C H A P T E R - IV

STUDIES ON MITOCHONDRIAL GLUTAMINASE ACTIVITY IN DEVELOPING RAT BRAIN

In the last few years biochemical studies have been carried out in the developing brain with an idea to obtain proper 'criteria' which can correlate the functional activity of the brain with aging. These studies have revealed changes in chemical constituents and enzymic activities in brain during development. Folch Pi (122) studied the changes in structural components of mouse brain, namely, lipids and proteins are present at birth and they increase with age by a factor of about 1.6; cholesterol and phosphatides are also present at birth and their increase with age is more marked than that exhibited by proteins and strandin, but cerebrosides and proteolipids are essentially absent upto the age of about 7 days and increase markedly and steadily upto the age of 180 days.

Brain weight of rat increases dramatically until 21 days after birth and then at a slower rate (107). Body weight on the other hand gained slowly until 14 days of life and then proceeds at a rapid rate. In rat brain the activities of succinic dehydrogenase (111) and ATPase (123) are very low in the last part of gestation, the maximum rate of increase being at about 10 days of post natal life with an adult level being reached at 30 days. Naidoo (124,125) observed an uniform rate of increase in the activity of adenosine-5-phosphatase throughout life in the rat brain. The activities of DPNase (126), carbonic anhydrase (127, 128), acetylcholine esterase (129), glutamic decarboxylase (130)

are also rises with the maturation of function of the central nervous system. During development glutamine, GABA and aspartic acid progressively increased with age. The adult level of glutamic acid and glutamine were obtained at 21 and 14 days respectively whereas GABA and aspartic acid reached this point at about 30 days (108).

Though glutamine concentration in rat brain did not change significantly between birth and adulthood, when expressed in wet weight, yet glutaminase activity assayed were all lower in new born brain than in the adult, whether expressed relative to wet weight or protein content (104). The rate of increase in activity were greatest for period between birth and 15 days. In most cases values approximately those of adult brain were reached in the first month of life (104). Glutaminase activity is mostly located in brain cortex mitochondrial fraction (109). In view of the fact that brain mitochondrial enzymic activity undergoes a change during development, morphological change may also be expected, as cerebrosides and proteolipids, which are known to undergo change during development are closely connected with the morphological phenomena of myelination (122).

It has been reported that in the guinea pig, brain weight increases most rapidly during fetal life, whereas in other species the greatest increment of brain weight occurs in postnatal

life (131). Enzymatic study of guinea pig brain by Flexner et.al. (132,133) have revealed that an adult level in most of the enzymic activities studied were attained in the latter part of gestation period. Increase in DNA content in rat brain after birth occurs but in case of guinea pig brain there is no significant difference in total DNA content after birth (134,135). The RNA content in rat brain shows nearly a five fold increase from birth to 19 days of age, after which more or less constant level is maintained. The guinea pig on the other hand shows a slight decrease in total RNA content. The slight increase in brain weight after birth may be due to further increase in cell processes while the RNA content per cell remains constant. This may explain the apparent fall in the total RNA content of the brain.

RESULTS

Glutaminase activity in presence of phosphate and different carboxylic acids in mitochondrial fraction of developing rat brain

Mitochondrial glutaminase activity in rat brain increases with development and aging, in presence of phosphate or carboxylic acids. The ratio of activities in presence of different carboxylic acids with those in presence of phosphate, increases in developing rat brain mitochondria (Table - VIII). This indicates glutaminase of rat brain mitochondria undergo change in developing brain or in other words, with development mitochondrial morphology changes to produce a secondary effect on glutaminase.

Effect of different swelling agents and 2:4 DNP on glutaminase activity of developing rat brain mitochondria

Mitochondrial swelling agents like L-thyroxine, Ca^{++}, GSH do not produce any significant difference of mitochondrial glutaminase activity between young and adult rat brain (Table - IX). Fatty acids like oleic, palmitic and myristic, however, can distinguish between young and adult rat brain mitochondria by their differential effect produced at the level of glutaminase activity. Since response of brain mitochondrial glutaminase in presence of these fatty acids is significantly different in young and adult rat brain, morphological change in mitochondria in developing rat brain may be expected.

Table - VIII

Ratio of glutaminase activity in presence of carboxylic acids and in presence of phosphate in developing rat brain mitochondria.

Age in days	Activity*				Ratio		
	0.02 M formate	0.2 M oxalate	0.02 M citrate	0.02 M phosphate	formate phosphate	Oxalate phosphate	citrate phosphate
1-2	6.6 ± 0.4	18.0 ± 0.8	19.6 ± 1.1	61.0 ± 4.2	0.11	0.30	0.32
3-8	8.5 ± 0.6	28.9 ± 1.3	35.1 ± 1.5	77.1 ± 5.4	0.11	0.37	0.45
9-14	12.7 ± 0.8	39.2 ± 2.2	49.0 ± 2.2	88.0 ± 4.6	0.14	0.45	0.56
15-21	13.8 ± 0.7	41.6 ± 2.0	56.4 ± 4.1	89.1 ± 6.2	0.15	0.47	0.63
22-30	15.2 ± 0.8	47.6 ± 3.2	63.9 ± 3.5	90.3 ± 6.8	0.17	0.53	0.71
Adult	16.5 ± 1.1	53.2 ± 2.8	70.0 ± 4.4	94.8 ± 7.2	0.175	0.56	0.74

Additions were made according to the legent to Table - IV.

Mitochondria from brains of rats of different age were used.

*Activity = μg of ammonia produced per mg of protein per hour.
Mean ± S.D. from five separate experimental preparations.

Ratio calculated on the basis of mean activity.

Table - IX

Effect of different mitochondrial swelling agents and 2:4 DNP on young and adult rat brain mitochondrial glutaminase activity

Agents	Concentration	Activity*	
		1-2 days old	Adult
Complete system -	-	68.0 ± 4.1 (100)	94.8 ± 7.2 (100)
" " plus L-thyroxine	0.2 mM	151.6 ± 6.8 (223)	213.2 ± 10.3(225)
" " " Ca^{++}	0.2 mM	95.9 ± 7.0 (141)	128.9 ± 3.8(136)
" " " GSH	10 mM	170.7 ± 9.5 (251)	208.5 ± 8.8(220)
" " " Oleic acid	0.01 mM	159.8 ± 9.1 (235)	136.6 ± 9.6(144)
" " " Palmitic "	1 mM	191.7 ± 11.5(282)	148.8 ± 7.2(157)
" " " Myristic "	1 mM	142.8 ± 8.3(210)	135.5 ± 7.4(143)
" " " 2:4 DNP	0.2 mM	36.0 ± 2.7(53)	49.0 ± 3.5(52)

The complete system contained 0.005 M L-glutamine; 0.02 M tris-HCl buffer, pH 7.4; 0.02 M Na-phosphate (pH 7.4); different concentrations of swelling agents as indicated; 0.5 ml of mitochondrial suspension containing 1 mg protein in 0.32 M sucrose and distilled water to a final volume of 3 ml. Mitochondria from 1-2 days old rat brain and adult rat brain were used. Incubation time was 60 minutes at 37°C.

*Activity = μg of ammonia formed per mg of protein per hour.
Mean ± S.D. from five separate experimental preparations.

The concentration at which maximum activation observed was used (as indicated).

Values in parenthesis indicates percent value with respect to that in complete system as 100.
Percent values are calculated on the basis of mean activity.

Fig. 15. Effect of fatty acids on glutaminase activity of young and adult rat brain mitochondria.

Control activity was designated as 100 where no fatty acid was added. Other additions were made according to legend to Table - III, containing 0.02 M phosphate to a final volume of 3 ml.

Percent activity is calculated on the basis of mean activity of five separate experimental preparations.

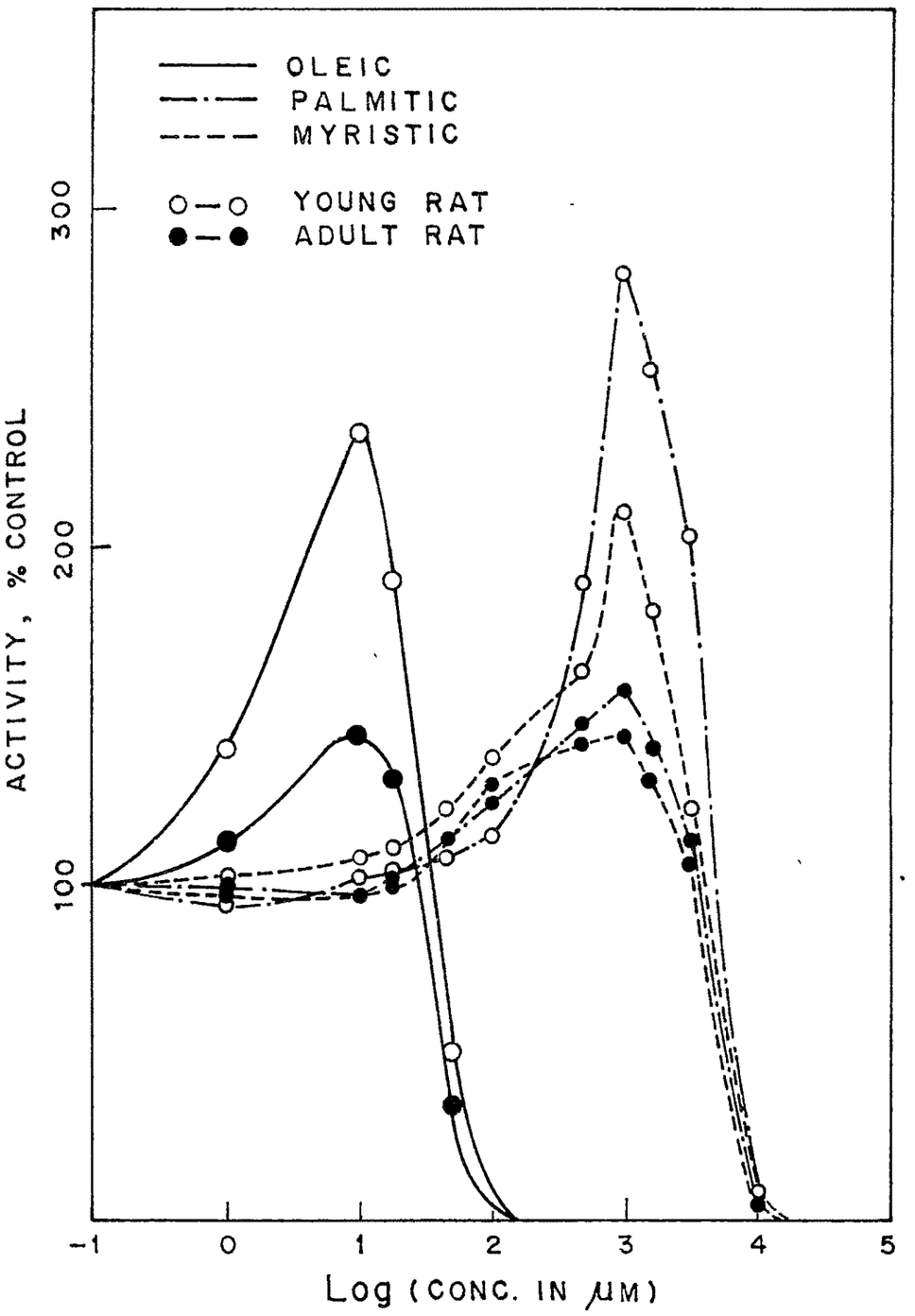
OLEIC
PALMITIC
MYRISTIC
YOUNG RAT
ADULT RAT
ACTIVITY, % CONTROL
100
200
300
-1
0
1
2
3
4
5
Log (CONC. IN μM)

Fig. 15

Fig. 16. Effect of oleic acid on glutaminase activity of aging mitochondria of young rat brain.

Control activity was designated as 100 where no oleic acid was added. Besides oleic acid other additions were made according to the legend to Table - III, containing 0.02 M phosphate to a final volume of 3 ml.

Percent activity is calculated on the basis of mean activity of five separate experimental preparations.

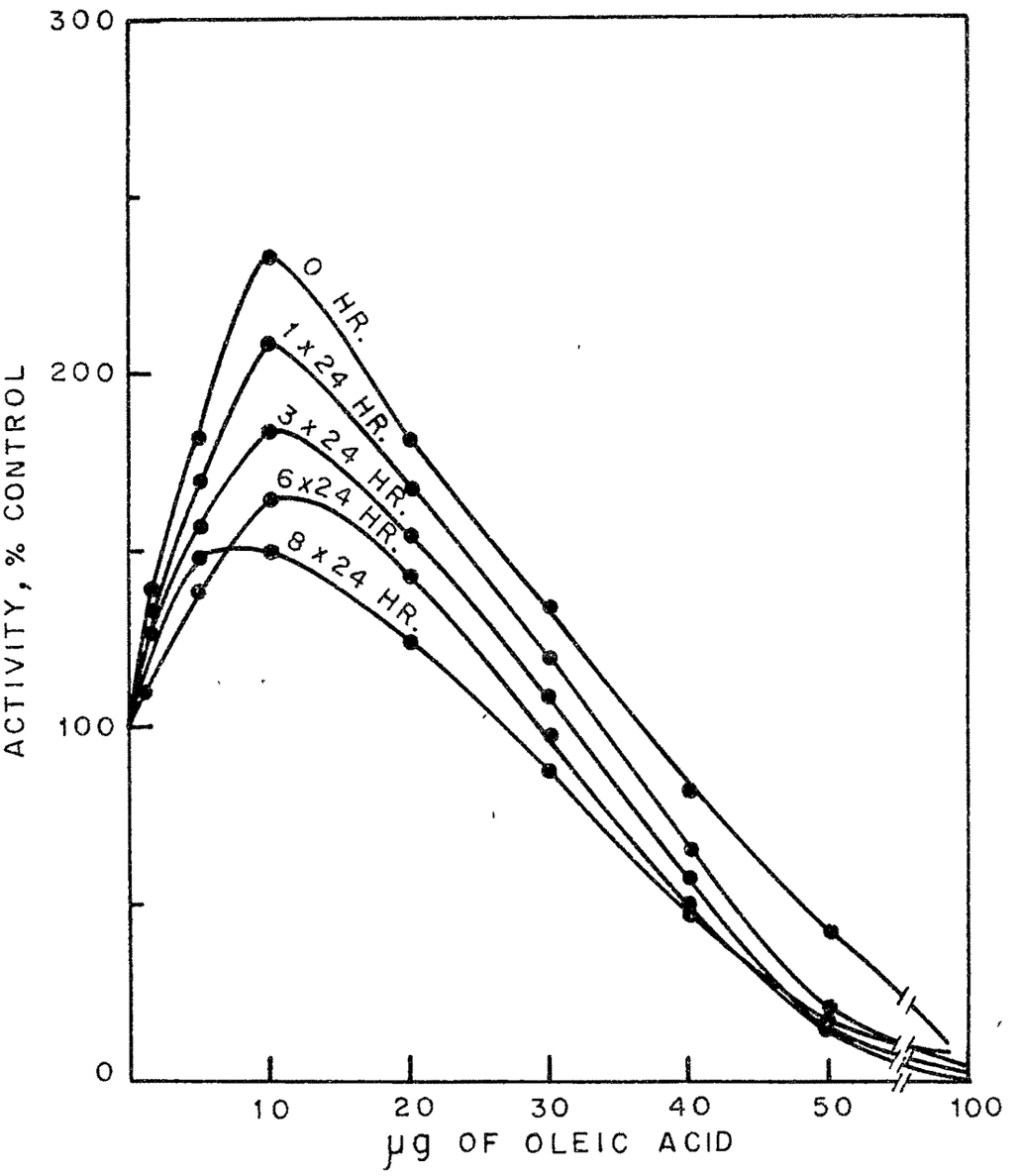
300
200
100
0
ACTIVITY, % CONTROL
0 HR.
1 x 24 HR.
3 x 24 HR.
6 x 24 HR.
8 x 24 HR.
10
20
30
40
50
100
µg OF OLEIC ACID

Fig. 16

Fatty acid induced activation of glutaminase in mitochondria of developing rat brain

Percent activation of glutaminase by fatty acids is higher in young rat brain mitochondria than that in adult rat brain mitochondria. This difference reflects functional difference of mitochondria in young and adult rat brain (Fig.15). The maturation of rat brain is characterized by a progressive increase in long chain fatty acids of cerebrosides and sulphatides (137). It is very plausible that young rat brain mitochondria should be very sensitive to fatty acid induced modification and adult rat brain mitochondria exposed to an environment in vivo where availability of long chain fatty acids is higher from the sources like cerebrosides and sulphatides, is expected to be less susceptible to fatty acid induced change.

Effect of oleic acid on glutaminase activity of aging mitochondria of young rat brain

With aging young rat brain mitochondria become less sensitive to oleic acid induced activation and percent activation diminishes from a peak value of 135% to 50% with 8 days of aging at $4^{o}C$ in 0.32 M sucrose (Fig. 16). Young rat brain mitochondria had been used as they are very sensitive to fatty acid induced activation. Percent activation of glutaminase activity by oleic acid diminishes with the aging of mitochondria which may be due to the fact that increased pool size of endogenously synthesized FFA with aging make the mitochondria less sensitive to the action of fatty acids (112, 70).

Comparative study of glutaminase activity in developing rat and guinea pig brain mitochondria

Since in guinea pig, brain weight increases most rapidly during fetal life, whereas in case of rat the greatest increment of brain weight occurs in postnatal life (107), a comparative study was made at the level of glutaminase activity in brain of those two animals with development. There is little difference in glutaminase activity between 4-5 days' old and adult guinea pig brain mitochondria. A considerable difference of glutaminase activity in developing rat brain mitochondria has already been noted (Table - IX). Another interesting finding is that the ratio of glutaminase activity in presence of a particular carboxylic acid and that in presence of phosphate, shows marked difference between young and adult rat brain mitochondria but the ratio remains almost unchanged in case of guinea pig brain mitochondria (Table - X). This indicates with development mitochondrial morphology of guinea pig brain does not undergo the change, which ~~might~~ is reflected in differential values of the ratio in young and adult brain, as in the case of rat brain.

Table - X

Glutaminase activity in brain mitochondria of young and adult rat and guinea pig

Substance	Concentration	Activity*			
		Rat		Guinea pig	
		4-5 days	Adult	4-5 days	Adult
Na-phosphate	0.02 M	77.1 ± 5.4 (100)	94.8 ± 7.2 (100)	74.2 ± 3.8 (100)	78.0 ± 4.5 (100)
Na-formate	0.02 M	8.5 ± 0.6 (11)	16.5 ± 1.2 (17)	8.9 ± 0.8 (12)	10.1 ± 0.6 (13)
Na-oxalate	0.02 M	28.9 ± 1.3 (37)	53.2 ± 2.7 (56)	22.3 ± 1.8 (30)	25.7 ± 1.7 (33)
Na-citrate	0.02 M	35.1 ± 1.5 (45)	70.0 ± 4.4 (74)	30.4 ± 2.1 (41)	35.1 ± 2.4 (45)

Additions were made according to the legend to Table - IV. Mitochondria isolated from the brains of young and adult animals were used as indicated.

*Activity = μg of ammonia formed per mg protein per hour.
Mean ± S.D. from five separate experimental preparations.

Values in parenthesis indicates percent value with respect to that in presence of phosphate as 100.
Percent activity is calculated on the basis of mean activity.

DISCUSSION

The age at which the deoxypentose nucleic acid in the brain of the rat attained its maximum value was 16 days after birth. The corresponding time, presumably those of attaining adult cell numbers were, in the guinea pig, before birth and in the rat at 2-3 weeks (101,102). This has been reflected in the activity of glutaminase in developing rat and guinea pig brain mitochondria. The ratio of activities of glutaminase in presence of phosphate and different carboxylic acids undergoes prominent change in the case of developing rat brain mitochondria but no such remarkable change was noticed in case of young and adult guinea pig.

Among the agents, which are known to stimulate glutaminase activity, investigated in the present case, only long chain fatty acids produced marked difference of glutaminase activity between young and adult rat brain mitochondria. Brain mitochondria of adult rat have been shown to be less sensitive to fatty acid induced activation as compared to that of young rat at the level of glutaminase activity. This differential response can be attributed to difference in natural cellular environment to which mitochondria were subjected in vivo, during young and adult stage.

Maturation of brain is known to be accompanied by progressive increase in long chain fatty acids of cerebrosides and sulphatides(137). Since during aging of mitochondria, a small amount of free fatty acids accumulates along with the accumulation of lysophosphatides (112), it is expected that aged mitochondria should be less susceptible to long chain fatty acid induced activation. The percent activation of glutaminase by oleic acid diminishes from 135 to 50 percent within eight days of *in vitro* aging at $4^{\circ}C$ in 0.32 M sucrose. Considering above two findings it seems that related modification of mitochondrial structure may occur either when mitochondria were aged for a short time under *in vitro* condition or by the natural changes which occur under *in vivo* condition for a longer period of time i.e. the period of development of brain to adult stage.
