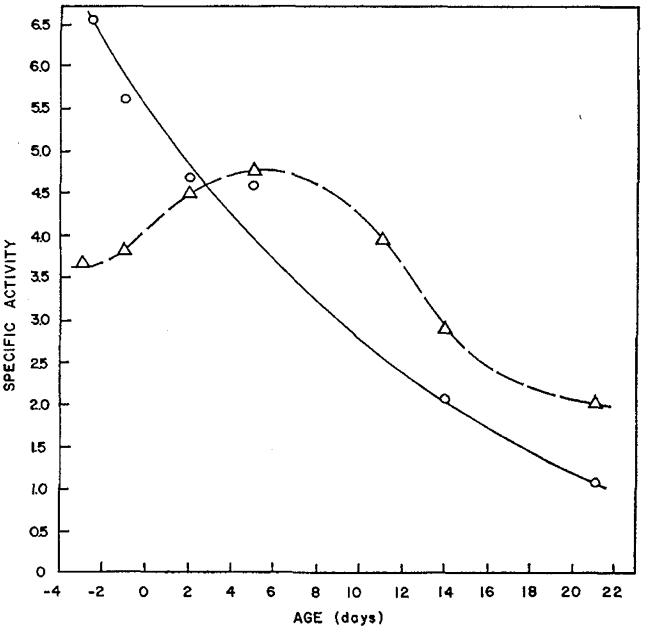

Fig. 3. Developmental curves for aspartate transcarbamylase from cerebellum (△——△) and cerebral cortex (○——○). Enzyme assays were run as described in *Materials and Methods* except that homogenates were used rather than high speed supernatants. One unit of enzymatic activity equals 1 mμmole carbamyl aspartate formed/min/mg protein.

Neurospora are present as a single complex has been presented [8, 15, 26] and supports the suggestion that CPS and ATC of eukaryotes are present as a bifunctional complex.

Altman [1, 2], using tritiated thymidine, demonstrated a very high rate of cellular multiplication in the external granular layer of rat cerebellum that persisted for two weeks from birth. Our measurements of the incremental increase in DNA content of cerebellum (Fig. 2) also indicate a high rate of cell multiplication and considerable synthesis of DNA, which is in agreement with the data of Fish and Winick [9]. Activities of the first two enzymes of the *de novo* pyrimidine pathway, CPS and ATC, closely paralleled this pattern of growth. Activities of the enzymes were elevated during the stages of accumulation of DNA. They decreased in activity when the rate of increase of DNA dropped. However, activity of UK in the cell dropped between the third and sixth day after birth while cellular multiplication continued at a rapid rate. At 9 days, UK activity was still relatively high when the amount of cerebellar DNA had become constant.

When the developmental pattern for ATC from cerebellum was compared with that for the same enzyme in cerebral cortex, very different curves were obtained (Fig. 3). In rat, cerebral hemispheres develop much earlier than cerebellum. Except for small areas in the hippocampus of the temporal lobe, maximal cellular replication of cerebral neurons occurs early in gestation. Activity of ATC was most elevated when first measured at 19-day gestational age and then decreased continually thereafter. Activity of the *de novo* pyrimidine biosynthetic enzyme, ATC, reflected the difference in developmental anatomy of the cerebral hemispheres and the later-developing cerebellum.

A relation has been established between the rate of cellular proliferation and the activities of the first two enzymes in the *de novo* pyrimidine pathway. Changes in the activity of an enzyme are of biological importance if: (*1*) the enzyme is subject to metabolic or genetic control; (*2*) the enzyme is or can become rate-limiting in a reaction sequence; and (*3*) it is directly involved in an important biologic process. The first enzyme of the *de novo* pyrimidine pathway, CPS, has these characteristics: (*1*) it is subject to feedback inhibition by the end product of the pathway, UTP [16]; (*2*) it is the rate-limiting enzyme in the synthetic pathway; and (*3*) activity of CPS is correlated with the rate of cellular proliferation. Although activity of the second enzyme of the pathway, ATC, is correlated with the rate of cellular proliferation, it is not rate-limiting and is not subject to feedback regulation by pyrimidine nucleotides [7, 14]. Thus, CPS might serve as an indicator of the effect of any stimulus or environmental stress on the rate of cellular proliferation.

Summary

In the present paper, a close correlation has been shown to exist between the first two enzymes of the *de novo* pyrimidine pathway, CPS and ATC, and cellular proliferation in cerebellum of the developing rat. This association of the enzymes with cellular proliferation was further demonstrated by comparing activity of ATC of cerebral cortex and cerebellum. Two distinctly different developmental curves were obtained for portions of the brain which are well known to have different time courses of histological development. These enzymes can then serve as markers for the rate of cellular division.

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