Postnatal Changes in Protein Metabolism of Brain

I. Studies in Newborn Miniature Pigs at Varying Conceptual Ages

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Extract

The incorporation of L-phenylalanine-^{14}C into protein of several brain areas and into liver of the miniature pig has been studied *in vivo*. A sequential decrease (approximately 50 %) in incorporation of labeled phenylalanine occurred during the first day of life in all brain regions (fig. 1). There was no important difference in degree of incorporatn ioin the three brain regions examined. Labeling of liver protein was roughly threefold higher than in brain protein. A considerably smaller postnatal decrease of labeling of liver protein occurred in proportion to the total initial count.

Premature delivery by hysterectomy at two different conceptual ages resulted in a similar reduction of labeling of brain protein fractions, suggesting that birth rather than the conceptual age of the animal was the relevant factor (figs. 2 and 3).

Radioactivity in the trichloracetic acid-soluble fraction (free amino acids) of all brain areas increased during the first day of life, suggesting that the decreased incorporation of labeled amino acid was not a function of failure of the tracer to enter into brain substance (fig. 4).

Expression of the radioactivity of protein in the brain regions as a function of radioactivity of liver protein established that while there is some postnatal decrease of incorporation into liver protein, there is a relatively greater decrease of incorporation into various brain regions (table I).

Speculation

The timing of the abrupt decrease of amino acid incorporation into brain protein that occurs postnatally may be an important factor in determining subsequent development of the central nervous system. Prematurely induced birth apparently is associated with a premature decrease of incorporation rate. This may have significance in regard to the high incidence of neurological complications found in prematurely born human infants.

Introduction

Studies of protein metabolism using radioactive amino acids administered to intact organisms have revealed that protein turnover in brain is more rapid in immature than in adult animals [4]. Observations *in vivo* by

SCHREIER *et al.* [8, 9] indicated that the major decrease of incorporation of labeled glycine into brain protein occurred during the first few days of life. It is unclear, however, whether these changes were associated with the event of birth itself or with maturational factors determined by the conceptual age of the animal. The

present studies were undertaken in order to determine whether the reported postnatal changes of amino acid incorporation into brain protein are related to the birth process, and to investigate these changes in several regions of the brain. A preliminary report of these studies has been previously published [7].

Methods

Experiments were performed on miniature pigs [13]. These animals were chosen because the gestational ages were known and the brain was of a sufficient size at birth to ensure adequate amounts of tissue. The gestational period of the miniature pig is 113–114 days, with little variation.

The piglets were procured by hysterectomy following electroshock anesthesia to the sow or by permitting the sow to farrow spontaneously. Animals obtained by hysterectomy were immediately placed in a Gordon-Armstrong incubator maintained at 30–33°. The piglets were allowed to feed *ad libitum* on a sow's milk replacement formula [14]. Animals delivered near term were able to successfully pan feed on this formula within 24 hours after birth. Litters delivered spontaneously by farrowing were nursed by their dam in an ordinary stall.

Tracer doses of uniformly labeled L-phenylalanine-^{14}C in a solution of isotonic saline (specific activity 300 μc/mmole) were administered to the piglets by intraperitoneal injection of 20 μc/kg of body weight. One hour after injection, the animals were exsanguinated and the brain and liver removed. The brain was washed with saline and divided into cortical mantle, cerebellum, and pons medulla. Trichloracetic acid (TCA)-soluble and insoluble fractions were prepared [5].

Tissue samples were homogenized in 9 volumes of 5 % TCA and centrifuged. The residue was resuspended twice in 5 % TCA, homogenized, and centrifuged. The combined supernatants represented the TCA-soluble fraction. The residue was again homogenized in 5 % TCA and allowed to stand in a water bath for 15 minutes at 90° to destroy the nucleic acids. The residue was then extracted three times with acetone and three times with ether, dried first at room temperature and finally at 110° for 15 minutes. The fat-free protein powder was used for radioactivity determinations.

TCA was removed from the soluble fraction by passage through a Dowex-2 column; the effluent was freeze-dried. Individual samples were counted by conventional liquid scintillation techniques. Counts obtained were corrected for quenching and expressed as disintegrations per minute (DPM) per mg of protein or, in the acid-soluble fraction, per gram of wet weight tissue.

The identity of radioactivity in representative TCA-soluble fractions was determined by chromatographing this fraction on a Spinco Amino Acid Analyzer and passing the effluent through a Nuclear-Chicago flow cell attached to a scintillation spectrometer. Virtually all of the activity in the free amino acids existed as phenylalanine and/or tyrosine. It was difficult to distinguish which amino acid was more highly labeled since these two amino acids are eluted very close to one another during chromatography.

All measurements of radioactivity in tissue were corrected by a factor (F) in order to adjust plasma radioactivity to a constant, 30,000 DPM/ml. The factor F equals 30,000 per observed plasma sample. This minimized the variation caused by ununiform absorption of the radioactive phenylalanine from the peritoneal cavity.

Results

Figure 1 illustrates the labeling of protein in various brain regions and in the liver during the first 28 hours of life in animals obtained by hysterectomy at conceptual ages of 110–111 days. Experiments on one litter were performed for the first 72 hours and the results of this single experiment are also plotted on the graph. A decrease (approximately 50 %) in incorporation of labeled phenylalanine occurred during the first day of life in all regions of brain. There was no important
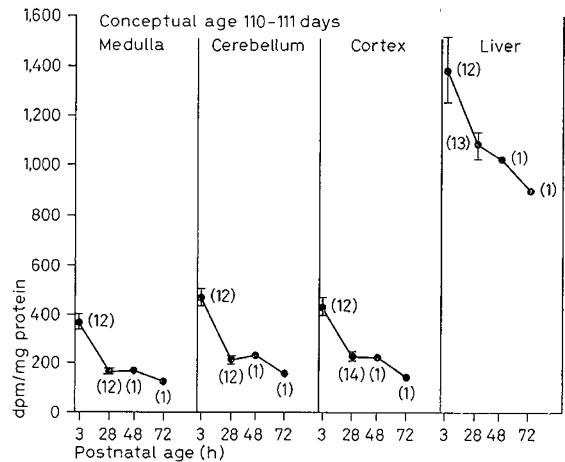

Fig. 1. Incorporation of ^{14}C labeled phenylalanine into protein from various brain regions and liver. Solid symbols refer to means; vertical lines refer to standard error of the mean. Figures in parenthesis refer to number of animals. Probability values of differences between means at 3 and 28 hours:
Pons-medulla, cerebellum, cortex: p < 0.001
Liver: p < 0.05

difference in degree of incorporation in the three regions examined. Labeling of protein in liver was threefold higher than that in brain. A considerably smaller postnatal decrease of labeling of liver protein occurred in proportion to the total initial count.

The animals obtained by hysterectomy at conceptual ages 110–111 days appeared mature at birth, rooted vigorously, and attempted to walk shortly after placement in the incubator. Pan feeding was established within 6–12 hours. The appearance and behavior of these newborn piglets was similar to that of the animals delivered spontaneously.

Figure 2 illustrates radioactive labeling of protein in brain and liver of newborn animals in which the sow was allowed to deliver spontaneously. These experiments were carried out to compare the effects of hysterectomy with normal partuition. The decrease of incorporation into protein in all regional areas was again roughly 50 %, although the initial levels of radioactivity in protein were lower than those in the hysterectomy-delivered group. When the experiments were extended until 3 days of age, leveling of the postnatal drop of phenylalanine-^{14}C incorporation into protein was found.

The initial low levels of brain protein labeling in the spontaneously delivered piglets (conceptual age 112–114 days) raised doubt as to whether the postnatal changes might be due to maturational changes unassociated with the birth. A group of animals was then

delivered by hysterectomy at conceptual ages of 100–103 days. These animals appeared immature at birth and showed poorly developed motor functions. Figure 3 illustrates the labeling of brain protein in these animals. The initial levels of labeling were higher than those in the preceding experiments; however, the postnatal fall in incorporation was more abrupt, dropping to one-third of that of the initial levels.

Figure 4 illustrates radioactivity in the TCA-soluble (free amino acid) fraction of all of the animals born spontaneously or by hysterectomy at conceptual ages 110–114 days. In all brain regions and in liver, the radioactivity of the TCA-soluble fraction increased during the first day of life. Most of the radioactivity (about 70 %) in this fraction was phenylalanine or tyrosine. Although there was a large standard error in these determinations, the results suggest that the decreased incorporation of labeled amino acid into protein during this time period was not a function of failure of the tracer to enter into brain substance.

Expression of the radioactivity of protein in brain regions as a function of that in liver is given in table I. The data establish that while there is some postnatal decrease of incorporation into liver protein, there is a relatively greater decrease of incorporation into various brain regions.
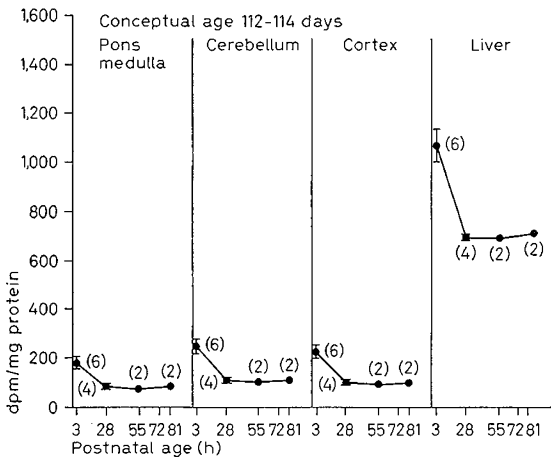

Fig. 2. Incorporation of ^{14}C labeled phenylalanine into protein from various brain regions and liver. Solid symbols refer to means; vertical lines refer to standard error of the mean. Figures in parenthesis refer to number of animals. Probability values of differences between means at 3 and 24 hours:
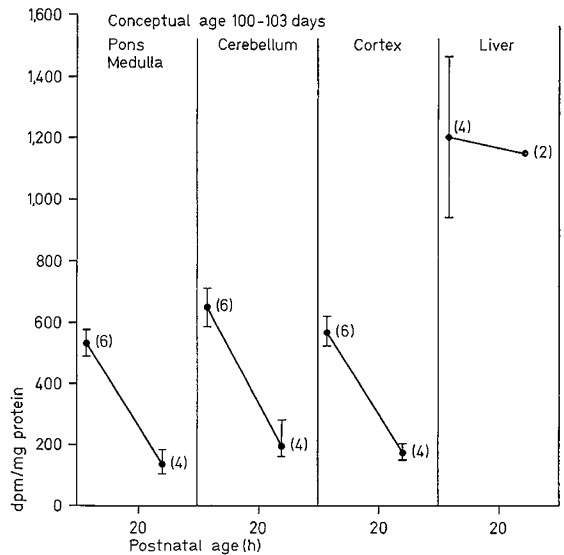Pons-medulla, cerebellum, cortex: p < 0.01
Liver: p < 0.001

Fig. 3. Incorporation of ^{14}C labeled phenylalanine into protein from various brain regions and liver. Solid symbols refer to means; vertical lines refer to standard error of the mean. Figures in parenthesis refer to number of animals. Probability values of differences between means at 3 and 24 hours:
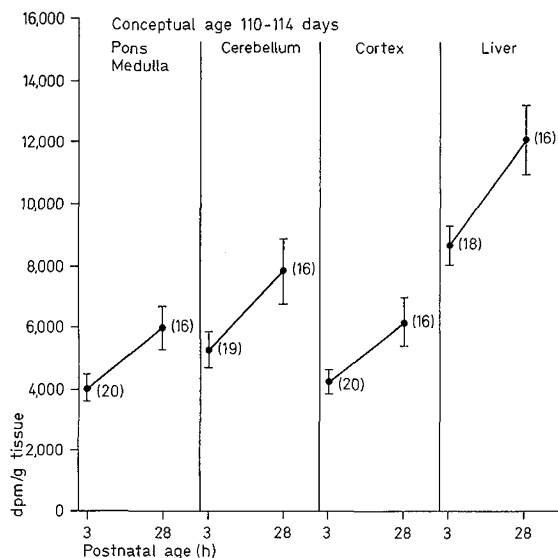Pons-medulla, cerebellum, cortex: p < 0.001
Liver: p not significant

Fig. 4. Radioactivity in free amino acid (FAA) fraction from brain regions and liver. Remainder of legend as in figure 1.

Table I. Relation of labeling of brain protein to liver protein[1]

Postnatal age (h)	Cortex	Cerebellum	Pons-medulla
0– 6 (21)[2]	33.5 ± 3.5	36.6 ± 3.8	28.5 ± 3.1
24–33 (17)	17.8 ± 0.9	18.9 ± 0.8	14.5 ± 0.9
48–87 (6)	15.5 ± 1.4	16.9 ± 1.3	12.9 ± 1.1

[1] Values represent means and SEM of the ratio:
$$\frac{dpm/mg \text{ brain protein}}{dpm/mg \text{ liver protein}} \times 100.$$
[2] Numbers in parentheses refer to number of animals studied.

Discussion

These results demonstrate that an abrupt reduction in incorporation of intraperitoneally administered L-phenylalanine-^{14}C into protein fractions of several brain regions occurs shortly after birth. Premature delivery at two different conceptual ages is associated with a similar reduction, suggesting that the event of birth rather than the conceptual age of the animal is the relevant factor.

The simplest explanation of the reduced labeling of brain protein is the decrease in the rate of protein turnover. Two alternate possibilities need to be con-

sidered, however. The first is the question of decreased entry of the isotope into brain substance *per se*. The ability of substances, including amino acids, to penetrate the blood brain barrier is known to diminish with maturity [11]. The data presented in figure 4 argue against this possibility, at least during the relatively short postnatal time interval covered by these experiments. Nevertheless, data on radioactivity in the acid-soluble fraction of the brain do not rule out changes in functional compartmentalization of the free amino acid pools [10]. The possible decreased flux of tracer into the specific precursor pool for protein synthesis might conceivably explain the reduced labeling of brain protein.

A second possibility is that an increase in the size of the precursor pool might reduce protein labeling by diluting the tracer within the pool. This seems unlikely since free phenylalanine concentrations in the plasma of these animals steadily fall after birth [7]. Experiments with infant rats have suggested a fall in levels of free phenylalanine in brain during the immediate postnatal period [1]. A definite answer to this question is impossible under *in vivo* conditions, however, because of the uncertainties regarding the dimensions of precursor amino acid pools leading to protein synthesis.

In vitro investigations have revealed that protein turnover is more rapid in immature brain tissue than in adult animals [3, 4, 6]. Studies in which longitudinal data have been obtained have revealed a progressive diminution of rate in incorporation of labeled amino acids into protein over a period of weeks or months. Our data also indicate a gradual reduction in incorporation with increasing conceptual age in animals three hours old. *In vitro* studies, however, have not indicated abrupt postnatal reduction of incorporation analogous to that observed under *in vivo* conditions.

It is probable that these are two different phenomena with separate causes. The gradual decrease with maturation in the ability of brain tissue to incorporate amino acids may be explained by intrinsic changes in nuclear RNA synthesis resulting in decreased protein synthesizing ability of brain ribosomal systems [12]. The abrupt reduction of brain protein labeling during the immediate postnatal period occurring in animals studied *in vivo* are more likely to be related to external factors associated with the transition to extrauterine life. These factors would not be present under *in vitro* conditions.

The overall significance of an abrupt change in protein metabolism of brain subsequent to birth may be related to the question of critical periods of brain development. The concept of the time of myelination as a vulnerable period in brain development has been advanced [2]. The period of rapid protein turnover in brain may represent another physiological time period

during which the brain is vulnerable to adverse circumstances. Untimely alterations in protein metabolism during this period could permanently affect subsequent central nervous system development. Prematurely induced birth is apparently one circumstance resulting in an untimely alteration of protein turnover in brain. This may have significance for prematurely born human infants, in whom are found a disproportionately high incidence of neuropsychological disorders in later life.

Surprisingly similar patterns of incorporation of labeled phenylalanine into protein were found in the brain regions examined. There are many differences in rate of protein turnover [4] in different cell types and in smaller regions, but these are not reflected in the relatively large regions studied. The measurement of radioactivity in protein of such regions is a resultant of the incorporation into the numerous protein fractions existing in the brain. This type of data is perhaps analogous to the metabolic rate of whole organisms. As such, it can provide meaningful information regarding the effect of external or physiological factors on tissue protein metabolism under *in vivo* conditions.

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13. Obtained through the courtesy of the Department of Veterinary Medicine, University of Nebraska.

14. SPF-lac, Borden.

15. Presented in part at the 38th Annual Meeting of the Society for Pediatric Research, Atlantic City, N.J. 1968.

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