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Changes in DNA, RNA, protein and the activities of acid and alkaline DNases in developing and aging rat cerebellum

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Abstract. The content of DNA, RNA and protein in cerebellum at different stages of the life span of rat as well as the ratios of protein to DNA, showed-that in this region extensive cell proliferation occurs between the 1st and 7th day after birth and once again between the ages of 225 and 750 days. The putative DNA degrading enzymes, acid and alkaline DNases, showed a positive correlation with the rapid DNA accretion noticed during developmental stages as well as during old age. From these results, it could be presumed that there was a second bout of glial cell multiplication in aging cerebellum and that DNases must be playing some important role in the process.

Keywords. DNA; DNases; aging; rat; cerebellum.

Introduction

This laboratory, for the past several years, has been engaged in studying biochemical aspects of developing and aging brain in different species (Subba Rao and Janardana Sarma, 1972; Subba Rao, 1973; Shrivastaw and Subba Rao 1975; Subba Rao and Shrivastaw, 1976; Subba Rao and Janardana Sarma, 1976; Subba Rao and Shrivastaw, 1979). During our studies on chick brain it was observed that the DNA content of the brain continues to increase even beyond the adult stages indicating that there was cell proliferation occurring even in that adult brain. It also became clear during these studies that the putative DNA degrading enzymes, the acid and alkaline DNases, exhibited maximum activity during the embryonic stages of the brain. Thus these enzymes, although degradative in nature, showed a positive correlation with the DNA synthesis.

This study was undertaken to check whether a similar pattern of biochemical changes would be observed in another species, the rat. In view of the complexity of the type of cells that are present in the brain (Dunn and Bandy, 1974) it was considered appropriate that different regions of the rat brain be examined separately.

Materials and methods

Rats of specified age (Wistar strain) were obtained from Indian Drugs and Pharmaceuticals Limited Animal House, Hyderabad. Highly polymerized calf

thymus DNA, yeast RNA and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. All the reagents used were of the Analytical grade.

Cerebellum was separated from the brain and 10% (w/v) homogenates were prepared in glass distilled water using Potter-Elvehjem homogenizer. A portion was immediately taken for the estimation of DNA and RNA (Schmidt and Thannhauser, 1945, Munro 1966). DNA was estimated by the diphenylamine method while RNA was measured by the orcinol reaction. Acid and alkaline DNases were assayed as described by McDonald (1955), by measuring the acid soluble deoxyribosei at the end of incubation. The activity was also checked by following the increase in ultra violet absorption at 260 nm of the acid soluble fraction. Protein was estimated by the biuret method (Gornall *et al.*, 1949). Phosphorus was measured by the procedure of Bartlett (1959).

Results and discussion

There was a steady growth in the wet weight of the cerebellum with advancing age upto 60 days. This weight was maintained constant upto 225 days but between 225 and 750 days there was again a significant increase in the weight (table 1).

Table 1. Changes in wet weight, DNA, RNA and protein levels in aging and developing rat cerebellum

Age (days)	Wet wt. (mg)	DNA/g (mg)	Total DNA (mg)	RNA/g (mg)	Total RNA (mg)	Protein/g (mg)	Total protein (mg)
1(6)	24±1	5.75±0.2	0.13±0.01	5.73±0.50	0.13±0.01	69.8± 3.6	1.62±0.12
7(12)	55±4	9.53±0.9	0.53±0.07	6.62±0.49	0.37±0.03	75.3± 4.3	4.18±0.32
15(6)	210±40	5.32±1.2	1.20±0.30	4.03±1.10	0.80±0.20	104.6±13.5	22.50±3.60
60(10)	240±40	4.40±1.1	1.10±0.28	1.98±0.22	0.49±0.05	126.4±25.4	32.00±6.20
225(16)	240±50	2.70±0.5	0.82±0.28	2.40±0.25	0.59±0.09	107.4±17.3	25.20±4.60
750(17)	290±30*	5.41±0.9*	1.60±0.16*	2.76±0.26	0.79±0.07*	114.5±15.4	32.62±3.01*

All the values are expressed as mean ±S.D. and the number of samples analyzed is indicated in parenthesis. For other details see text.

* These values are significantly different from the corresponding values at 225 days of age ($P<0.001$)

It can be seen from the table that total DNA content of cerebellum increased 4-fold between day 1 and 7 after birth, while during the same period the increase in protein content was only 2.5 fold and that of RNA was about 3 fold. This pattern suggests higher rate of cell proliferation as compared to the increase in cell size during this period. Similar higher rate of cell proliferation (as indicated by DNA increase) in the cerebellum during the few days after birth in rat was observed by

earlier workers (Balazs and Patel, 1973; Weichsel, 1974; Griffin *et al.,* 1977; Clark and Weichsel, 1977; Gaitonde *et al.,* 1978; Clark *et al.,* 1978; Litteria, 1980). However, from the 7th day onwards the rate of increase in protein was significantly higher than that of DNA indicating that beyond the 7th day the extent of cell size increase was greater than that of cell proliferation. From the 15th day the levels of DNA remained essentially constant upto 225 days but between 225 and 750 days there was once again a significant increase in DNA content (expressed either as total content or per *g* of the region). In fact the values found in old cerebellum were the highest compared to the values at any other earlier ages. Even in the case of RNA and protein, there was a significant increase in their total content between 225 and 750 days although the values in old cerebellum were not the highest as compared to the values at earlier ages. It should also be noted that the magnitude of accumulation of DNA (100%) between adult and old ages was much greater than those in the case of either RNA or protein (30% and 20% respectively) during the period. These observations point to a second peak of cell proliferating activity during the life span of rat cerebellum confirming our earlier similar findings in the case of chick cerebellum (Subba Rao and Shrivastaw, 1976). The above observation is also in agreement with the other findings with mouse cerebellum (Caron and Unsworth, 1978).

Highest specific activity of acid DNase was found on the 1st day postnatal. The values decreased steadily with increasing age upto 225 days. But there was a significant increase in this value by the time the animal became 750 days old, although this value was much lower than the activities observed during early developmental stages (days 1 and 7). A similar increase in the total activity of acid DNase between 225 and 750 days could also be seen and the value at 750 days was the highest (table 2). Previous studies from this laboratory with chick cerebellum showed that there was no increase of either the specific activity or of total activity of acid DNase in old age (Subba Rao and Shrivastaw, 1976). It thus appears that there is some species variation as far as the activity of acid DNase in aging brain is concerned.

On the other hand, highest specific activity and total activity of alkaline DNase were observed in aging cerebellum. The specific activity remained at the same level from day 1 to 225 days with an approximately 100% increase between 225 and 750 days. A similar magnitude of increase is also found in total activity during this period. The values between 15 days and 225 days showed some fluctuations but it should be noted that the value at 750 days was significantly higher even when compared to the earlier highest activity found at 60 days. It, therefore appears that alkaline DNase may have an important role, in aging cerebellum. These observations are once again in line with our results with chick brain (Shrivastaw and Subba Rao, 1975; Subba Rao and Shrivastaw, 1976).

The ratios of protein, RNA and the activities of DNases to DNA are presented in table 3, since such an expression would indicate indirectly the increase in cell size and the enzyme activities per cell. There was actually a decrease in protein/DNA and RNA/DNA values between days 1 and 7 thereby indicating once again (table 1)

Table 2. Acid and alkaline DNase activities in aging and developing rat cerebellum.

Age (days)	Acid DNase		Alkaline DNase	
	Sp. activity	Total activity	Sp. activity	Total activity
1(6)	17.0±1.4	27.7±3.4	8.7±0.3	14.0±1.0
7(12)	15.7±1.5	65.7±5.3	9.3±1.0	38.9±5.9
15(6)	4.9±0.6	110.0±27.0	7.6±2.3	204.2±50.9
60(10)	3.0±0.7	92.5±12.4	8.1±1.3	264.8±47.2
225(16)	3.2±0.6	81.3±1.6	7.8±1.6	200.4±36.6
750(17)	5.5±0.7*	176.5±23.0*	14.6±3.1*	445.0±107.0*

For the assay of acid DNase the reaction mixture consisted of 2 mg of highly polymerized calf thymus DNA, in 1 ml of water, 1.5 to 1.7 ml. of 0.1M acetate buffer, pH 5.1, and 0.3 to 0.5 ml of the cerebellar homogenate. At the end of a 2 hour incubation at 37°C the reaction was terminated by adding 2 ml of 1.4M perchloric acid and immediate chilling. The whole mixture was filtered through Whatman 42 filter paper and deoxyribose content/increase in UV absorption at 260 nm was estimated in the filtrate. For measuring alkaline DNase activity the procedure is same as for acid DNase except that the reaction was carried out at pH 8.2 (0.05M Tris-Hcl buffer) and denatured DNA (2 mg) instead of Native DNA was used as substrate. Specific activity is expressed as µg of DNA-P liberated per 2 h per mg of protein. Total activity is obtained by multiplying the specific activity with the total amount of protein in mg. All the values are expressed as means ±S.D. and the number of samples are indicated in the bracket.

* These values are significantly different from the corresponding values at 225 days of age ($P<0.001$)).

Table 3. Protein/DNA, RNA/DNA and activities of DNases/mg of DNA in developing and aging rat cerebellum.

Age (days)	Protein/ DNA	RNA/DNA	Acid DNases/ mg DNA	Alkaline DNase/ mg DNA
1(6)	11.9±0.6	1.0±0.1	203.50±22.2	102.2±3.9
7(12)	8.0±0.06*	0.7±0.06*	131.8±14.1*	73.6±7.3*
15(6)	20.7±4.8	0.8±0.2	98.2±16.0	139.0±40.2
60(10)	30.7±4.1	0.7±0.2	94.8±13.4	258.6±64.6
225(16)	28.8±5.0	0.7±0.2	106.0±22.0	213.9±65.0
750(17)	21.1±2.4**	0.5±0.1	112.6±14.5	316.6±41.7**

All the values expressed as means ±S.D. and the number of samples are indicated in the bracket.

* These values are significantly different from the corresponding values at 1 day of age ($P<0.001$)

** These values are significantly different from the corresponding values at 225 days of age ($P<0.001$)

that the rate of DNA accumulation (hence cell proliferation) was higher than the rate of either protein or RNA accumulation (cell size increase) during this period. From the 7th day onwards both protein/DNA and RNA/DNA increased and these adult levels were maintained upto 225 days. Between 225 and 750 days there was a small but statistically significant decrease in protein/DNA value whereas the RNA/DNA ratio showed no change (although there was an apparent difference between the values, they were not statistically significant). The absence of a difference in RNA to DNA ratio between adult and aging cerebellum was in agreement with the earlier findings with mouse cerebellum (Chaconas and Finch, 1973; Somarjski and Roisten, 1973). However, the studies on RNA/DNA ratios in different regions (except Corpus striatum) of rat brain by Shaskan (1977) showed an increase in this ratio between the adult and senescent rat brains. But, in this study the cerebellum was not analyzed while the present investigation deals exclusively with the changes in cerebellar region and it is therefore difficult to compare the two data. The decreased protein/DNA value with a simultaneous increase in total DNA content observed in the present studies (table 1) in aging cerebellum strongly suggest a rapid cell proliferating activity during this period.

Acid DNase activity per mg of DNA was highest at the earliest age period studied with a decrease of this value to a lower adult level subsequently. No change in this adult level activity could be noticed during old age (table 3). However, alkaline DNase activity expressed per mg of DNA was highest in old age (750 days). There was a significant increase in this activity between 225 and 750 days, thereby indicating that this enzyme protein is probably synthesized in larger amounts in aging cerebellum. On the basis of these results, it is tempting to speculate that acid and alkaline DNases, in particular the latter, may be playing a vital role in aging rat cerebellum. Further work could throw some light on this intriguing aspect.

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