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Distribution of acid and alkaline deoxyribonucleases in white and grey matter regions of growing and aging chick brain

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Abstract. The activities of acid and alkaline deoxyribonucleases in the white and grey matter areas of growing and old chick cerebrum were measured. Two marker enzymes for glial cells, butyrylcholinesterase and carbonic anhydrase were also measured in these regions. Higher specific activities of both butyrylcholinesterase and carbonic anhydrase were found in the white matter region at all the stages studied. Acid and alkaline deoxyribonuclease activities were observed in both white and grey matter. The decrease in the specific activity of acid deoxyribonuclease with advancement of age was more pronounced as compared to the alkaline deoxyribonuclease Marked reduction in total acid deoxyribonuclease activity in white matter, beyond the age of 130 days, was observed. On the other hand, total alkaline deoxyribonuclease activity in both white and grey matter continued to increase with age Further, the activity per mg of DNA also increased in white matter of the old brain. These results indirectly suggest a continued role for alkaline deoxyribonuclease in glial cells formed at a later age.

Keywords. Acid deoxyribonucleases; alkaline deoxyribonucleases; aging; chick brain.

Introduction

Recent investigations in this laboratory concerning the metabolism·of DNA in developing and old chick brain have revealed high activities of both acid and alkaline deoxyribonucleases (E.C. 3.1.4.5. and 3.1.4.6; DNases) during the early embryonic development. With the increase in the age of the animal, both the activities decreased and in the old brain, acid DNase could hardly be detected although alkaline DNase remained at a significant level (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976). These results tempted us to speculate that the physiological role of acid DNase may be in those types of cells proliferating during the early part of the brain development (neuronal cells), whereas alkaline DNase may have some role both in the early forming (neuronal) as well as in late forming cells (glial cells). In order to test this possibility, we have now studied the activities of both acid and alkaline DNases in the white and grey matter regions of

growing and old chick brain with a hope of finding some changes in the distribution of the DNases.

Materials and methods

Chicks of specified age (white leg-horn) were obtained from Uttar Pradesh Government Poultry Farm, Varanasi. Highly polymerised calf thymus DNA, butyryl-thiocholineiodide were purchased from Sigma Chemical Company, St. Louis,MO, USA. All the reagents used were of analytical grade.

The cerebral hemispheres were carefully removed from the dissected brains and the white and grey matter regions were carefully separated. Ten per cent homogenates of the appropriate regions were prepared in glass-distilled water using Potter Elvehjem homogeniser. Whenever necessary, the homogenate was further diluted with water to 1%. DNA, protein acid and alkaline DNases were estimated as described earlier (Shrivastaw and Subba Rao, 1975).

Butyrylcholinesterase activity was determined colorimetrically by the rate of hydrolysis of the substrate, butyryl thiocholineiodide using an Eppendorf spectrophotometer (Netheler and Hinz GmbH, Hamburg, Germany), according to the method of Ellman *et al.* (1961). The selective acetylcholinesterase inhibitor, 1, 5-bis-(4-trimethyl ammonium-phenyl) phentan-3-one diodide (62c47) was used at a final concentration of 20μM (Bayliss and Todrick, 1956). The final reaction mixture for determining the butyrylcholinesterase activity consisted of 0·2 to 0·4 ml of 1% homogenate (w/v) made upto a volume of 2·0 ml by phosphate buffer, pH 8·0 (0·07 M), 0·5 ml of 62c47 (12μM), 0·1 ml of dithiobisnitrobenzoic acid (0·01 M) and 0·5 ml of butyrylthiocholine iodide (33 mM). Tubes without the homogenate served as controls. Enzyme activity was expressed as nmol of substrate hydrolysed per min per mg of protein.

Carbonic anhydrase activity was followed by the manometric method of Krebs and Roughton (1948) Two ml of 0·1 M Na_2HPO_4 and 0·1 M KH_2PO_4 in the proportion of 3:2 was placed in the main compartment of Warburg flask with 0·2ml of 1% homogenate (w/v). One ml of 0·1 M $NaHCO_3$ was kept in the side arm and equilibrated at 4° C. The reaction was started by mixing the two solutions shaken at uniform speed and the CO_2 released was measured at 30 sec intervals over a period of 5 min. One unit of enzyme was defined as 1 μl of CO_2 liberated per min per mg of protein

Results and discussion

As the main purpose of this investigation was to examine whether there is any differential distribution of DNases in brain at early and late stages of life, three stages of the life span of chick, young, adult and old, were selected. Table 1 shows the wet wt, DNA and protein content of the white and grey matter of chicken brain at three different ages. It is evident that the weights of these regions increase with the advancing age. Similarly both protein and DNA show an upward trend with age. It can further be seen that in the old brain, on the basis of wet wt the protein percentage has gone up beyond 20, a value considerably higher than that noticed at young ages. However, such increased percentage of protein in old

Table 1. Wet weight, DNA and protein content in the white and grey matter of chick brain at different ages.

Age (days)	White matter			Grey matter		
	Wet wt (mg)	Total protein (mg)	Total DNA–P (μg)	Wet wt (mg)	Total protein (mg)	Total DNA–P (μg)
13	155 ± 18	12·3 ± 1·2	10·1 ± 0·6	405 ± 31	47·6 ± 4·9	35·8 ± 2·2
130	450 ± 52	57·0 ± 8·4	33·7 ± 4·4	810 ± 25	138·0 ± 18·2	74·7 ± 5·5
422	480 ± 24	110·3 ± 6·6	49·3 ± 4·4	1010 ± 10	229·1 ± 19·1	128·5 ± 13·1

All values are averages ± S·D·of six experiments·

chick brain has been observed in our earlier studies also (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976) and it is not known at this time whether this is a characteristic phenomenon of aging chick brain.

Tables 2 and 3 show the activities of butyrylcholinesterase and carbonic anhydrase in white and grey matter of young and old chick brain. It is known that

Table 2. Butyrylcholinesterase activity in the white and grey matter of chick brain at different ages

Age (days)	White matter			Grey matter		
	Total activity	Activity per mg protein	Activity per mg DNA	Total activity	Activity per mg protein	Activity per mg DNA
13	300 ± 38	24·5 ± 1·8	1909 ± 277	373 ± 52	7·9 ±1·6	667 ± 93
130	841 ± 111	14·9 ± 2·2	1520 ± 237	809 ± 106	6·0 ±1·4	701 ±134
422	1237 ± 107	11·2 ± 0·8	1614 ± 175	1341 ± 273	5·8 ±0·8	665 ± 98

Assay of butyrylcholinesterase was carried out according to the method of Ellman *et al.* (1961). Activity expressed as nmol of substrate hydrolysed per min. All values are averages ± S.D. from six experiments· Total activity is obtained by multiplying the specific activity (per mg of protein) with the total amount to protein in mg in the region.

these two enzymes are largely located in glial cells and in fact could be used as marker enzymes to check the purity of glial fraction (Giacobini, 1961; 1964; Vernadakis, 1973). Higher specific activities of both butyrylcholinesterase and carbonic anhydrase were found in the white matter as compared to the grey matter irrespective of the way in which the activities were expiessed i.e., whether per mg of protein or per mg of DNA. However, the total activities of these two enzymes compared well in the two fractions of the brain studied, apparently because of the large quantity of grey matter in comparison with the white (table 1). These results thus indicate indirectly that white matter is rich in glial cells.

The above experiments were continued to estimate the acid and alkaline DNase activities in these two fractions. If the assumption with which the present study was started was correct, then white matter should show little activity of acid DNase whereas grey matter should have considerable amount of this enzyme.The picture with regard to alkaline DNase would be reversed. However, the results obtained (tables 4 and 5) were quite contrary to such expectations. The specific activity of both the enzymes in the two tissues at any age are comparable. In fact, slightly higher specific activities of acid DNase were (tables 4 and 5) found in white matter as compared to the grey matter. Nevertheless, the data presented in table 4 would also reveal that the total acid DNase activity declines markedly between 130 and 422 days of age in white matter while in grey matter this activity remained constant beyond 130 days of age. However, when the activity per mg of DNA or per mg of protein was considered, significant reduction

Table 3. Carbonic anhydrase activity in the white and grey matter of chick brain at different ages.

Age (days)	White matter			Grey matter		
	Total activity	Activity per mg protein	Activity per mg DNA	Total activity	Activity per mg protein	Activity per mg DNA
13	923 ± 145	75·0 ± 5·8	5895 ±1127	1112 ± 88	23·4 ± 0·9	1986 ± 79
130	3810 ± 653	67·2 ± 8·4	6829 ± 855	2425 ± 476	17·7 ± 3·5	2080 ± 375
422	5306 ±1104	47·9 ± 8·6	6849 ±1100	6293 ±1386	27·6 ± 6·4	3158 ± 766

Carbonic anhydrase was assayed as described by Krebs *et al.* (1948) and the activity expressed as μ1 of CO_2 liberated per min. All values are averages ± S.D. from six experiments.

was observed in both white and grey matter in the old age. Alkaline DNase, on the other hand, has shown a slightly different pattern. Its specific activity has declined while the total activity increased markedly with the advancing age, in both the regions. Further, the activity per mg of DNA has increased in white

Table 4. Acid DNase activity in the white and grey matter of chick brain at different ages.

Age (days)	White matter			Grey matter		
	Total activity	Activity per mg protein	Activity per mg DNA	Total activity	Activity per mg protein	Activity per mg DNA
13	68 ± 13	5·5 ±0·6	431 ± 84	168 ± 15	3·6 ±0·4	306 ± 31
130	246 ± 60	4·3 ±0·5	439 ± 79	412 ± 76	3·0 ±0·6	354 ± 67
422	180* ± 22	1·6* ±0·2	235* ± 34	396 ± 58	1·7* ±0·3	200* ± 42

Activity expressed as μg of DNA-P liberated per 2 h. All values are averages ± S.D. from six experiments.

* This value is significantly different from the corresponding value at 130 days of age ($p < 0{\cdot}005$)

Table 5. Alkaline DNase activity in the white and grey matter of chick brain at different ages.

Age (days)	White matter			Grey matter		
	Total activity	Activity per mg protein	Activity per mg DNA	Total activity	Activity per mg protein	Activity per mg DNA
13	297 ± 19	24·3 ± 4·0	1896 ± 205	991 ± 111	20·8 ± 1·1	1767 ± 131
130	1066 ± 169	18·0 ± 0·5	1839 ± 139	2347 ± 356	17·0 ± 0·7	2115 ± 261
422	1540 ± 26	14·0+ ± 0·6	2011+ ± 148	3015* ± 208	13·2* ± 1·0	1511* ± 183

Activity expressed as μg of DNA-P liberated per 2 h. All values are averages ± S.D. from six experiments.

* These values are significantly different from the corresponding values at 130 days of age ($p < 0{\cdot}005$).

\+ This value is significantly increased as compared to the value at 130 days of age ($p<0{\cdot}05$).

matter but a reduction was observed in the case of grey matter, between 130 and 422 days (table 5) .

In the grey matter, the alkaline DNase activity continues to increase with respect to accumulating protein and increase in age (compare column 5 in table 1 and column 6 in table 5). Acid DNase, on the other hand, increases upto a total protein level of 138 mg (corresponding to the age of 130 days) and thereafter the activity showed little change in spite of the increasing levels of total protein (compare column 5 in table 1 and column 6 in table 4). In the white matter also alkaline DNase activity increases with age but acid DNase, after being at its highest level at 130 days of age (corresponding to 57 mg of total protein level) declines significantly with further advancement of age although the protein level continues to increase.

White matter is thought to be made up of predominantly oligodendroglial cells, while grey matter could contain both neuronal and glial (astroglia) cells (Deshmukh *et al*., 1974; Poduslo and Norton, 1975 ; Iqbal *et al*., 1977). If this assumption is correct, the present results along with our earlier results (Shrivastaw and Subba Rao, 1975 ; Subba Rao and Shrivastaw, 1976) where very high activities of both these enzymes were noticed during early embryonic stages but decreased with age could probably be interpreted as follows : Both these enzymes have some important role in all the regions of developing as well as aging brain and. no exclu sive localisation of either of the enzymes in any specific region could be observed The increased activity, total as well as per mg of DNA, of alkaline DNase in the white matter of old chick brain may be taken as an indirect indication that this enzyme may be localised more in those cells predominantly present in the white matter region. A direct approach to test this interesting possibility would be to isolate different enriched fractions of various cell types at different ages of chick brain and to measure these enzyme activities in such isolated cell populations. Such experiments are being initiated now.

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