MORPHOLOGY AND PATHOMORPHOLOGY

Age-Related Changes in the Dopaminergic System of Rat Striatum

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The elucidation of specific features of neurotransmitter system aging is of interest not only as a tool for studying fundamental mechanisms of age-related changes in the brain, but primarily as an opportunity for analysis of the general pathways of pharmacological regulation under the conditions of a gradually deteriorating complex of functional interrelationships between neurotransmitter-mediated processes [2]. Recently, a number of data appeared that enable us to consider the changes in the dopaminergic (DA) regulation of the brain in old age as one of the main mechanisms of brain aging [2,11]. In this respect the DA system is today one of the most thoroughly studied systems from the point of view of aging. However, most investigations in this field have been aimed at the evaluation of changing DA activity using solely static parameters (tissue content of DA and its metabolites) or solely functional parameters (release, biosynthesis, or capture), thereby hindering the interpretation of the information concerning the state of DA-ergic neurotransmission during natural aging.

The present study was carried out in order to examine the interrelationships between the static and functional neurochemical parameters of the DA-ergic system in the striatum of the aging brain.

MATERIALS AND METHODS

Experiments were carried out on outbred male rats of two age groups: 26 and 3 months. The content of DA, 3,4-dioxyphenylacetic acid (DOPAA), and homovanillic acid (HVA) in the rat striatum was recorded using high-performance liquid chromatography with electrochemical detection (HPLC ED) [1]. The release of DA was studied *in vitro* on specimens taken from the same rats. After decapitation, the striatum (80-90 mg) was extracted from both cerebral hemispheres under cooling. Each striatum was dissected transversely and placed in a carbogenized buffer solution of the following composition: 111 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.64 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 0.054 mM Na_2EDTA, and 0.28 mM ascorbic acid. The striatum of each rat was manipulated in individual thermostat chamber (at 37°C). The first incubation lasted 1 hour, after which the buffer solution was renewed. A specimen of solution was taken after 10 min, and following adsorption on aluminum oxide DA was detected by HPLC ED. Adsorption of DA was performed using preactivated aluminum oxide of the "Katekholkhrom" kit (DIA-M, Moscow) in the pres-

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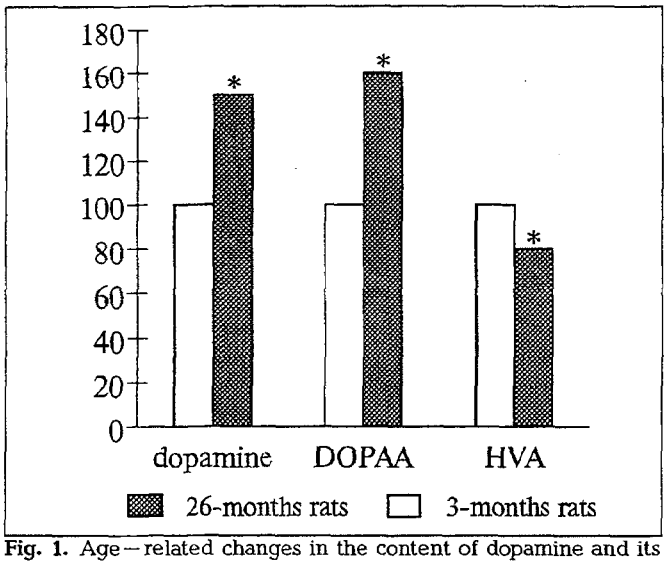

Fig. 1. Age-related changes in the content of dopamine and its metabolites in homogenates of rat striatum. Here and in Fig. 2 ordinate: changes in the concentration in 26-month rats (dark bars) in percentage to that in 3-month rats (open bars). Asterisk: statistically significant difference ($p<0.05$, Student t test).

ence of 1 M Tris buffer at pH 8.6. After shaking for 10 min and three washings of aluminum oxide with deionized water, DA was eluted with 0.1 M $HClO_4$. The samples were analyzed on a reverse-phase column (3×150 mm, C_{18}, 5 μ, Elsiko, Moscow) using 0.1 M citrate-phosphate buffer solution containing 0.25 mM Na octanesulfonate, 0.1 mM EDTA, and 8.5% acetonitrile, pH 3.6. Detection was performed on a glass-carbon electrode at +0.8V (LC-4B, BAS, USA). The significance of the results was estimated according to the Student t test.

RESULTS

The determination of the DA and metabolite level in the striatum of young (3 months) and old (26 months) rats revealed (Fig. 1) that the neurotransmitter content increases with age from 38.3±1.9 up to 58.5±1 pM per mg tissue ($p<0.05$). The change in the DOPAA concentration shows the same pattern and increases from 4.3±0.5 to 7.1±0.9 pM per mg tissue ($p<0.05$). However, the level of HVA reliably decreases in old rats (from 4.3±0.3 to 3.3±0.4, $p<0.05$). Experiments on superfusion of striatum fragments from the same rats showed (Fig. 2) that the basal release of DA in 26-month animals drops to 25±5 pM per min per mg as compared to 55±11 pM per min per mg in young rats ($p<0.05$).

A pronounced inverse correlation between the concentration and basal release of DOPAA was revealed in old animals ($r=-0.90$, $p<0.05$), whereas in the striatum of young rats this relation was negligible ($r=0.11$).

Today the notion regarding the decline in the DA concentration in the nigrum-striatum region in aging is becoming more broadly accepted [5,8,9], although some researchers do not confirm the existence of such a trend [3]. In our experiments the DA content in the striatum of old rats was much higher than in that of the young animals. It is thought that possible causes of discrepancies in the results may be species-specific differences, influence of seasonal fluctuations, and specific features of animal maintenance [5,6,11]. In this connection it may be assumed that in the course of aging the basal ganglia may contain an excess of DA as well. In that case the described biochemical phenomenon of increased activity of MAO-B in the nigrum-striatum region in aging [7] can be explained as a compensatory response to the excess of intracellular DA.

It is accepted that the bulk of DOPAA is produced by intraneuronal metabolism of unreleased DA [12]. The increase in the concentration of this metabolite detected by us in the striata of old rats may be evidence of an accumulation of DA directly in the terminals and, therefore, indicate hampered release. This notion was corroborated by our results concerning DA basal release *ex vivo* and is in agreement with data of other authors [4,5,10]. Moreover, such an interpretation of the aggregate of data is consistent with the described reduction of the concentration of HVA, another metabolite to which a mostly extraneuronal localization is ascribed [13], a fact which also indicates diminished DA release.

Thus, on the one hand, an increase in DA content in the striatum of old rats and, on the other, a diminishment of its basal release, as well as corresponding changes in metabolite levels and an inverse relationship between the basal release of DA and the level of its intraneuronal metabolite DOPAA - all these attest to a disturbance in neurotransmitter me-

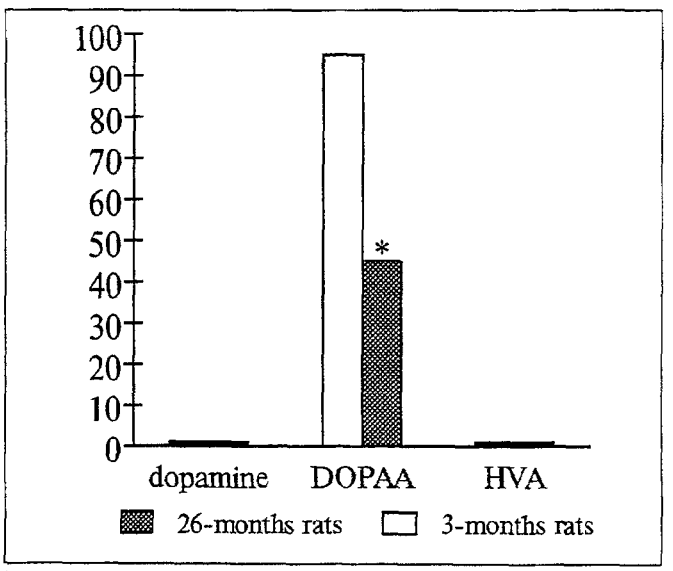

Fig. 2. Age-related changes in the release of dopamine from isolated rat striatum in the course of perfusion.

tabolism and weakening of its regulatory role in the central nervous system during the aging process.

REFERENCES

1. I. I. Miroshnichenko, V. S. Kudrin, and K. S. Raevskii, *Farmakol. Toksikol.*, No. 2, 26-29 (1988).
2. V. V. Frol'kis *et al.*, *Aging of the Brain* [in Russian], Leningrad (1991).
3. I. Date, D. L. Felten, and S. Felten, *Brain Res.*, **519**, 266-276 (1990).
4. D. E. Dluzen, J. L. McDermott, and V. D. Ramirez, *Exp. Neurol.*, **106**, 259-264 (1989).
5. F. Godefroy, M. H. Bassant, Y. Lamour, and J. Weil-Fugazza, *J. Neural Transmis.*, **83**, 13-24 (1991).
6. I. M. Henry, G. S. Joseph, K. Kochman, *et al.*, *Brain Res.*, **418**, 334-342 (1987).
7. L. Oreland, in: *Normal Aging, Alzheimer Disease, and Senile Dementia*, Brussels, (1985), pp. 129-134.
8. H. H. Osterburg, H. G. Donahue, J. A. Severson, *et al.*, *Brain Res.*, **224**, 337-352 (1981).
9. F. Ponzio, G. Gladerini, G. Lomuscio, *et al.*, *J. Neurobiol. Aging*, **3**, 23-29 (1982).
10. J. L. Venero, A. Machado, and J. Cano, *Brain Res.*, **557**, 109-114 (1991).
11. J. J. Woodward, S. W. Leslie, J. A. Severson, and R. E. Wilcox, *Neurosci. Lett.*, **97**, 191-197 (1989).
12. T. Zetterstrom, T. Sharp, A. K. Collin, and U. Ungerstedt, *Ibid.*, **148**, 327-334 (1988).
13. T. Zetterstrom, T. Sharp, and U. Ungerstedt, *Europ. J. Pharmacol.*, **132**, 1-9 (1986).

Three-Factor Correlation Analysis of Morphofunctional Changes of Mitochondria during Irradiation

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Key Words: *morphofunctional changes of mitochondria; correlation analysis; correlation coefficient; closeness and connection of correlation; regression*

There have been many studies of structural and functional changes of animal and plant mitochondria (Mt) under conditions of dosed exposures at different time intervals. However, strict mathematical analysis has rarely been used, and the results are often subjective, only to be taken as hypotheses requiring further investigation and corroboration. One proof can be the establishment of a correlation between test variables such as doses of exposures, time intervals, and morphological and functional characteristics of the biological object. Multifactorial correlation analysis and calculations of the correlation coefficients should be performed, to reveal these connections. For instance, due to the results of previous two-factor correlation analysis between known morphofunctional mitochondria types and redistribution of Mt sizes, we obtained supplementary proofs of our earlier conclusions [3-5]: knowing the areas of Mt, it is possible to determine their functional properties with a high degree of reliability, and, conversely, knowing the functional properties, it is possible to judge the areas using regression lines to estimate the whole population of Mt [8].

The present study aims at proving the existence of a correlation between such variables as time period, rate of respiration and redistribution of Mt ar-

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