ACTIVITY OF DEOXYRIBONUCLEASES FROM FUNCTIONALLY AND MORPHOLOGICALLY DIFFERENT REGIONS AND TISSUES OF THE BRAIN

A. D. Reva and G. P. Chernenko

UDC 612.822.1.015.12:577.155.2

Differences between the distribution of activities of acid and alkaline deoxyribonucleases (DNases) in the tissues of the gray and white matter of the cerebral hemispheres, medulla, and cerebellum were discovered in cats. The highest acid DNase activity was found in the cerebellum and cerebral cortex, followed in descending order by tissues of the medulla and the white matter of the cerebral hemispheres. Alkaline DNase in the cerebellar tissues is almost twice as active as in the cortex, the medulla, and the white matter of the cerebral hemispheres. In all regions and tissues of the brain investigated, activity of acid DNase is two to four times greater than that of the alkaline enzyme.

Investigations on the whole brain have shown that nervous tissue exhibits both acid [1, 4, 9, 14] and alkaline DNase activity [4, 12]. However, there are few data in the literature on DNase activity in the various regions and tissues of the central nervous system. It has been shown, for instance, that the activity of acid and alkaline DNases differs in the various tissues of the lumbar enlargement of the spinal cord in cats and cattle [4]. Attempts have been made to determine the activity of DNases in various parts of the rabbit brain [5].

Since nucleic acid metabolism in nerve tissue is differentiated three-dimensionally [2], it was decided to investigate activity of the DNases in different regions and tissues of the central nervous system in cats in order to determine any biochemical differences in the distribution of their activity in functionally and morphologically different tissues of the cortex and white matter of the cerebral hemispheres, the medulla, and the cerebellum.

EXPERIMENTAL METHOD

Experiments were carried out on cats. Activity of the DNases was determined by the method of Schneider and Hogeboom [13] at pH 7.3 (Tris-HCl buffer) for alkaline DNase and pH 5.25 (Na–acetate buffer) for acid DNase. The substrate was high-polymer DNA from chicken blood (Reanal, Hungary). The enzyme preparation was an aqueous extract of a 10% homogenate, centrifuged at 5,000 g for 10 min. The final protein concentration in the extract and reaction mixture was as follows for the different tissues: cerebral cortex 13.3 μg, white matter of cerebral hemispheres 8 μg, medulla 10 μg, cerebellum 13.4 μg. The reaction mixture (1.5 ml), containing 0.033 mole of the corresponding buffer, 10 μg/ml DNA, 4×10^{-3} mole $MgCl_2$, and aqueous extract of the enzyme, was incubated for 30 min at 37°C. The enzyme reaction was stopped by the addition of 0.5 ml 25% $HClO_4$ solution to the sample. The quantity of enzyme causing an increase in the optical density by unity at 260 nm after incubation for 30 min at 37°C was taken as the unit of activity. Activity of the DNases was calculated per milligram protein per gram fresh tissue. Protein was determined by Lowry's method [11].

Department of Biophysics and Biochemistry, Dnepropetrovsk University. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 5, pp. 44-46, May, 1972. Original article submitted March 9, 1971.

TABLE 1. Activity of DNases in Tissues From Different Parts of the Brain (M + m for 6-7 experiments)

Name of tissue and part of central nervous system	DNase I		DNase II	
	act. per mg protn. extract	act. per gram fresh tissue	act. per mg protn. extract	act. per gram fresh tissue
Cerebral cortex	0,532±0,025	21,0±0,38	2,086±0,07	79,4±1,2
White matter of cerebral hemispheres	0,682±0,014	16,26±0,15	1,186±0,04	28,26±0,43
Medulla	0,552±0,032	17,4±0,13	1,614±0,05	50,4±0,75
Cerebellum	1,206±0,024	50,0±0,4	1,968±0,08	78,2±1,3

EXPERIMENTAL RESULTS

The results in Table 1 show that the activities of the DNases differed in functionally and morphologically different tissues of the central nervous system. This difference was particularly marked in the case of acid DNase (DNase II). The highest activity of this enzyme, calculated per milligram protein, was found in the tissues of the cerebral cortex and cerebellum, activity was slightly lower in the tissues of the medulla, and lowest of all in the white matter of the cerebral hemispheres. All these differences are statistically significant. Activity of DNase II calculated per gram fresh tissue was identical in the tissues of the cerebellum and cerebral cortex, while in the medulla and cerebellun it showed a successive and significant decrease. Activity of alkaline DNase (DNase I), calculated per milligram protein, was almost twice as high in the cerebellum as in the cortex and white matter of the cerebral hemispheres and in the medulla. When calculated per gram fresh tissue,the activity of DNase I in the cerebellum was almost 2.5 times higher than in the tissues of the cerebral cortex, and three times higher than in the tissues of the white matter of the hemispheres and in the medulla.

Comparison of the activities of acid and alkaline DNases in the tissues of the CNS investigated, expressed per milligram protein and per gram fresh tissue, shows the following results: activity of DNase II was almost four times as high as that of DNase I in the tissues of the cerebral cortex and almost twice as high in the tissue of the white matter. Activity of DNase II was almost three times as high as that of DNase I in the medulla and almost twice as high in the cerebellum.

In all the brain tissues investigated, activity of acid DNase was thus much stronger than that of the alkaline enzyme. This may perhaps be due to the presence of specific inhibitors of DNase I in nerve tissue [8, 10] and, possibly, to differences in the molecular structure of these enzymes in the character of their interaction with the substrate [7].

These findings confirms the view that differences exist in the activity of the DNases in functionally and morphologically different tissues of the CNS [3].

LITERATURE CITED

1. M. S. Kucherenko, Ukr. Biokhim. Zh., No. 2, 174 (1967).
2. L. Z. Pevzner, in: Morphology of Tracts and Connections of the Central Nervous System [in Russian], Moscow (1965), p. 144.
3. A. D. Reva, Biochemical Characteristics of the Spinal Cord under Normal Conditions and after Radiation Injury. Doctoral Dissertation, Dnepropetrovsk (1970).
4. A. D. Reva, M. F. Starodub, and R. I. Kukharenko, Ukr. Biokhim. Zh., No. 1, 60 (1967).
5. E. B. Skvirskaya and T. P. Silich, in: Biochemistry of the Nervous System [in Russian], Kiev (1964), p. 59.
6. V. S. Shapot, I. A. Chudinova, and G. D. Krechetova, in: Modern Methods in Biochemistry [in Russian], Vol. 1, Moscow (1964), p. 267.
7. A. I. Barrett, in: Lysosomes in Biology and Pathology, Vol. 2, Amsterdam (1969), p. 261.
8. Festi and Paoletti, in: V. S. Shapot, Nucleases [in Russian], Moscow (1968), p. 138.
9. R. Howk and T. Y. Wang, Arch. Biochem., 113, 238 (1969).
10. U. Lindberg, Biochem. J., 92, 27 (1965).
11. O. H. Lowry, J. N. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
12. P. I. O'Connor, Biochem. Biophys. Res. Commun., 35, 805 (1969).
13. W. S. Schneider and J. N. Hogeboom, J. Biol. Chem., 198, 63 (1952).
14. T. Y. Wang, Arch. Biochem., 122, 629 (1967).