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Vulnerability of Developing Brain. V. Effects of Fetal and Postnatal Undernutrition on Regional Brain Enzyme Activities in Three-Week-Old Rats

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Extract

Normal and undernourished 3-week-old rats were examined for activity of crude mitochondrial acetylcholinesterase (a marker for nerve-ending particles) in four brain areas. Littermates of these animals were used for determination of four other brain enzymes which are known to increase in activity during rat brain growth. Significant deficits (in units per gram wet weight) in crude mitochondrial acetylcholinesterase activity were found in the forebrain (normal = 2085, malnourished = 1444), brainstem (normal = 709, malnourished = 536), and olfactory lobes (normal = 74, malnourished = 46) of undernourished animals. When enzyme activities were expressed in terms of tissue wet weight, whole brain butyrylcholinesterase, fumarate hydratase, and β-galactosidase were found to be unaffected by undernutrition, whereas 5′-nucleotidase activity was higher in undernourished (730 units/g dry weight) than in control animals (660 units/g dry weight). The results lend support to the hypothesis that those constituents of the brain which show a large increase in concentration during brain growth are those most likely to be affected by growth retardation in early life.

Speculation

Undernutrition during the period of rat brain growth when interneuronal connectivity is being established may result in a deficit in the number of nerve-ending particles in the brain. This could imply that an impaired formation of synaptic connections may account for some of the functional changes which result from growth restriction at this time.

Introduction

We have previously reported [2, 3] that the development of whole brain acetylcholinesterase, EC 3.1.1.7, in suckling rats is somewhat retarded by underfeeding the mother during pregnancy and lactation. Acetylcholinesterase is often considered to be a marker for nerve-ending particles, and, by implication, synapses. However, inasmuch as only 50–60% of the brain enzyme is present in isolated nerve-ending particles [6, 12], whereas considerable activity resides in the microsomal fraction [1, 6, 20], it cannot be considered a very specific index of these structures in whole homogenates. It may be reasonable to use the enzyme activity in the crude mitochondrial fraction as a measure of the activity in nerve endings in view of the following three considerations: (*a*) nerve-ending particles sediment largely in the crude mitochondrial fraction [12, 20]; (*b*) purified mitochondria contain very little ace-

tylcholinesterase [1,6]; and (*c*) most of the enzyme activity of a crude mitochondrial fraction is recoverable in a relatively pure preparation of nerve-ending particles [6, 12].

We have examined normal and undernourished 3-week-old rats for activity of crude mitochondrial acetylcholinesterase in four brain areas. Littermates of these animals have been used for determination of four other brain enzymes which are known to increase in activity during rat brain growth: butyrylcholinesterase, EC 3.1.1.8 [14], fumarate hydratase, EC 4.2.1.2 [4], 5′-nucleotidase, EC 3.1.3.5 [6], and β-galactosidase, EC 3.2.1.23 [8].

Methods

Animals

Rats were of a hooded strain. The maternal females had successfully reared one litter. The day of mating was assessed by a daily examination of vaginal smears for sperm. Control dams were given food [21] and water *ad libitum* throughout. Undernourished dams were given the same food in restricted quantity, with water *ad libitum,* from the 7th day of gestation; the diet represented approximately 50% of the food intake of control rats and consisted of 10 g of food per day during pregnancy, followed by 15, 20, and 25 g of food daily during successive postnatal weeks of the suckling period.

All litters were reduced to eight animals (four males, four females) at birth. Any litter which subsequently numbered fewer than six was discarded. A total of five control and five undernourished litters were reared to 21 days in these experiments. All animals were then killed by an overdose of ether. In addition, a number of newborn animals were used as indicated in the footnotes to *Tables I* and *II.*

Acetylcholinesterase

Crude mitochondrial acetylcholinesterase was assayed in the brains of four animals (two males, two females) from each litter on the day of killing. Brains were separated from the spinal cord at the foramen magnum, and were dissected into brain stem, cerebellum, forebrain, and olfactory lobes. Brainstem and forebrain were separated rostral to the superior colliculi. Individual brain regions were pooled from two animals of the same sex, and a crude mitochondrial fraction was prepared [11]. Acetylcholinesterase was assayed at pH 7.4 [3], using acetylthiocholine [22] as substrate [15] and an incubation temperature of 25°.

Other Enzymes

Whole brains from the remaining animals (18 controls, 15 undernourished) were stored at −20° for 1 month. It was established in preliminary experiments that this storage does not affect the activities of the enzymes examined. On thawing, the tissue was homogenized in 0.32 M sucrose. *Fumarate hydratase* was immediately assayed [19] in Tris-HCl buffer (pH 7.4, final concentration 0.05 M) at 25°. Other enzymes were assayed after storage of the homogenates for 1–4 days. *Butyrylcholinesterase* activity was determined at 25°, using butyrylthiocholine [22] as substrate [15] in Tris–HCl buffer (pH 7.4, final concentration 0.05 M). *5′-Nucleotidase* was determined by measuring the inorganic phosphate released [16] after incubation (37°) of adenosine 5′-monophosphate (5.0 mM) with tissue in Tris-HCl buffer (pH 7.4, final concentration 0.05 M). *β-Galactosidase* was determined with *p*-nitrophenyl-β-D-galactoside as substrate, using the method previously described for β-*N*-acetylglucosaminidase [3]. The enzyme activities examined exhibited linear kinetics with respect to tissue concentration and time of incubation.

Protein was determined by the method of Lowry *et al.* [18]. The nonprotein solids were calculated by difference.

Results

Brain Weights and Gross Composition

Maternal undernutrition during pregnancy and lactation resulted in 3-week-old young having a brain weight deficit of 20–22% compared with control rats. The corresponding body weight deficit was 64%.

The cerebellum was the region of the brain most affected (Table I). When the deficits in each individual undernourished animal were expressed in terms of the mean control value, the percentage reduction in cerebellar weight was significantly ($P < 0.02$) greater than that of either the forebrain or the brainstem.

The concentration of solids was significantly less in the brains of undernourished than of control animals (Table II). When solids were divided into protein and nonprotein components, the concentration of the latter, but not the former, was significantly reduced in undernourished animals (Table II).

Acetylcholinesterase in the Crude Mitochondrial Fraction

Significant deficits in crude mitochondrial acetylcholinesterase activity (expressed per gram wet weight of tissue) were found in the forebrain, brainstem, and

Table I. Acetylcholinesterase (ACE) activity in the crude mitochondrial fractions[1]

	Control at birth[2]	Control at age 21 days[3]	Undernourished at age 21 days[3]	Percentage deficits in ACE activity in undernourished[4]
Body wt, g	5.7	45.7 ± 5.4	16.5 ± 2.9	−64%[5]
Brain wt, g	0.227	1.435 ± 0.034	1.153 ± 0.034	−20%[5]
Forebrain				
Wt, g	0.1378	0.9501 ± 0.0237	0.7783 ± 0.0701	−18%[5]
ACE, units/g wet wt	196	2193 ± 118	1843 ± 253	−16%[5]
ACE, units/region	27.0	2085 ± 125	1444 ± 279	−31%[5]
Cerebellum				
Wt, g	0.0123	0.1905 ± 0.0074	0.1357 ± 0.0212	−29%[5]
ACE, units/g wet wt	258	716 ± 156	634 ± 121	−11%[6]
ACE, units/region	3.0	136.5 ± 29.3	84.5 ± 13.6	−38%[5]
Brainstem				
Wt, g	0.0808	0.2394 ± 0.0047	0.1960 ± 0.0131	−18%[5]
ACE, units/g wet wt	426	2965 ± 179	2742 ± 123	−8%[7]
ACE, units/region	34.4	709.5 ± 42.7	536.4 ± 26.4	−24%[5]
Olfactory lobes				
Wt, g	0.0058	0.0547 ± 0.0035	0.0430 ± 0.0075	−20%[5]
ACE, units/g wet wt	174	1366 ± 156	1061 ± 156	−22%[5]
ACE, units/region	1.0	74.7 ± 11.4	46.0 ± 12.5	−38%[5]

[1] One unit of enzyme activity = 1 mμmole substrate consumed/min.

[2] Based on 12 animals from 2 litters. Brain regions from animals of each litter (three males, three females) were pooled for assay. Results are expressed as the mean of two litters.

[3] Based on 10 litters, 5 control and 5 undernourished, with 4 animals (2 male, 2 female) from each. Brain regions from animals of the same sex of each litter were pooled. Since equal numbers of males and females were used, results were pooled for statistical analysis and are expressed as mean ± SD.

[4] Significance tests compare data in control and undernourished at 21 days of age.

[5] $P < 0.001$.

[6] Not significant. [7] $P < 0.01$.

olfactory lobes of undernourished animals when compared with control animals (Table I). Dry weight determinations on forebrain permitted expressing the enzyme activity in terms of tissue dry weight. Expressed in this way, activity remained significantly less ($P < 0.001$) in the undernourished animals.

Undernutrition resulted in significant deficits in crude mitochondrial acetylcholinesterase activity per whole region in the four regions examined (Table I). Deficits were greatest in the cerebellum and olfactory lobes.

Other Enzymes in Whole Brain

Other enzyme activities were expressed in terms of wet weight, tissue protein, and dry weight (Table II). Differences between undernourished and control 3-week-old animals were found only for fumarate hydratase and 5′-nucleotidase activity. For the former, enzyme activity per gram dry weight was higher in the undernourished group. 5′-Nucleotidase activity expressed in terms of any of the three parameters was slightly but significantly raised in the brains of undernourished animals. The results on 5′-nucleotidase differ somewhat from those of Banik and Davison [6] who found lower enzyme activity at 21 days of age but a 3.5-fold increase from 12 to 21 days of age. This is compared with a much smaller increase between birth and 21 days (Table II) and suggests that the enzyme activity may pass through a trough. It should be noted, however, that the assay systems differed in that potassium and magnesium ions were omitted in the present study.

Discussion

It is well known [17] that there are large regional variations in acetylcholinesterase activity throughout the brain. This may represent the varying importance of cholinergic neurons relative to other types. It is apparently possible to distinguish cholinergic nerve endings from noncholinergic nerve endings [12] as the latter contain negligible amounts of acetylcholine or acetylcholinesterase. We suggest that acetylcholinesterase in a crude mitochondrial fraction may be used as an approximate marker for nerve endings, or, more probably, cholinergic nerve endings.

Table II. Activity of four enzymes[1] in whole brains of control and undernourished 3-week-old animals and of control animals at birth

	Control at birth[2]	Control at age 3 wk[3]	Undernourished at age 3 wk[3]
Body wt, g	5.0 ± 0.8	47.2 ± 6.2	16.9 ± 3.3[4]
Brain wt, g	0.202 ± 0.025	1.450 ± 0.053	1.130 ± 0.106[4]
Brain solids, mg/g wet wt	127.0 ± 2.0	185.8 ± 3.1	178.6 ± 6.7[4]
Brain nonprotein solids, mg/g wet wt	43.0 ± 4.8	84.5 ± 3.9	79.7 ± 4.3[5]
Brain protein, mg/g wet wt	83.9 ± 4.8	100.8 ± 2.1	99.1 ± 3.3
Butyrylcholinesterase			
Units/g wet wt	5.50 ± 0.40	15.14 ± 0.96	14.73 ± 1.28
Units/100 mg protein	6.63 ± 0.63	15.04 ± 1.12	14.86 ± 0.96
Units/g dry wt	43.3 ± 2.5	81.8 ± 5.1	82.4 ± 5.5
Fumarate hydratase			
Units/g wet wt	95 ± 5	255 ± 16	258 ± 15
Units/100 mg protein	113 ± 8	253 ± 18	260 ± 12
Units/g dry wt	758 ± 63	1377 ± 76	1442 ± 65[6]
5′-Nucleotidase			
Units/g wet wt	77.0 ± 3.8	122.8 ± 9.3	130.4 ± 7.3[6]
Units/100 mg protein	92.0 ± 7.2	122.2 ± 8.4	131.6 ± 6.5[5]
Units/g dry wt	607 ± 27	660 ± 53	730 ± 41[4]
β-Galactosidase			
Units/g wet wt	4.32 ± 0.39	7.93 ± 0.63	7.75 ± 0.83
Units/100 mg protein	5.13 ± 0.58	7.86 ± 0.61	7.83 ± 0.86
Units/g dry wt	33.8 ± 3.1	42.7 ± 3.5	43.5 ± 4.8

[1] One unit of enzyme activity = 1 μmole of substrate consumed/hr.
[2] Based on six animals (three male, three female), each from a different litter. Results are expressed as mean ± SD.
[3] Based on 18 control animals (11 male, 7 female) and 15 undernourished (7 male, 8 female) animals. Results are expressed as mean ± SD.
[4] $P < 0.001$.
[5] $P < 0.01$.
[6] $P < 0.05$.

We have found that undernutrition results in deficits in the crude mitochondrial acetylcholinesterase activity of brainstem, forebrain, and olfactory lobes. If the enzyme activity in the crude fraction predominantly represents synaptosomal acetylcholinesterase, this might suggest a decreased concentration of nerve endings, or, alternatively, a normal complement of nerve endings with each having less enzyme activity.

This result should be considered in relation to a recent histologic study [7] of the effects of neonatal undernutrition on the rat somatosensory cortex in which reduced formation of synapses was reported. It has also been shown that neonatal hypothyroidism, some of whose effects on the developing brain are similar to those of undernutrition, causes a deficit in the activity of glutamate decarboxylase, an enzyme located in nerve endings [5], whereas lactate dehydrogenase (cytoplasmic) and glutamate dehydrogenase (mitochondrial) are unaffected.

It is at first surprising that undernutrition had no effect on the cerebellar acetylcholinesterase activity since, in terms of weight, the cerebellum was more affected than any other region. On the other hand, the absolute enzyme activity was relatively low in the cerebellum (at 3 weeks), and showed a relatively small increase (approximately 3-fold) during the first 3 weeks of life. This might support the hypothesis that the vulnerability of the levels of a brain constituent to alteration by undernutrition may be related to the degree of increase of that constituent during the brain growth spurt [13]. For example, the increase in acetylcholinesterase activity in the first 21 days (6–10-fold) is large in three of the four regions, excluding the cerebellum, and only in these regions is there substantial reduction caused by undernutrition. By contrast, butyrylcholinesterase, fumarate hydratase, and β-galactosidase show only moderate developmental increase (2–3-fold) and these are all unaffected by undernutrition. 5′-Nucleotidase, which normally has a comparatively small increase at this time, even shows *excess* activity in the undernourished brain.

The finding that undernutrition during development results in a reduced concentration of nonprotein solids (presumably largely lipid), whereas the protein concentration is unaffected, is consistent with the findings of Culley and Mertz [10]. Those workers found

that in brain the concentration of total lipid, but not the concentration of nonlipid solids, was adversely affected by undernutrition. These results may also be considered in terms of the possible vulnerability of substances whose concentrations increase most markedly during brain growth. Whereas nonprotein solids increased in concentration by 97% between birth and 3 weeks, the protein concentration increased by only 20% during this same period.

In general, the results confirm [3, 9] that undernutrition in early life may alter the enzymic composition of the brain of the young rat. The changes are, however, relatively small, and it is at present impossible to state whether they may have functional significance.

Summary

Fetal and postnatal undernutrition resulted in changes in enzyme activities, but not in total protein concentration, in the brains of 3-week-old rats. Crude mitochondrial acetylcholinesterase activity was less in three regions of the brain of undernourished animals than in control animals, whereas the undernourished group had higher whole brain 5′-nucleotidase activity. Fumarate hydratase activity also increased in the undernourished animals when expressed in terms of tissue dry weight, although it was unchanged in terms of wet weight. The results lend support to the hypothesis that those constituents of the brain which show a large increase in concentration during brain growth are those most likely to be affected by growth retardation in early life.

References and Notes

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