EFFECT OF VITAMIN B₆ ON L-GLUTAMATE DEHYDROGENASE ACTIVITY IN MICE BRAIN

Gilberto G. VILLELA and Ana Maria CALCAGNOTTO

*Laboratory of Biochemistry, Instituto Oswaldo Cruz,
Rio de Janeiro, Brazil*

(Received July 15, 1976)

Summary Investigations were carried out to obtain information on the effect of vitamin B₆ level on L-glutamate dehydrogenase activity in mice brain. Subcutaneous and intracerebral injection of pyridoxal phosphate or pyridoxamine resulted in a significant enhancement of the L-glutamate dehydrogenase activity. In the case of pyridoxine, much larger doses and more prolonged time were necessary to exhibit the effect. The above effect of vitamin B₆ was much more evidently observed in vitamin B₆-deficient animals.

The role of vitamin B₆ in the metabolism of amino acids has been well established. The central position of glutamic acid in the metabolism of the brain and the part played by L-glutamate dehydrogenase (GDH) [L-glutamate: NAD oxidoreductase, EC 1.4.1.2] suggested to us to investigate the effect of the vitamins of the B₆ group on its activity. The present study is an attempt to know the possible integration of these vitamins in the glutamic acid metabolism of the brain.

MATERIALS AND METHODS

Pyridoxine hydrochloride was a gift of Hoffmann-La Roche Laboratories, Basel, Rio de Janeiro Branch; pyridoxal phosphate and pyridoxamine dihydrochloride-H₂O were purchased from Calbiochem; β -nicotineamide adenine dinucleotide reduced form (NADH) from Sigma and α -ketoglutarate from E. Merck, Darmstadt. Other chemicals were obtained from commercial sources.

Normal white mice of known pedigree were used throughout this study. The mice were injected subcutaneously with 0.1 ml to 0.2 ml of the vitamin B₆ solutions of the following concentrations: pyridoxine hydrochloride, 4.86×10^{-4} M; pyridoxamine dihydrochloride-H₂O, 3.85×10^{-4} M and pyridoxal phosphate, 4.04×10^{-4} M. If necessary, more diluted solutions were used. The animals were injected intracerebrally with 0.1 ml of the vitamin B₆ solutions after 10 times dilution.

At a definite period after the injection, the animals were killed by cervical dislocation, the skull was opened, and the brain was immediately removed, blotted, weighed and immersed in ice-cold distilled water. The homogenates were prepared in a glass Potter-Elvehjem homogenizer with a Teflon pestle for 3 min and sufficient amounts of ice-cold water were added to the homogenates to dilute 5 times. The diluted homogenates were centrifuged at 2°C in a Servall RC-2 automatic refrigerated centrifuge for 15 min at $3,090\times g$. The resulting cell-free supernatant was used for the assay of GDH activity.

The activity of GDH was measured according to the method of OLSEN and ANFINSEN with a slight modification (1, 2). The reaction mixture contained 1.6×10^{-3} M α -ketoglutarate, 1.4×10^{-3} M NADH and 0.2 ml of the extract diluted 20 times. The change in the absorption at 340 nm due to the enzyme reaction was recorded using a Gilford 240 digital spectrophotometer. The enzyme activity was expressed as μ M of NADH oxidized per mg protein per minute. Protein concentrations were determined by the method of LOWRY *et al.*, using crystalline bovine serum albumin as standard (3).

A group of mice were fed on a vitamin B₆-deficient diet. The composition of the diet was as follows: casein, 20 parts; sucrose, 73 parts; the salts mixture of Osborne and Mendel, 4 parts; bacto-agar, 2 parts; vitamin A, 1 part and 40 μ g of riboflavin and 40 μ g of thiamine, daily (4). The amount of diet was 3.6 g per mouse per day. The sets of experiments were carried out: one for 14 days and another for 37 days. After the periods, the mice of each group were divided into two: the one was control and the other was injected with 2.50 and 4.56 mg of vitamin B₆ per mouse during 4 days.

RESULTS AND DISCUSSION

Subcutaneous injection of pyridoxal phosphate and pyridoxamine to mice

Table 1. Effect of injected vitamin B₆ on the GDH activity in mice brain.

Pyridoxine		Pyridoxal phosphate		Pyridoxamine	
Dose in 24 hr (μ g)	GDH units ($\times 10^3$)	Dose in 24hr (μ g)	GDH units ($\times 10^3$)	Dose in 24hr (μ g)	GDH units ($\times 10^3$)
1.25	2.72	10	4.57	10	3.85
2.50	2.45	20	3.89	20	4.46
5.00	2.59	20 ^a	4.74	40 ^b	5.80
10.00	2.57	40 ^a	5.25	80 ^b	4.31
20.00	2.37				
40.00 ^a	4.23				
80.00 ^b	3.74				
Normal control: $(2.45\pm 0.28)\times 10^{-8}$ units					

^a Total doses injected in 48 hr. ^b Total doses injected in 96 hr. Units: μ M of NADH oxidized/min/mg protein. Numbers of mice injected in 24 hr: 31. Numbers of mice injected in 48 and 96 hr: 27. Numbers of mice of normal control: 22.

resulted in a significant increase in the GDH activity of mice brain after 1, 2, and 4 days of the vitamin administration (Table 1). However, no effect was observed after 2 hr of the injection. In the case of pyridoxine, much larger amounts and prolonged time were required for the enhancement of mice brain GDH as compared with pyridoxal phosphate or pyridoxamine.

Intracerebral administration of pyridoxine caused the appreciable increase in the GDH activity after 1 hr of the injection (Table 2).

Table 2. Effect of intracerebrally injected pyridoxine on mice brain GDH activity.

Dose (μ g)	Time after injection (hr)	GDH units ($\times 10^3$)
1,000	1	4.96
500	1	4.01
25	24	2.40

Mice fed on the vitamin B₆-deficient diet showed a decrease in the brain GDH activity after 14 days and a significant increase in the enzyme activity was observed when pyridoxine was supplemented to the diet (Table 3).

Table 3. Effect of vitamin B₆ on the GDH activity of vitamin B₆-deficient mice brain.

Period fed on vitamin B ₆ -deficient diet (days)	Pyridoxine added (mg/mouse/day)	GDH units ($\times 10^3$)
14	—	1.13
14	2.50	4.08
37	—	3.88
37	4.56	5.49

The GDH activity of control fed on normal diet: $(2.45 \pm 0.28) \times 10^{-3}$ units.

When the extract was incubated *in vitro* with graded amounts of pyridoxal phosphate, pyridoxamine or pyridoxine, pyridoxal phosphate as well as pyridoxamine showed a slight enhancing effect on the activity of GDH.

The results obtained here would indicate that the GDH activity in mice brain could be increased both *in vivo* and *in vitro* treatment with vitamin B₆. The experiment using vitamin B₆-deficient animals would substantiate the effect of vitamin B₆.

The authors are grateful to the Brazilian Research Council (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support and to Hoffmann-La Roche Laboratories, Rio de Janeiro Branch, for a generous gift of pyridoxine.

REFERENCES

- 1) OLSEN, J. A. and ANFENSEN, C. B., *J. Biol. Chem.*, **197**, 67 (1952).
- 2) VILLELA, G. G. and CALCAGNOTTO, A. M., *Biochem. Pharm.*, **19**, 2176, 2179 (1970).
- 3) LOWRY, D. H., ROSEBROUGH, N. J., FARR, A. L., and RANDALL, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
- 4) HAWK, P. B., OSLER, B. L., and SUMMERSON, W. H., *Practical Physiological Chemistry*, 13th edition, p. 1375 (1954).