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IDENTIFICATION OF CHANGES IN THE CONTENT OF GUINEA-PIG BRAIN PROTEINS BETWEEN THE 30th PRENATAL AND THE 75th POSTNATAL DAY

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The whole guinea-pig brain was studied during prenatal, as well as postnatal development. The percentage of total proteins and their electrophoretic pattern on polyacrylamide gel were determined for each brain.

From the 30th until the 48th day of gestation, when guinea-pig brain mass rapidly increased, the protein concentratton decreased 1.4 times. From the 48th until the 63rd day (parturition), when the increase of the brain mass was relatively slow, protein concentration increased 1.4 times. During the postnatal period, from parturition until adult hood, the total protein level increased by 0.5% only.

Electrophoretic patterns of the total brain proteins showed that, from the 30th until the 48th day, the levels of all histone fractions (H_1, H_2A, H_{2B}, H_3 H_4) decreased, at a rather high rate. From the 48th until the 63rd day, the level of mast histone fractions changed remarkably little. However, H_1 histone level increased during this period more than any other protein fraction - the increment being sixfold. The myelin basic protein (MBP) level began to increase from the 48th day and continued to increase until parturition, at the same or approximately the same rate as the H_1 histone. Following parturition MBP gain accelerated. MBP level in the adult brain was up to eight times higher than in the brain of new-born guinea-pigs.

Key words: Brain, guinea-pig, protein, myelin, histone, parturition, content, change, prenatal, postnatal.

INTRODUCTION

There are few data, in the references available, regarding mutual relationships of dynamic synthetic processes concerning total brain proteins, histone proteins of the brain and the proteins constitutin part of the brain myelin. The initial idea of these studies was based upon the following regarding their chemistry, histone proteins and MBP hawe similar protein structures; histone H_1 is the least tightly bound to DNA (Butker, Jons and Phillips, 1968), while histone H_4 and MBP are phylogenetically kindred proteins (Bauer, 1972).

Guinea-pig brain matures similarly to the brains of other precocious types of animals. Together with the formation of myelin components myelination is initiated prior to birth and does not appear to continue throughout life (Davison, 1970). The percentage of total protein in guinea-pig brain starts to increase from the 48th prenetal day (Flexner and Flexner,1950; Davis and Himwich, 1973). Histone content also changes during brain development, because histones repressively affect active functions of genes, inhibiting protein synthesis (Klimenko, 1970; Johns et al., 1975).

Similar processes have recently been studied in the rat brain, as a non-precocious mammal, as well. (Ćirović and Đurica, 1994).

MATERIAL AND METHODS

Female guinea-pigs were decapitated and the brains of animals of different age (prenatal and postnatal) were immediately removed, blotted on filter paper and frozen at -30^{0}C. Except for myelin isolation, all of the procedures described below were performed at room temperature.

The proteins were extracted by homogenizing samples of whole brain with phenol solution in the ratio 1:100 (w/v) (phenol: concentrated acetic acid: 6 mol/L urea, 2:1:1, w/v/v. More than 95% of the brain proteins were thus solubilized (Gall et al., 1980).

For polyacrylamide gel electrophoresis (PAGE), a solution containing 7.5% (or 10.0%) of acrylamide was initially prepared (Ćirović and Đurica, 1993). Glass tubes (16 cm long and 0.8 cm in diameter) were filled with 7 ml of acrylamide solution. Aliguots containing 0.2 ml of the prepared protein solution were applied on to the upper flatpolyacrylamide layer. The electrophoresis lasted 24 hours at 3 mA per tube, in dilute acetic acid. Gels were stained with amido black, clarified with acetic acid and scanned (by densitometry) at 580 and 690 nm.

The percentage was determined by measurement of the peak areas of each densitometric tracing obtained by scanning the electrophoregrams.

The first amounts of MBP (on the gel) were recorded by densitometric processing of the electrophoregrams at 580 and 690 nm because the greenish-stained MBP (when proteins on the gel were stained by amido black) absorbed red light (690 nm) most intensely, whereas most of the other proteins absorbd yellow light (580 nm) preferentially (Martenson et al., 1971b).

The percentage of total proteins in fresh brain tissue was determined by Lowry's method (Lowry et al., 1951).

Total brain histones were prepared as described and modified by Johns (1967).

RESULTS

The percentage of total proteins in guinea-pig brain decreased from the 30th until the 48th prenatal day (9.45% - 6.85%). From the 48th day until parturition, the percentage of total proteins in the brain increased (6.85% - 9.45%). This

increase continued after parturition, but the rate of postnatal protein gain was rather small. Thus, only about 0.5% more protein is present in the brain of adult guinea-pigs than in the brain of new-born animals (Table 1, Fig. 3).

Table 1. Total protein level during the development of guinea pig whole brain[1]

Age in days	% of proteins in fresh tissue ($\bar{X}\pm SD$)
-30	9,45±0.13
-35	8,20±0.94
-40	7,50±0.24
-43	7,20±0.20
-48	6,85±0.33
-55	7,50±0.26
-59	7,85±0.33
birth	9,45±0.61
15	9,80±0.30
adult	9,95±0.15

[1] The percentage with standard deviation (SD) calculated from the mean values ($\bar{X}$) of three brains.

Electrophoretic patterns of the total brain proteins (Fig. 1) revealed significant changes in the period from the 30th until the 63rd day gestation. Protein fractions with large molecular mass exhibiting slow cathodic mobility (structural proteins, membrane globulins) during gel electrophoresis, were subject to rather minor alterations from the quantitativ standpoint. Protein fractions (at the frontal part of the gel) exibiting relatively high cathodic mobility, during electrophoresis

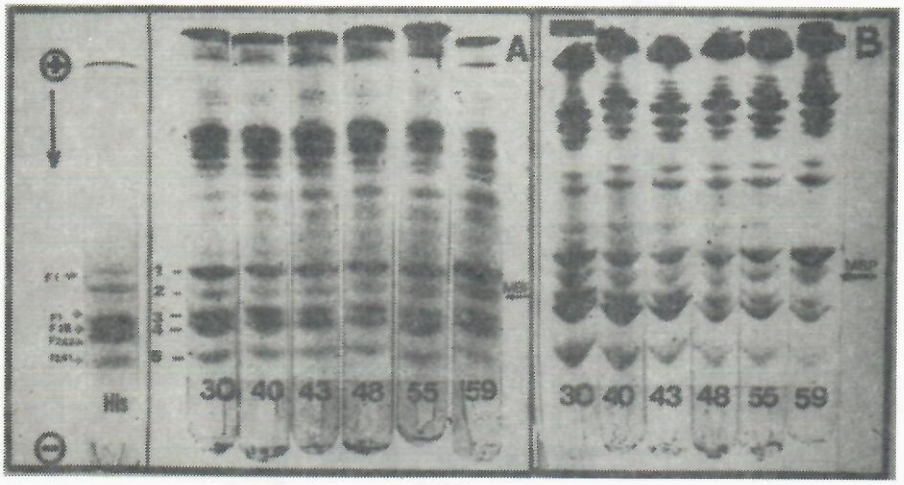

Figure 1. 7,5% (A) and 10% (B) polyacrylamide gel electrophoresis of proteins of the whole guinea-pig brain. The brains were removed on the 30th, 40th, 43rd, 48th and 59th day of gestation. The same amounts of protein (0.2 mg) from homogenized whole guinea-pig brain tissue were applied on to gels (0.2 ml). Histones isolated from an adult rat brain and electrophoretically separated on the gel, served for comparison purposes. The arrows indicate the positions of myelin basic protein (MBP) and" $H_1(F_1)$, $H_3(F_3)$, $H_2B(F_2B)$, $H_2A(F_2A_2)$, as well as $H_4(F_2A_1)$ histone.

underwent significan quantitative alterations from the 30th until the 48th day. These are fractions with relatively small molecular masses, the following being quantitatively the most import: MBP fraction and the histone protein fractions. From the 30th until the 48th day, the amount of each of these five fractions decreased five times (Fig. 1B). However, the quantitative ratios of these fractions within the period remained unchanged. Relatively high levels of the histone fractions were dominant in the brain at the middle of gestation and represent a characteristic feature in brain development of the quinea-pig. The level of the fraction located at the position of H_1 histone (MM:21500) markedly increased in the period from the 48th day until parturition, while the levels of the remaining four fractions (H_2,A, H_2B, H_3, H_4) under went rather mior alterations in this period. MBP level (MBP level (MM:18500) began to increase from the 48th day.

The first increase of MBP content, recorded on the densitograms (690 nm), appeared on the 48th (or 49th) prenatal day. Thus dalong with the initial increase of MBP content on the gel, absorption values showed a proportional increase, too). MBP content (the peak area in the densitogram), expressed as the percentage of the total protein (the total densitogram area), rapidly increased at parturition. The fraction at the H_1 histone position and the fraction at the MBP position were accumulated at identical or very similar rates from the 48th day until partirution (Fig. 2).

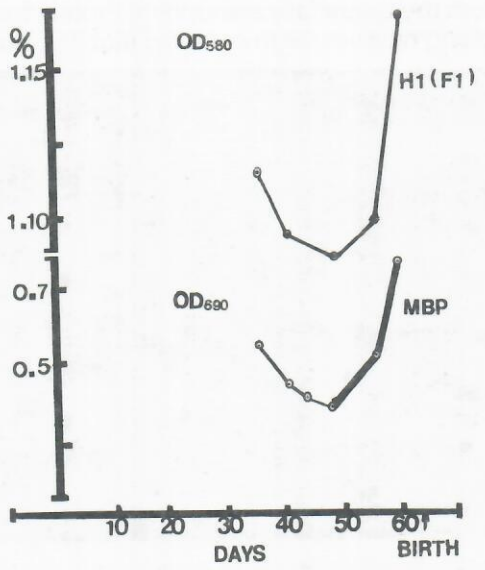

Figure 2. Alterations in the content of two characteristic protein fractions: the fraction at H_1 histone position (F_1) and the fraction at myelin basic protein (MBP) position. The values are expressed as percentages determined by measurement of peak areas of each densitometric tracing obtained by scanning of. the electrophoregrams.

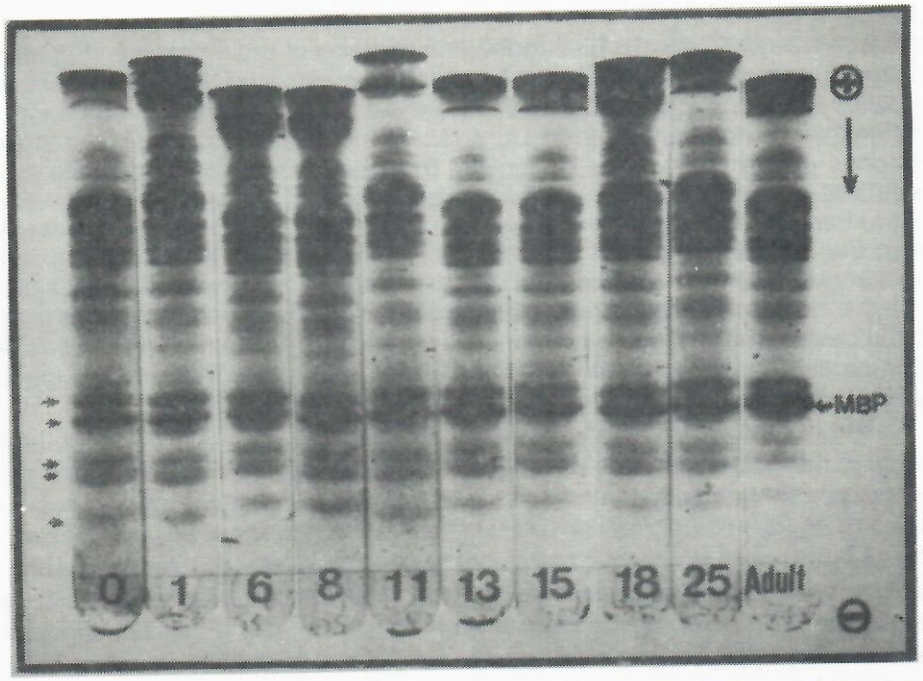

Figure 3. Polyacrylamide gel electrophoresis of proteins of the whole guinea-pig brain. The brains were removed on: 0,1st, 6th, 8th, 11th, 13th, 15th, 18th, 25th, and 75th day (adult) of age. The same amount of protein of the homogenized whole guinea pig brain tissue (2 mg) was applied on to the gels (0.2 ml). The arrows indicate the position of myelin basic protein (MBP) and histone proteins.

Changes in the electrophoretic patterns of brain proteins during the postnaal period were minor (Fig.3). MBP was the most characteristic light, rapidly mobile fraction. Its content in the brain of an adult animal (75 days old) was increased eight times, compared to the content in the brain of a new-born guinea-pig (0 days). The level of all histone fractions during this developmental period decreased, slowly, but their quantitative ratio remained unchanged. Thus the level in the brain of an adult animal (75 days) was 2.3 times lower than that found in the brain of a now-born guinea-pig (0 days) (Fig. 3).

DISCUSSION

Alterations in protein levels were studied during three time periods (stages) in the course of guinea-pig brain development from the 30th until the 48th prenatal day, from the 48th until the 63rd(parturition) prenetal day, as well as from day 0 (birth) until the 74th (adult) day.

During the first stage (30 to 48 days) when guinea-pig brain mass, due to water accumulation, increases more than eight times (Davis and Himwich, 1973) the protein concentration decreased approximately 1.4. times (from 9.45±0.13%

to 6.85±0.33%) (Table 1). Electrophoretic patterns of brain proteins (Figure 1) showed that the levels of slowly mobile protein fractions membrane and structural proteins with large MM and, globulins were subject to relatively minor changes during this developmental period. Light, rapidly mobile protein fraction, especially histones (MM:21000-11300), underwent the most pronounced quantitative alterations. Electrophoregrams (and their densitograms) showed that the levels of all five fractions in the positions of histone proteins (H_1, H_2A, H_2B, H_3) decreased from the 30th until the 48th day, but that their quantitative rations remained unchanged. Alterations in the level of these proteins are essentially associated with the multiplication and proliferation of cells. It has been shown that this stage in the development of guinea-pig brain is characterizid by a high rate of cellular multiplication because of neuroblast proliferation (with resulting neuron formation) (Benjamin and McKhann, 1972; Gunnar and Thomas, 1992).

During the second stage (from the 48th until the 63rd day) when the brain mass in the guinea pig, due to water loss, increases 1,7 times only (Davis and Himwich, 1973), the concentration of total proteins increased approximately 1.4 times (6.85±0.33% up to 9.45±0.61%) (Table 1). The process of water loss after the 48th day is a characteristic feature in the development of the guinea-pig brain, and occurs in parallel with and myelination (Donaldson and Hatai, 1931). Formation of the first significant amounts of MBP on the 49th day (Figure 2) correlates with intense multiplication of glial cells, i. e. of spongioblasts resulting in glia formation) (Benjamin and McKhann, 1972). At the beginning of myelination, the number of glial cells is four to five times lower that the number of neurons; but along with the intensification of myelin deposition, the number of glial cells increases while the number of neuronal cells decreases (Brizzee et al., 1964). Electrophoretic patterns of guinea-pig brain proteins (Figure 1) (as well as the densitograms) showed that the basic protein fractions undergo the maximum alterations in the period from the 48th until the 63th day, if the quantitative aspect is concerned. The levels of three basic protein fractions highly enriched by histones (H_2A, H_2B, H_3) as well as the level of one (on the gel) pure histone fraction (H_4) showed a continual decrease until parturition without changes in their quantitative ratios. Hawever the level of the first, slowest fraction highly enriched with H_1 histone (MM: 21500) exhibited quite different behaviour, i. e. it drastically increased, exhibiting a sixfold increment by the end of the gestation period. However, until the 59th day of gestation, the quantitative relation between MBP and H_1 fractions did not change or showed minimal changes. Thus the increments of both fractions wereclose or identical (Figure 2). The levels of H_1 fraction on the 59th and the 30th gestation day were approximately the same. There are few data on the biological role of this fraction in the function of brain development. However, it has recetly been shown that an increase in the concentration of H_1 hisone correlates with differentiation (Di Liegro and Cestelli, 1990).

During the third (postnatal) stage of guinea pig brain development there were no marked alterations (Figure 3).The accumulation of total proteins in brain

is a slow and mild process. The total protein concentration from birth (0 days) adult hood (75 days) increased by 0.5% only (from 9.45% up to 9.95%) (Table 1). This period is also characterized by a mild and moderate decrease of the basic proteins, except for MBP. Thus, the brain of an adult animal hasonly 2.3 times lowever levels of H_4 histone than the brain of a new-born guinea pig, whereas the MBP level increased by more than eight times.

REFERENCES

1. *Bauer Klausdieter.* 1972. Evidence for the homology of the main determinant of the human encephalitogenic protein and an ancestral histone IV sequence. *Biochem. J., 126, p.*1245-1248.
2. *Benjamins, J. A. and McKhann, G.M.* 1972. Neurochemistry of development, in: Basic neurochemistry (R. Wayne Albers, George J. Siegel, Robert Katzman and Bernard W. Agronoff, eds.) *Litlle Brown and Company, Boston, 14,* 269-298.
3. *Brizzee, K. R., Vogt, J. and Kharetchko, X.* 1964. Postnatal changes in glial neuron index with a comparison of methods of cell enumeration in the white rat. *Progr. Brain. Res.*, 136-149.
4. *Ćirović, M. and Đurica, S.* 1994. The H4 histone content change in the postnatal rat brain maturation. *Revue Roumaine de Biochimie, tome 31, No. 2-3, p.* 121-125.
5. *Davis, J. M. and Himwich, W. A.* 1973. Amino acids and proteins of developing mammalian brain in: *Biochemistry of the developing brain (Williamina Himwich, ed.), Marcel Dekker, Inc. New York, vol 1,* 55-112.
6. *Davison, A. N.* 1970. The biochemistry of the myelin sheath. In: Myelination (A.N.Davison and A. Peters, eds.), *Charles C. Tomas, Illinois,* 80-161.
7. *Di Liegro, I. and Cestelli, A.* 1990. The relative proportion of H1 degrees and A24 is reversed in oligodendrocytes during rat brain development. *Cell. Mol. Neurobiol., 10(2),* 267-274.
8. *Donaldson, H.H. and Hatai, S.* 1931. On the weight of the parts of the brain and on the percentage of water in them according o brain weight and to age, in albino and in wild Norway rats, *J. Comp. Neurol., 53,* 263-307.
9. *Flexner, J. B. and Flexner, L. B.* 1950. Biochemical and physiological differentiation during morphogenesis. The effect of growth on the amount and distribution of water, protein and fat in the liver and cerebral cortex of the fetal guinea pig. *Anat. Rec., 106,* 413-427.
10. *Gall, O., Medgyesi, G. A. and Verczkey, L.* 1980. Electrophoretical separation of some groups of proteins. In: Electrophoresis in the separation of biological macromolecules. A. Wiley-interscience publication (John Wiley and Sons, eds.), Chichester. New York. Brisbane, Toronto.
11. *Gunner, J. and Waehneldt. Kreysing. Thomas V. B.* 1992. An evolutionary approach to myelin proteins and myelin-forming cells in the vertebrate brain. *Biochem. Soc. Trans., 20,* 617-621.
12. *Johns, E. W.* 1967. The electrophoresis of histones in polyacrylamide gel and their quantitative determination. *Biochem. J., 104,* 78-82.
13. *Johns, E. W., Goodowin, G. H., Walker, J. M. and Sanders, C.* 1975. Chromosomal proteins related to histones, in: The Structure and Function of Chromatin. CIBA Foundation Symp. *Associsted Scientific Publishrs, Amsterdam, 28,* 95-108.
14. *Klimenko, A. I.* 1970. Nukleinovie kisloti i gistoni jader Kletok, in: Molekularnie i funkcionalnie osnovi ontogeneza (V. I. Mahinko, E. A. Gordienko, E.M. Kreps, A. A. Markosjan, eds.). "Medicina", *Moskva,* 89-109.
15. *Lowry, O. H., Rosebrough, M., Forr, A. and Rondall, R.* 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem., 193,* 265-275.

IDENTIFIKOVANJE PROMENA U SADRŽAJU UKUPNIH PROTEINA MOZGA ZAMORČETA IZMEĐU 30. PRENATALNOG I 75 POSTNATALNOG DANA

M. ĆIROVIĆ

SADRŽAJ

Karakterišući prenatalni i postnatalni razvoj mozga zamorčeta, posmatrane su kvantitativne i kvalitativne promene proteinskog sadržaja mozga. Prenatalni period smo ograničili na drugu polovinu gestacionog perioda, a postnatalni - na period koji obuhvata vreme od rođenja do adulta.

Od 30. do 48. prenatalnog dana, kada se masa mozga zamorčeta akumulirajući vodu, uveća za više od 8 puta, koncentracija proteina se smanji za približno 1,4 puta. Elektroforetske slike proteina mozga pokazuju da najdublje i najizražajnije kvantitativne promene, u ovoj fazi razvoja mozga, nastaju kod proteinskih frakcija koje imaju relativno male molekulske mase. Među njima (po kvantitetu) histoni zauzimaju primarno mesto. Od 30. do 48. dana, koncentracija svih histonskih frakcija (H1, H2A, H2B, H3, H4) drastično se smanji. Ali, za to vreme njihov kvantitativni odnos se ne menja.

Od 48. do 63. dana, kada se masa mozga zamorčeta gubeći vodu uveća za samo 1,7 puta, koncentracija totalnih proteina se uveća za približno 1,4 puta. U ovoj fazi razvoja mozga, koncentracije 4 histona (H2A, H2B, H3, H4) se promene, ali vrlo malo. Koncentracija histona H1 izuzetno oštro raste. Raste istom brzinom kojom raste i MBP. To je zajedničko svojstvo ove dve funkcije baznih proteina.

Od rođenja (0 dana) do adulta (75 dana), ukupna masa proteina poraste za samo 0,5%. Elektroforetske slike pokazuju blag i usporen tok promena. Koncentracije svih histonskih frakcija se smanjuju, ali blagim i usporenim tempom. Mozak adulta ima 2,3 manju koncentraciju H4 histona nego mozak novorođenog zamorčeta. Koncentracija MBP raste intenzitetom akumulacije mijelina u mozgu.