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Age-Dependent Modifications of Mitochondrial Proteins in Cerebral Cortex and Striatum of Rat Brain

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The protein composition of free mitochondria purified from cerebral cortex and striatum during aging was analyzed by gel electrophoresis. Mitochondria were isolated from cerebral cortex and striatum of 4-, 12-, and 24-month-old rat brain. The percent amount of mitochondrial proteins after gel-electrophoretic separation was determined densitometrically. A significant decrease in the amount of two polypeptides (with molecular weights of 20 and 16 kDa, respectively) in both brain regions during aging was found. The decrease was higher in the striatum indicating a greater vulnerability of this brain area to the aging process. The age-dependent modifications of mitochondrial proteins observed may play an important role in several mitochondrial functions, such as energy transduction and transport processes as well as in structural changes occurring with age, causing altered membrane permeability and fluidity.

KEY WORDS: Mitochondrial proteins; aging; cortex; striatum.

INTRODUCTION

Brain metabolism during aging has been the subject of several studies with the aim of understanding the molecular mechanism underlying the structural and functional alterations observed during senescence. Many studies (1-7) have been focused on brain protein synthesis in different regions and subcellular fractions but the results obtained are very often contradictory. However, few studies have been performed on specific proteins with particular function in brain.

Changes in mitochondrial structural components with a concomitant impairment of mitochondrial function have been observed (8). A decrease in the content of cytochromes and modifications of cytochrome oxidase activity during aging (9-15) suggest that the mitochondria from old animals contain less respiratory units than those of young animals, resulting in an impairment of energy transduction during aging.

It has been hypothesized that a disorganization of proteins and lipids forming the structurally and functionally active membranes, with a consequent decrease in membrane fluidity, may influence the function of membrane proteins involved in mitochondrial enzyme activities or in transport processes (16). While there are many reports dealing with the morphology, the number of mitochondria (by electronmicroscopy or histology) in aging tissues, and with lipid composition or enzyme activities (11, 15), very little is known about the possible quantitative modifications of mitochondrial membrane proteins with advancing age (17).

In recent studies we have determined the in vivo rate of protein synthesis in different brain regions and in subcellular fractions during aging (7). We did not observe statistically significant modifications in the specific radioactivity of proteins or in the percent incorporation rate of the labeled precursor into proteins. In the present study we have investigated the possible

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modifications in the protein composition of non-synaptic mitochondria obtained from cortex and striatum of rats at different ages.

EXPERIMENTAL PROCEDURE

Animals Used. The experiments were performed using 4-, 12-, and 24-month-old male Wistar (IFFA-Credo Lyon) rats. The animals were kept from birth under standard cycling and caging conditions (temperature: 22 ± 1 °C; relative humidity: 60 ± 3%; light cycle: 12 h light and 12 h darkness; low noise disturbances), fed with a standard pellet diet and water ad libitum and housed three and subsequently two per cage.

Chemicals. The reagents for electrophoresis (acrylamide, N,N'-methylen-bisa-crylamide, 2-mercaptoethanol, ammoniumpersulfate, N,N,N',N'-tetra-methylethylendiamine, sodium dodecylsulfate) were purchased from BioRad Laboratories, Richmond, California. Coomassie Brilliant Blue R-250 was obtained from E. Merck, Darmstadt. Low molecular weight kit used for molecular weight determination was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

Preparation of Non-synaptic Mitochondria. Free (non-synaptic) mitochondria were prepared according to the methods of De Robertis et al (18) and Whittaker (19) as described: the whole, frozen tissue was homogenized with 9 vol of 0.32 M sucrose. Nuclei were separated by centrifugation at 1,000 *g* for 10 min, nuclear pellet was washed once and the supernatant was centrifuged at 10,000 *g* for 30 min to obtain the crude mitochondrial pellet. The pellet was washed twice and layered on the top of a discontinuous sucrose gradient (0.8, 1.0, 1.2 and 1.4 M sucrose solutions) to obtain the purified mitochondrial pellet after centrifugation at 75,000 g for 2 hours.

Electrophoresis and Quantitative Determination of Proteins. Gel-electrophoretic separation of various mitochondrial proteins was carried out in SDS-polyacrylamide gels according to the method of Laemmli (20). For electrophoresis 40 μg of mitochondrial proteins were used.

The separation gel contained 12% polyacrylamide (pH 8.8) and the stacking gel 4.5% polyacrylamide (pH 6.8). The running buffer was 25 mM Tris-HCl, 192 mM glycine, 0.1% SDS, pH 8.3; the running time was 1 h with 150 V in the stacking gel and 3.5 h with 350 V in the separation gel. After electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 in 40% methanol/10% acetic acid and destained with 10% methanol/10% acetic acid. Gels were scanned at λ-600 nm with a Gelman densitometer (model ACD-18), which calculated automatically the peak area and the percentage value of each protein band. The total protein content was determined by the method of Lowry et al. (21). The quantity of the mitochondrial proteins has been calculated from the total amount used for electrophoresis (40 μg) and from the percent values obtained densitometrically from destained gels after electrophoresis. The results are expressed as μg of protein per mg total mitochondrial proteins. The values were obtained from 4 independent electrophoretic separations of mitochondrial proteins from two pooled brain regions (number of animals = 8).

RESULTS

The results obtained are reported in Table I and in Figures 1 and 2. Table I reports the amounts of the two mitochondrial proteins at different ages, calculated as μg per mg of total mitochondrial proteins. In Figures 1 and 2, the results are expressed as percent values (obtained densitometrically) of the two protein bands compared with the proteins separated on the gel.

There is a statistically significant decrease in the amount of the two mitochondrial proteins, with molecular weights 20 kDa and 16 kDa, in both brain regions examined. For the 20 kDa protein the diminution in striatum is 69% at 12 months and 80% at 24 months compared to the values observed at 4 months of age. In the cerebral cortex the decrease is significant (−93 %) only in the oldest animals (24 months).

The decrease in the quantity of 16 kDa protein is 41% in cerebral cortex and 61% in striatum at 12 months and about 50% in both areas at 24 months.

DISCUSSION

Our results demonstrate that some specific mitochondrial proteins undergo significant quantitative modifications during aging. According to the actual knowledge about mitochondrial protein composition, it is probable that the protein with molecular weight 20 kDa may represent one subunit of the complex V of respiratory chain and the protein with molecular weight 16 kDa the subunit IV of cytochrome oxidase. However an exact characterization of these proteins cannot be done only on the basis of the molecular weight, but needs further investigations. As it has been shown in several studies it is difficult to find striking age-related differences by measuring the rate of brain protein synthesis or analyzing the proteins of whole brain regions (1, 2, 5, 7, 22). Instead it is of great importance to evaluate the possible modifications caused by aging on particular proteins from different brain regions or specific subcellular fractions.

As it has been demonstrated in our previous studies using mitochondrial and synaptosomal preparations from cerebellum (17, 23–24), some of the proteins examined are very vulnerable to the aging process.

The results obtained in the present study demonstrate that also in other brain areas, such as the cortex and the striatum, the mitochondrial proteins are affected by aging and that the striatal mitochondrial proteins are influenced to a greater extent. The vulnerability of the striatum to the aging process has been demonstrated in previous studies as well (25).

We are aware of the limits of the methods used in the present study to quantify the changes in the two proteins at different ages (due to the binding capacity of various proteins, and the one-dimensional separation

Table I. Changes in the Amounts of Mitochondrial Proteins During Aging

Brain regions	Mw	4 months	12 months	% difference*	24 months	% difference*
cerebral cortex	20 kDa	15.0 ± 1.6	11.0 ± 0.8	−27%	1.0 ± 0.5	−93%
striatum	20 kDa	35.0 ± 3.5	11.0 ± 5.2	−69%	7.0 ± 1.0	−80%
cerebral cortex	16 kDa	107.0 ± 10.0	63.0 ± 6.5	−41%	54.0 ± 5.3	−50%
striatum	16 kDa	102.0 ± 2.0	40.0 ± 4.5	−61%	47.0 ± 3.5	−54%

The results are expressed as μg protein/mg total mitochondrial proteins and are the mean ± SEM of four independent experiments.
*Percent differences compared to 4-month-old rat values.

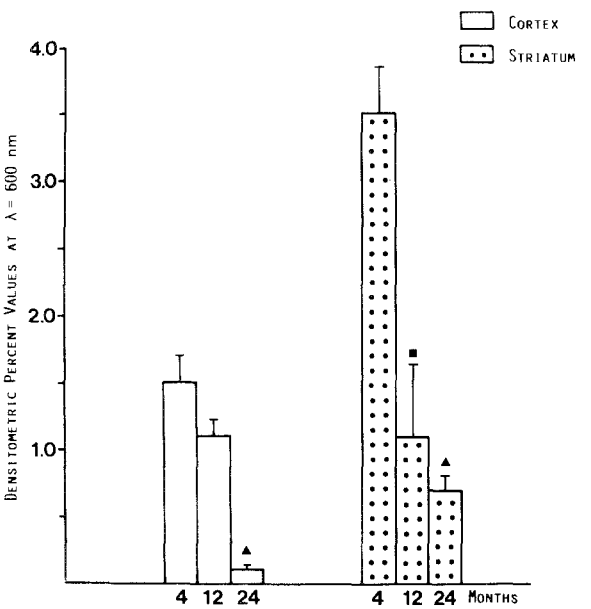

Fig. 1. Densitometric percent values of the 20 kDa protein at different ages. Mean ± SEM of 4 different electrophoretic separations (8 animals). Statistical significance (by Student's *t* test) compared with the values at 4 months: •$P<0.05$.; ■$P<0.01$; ▲$P<0.001$.

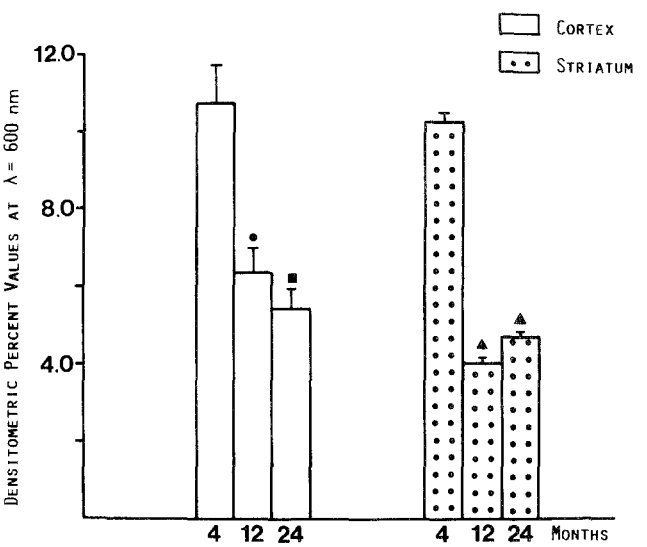

Fig. 2. Densitometric percent values of the 16 kDa protein at different ages. Mean ± SEM of 4 different electrophoretic separations (8 animals). Statistical significance (by Student's *t* test) compared with the values at 4 months: •$P<0.05$; ■$P<0.01$; ▲$P<0.001$.

performed), therefore the amounts calculated by our method may not represent the true values of these two polypeptides in mitochondria. However, the significant decrease obtained in the amount of the two protein groups, which may have a crucial role in the mitochondrial respiratory chain, is in good agreement with the impairment of energy transduction observed in aging brain (14-15).

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REFERENCES

1. Gordon, S. M., and Finch, C. E. 1974. An electrophoretic study of protein synthesis in brain regions of senescent male mice. Exp. Gerontol. 9:269–273.
2. Dunlop, D. S., van Elden, W., and Lajtha, A. 1977. Developmental effects on protein synthesis rates in regions of the CNS in vivo and in vitro. J. Neurochem. 29:939–945.
3. Dwyer, B. E., Fando, J. L., and Wasterlain, C. G. 1980. Rat brain protein synthesis declines during postdevelopmental aging. J. Neurochem. 35:746–749.
4. Fando, J. L., Salinas, M., and Wasterlain, C. G. 1980. Age-dependent changes in brain protein synthesis in the rat. Neurochem Res 5:373–383.

5. Shahbazian, F. M., Jacobs, M., and Lajtha, A. 1986. Regional and cellular differences in rat brain protein synthesis in vivo and in slices during development. Int. J. Devel. Neurosci. 4:209–215.
6. Giuffrida Stella, A. M., and Lajtha, A. 1987. Macromolecular turnover in brain during aging. Gerontology 33:132–144.
7. Avola, R., Condorelli, D. F., Ragusa, N., Alberghina, M., Renis, M., and Giuffrida Stella, A. M. 1988. Rate of protein synthesis in various brain regions and subcellular fractions during aging. Neurochem. Res. 13:337–342.
8. Vorbeck, M., Martin, A. P., Lang, J. W. J., Smith, J. M., and Orr, R. Jr. 1982. Aging dependent modification of lipid composition and lipid structural order parameter of hepatic mitochondria. Arch. Biochem. Biophys. 277:351–361.
9. Abu-Erreish, C. M., and Sanadi, D. R. 1978. Age-related changes in cytochrome concentration of myocardial mitochondria. Mech. Ageing Devel. 7:425–432.
10. Benzi, G., Arrigoni, E., Dagani, F., Marzatico, M., Curti, D., Raimondo, S., Dossena, M., Polgatti, M., and Villa, R. F. 1980. Age-dependent modifications of drug interference on the enzymatic activities of the rat brain. Exp. Gerontol. 15:593–603.
11. Hansford, R. G. 1983. Bioenergetics in aging. Biochim. Biophys. Acta 726:41–80.
12. Starnes, J. W., Beyer, R. E., and Edington, D. W. 1983. Myocardial adaptations to endurance exercise in aged rats. Am. J. Physiol. 245:4560–4566.
13. Starnes, J. W., Edington, D. W., and Beyer, R. E. 1983. Myocardial protein synthesis during aging and endurance exercise in rats. J. Gerontol. 33:802–810.
14. Benzi, G., and Giuffrida, A. M. 1985. Bioenergetics of hypoxic brain during aging. Mol. Physiol. 8:535–547.
15. Benzi, G., and Giuffrida, A. M. 1987. Changes of synaptosomal energy metabolism induced by hypoxia during aging. Neurochem. Res. 12:149–157.
16. Vitorica, J., Clark, A., Machado, A., and Satrestequi, J. 1985. Impairment of glutamate uptake and absence of alterations in the energy transducing ability of old rat brain mitochondria. Mech. Ageing. Devel. 29:255–266.
17. Turpeenoja, L., Villa, R. F., Magri, G., and Giuffrida Stella, A. M., 1988. Changes in mitochondrial membrane proteins of rat cerebellum during aging. Neurochem. Res. 13:859–865.
18. De Robertis, E., de Iraldi, A. P., deL Arnaiz, G. R., and Salganicoff, L. 1962. Cholinergic and non-cholinergic nerve endings in rat brain. 1. Isolation and subcellular distribution of acetylcholine and acetylcholine esterase. J. Neurochem 9:23–35.
19. Whittaker, V. P. 1969. The synaptosome. Pages 327–364, *in* Lajtha, A. (ed.): Handbook of Neurochemistry, Vol. 2. New York: Plenum Press.
20. Laemmli, U. K. 1970. Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680–685.
21. Lowry, O. M., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
22. Cosgrowe, J. W., Atack, J. R., and Rapoport, S. I. 1987. Regional analysis of rat brain proteins during senescence. Exp Gerontol 22:187–198.
23. Turpeenoja, L., Lahdesmaki, P., Villa, R. F., and Giuffrida, A. M. 1986. Modifications of synaptosomal plasma membrane proteins during aging. Page 223. *in* Tucek, S., Stipek, S., Stastny, F., Krivanek, J. (eds), Molecular Basis of Neural Function, Prague: Proceedings of the European Society for Neurochemistry.
24. Turpeenoja, L., Villa, R. F., Benzi, G., Lahdesmaki, P., and Giuffrida-Stella AM. 1987. Changes in cerebellar mitochondrial proteins during aging. (Abstr.) J. Neurochem. 48 (Suppl.):S155.
25. Condorelli, D. F., Renis, M., Avola, R., Surrentino, S., Costa, A., and Giuffrida Stella, A. M. 1987. DNA and RNA labeling in different brain regions and subcellular fractions during aging. Gerontology 33:269.