THE EFFECT OF ANTIVITAMIN B₆ ADMINISTRATION ON γ -AMINOBUTYRIC ACID METABOLISM IN RETINA AND ELECTRORETINOGRAM

Aritake MIZUNO, Yoshio KAMADA, Masanori KUNITA,
and Makoto MATSUDA¹

*Department of Ophthalmology and Department of Biochemistry,
The Jikei University School of Medicine,
Minato-ku, Tokyo 105, Japan*

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Summary The effect of several antivitamin B₆ on γ -aminobutyric acid (GABA) metabolism was studied in the rat retina. The rat electroretinogram (ERG) was also recorded after administration of these drugs. Aminooxyacetic acid (AOAA) and hydrazine administration increased the GABA content and inhibited the GABA degrading enzyme, GABA transaminase in retina. In addition, these drugs elongated the peak latency of the oscillatory potential in the rat ERG. In contrast, 4-deoxypyridoxine (DOP) or isonicotinic acid hydrazide (INAH) administration decreased the GABA content and inhibited the GABA synthesizing enzyme, glutamic acid decarboxylase in retina, and administration of these drugs together with AOAA lessened the degrees of elevation of GABA content and of the elongation of the peak latency produced as compared with AOAA alone, though neither of the former drugs had a significant effect on ERG. The retinal GABA seems to play an important role in relation to the oscillatory potential of ERG.

Keywords GABA, rat retina, antivitamin B₆, glutamic acid decarboxylase, GABA-transaminase, electroretinogram

It is known that γ -aminobutyric acid (GABA) may act as an inhibitory neurotransmitter mediating the visual process in the retina (1–4). GABA is synthesized from glutamic acid by glutamic acid decarboxylase (GAD) [L-glutamate 1-carboxy-lyase; EC 4.1.1.15] and is catabolised to succinic semialdehyde by the enzyme so-called GABA transaminase (GABA-T) [4-aminobutyrate: 2-oxoglutarate aminotransferase; EC 2.6.1.19]. Since pyridoxal phosphate is essential as the coenzyme to show the activities of these enzymes, antivitamin B₆

¹ 水野有武, 鎌田芳夫, 國田正矩, 松田 誠

Abbreviations: GABA, γ -aminobutyric acid; GAD, glutamic acid decarboxylase; GABA-T, GABA transaminase; ERG, electroretinogram; AOAA, aminooxyacetic acid, DOP, 4-deoxypyridoxine; INAH, isonicotinic acid hydrazide; OP₁, first oscillatory potential.

are expected to alter the levels of GABA in the retina. We investigated the effect of these drugs on the GABA levels and the GABA-related enzyme activities in the rat retina. At the same time, the electroretinogram (ERG) of a rat treated with these drugs was recorded as some reports have suggested that GABA could affect the ERG pattern (5-8).

MATERIALS AND METHODS

NADP was purchased from Kohjin chemicals (Tokyo, Japan). Aminoxyacetic acid (AOAA), Sigma (St. Louis, Missouri), 4-deoxypyridoxine (DOP), Nakarai chemicals (Kyoto, Japan), isonicotinic acid hydrazide (INAH), Tokyo Kasei (Tokyo), Scintisol 500, Dojin chemicals (Kumamoto, Japan). The radioactive substances from New England Nuclear (Boston, Massachusetts). GABase was kindly presented by Dr. Y. Okada.

Male SD (CRJ:CD) strain rat aged 5 to 6 weeks were used for all the experiments. The drugs were dissolved in 0.9% NaCl solution and injected intraperitoneally. The animal was decapitated at an appropriate time after drug administration. The eyeball was immediately enucleated and cut on a filter paper with a razor 1 mm posterior to the corneal margin in ice cold. The retina was dissected with an ophthalmic spatula and weighed on a chemical balance.

The GABA content was determined by the enzymic method of Okada *et al.* (9). The dissected retina was homogenized in 0.5 ml of distilled water and the homogenate was heated in boiling water for 10 min and then centrifuged at 3,000 rpm for 10 min. The supernatant (100 μ l) was added to 200 μ l of 100 mM Tris-HCl buffer containing 15 mM α -ketoglutaric acid, 5.25 mM NADP, 2.3 mM mercaptoethanol and 0.02 unit of GABase. The incubation was carried out for 30 min at 37°C. After 2 ml of ethanol and 0.7 ml of distilled water had been added to the incubation mixture, the fluorescence of the reduced NADP was measured with a spectrofluorophotometer. In some cases, 10 nmoles of GABA were added to the extract of retina and this was all subjected to the entire procedure to determine the degree of inhibition of drug taken up in the retina on GABase system used for GABA assay.

GAD activity was determined by modifying the method of Molinoff and Kravitz (10). The dissected retina was homogenized in 20 volumes of 0.25% (v/v) Triton X-100 containing 0.02 M mercaptoethanol. Enzyme activity was estimated by measuring the radioactive GABA formed from 14 C-glutamic acid. The incubation mixture contained 50 μ l of the homogenate and 50 μ l of 100 mM potassium phosphate buffer (pH 6.5) using a substrating 40 mM [U- 14 C]-L-glutamic acid (0.1 μ Ci). After being incubated at 37°C for 120 min, the mixture was adjusted to pH 7.0 with 0.2 M K_2HPO_4 solution and then passed through a Dowex 1 $\times$ 8 acetate form column (8 $\times$ 30 mm). The 14 C-GABA formed was eluted with 3 ml of 1 mM GABA solution into 10 ml of Scintisol 500 and counted in a scintillation spectrometer.

GABA-T activity was measured using a modification of the method of Waksman and Roberts (11). The retina was homogenized in the same manner as for the GAD assay. The incubation mixture consisted of 50 μ l of the homogenate and 50 μ l of 100 mM potassium phosphate buffer (pH 8.0) containing 100 mM GABA and 20 mM [1-¹⁴C]- α -ketoglutaric acid (0.03 μ Ci). After being incubated at 37°C for 30 min, the reaction was stopped by adding 50 μ l of 1.2 N HCl to the mixture. The reaction mixture was passed through a Dowex 50W $\times$ 8 H⁺ form column (8 $\times$ 15 mm) and the column was washed with 10 ml of distilled water to remove the remaining ¹⁴C- α -ketoglutaric acid and the ¹⁴C-glutamic acid produced from ¹⁴C- α -ketoglutaric acid was eluted with 3 ml of 2 N NH₄OH solution. The radioactivity of ¹⁴C-glutamic acid was counted in a liquid scintillation system in the same manner as for the GAD assay.

ERG was recorded at intervals of 20 min after administration of the drugs. The unanesthetized animal was placed in a specially designed holder and the head was immobilized by screws placed in the external auditory meati. The pupil was dilated by a mydriatic, Mydrin-P (*N*-ethyl-2-phenyl-*N*-(4-pyridylmethyl)-hydracylamide) and eyelids were held open with silk threads. The ERG was obtained from the apex of cornea with an active electrode, silver-silver chloride wrapped in black cotton thread, against a reference electrode that had been implanted under the scalp. The evoked activity was obtained by photic activation of the eye. The stimulus was provided by a Xenon flash lamp (20 joules, Nihonkoden MSP-II, Tokyo) placed 20 cm from the rat's eye, parallel with the optic axis. The recording system consisted of an amplifier (Saneisokki 1205S, Tokyo), an oscilloscope (Saneisokki signal processor 7S06) and an X-Y recorder (Watanabe-sokki WX 132, Tokyo). The time constant was 5 msec. Each recording was preceded by 5 min of dark adaptation. The rectal temperature and electrocardiogram were monitored throughout the preparatory and experimental periods.

RESULTS

Table 1 shows the effects of various drugs on the GABA content, GAD and GABA-T activities in the rat retina. Three types of drugs appear to be recognized by the effect on the retinal GABA level: the drugs increasing GABA content, those decreasing GABA content and those having no significant effect on the GABA content. The first group, AOAA and hydrazine, inhibited both GAD and GABA-T activities, while the second group, DOP and INAH, inhibited exclusively GAD activity. The third group consisted of DL-penicillamine, semicarbazide thiosemicarbazide, castrix (2-chloro-4-dimethylamino-6-methyl pyrimidine) or γ -glutamyl hydrazide plus pyridoxal phosphate (12) (not shown).

Figure 1 and Table 2 represent the effect of AOAA, hydrazine, DOP and INAH on the ERG of the rat. The average peak latency of the first oscillatory potential (OP₁) on b-wave was 29.3 ± 0.3 msec ($\pm$ S.E.M.) for 19 experiments.

Table 1. Effect of various drugs on GABA content, GAD and GABA-T activities in rat retina.

Drugs	Dose (mg/kg)	Time after inj. (hr)	GABA ($\mu\text{mol/g}$)		GAD ($\mu\text{mol/hr/g}$)		GABA-T ($\mu\text{mol/hr/g}$)	
			Control	Treated	Control	Treated	Control	Treated
AOAA	25	1	4.26 $\pm$ 0.17	6.84 $\pm$ 0.21 ^a	5.68 $\pm$ 0.20	2.68 $\pm$ 0.29 ^a	10.82 $\pm$ 0.92	5.05 $\pm$ 0.14 ^a
Hydrazine	100	1	4.59 $\pm$ 0.16	6.32 $\pm$ 0.26 ^a	5.73 $\pm$ 0.18	2.52 $\pm$ 0.22 ^a	11.90 $\pm$ 0.51	8.15 $\pm$ 0.37 ^a
DOP	50	1	5.04 $\pm$ 0.22	3.93 $\pm$ 0.10 ^a	4.40 $\pm$ 0.17	3.08 $\pm$ 0.14 ^a	10.45 $\pm$ 0.49	10.26 $\pm$ 0.61
INAH	100	1	4.53 $\pm$ 0.09	3.76 $\pm$ 0.06 ^a	4.74 $\pm$ 0.29	3.66 $\pm$ 0.26 ^d	9.42 $\pm$ 0.28	10.03 $\pm$ 0.47
AOAA	25	1	4.62 $\pm$ 0.17	5.87 $\pm$ 0.22 ^a	5.42 $\pm$ 0.41	2.59 $\pm$ 0.31 ^a	10.82 $\pm$ 1.83	3.02 $\pm$ 0.37 ^b
+ DOP*	50	2						
AOAA	25	1	4.32 $\pm$ 0.14	6.08 $\pm$ 0.37 ^a	5.23 $\pm$ 0.41	3.28 $\pm$ 0.38 ^c	10.82 $\pm$ 1.83	3.11 $\pm$ 0.36 ^b
+ INAH*	100							

Each value represents mean $\pm$ S.E.M. of 4-10 experiments. ^a $p < 0.005$, ^b $p < 0.01$, ^c $p < 0.025$, ^d $p < 0.05$.

* DOP was injected 1 hr prior to AOAA, and INAH was simultaneously injected with AOAA.

Table 2. Effect of various drugs on peak latency of the first oscillatory potential.

Drugs	Dose (mg/kg)	Time after inj. (min)	$T_t - T_c$ (msec)	$(T_t - T_c)/T_c \times 100$	Changes of GABA content ^a (%)
AOAA	25	20	+4.6	+15.4	+60.6
		60	+7.2	+24.2	
Hydrazine	100	20	+2.2	+7.9	+37.7
		60	+6.8	+24.3	
DOP	50	20	-1.0	-3.3	-22.0
		60	± 0	± 0	
INAH	100	20	+1.0	+3.5	-17.0
		60	+1.0	+3.5	
AOAA + DOP*	25	20**	+2.0	+6.7	+27.1
	+50	60**	+3.2	+10.7	
AOAA + INAH	25	20	± 0	± 0	+40.7
	+100	60	± 0	± 0	

T_t , represents the peak latency of OP₁ after administration; T_c , represents the peak latency of OP₁ before administration.

* DOP was administered 1 hr prior to AOAA injection. ** Time after AOAA injection. ^a Calculated from the data in Table 1.

Fig. 1. Effect of intraperitoneally administered AOAA and DOP on rat ERG. Time constant was 5 msec. A, AOAA 25 mg/kg of body weight; B, DOP 50 mg/kg of body weight.

AOAA and hydrazine which were found to increase the retinal GABA elongated the peak latency of OP_1 significantly, while DOP and INAH produced no effect on the ERG pattern until at least 140 min after drug administration. AOAA began to delay the peak latency of OP_1 from about 20 min after AOAA treatment and the delay continued to about 60 min after that time. The characteristic effect of AOAA began to disappear after about 80 min. Hydrazine also produced the same effect on the peak latency of OP_1 as AOAA. The administration of DOP or INAH, a drug decreasing the retinal GABA, together with AOAA lessened the degree of elevation of GABA level and of the elongation of the peak latency of OP_1 by AOAA alone, though DOP or INAH treatment itself could hardly change the events with respect to ERG.

DISCUSSION

Since the retinal GABA content would appear to reflect the activity of enzymes synthesizing and degrading GABA (GAD and GABA-T, respectively) and both the enzymes are pyridoxal phosphate-dependent, some drugs including antivitamin B₆ may in turn affect the GABA content. In this study, an attempt has been made to relate the retinal GABA content to GAD and GABA-T activities in retina. The retinal GABA was significantly decreased by DOP and INAH while being considerably increased by AOAA and hydrazine. The fall or rise in GABA levels appears to depend on the different rates of inhibition of GAD and GABA-T. The administrations of DOP and INAH markedly inhibited GAD activity, but not GABA-T activity. The administration of AOAA and hydrazine greatly inhibited both the enzyme activities, but the inhibition of GABA-T was possibly the dominant factor and the GABA levels rose accordingly. Since retinal GABA, GAD and GABA-T have been known to be mainly localized in the inner plexiform layer of the retina (13-16), the changes in these parameters in GABA metabolism might reflect changes in those in the inner plexiform layer.

On the other hand, evidence that GABA is an inhibitory neurotransmitter within the central nervous system continues to accumulate (17, 18) and is supported by the effects of drugs known to interfere with the metabolism of the amino acid. For example, inhibition of GABA-T *in vivo* by AOAA elevates brain GABA levels with such a concomitant sedation of the nervous system as to inhibit the glutamate-evoked potential (19). The present study showed that the administration of AOAA and hydrazine to rats altered the photically evoked potential recorded from ERG, and in particular temporarily elongated the peak latency of OP_1 . Moreover, the administration of AOAA together with DOP or INAH lessened the degree of elevation of GABA content and elongation of the peak latency of OP_1 produced by AOAA alone. These results suggest that the elevation of retinal GABA content might produce a delay in the appearance of the oscillatory potential. Since the oscillatory potential has recently been suggested to relate to the activities of the amacrine cells in the retina (20), the alteration of the peak latency of OP_1 reported

in this paper might be due to a change of GABA content in the amacrine cells.

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