

Dietary Folic Acid and The Activity of Brain Cholinergic and γ -Aminobutyric Acid (GABA) Enzymes

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SUMMARY: *Charles River CD male rats were randomly divided into 3 groups of five each and placed on folate deficient, folate excess, and control diets respectively. Glutamate decarboxylase GAD γ -aminobutyrate aminotransferase (GABA-T), choline acetyltransferase (ChAc), and acetylcholinesterase (AChE) were assayed in the rat brains after 6 weeks of dietary treatment. Neither folate deficiency nor folate supplementation influenced the enzymes associated with GABA and acetylcholine metabolism.*

RÉSUMÉ: *Cette étude a été réalisée sur des rats mâles (Charles River CD) âgés de 21 jours et répartis en 3 groupes de 5 animaux soumis à l'un des régimes alimentaires suivants: une diète totalement carencée en acide folique, une diète contrôle contenant 2 μ g/g d'acide folique et une diète avec surdosage en acide folique (100 μ g/g). Les animaux furent maintenus pendant 6 semaines sous ces différentes diètes après quoi les enzymes suivantes furent analysées au niveau du cerveau: la glutamate décarboxylase (GAD), la γ -aminobutyrate aminotransférase (GABA-T), la choline acétyltransférase (ChAc) et l'acétylcholinestérase (AChE). Les résultats ont montré que la déficience ou la surcharge en acide folique n'influencent en aucune manière les enzymes associées au métabolisme du GABA et de l'acétylcholine.*

The need for an explanation of folate-responsive neuropsychiatric symptoms (Reynolds, 1976; Manzoor and Runcie, 1976; Botez et al., 1978; 1979) prompted us to develop an animal model for the study of the effects of folate on behavior and brain chemistry (Bachevalier and Botez, 1978; 1978a).

Recently we have found that the concentration of 5-hydroxyindoleacetic acid is low in cerebral spinal fluid of folate-deficient patients who exhibit neuropsychiatric symptoms that respond to folic acid (Botez et al., 1979). In the same preliminary study we reported low 5-hydroxytryptamine (5-HT) levels in the brains of rats fed either a folate-deficient diet or a diet containing excess folate. This is in agreement with previous findings (Koorevar et al., 1973) showing that the regional distribution of 5-methyltetrahydrofolate in the brain is similar to that of 5-hydroxytryptamine (5-HT). In earlier studies, Davies and Watkins (1973) found evidence that folate could block the inhibitory effects of GABA by up to 80 per cent.

There is a close relationship between the influence of folic acid and thiamine on the nervous system (Thomson et al., 1972). Plaitakis and coworkers (1978) demonstrated in thiamine-deficient rats a selective impairment of the cerebellar serotonergic system.

It has been reported that rats maintained on a thiamine-deficient diet show reduced brain γ -aminobutyric (GABA) acid levels (Gubler et al., 1974). However, the activities of glutamic decarboxylase (GAD) and γ -aminobutyrate aminotransferase (GABA-T) were unaffected. Reports about the effect of thiamine deficiency on the levels of acetylcholine are conflicting (Heinrich et al., 1973;

Reynolds and Blass, 1975).

The present study was carried out to determine whether a folate-deficient diet or a diet containing excess folate in rats could influence the enzymes associated with GABA and acetylcholine metabolism. Our results suggest that they do not.

Methods

Charles River CD male rats (60-70g) were randomly divided into 3 groups of five each and placed on the folate diets as shown in Table 1. The composition of the different diets has been described (Bachevalier and Botez, 1978, 1978a).

After 6 weeks the rats were killed by a sharp blow to the back of the neck. Brains were quickly removed and homogenised in 9 vol of ice-cold water.

GAD was measured by the procedure of Wu et al. (1973). However, no exogenous pyridoxal phosphate was added to the assay system. We verified that, under our conditions, the assay was linear with time and proportional to tissue concentration. GABA-T was assayed as previously described (Ngo and Tunnicliff, 1978). ChAc was measured by the procedure of McCaman and Hunt (1965); AChE was assayed as described by McCaman et al. (1968). Blood folic acid concentrations were measured as previously described (Bachevalier and Botez, 1978; 1978a).

Results and Discussion

The folic acid diets were effective in altering the blood levels of folate (Table 1). During the 6 weeks the animals were maintained on the diets, the rats from each group gained about the same weight (almost 300g). Therefore, folic acid has no effect on rats ability to gain weight.

The activity of the brain enzymes

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This work was supported by a grant from C.O. Monat Foundation (Hôtel-Dieu Hospital).

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TABLE I
Folate and Enzyme Determinations.

DIET	WEIGHT GAINS (gr) x ± S.E.	SERUM FOLATE ng/ml x ± S.E.	RBC FOLATE ng/ml x ± S.E.	Determinations of brain enzymes in μmoles/g/hr			
				GAD	GABA-T	ChAc	AChE
Control (folate deficient + 2 μg folate per g diet)	293.3±13.60	92.8±5.49	546.7±8.70	27.0±3.2	101.0±11.2	0.27±0.04	549.9±55.5
Folate deficient	288.6±11.18	21.12±2.10	245.7±27.40	26.4±2.1	96.6±10.5	0.30±0.04	560.6±61.3
Folate excess (folate deficient + 100 μg folate per g diet)	292.7±10.93	229.4±18.17	1424.7±341.59	28.1±3.5	95.7±13.6	0.25±0.05	551.8±48.4

showed no differences between the dietary groups (Table 1). This is surprising in light of our previous work in which we demonstrated that folic acid can inhibit the activity of GAD *in vitro* (Tunnicliff and Ngo, 1977). If the folic acid diets can alter the levels of brain folic acid, then the physiological significance of the inhibition of GAD is in doubt.

We have previously shown that folic acid deficiency and excess folate can produce alterations in brain 5-HT levels (Botez et al., 1978). In the same study, we found no evidence of changes in brain dopamine or nor-adrenaline metabolism. Thus, as a preliminary conclusion it seems that folic acid has an effect only on serotonergic transmitter system. Further studies will have to be carried out to establish whether or not the psychiatric symptoms that are folate responsive are related to alterations in 5-HT metabolism (Botez et al., 1979).

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