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Effects of early undernutrition on kinetic parameters of brain acetylcholinesterase from adult rats

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Abstract. The effects of undernutrition during early life on acetylcholinesterase (AChE) specific activity of cerebellum, hippocampus, hypothalamus and striatum were examined in rehabilitated adults rats. Undernourished rats were raised by dams maintained on a restricted food scheme from pup birth to pup weaning (day 31). The offspring were maintained on a restricted food schedule until day 38 when they started to have free access to food until 70-80 days of age. Control rats were raised by dams which had free access to food. The results showed that early malnutrition caused a significant increase in AChE specific activity in cerebellum (about 20%), striatum (about 40%), and hypothalamus (about 30%). No changes were found in the hippocampus. Undernutrition caused a significant increase in Vmax when compared to the control group without changes in Km both in cerebellum and striatum. These results suggest that early undernutrition changes AChE concentration in cerebellum and striatum and does not affect the affinity of the enzyme for the substrate.

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INTRODUCTION

Undernutrition may cause developmental, behavioural and neurochemical alterations in young animals (Frankova 1972, Morgane et al. 1978, Smart 1983, Crnic 1983, Wiggins et al. 1984, Represa et al. 1989, Rocha et al. 1990, Rocha et al. 1991). Various studies have demonstrated that perinatal undernutrition may permanently affect the activity of various enzymes involved in CNS functions (Morgane et al. 1978, Wiggins et al. 1984, Represa et al. 1989, Rocha et al. 1991). Among these enzymes, AChE has received exceptional attention. Perinatal undernutrition seems to cause an increase in the enzyme activity of adult rats (Adlard and Dobbing 1971, 1972, Eckhert et al. 1976a, 1976b, Im et al. 1976, Villescas et al. 1981). Using rats undernourished during gestation and lactation by reducing food availability to dams, Adlard and Dobbing (1972) reported an increase in forebrain and cerebellum AChE activity in nutritionally rehabilitated rats and in rats undernourished until enzyme assay. Using a low protein diet as the method to induce malnutrition, Eckhert et al. (1976a) reported that forebrain enzyme activity was lower in rats undernourished during gestation and rehabilitated for 7 weeks than in control animals. In contrast, forebrain enzyme activity of 49-day-old rats was unaltered by undernutrition during the suckling period (Eckhert et al. 1976a).

Although some studies have proposed that changes in AChE activity depend on nutritional deprivation, few of them have analyzed the effects of perinatal undernutrition on regional brain AChE activity (forebrain, brain stem, cerebellum, and cerebral cortex) of adult rats (Eckhert et al. 1976a, Villescas et al. 1981). In these studies, undernutrition was produced by giving dams a low protein diet. Different methods used to induce undernutrition alter maternal behaviour and other non-nutritional variables in distinct ways (Crnic 1983, Smart 1983, Rocha and Vendite 1990). This may help to explain discrepancies of some behavioural and biochemical studies (Wiggins et al. 1984). Another aspect that has not been assessed is whether alterations in AChE activity are a consequence of changes in Vmax and/or Km of the enzyme in undernourished animals. The aim of the present study was to examine the effects of undernutrition caused by reducing the quantity of food offered to dams and offspring during suckling and the early postweaning period on AChE activity in various brain regions (striatum, cerebellum, hippocampus and hypothalamus) in nutritionally rehabilitated adult rats. The kinetic parameters were examined in the cerebellum and striatum.

METHODS

Wistar-derived rats from our breeding stock were maintained in an air-conditioned room (16-20°C) on a natural light cycle. The breeding regimen consisted of grouping 3 virgin females (80-120 days of age) with one male for 20 days. After this period, pregnant rats were selected and housed individually in opaque plastic cages (48 x 22 x 18 cm). On the day of birth (day 0), litters were adjusted to 9 pups and half the dams were assigned at random to one of the nutritional groups. Control dams had free access to commercial pelleted rat chow (Guabilab, RS, Brazil) containing 20.5% protein (predominantly soybean), 54% carbohydrate, 4.5% fiber, 4% lipid, 7% ash and 10% moisture. Tap water was available to all rats. After birth (day 0), the following schedule of food availability was applied to the undernourished dams: from day 1 to 7 they received approximately 10 g/day of the rat chow, from day 8 to 14, 15 g/day, and from day 15 to 22, 20 g/day. From day 23 to 30 undernourished dams received food according to the number of pups they had (25 g/day plus approximately 3 g for each pup). This represents about 40% of the food ingested by control dams. Rats were weaned on day 31. Litters consisting of less than 7 pups were discarded. After weaning, the undernourished pups received approximately 5 grams of chow per day until day 38 when they started to have free access to food.

Since early handling may interfere with the effects of perinatal undernutrition on AChE activity of adult rats (Im et al. 1976), the animals designed for assays of enzyme activity were undisturbed from birth to weaning. To assess the growth of offspring and the general condition of undernourished dams, another set of rats was used and weighed on days 1, 12, 23 and 38. A total of 12 dams and litters were used (7 control and 5 undernourished).

Male offspring of both groups (70-80 day-old) were anesthetized with ether and decapitated. A total of 16 rats (8 in each nutritional group) were used for AChE activity and 8 rats (4 in each nutritional group) for dry weight and water content determinations. The brains were quickly removed, placed in ice and weighed. Hypothalamus, hippocampus, striatum and cerebellum were dissected on ice and homogenized (15 strokes at 1500 rpm) in 10 volumes of medium containing 0.32 M of sucrose and 10 mM Tris-HCl buffer, pH 7.5. The homogenates were stored at -20° C up to 15 days since no loss in enzyme activity was observed. AChE was determined by a modification of the method of Ellman et al. (1961) as described by Villescas et al. (1981), in the presence of 0.025 mM promethazine (10-(2-dimethyl amino propyl) phenothiazine-HCl) as a selective inhibitor of butyrylcholinesterase. The kinetic parameters (Vmax and Km) were determined at 5 to 9 different acetylthiocholine (AcThCh) concentrations ranging from 0.02 to 0.50 mM, and calculated using the Eadie-Hofstee plot. The enzyme assays were carried out at 25°C for 2 to 5 min depending on the brain area. All samples were run in duplicate or triplicate.

The dry weight and water content of brain regions of both groups was also determined. Wet brain regions were weighed and then maintained at 100°C until a constant weight was reached. Protein was determined by the method of Bradford (1976) with Comassie blue, using bovine serum albumin as standard.

Data were analyzed by multivariate analysis of variance (MANOVA) because the brain regions (striatum, hypothalamus, hippocampus and cerebellum) used for tissues wet and dry weight, protein content, and AChE activity determination were obtained from the same animal. A follow-up one way analysis of variance was conducted when a significant effect was observed. To avoid a litter effect (Abbey and Howard 1971, Denenberg 1984), we never used more than one rat from a given litter for enzyme or dry weight determination. Thus from each litter 7 to 8 pups were discarded and used for purposes not related to the present research. In the case of pup body weight, the mean of the litter was used in the analysis.

RESULTS

The body weights of control and undernourished dams and offspring are shown in Table I. On day 12 after delivery, undernourished dams had a body weight deficit of about 25% with no further change. On day 12 offspring raised by undernourished dams

TABLE I

Effects of food restriction on body weights (g) of lactating dams and pups

Day	Control		Undernourished	
	Pups	Dams	Pups	Dams
0	5.1±0.6	235.0±12.8	5.3±0.9	248.0±20.3
12	22.6±1.7	238.7±15.9	12.2±1.9*	191.3±12.5*
23	50.6±5.3	235.8±11.9	17.1±1.8*	187.4±14.3*
38	91.9±9.7	230.7±12.5	35.6±3.2*	189.7±13.7*

Mean ±SD of 7 (control) and 5 (undernourished) dams and litters. *$P<0.05$ compared to respective control by one way analysis of variance.

TABLE II

Wet and dry weight (in mg), water content (in %) and protein content (mg/g) of brain regions of control and undernourished adult rats (70 to 80 days)

Brain region	Control				Undernourished			
	wet weight	dry weight	water content	protein content	wet weight	dry weight	water content	protein content
cerebrum	1291.6±25.7				1104.4±44.0*			
cerebellum	301.6±9.1	63.0±2.9	79.3±0.2	63.0±6.8	238.9±19.3*	48.3±8.8*	79.9±0.9	65.1±5.1
striatum	162.4±17.9	31.7±1.9	77.4±0.3	48.8±6.0	132.3±8.6*	23.7±1.3	80.2±1.5	53.6±4.2
hippocampus	99.3±15.1	15.3±2.4	83.9±7.5	62.4±10.1	84.9±7.6$^{\#}$	13.0±0.3$^{\$}$	82.4±1.1	65.5±12.2
hypothalamus	36.9±6.4	4.9±2.2	84.0±1.4	37.8±4.7	34.7±5.0	36.9±6.4*	85.8±2.3	38.6±9.9

Mean ±SD of 12 (weights), 8 (protein content) and 4 (dry weight and % water content) animals. The body weight of control and undernourished rats were 321.3±11.9 and 234.2±9.4, respectively. *$P<0.01$, $^{\#}P<0.01$ and $^{\$}P<0.05$ when compared with respective value of controls by one way analysis of variance.

showed a 45% deficit in body weight. This deficit increased to about 65% on days 23 and 38. During adulthood (70 to 80 days), previously undernourished rats showed 27% deficit in body weight, suggesting that rehabilitation was only partial.

Table II shows the wet weight, the dry weight, the water content and protein content of brain regions in control and undernourished rats. Multivariate analysis of variance (MANOVA) of wet weight revealed a significant main effect of diet ($F(1,22)=87.3$, $P<0.01$) and diet x brain area interaction ($F(3,20)=34.3$, $P<0.01$). The wet weight of brain and brain areas of adult undernourished rats were reduced from 15 to 22%, excepting the hypothalamus. MANOVA of dry weight revealed a significant main effect of diet ($F(1,6)=16.8$, $P<0.01$) and a diet x brain area interaction ($F(3,4)= 13.3$, $P<0.01$).These results were identical to those obtained for wet weight data and were a consequence of the similar water content in brain of both nutritional groups. MANOVA of regional protein concentration demonstrated that nutritional treatment had no effect on this parameter.

Table III shows the AChE specific activity of four cerebral areas in control and undernourished rats. MANOVA revealed a significant effect of diet ($F(1,14)=98.73$, $P<0.01$) as a consequence of enhanced AChE activity in all brain regions examined of undernourished rats. MANOVA also revealed a significant effect of brain region ($F(3,12)=21.42$,

TABLE III

Brain AChE activity of control and undernourished adult rats (70- to 80-day-old)

	Control		Undernourished	
	Specific activity	Total activity	Specific activity	Total activity
cerebellum	1.19±0.11	22.48±1.97	1.42±0.14$^{\$}$	21.40±2.02
striatum	8.89±0.81	70.94±4.66	12.90±2.36*	82.82±7.18*
hippocampus	2.56±0.43	15.56±1.84	2.95±0.64	16.52±3.92
hypothalamus	2.60±0.43	3.68±0.39	3.48±0.49$^{\$}$	4.16±0.40^{+}

Mean ±SD for 8 animals. Specific activity is expressed as µmoles AcThCh hydrolysed/h/mg protein and total activity as µmoles AcThCh hydrolysed/h/brain area. $^{+}P<0.05$, $^{\$}P<0.005$ and *$P<0.001$ when compared with the respective value of controls by one way analysis of variance.

TABLE IV

Kinetic parameters for strial and cerebellar AChE of control and undernourished adult rats (70- to 80-day-old)

	Control		Undernourished	
	Km	Vmax	Km	Vmax
cerebellum	77.6	1.59	85.0	1.98$^{\#}$
	(62.0-93.1)	(1.45-1.73)	(66.6-103.8)	(1.82-2.09)
striatum	66.3	13.5	64.8	17.9^{+}
	(27.5-105.1)	(10.3-16.6)	(29.2-100.4)	(15.7-20.1)

Km is expressed as μM concentration of AcThCh. Vmax is expressed as μmoles AcThCh hydrolysed/h/mg protein. Values in parentheses correspond to 95% confidence limits for 4 (cerebellum) or 5 (striatum) animals. $^{\#}P<0.01$ and $^{+}P<0.05$ when compared with controls by one way analysis of variance.

$P<0.01$) mainly due to the higher AChE activity in striatum than in other areas. Furthermore, the diet x brain region interaction was also significant ($F(3,12)=19.45$, $P<0.01$), resulting from the differences in the effect of undernutrition as a function of cerebral region. The follow-up univariate analysis of variance (ANOVA) indicated that undernutrition increased AChE activity by about 45% in the striatum ($F(1,14)=32.04$, $P<0.01$), by about 30% in the hypothalamus ($F(1,14)=16.51$, $P<0.01$), by about 20% in the cerebellum ($F(1,14)=13.59$, $P<0.01$), and by about 15% in the hippocampus ($F(1,14)=2.01$; NS). The total AChE activity for each region is presented in Table III. MANOVA revealed a result similar to that obtained with specific activity. However, the follow-up univariate F-tests demonstrated that in cerebellum ($F(1,14)=0.19$; NS) and hippocampus ($F(1,14)=0.68$; NS). The total activity was similar in control and undernourished rats, while in striatum ($F(1,14)=8.46$; $P<0.01$) and hypothalamus ($F(1,14)=5.97$; $P<0.05$) the total activity was higher in undernourished animals.

The Eadie-Hofstee plot (Fig. 1) showed that in the striatum and cerebellum of undernourished rats there is an increase in Vmax without a change in Km (Table IV). For the striatum, the increase in Vmax

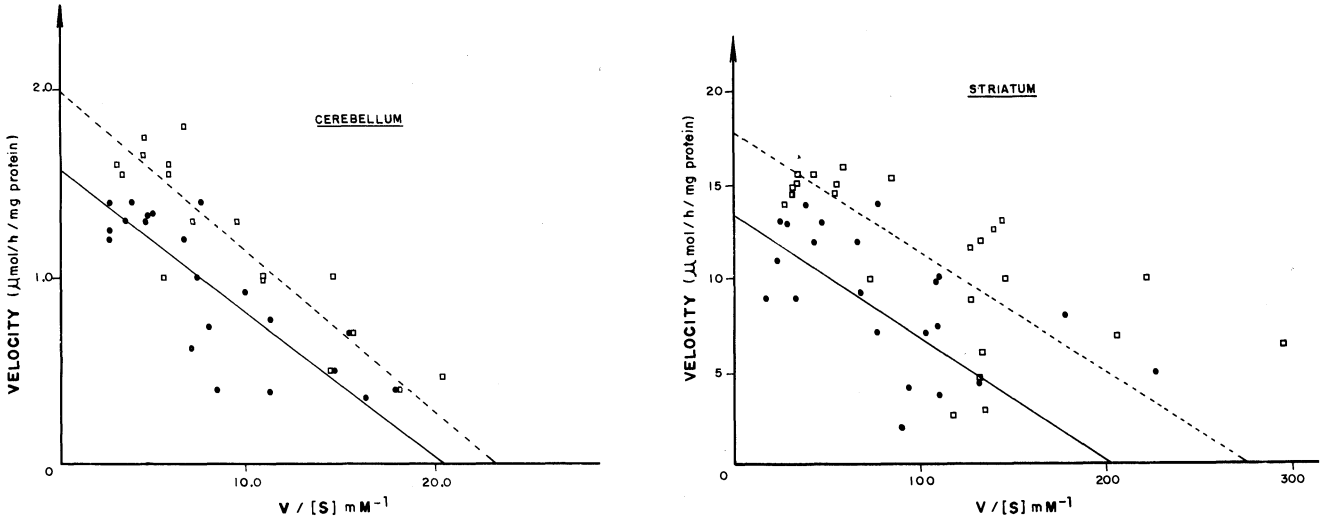

Fig. 1. Eadie-Hofstee plot of AChE activity from striatum and cerebellum of control (continuous line) and undernourished (broken line) rats.

was about 30% ($P<0.05$); and for the cerebellum about 20% ($P<0.01$).

DISCUSSION

The major objective of the present study was to examine the effects of undernutrition on AChE activity and to determine the kinetic parameters in brain regions which presented enhanced enzyme activity. The method for producing undernutrition used in the present report was similar to those used by various investigators (Adlard and Dobbing 1972, Smart 1983). However, the nutritional deprivation was more severe since the restricted food offered to malnourished dams amounted only to about 40% of the intake of control dams and resulted in the loss of about 25% of litters. During adulthood, undernourished rats showed a 27% deficit in body weight, suggesting that rehabilitation was only partial. This result is in agreement with previously published data (Adlard and Dobbing 1972, Im et al. 1976, Rocha et al. 1991) and indicates that the body weight deficit of rats undernourished during critical stages of development is long-lasting.

Undernourished animals showed an increase in AChE specific activity in cerebellum, hypothalamus and striatum that ranged from 20% to 40%. The increase in specific activity detected in cerebellum is rather similar to that reported in the literature for adult rats undernourished during the perinatal period (Adlard and Dobbing 1972, Villescas et al. 1981). As regards the other brain regions, to our knowledge, such studies have not been performed previously.

It has been hypothesized that the increase in specific activity could be a consequence of a reduced brain water content. However, a similar water content was found in brain regions of rats from both nutritional groups. Furthermore, the increase in specific activity could depend on a reduction of protein concentration in brain areas. However, the decrease in protein content in undernourished rats was proportional to the reduction in brain region weight. This is in agreement with data reported by others (Winick and Noble 1966, Crnic 1983).

In the present report, we show that the increase of AChE in cerebellum and striatum does not depend on changes in Km, but on an increase in Vmax. This may indicate an increase in the number of enzyme molecules. The phenomenon is somewhat similar to that reported for receptors of some brain neurotransmitters. In fact, a decrease or increase in binding capacity is due to changes in the density of binding sites (Bmax) rather than in affinity for the ligand (Wiggins et al. 1984). The overall data indicate that undernutrition may cause changes in concentration/density of macromolecules such as enzymes and receptors. It should also be considered that, in spite of an increase of AChE specific activity in the cerebellum, striatum and hypothalamus, the total enzymatic activity was only slightly increased in the striatum and hypothalamus. This suggest that the total capacity for hydrolyzing acetylcholine per region is somewhat less affected by undernutrition. The interpretation of the data is rather difficult, especially as regards the question whether the increase in specific activity of AChE represents a compensatory mechanism. The functional consequences of such an increase are unknown, but they may be related to behavioral abnormalities observed in rats following food deprivation during critical stages of brain development.

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