Concentrations of Histamine in Brain of Guinea Pig and Rat during Dietary Protein Malnutrition

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Protein malnutrition produced marked elevations in brain contents of histamine in guinea pigs (+142%) and rats (+257%) in comparison with findings in the control adequately fed animals. Associated with accumulation of this biogenic amine in each set of animals was an increase of similar magnitude in brain content of free histidine. It was concluded that the elevated brain content of histamine in protein malnutrition was due mainly to increased availability of the precursor amino acid, and that such alterations in amounts of neurotransmitter amines in the central nervous system might underlie some of the behavioural abnormalities seen in malnourished animals.

Defects in hepatic metabolism of histidine have been observed in protein-calorie-deficient children (Whitehead, 1964) and in experimental animal prototypes of the human nutritional syndrome (Rao *et al.*, 1965). The major degradative routes of histidine in the liver are the urocanic acid and the imidazolyl-pyruvic acid pathways, which do not appear to exist in the brain (Haavaldsen, 1962). Histidine crosses the blood-brain barrier more readily than do most other amino acids (Neame, 1964), and many studies have demonstrated a prominent increase in brain concentration of histidine in protein-deficient animals (Lajtha, 1968; Enwonwu & Glover, 1973; Enwonwu & Worthington, 1973, 1974). The primary metabolic routes of histidine in the brain are utilization of the amino acid for protein biosynthesis, formation of oligopeptides such as homocarnosine (γ -aminobutryl-L-histidine) and decarboxylation to histamine by a specific histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22) (Aures *et al.*, 1970; Schwartz *et al.*, 1970; Taylor & Snyder, 1972). Recent studies in our laboratory have shown a profound increase in brain content of homocarnosine in non-human primates fed on a low-protein diet (Enwonwu & Worthington, 1973, 1974).

Histamine is a pharmacologically active substance (Snyder & Taylor, 1972) and is involved in several behavioural phenomena (Aures *et al.*, 1970; Gerald & Maickel, 1972). In view of the well-documented behavioural changes often associated with protein malnutrition (Barnes, 1971; Platt & Stewart, 1971), it was decided to examine in detail the metabolic fate of the increased brain concentration of histidine found in protein-deficient animals. Results of the present study showed that, during protein malnutrition, marked elevations in brain concentrations of histamine occurred in both guinea pigs (+142%) and rats (+257%) in comparison with findings in pair-fed control animals of the same sex and age.

Materials and methods

Materials. Histamine (free base) and *o*-phthalaldehyde were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). All reagents used in the study were of analytical grade.

Animals and diets. Male Hartley guinea pigs (180–230 g) and Sprague-Dawley rats (100–250 g) were obtained from Simonsen Laboratory (Gilroy, Calif., U.S.A.) and housed in animal quarters equipped with regulated lighting. Lights were on between 06:00 and 18:00 h. Each set of animals was randomized into two groups and either fed on a low-protein diet *ad libitum* or pair-fed on an adequate-protein diet. For the guinea pigs, the protein-deficient diet contained 3% (w/w) casein, and the low-protein diet supplied to the rats contained 0.5% (w/w) lactalbumin. Details of the compositions of the various synthetic, adequate and deficient diets used in the studies have been reported in previous papers (Enwonwu, 1973a; Enwonwu & Sreebny, 1971). Drinking water was made constantly available to the animals, and for the guinea pigs the water was supplemented with ascorbic acid (500 mg/litre).

Assay of brain histamine. Each animal was killed by a sharp blow on the back of the neck, without any anaesthesia. Prompt removal of the whole brain was accomplished in a refrigerated room (0–4°C). In an attempt to eliminate possible complications resulting from diurnal alterations in brain histamine content (Friedman & Walker, 1968), the animals were killed at essentially the same time of the day (11:00–12:30 h) in each study. Spectrofluorimetric determination of histamine, after coupling of the amine with *o*-phthalaldehyde and subsequent purification to eliminate other *o*-phthalaldehyde-reactive substances such as spermidine, was by the method described by Håkanson *et al.* (1972). By this procedure, recovery of authentic histamine added

to brain extracts varied from 86 to 95% (C. O. Enwonwu, unpublished work). Fluorescence readings were made (in quartz micro-cells) in a Perkin-Elmer

Fig. 1. Typical excitation spectra of extract from rat brains

Histamine was extracted from 0.34g wet wt. of whole brain from protein-deficient (1) and control (2) rats. ----, Blank reading. The fluorescence intensity is given relative to histamine content of 0.34g wet wt. of whole brain.

spectrophotofluorimeter (model MPF-2A; Perkin-Elmer, Norwalk, Conn., U.S.A.) equipped with a Hitachi recorder (model QPD-33).

Determination of free histidine. Preparation of protein-free extract from brain for assay of free amino acids was as previously reported (Enwonwu, 1973b) and the chromatographic analysis was performed with a Beckman Automatic Analyser (model 121) with DC-2A resin (Durrum Instruments, Palo Alto, Calif., U.S.A.) in the short column.

Results

The concentrations of histamine in the brains of guinea pigs and rats were assayed after 3 and 7 weeks respectively on the low-protein diets. Previous studies (Enwonwu, 1973a; Enwonwu & Sreebny, 1970, 1971) have shown that these intervals of time are adequate for successful reproduction of some of the features associated with the human syndrome of protein-calorie malnutrition. Fig. 1 shows the typical excitation spectra of extracts from the same amount of brain tissue (0.34g wet wt.) in well-fed and malnourished rats. Findings in several rats and guinea pigs are summarized in Table 1. In both sets of animals, protein malnutrition produced no significant alteration in brain weight. Marked and significant elevations in brain histamine concentrations were observed in the protein-deficient guinea pigs (+142%) and rats (+257%) compared with values in the control animals (Table 1). Associated with the alterations in amounts of histamine were equally prominent increases in brain concentrations of the precursor amino acid, histidine.

Discussion

The concentrations of free histidine in the brains of control guinea pigs and rats noted in the present study (Table 1) were within the range of values

Table 1. Concentrations of histamine and histidine in brains of guinea pigs and rats

The protein-deficient guinea pigs received a 3%-casein diet *ad libitum* for 18–21 days, and the protein-deficient rats were fed on a 0.5%-lactalbumin diet for about 7 weeks. The control animals were pair-fed on adequate protein diets. Other details were as described under 'Materials and methods'. Values are expressed as means $\pm$ S.E.M., and the entries in parentheses indicate percentages of corresponding values for the well-fed control animals.

	Guinea pigs		Rats	
	Control (n = 12)	Protein-deficient (n = 11)	Control (n = 8)	Protein-deficient (n = 12)
Brain weight (g)	3.18 $\pm$ 0.05	3.10 $\pm$ 0.13	1.83 $\pm$ 0.05	1.77 $\pm$ 0.08
Histidine (μ mol/g)	0.07 $\pm$ 0.02	0.16 $\pm$ 0.01 (207) [†]	0.08 $\pm$ 0.05	0.30 $\pm$ 0.02 (391)*
Histamine (ng/g)	62.0 $\pm$ 2.7	150 $\pm$ 5 (242)*	56.1 $\pm$ 4.6	200 $\pm$ 6 (357)*

* Value significantly different from corresponding control, $P < 0.001$.

[†] Value significantly different from corresponding control, $P < 0.005$.

reported by other investigators (Perry & Hansen, 1973; Levi *et al.*, 1967). The rate of formation of histamine in the brain is almost solely dependent on the activity of a specific L-histidine carboxy-lyase (Taylor & Snyder, 1972; Aures *et al.*, 1970; Schwartz *et al.*, 1970), and studies have shown that, under normal conditions, the brain content of histidine is not sufficient to saturate this enzyme, whose K_m value has been reported to be about 4×10^{-4} M (Schwartz *et al.*, 1970). In support of the latter view is the finding of a close correlation between brain concentrations of histamine and histidine during development (Pearce & Schanberg, 1969; Schwartz *et al.*, 1971) as well as the marked elevation of brain histamine content in animals after administration of massive doses of histidine (Taylor & Snyder, 1972; Schwartz *et al.*, 1972). Observations in the present study lend further supporting evidence (Table 1). In the guinea pigs, protein deficiency elicited a 2.0–2.5-fold increase in brain histamine with a 2-fold increase in histidine. In the malnourished rats, a 3–4-fold increase in brain histidine was associated with the same magnitude of increase in histamine.

Histamine is considered to be a neurotransmitter (Snyder & Taylor, 1972), and there is evidence for a predominantly depressant or inhibitory effect of this amine on the firing of neurons in the medulla oblongata (Haas *et al.*, 1973) and the cerebral cortex (Godfraind *et al.*, 1973). Studies in experimental animals have implicated brain histamine in the modulation of water consumption (Leibowitz, 1973), continuous avoidance (Gerald & Maickel, 1972), self-stimulation (Cohn *et al.*, 1973) and thermal regulation (Shaw, 1971). Findings in the present study add to the increasing number of putative neuroregulatory substances whose concentrations in the brain are critically regulated by the quality and quantity of food intake (Fernstrom & Wurtman, 1971, 1974; Wurtman *et al.*, 1974). In view of the crucial role of these neurochemical substances in the modulation of behaviour and mood (Axelrod, 1974), extensive alterations in their normal concentrations in the nervous system may well underlie the neurological changes often found in protein-calorie-deficient children. The present study also suggests protein-deficient guinea pigs and rats as useful models for further evaluation of the relationship between brain function and histamine content.

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