GLUTAMIC DECARBOXYLASE AND γ -AMINOBUTYRIC- α -KETOGLUTARIC TRANSAMINASE ACTIVITIES DURING AND ON SUPPRESSION OF THE RUNNING FIT¹

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The studies of Namba (1), Yamanaka (2) and Konishi (3) suggest the importance of the metabolic pathway from glutamic acid to γ -amino-butyric acid to succinic semialdehyde in the biochemical elucidation of the running fit. Matsuo (4) and Moriya (5) have investigated the effect of 2-methyl-4-amino-5-hydroxymethylpyrimidine (OMP), as a toxopyrimidine group compound, and of 2-methyl-4-amino-5-aminomethylpyrimidine (MAAMP), as an atoxopyrimidine group compound, on the activity of glutamic decarboxylase and γ -aminobutyric- α -ketoglutaric (ABA-KGK) transaminase *in vitro*. In the present paper, the enzymic changes during the running fit and on suppression of the running fit were studied *in vivo* using OMP as a seizure-inducing agent and both pyridoxine and MAAMP as preventive agents.

EXPERIMENTAL

1. *Animals and Reagents*

Healthy male mice weighing 14–15 g were used. OMP was injected subcutaneously. Pyridoxine hydrochloride and MAAMP were given intraperitoneally.

2. *Determination of Brain Glutamic Decarboxylase Activity*

Measurements were made by the manometric method of Roberts and Franke (6). Mice were killed by decapitation, the brain removed as promptly as possible and homogenized with 5 volumes of pH 6.3, 1/10 M phosphate buffer under chilling.

Homogenate:	1.0 ml, main chamber.
Substrate:	0.3 M glutamic acid (aqueous solution, pH 6.3) 0.5 ml, side bulb.
Phosphate buffer (1/10 M, pH 6.3):	0.5 ml, main chamber.
Water:	1.0 ml, main chamber.
H ₂ SO ₄ (1 N):	0.5 ml, side bulb.

Reaction conditions: pH 6.3, N₂, 37.5°. After preliminary shaking for 10

¹ Studies on Substances of the Toxopyrimidine Group. XII.

minutes, the solutions were allowed to react for 80 minutes, H_2SO_4 then tipped in and the total CO_2 evolved measured.

3. Determination of Brain γ -Aminobutyric-Ketoglutaric Transaminase Activity

Measurements were made by the colorimetric method of Bessman (7). The brains of 3 mice, killed by decapitation, were homogenized with 9 volumes of pH 7.7, 1/10 *M* phosphate buffer under chilling.

Homogenate prepared as above: 3.0 ml.

α -Ketoglutaric acid (0.05 *M*, in pH 7.7 phosphate buffer): 1.0 ml.

γ -Aminobutyric acid (0.05 *M*, in pH 7.7 phosphate buffer): 1.0 ml.

For the control, γ -aminobutyric acid was replaced by an equal quantity of pH 7.7 phosphate buffer. The pH becomes 7.4 after mixing the solutions. The solutions were allowed to react for 60 minutes at 37.5° , pH 7.4 and in N_2 . The reaction was then stopped by adding 1.0 ml of 0.6 *N* acetic acid. A 3.0 ml-portion was deproteinized with 1.0 ml of 15 per cent trichloroacetic acid. To 2.0 ml of the filtrate was added 3.0 ml of 2,4-dinitrophenylhydrazine (5 mg/ml HCl solution) and the mixture left standing for 10 minutes under chilling. Then 8.0 ml of xylene was added, the mixture shaken well for 3 minutes, 5 ml of the xylene layer taken off, 5.0 ml of 1/5 *N* NaOH added, the solution shaken for 30 seconds and 3.0 ml of the aqueous layer was taken. To this was added 2.0 ml of 5 per cent alcoholic KOH and the solution measured colorimetrically at 425 $\text{m}\mu$. The succinic semialdehyde produced was determined by comparing with a standard curve of succinic semialdehyde-2,4-dinitrophenylhydrazone. The brain glutamic acid level was also measured in some cases besides the above two enzymic activities.

4. Determination of Brain Glutamate Levels

The brain of mice, killed by decapitation, was promptly frozen by placing in acetone-dry ice. The weight of the frozen brain was then measured and the organ was homogenized with 10 volumes of pH 4.3, 0.1 *M* acetate buffer. The homogenate was heated for 2 minutes at 100° , filtered and 2.0 ml of the filtrate used for the measurement. *Escherichia coli* C₄ (Osaka University Microbial Institute strain) acetone powder was used as glutamic decarboxylase and glutamic acid measured manometrically according to the method of Krebs *et al.* (8–10).

Homogenate filtrate: 2.0 ml, main chamber.

E. coli acetone powder (2 mg/ml, aqueous solution): 0.5 ml, side bulb.

Cetyltrimethyl ammonium bromide (11) (1.31 g/100 ml, pH 4.3 acetate buffer): 0.5 ml, main chamber.

Acetate buffer (0.1 *M*, pH 4.3): 0.5 ml, main chamber.

Reaction condition: pH 4.3, air, 37.5° .

After preliminary shaking for 10 minutes, the solutions were allowed to react and the CO_2 evolved measured until no further CO_2 was produced.

RESULTS

1. Change in Enzymic Activity during OMP-Induced Running Fit

In the animals in which the running fit occurred about 2.5 hours after

injection of 200 $\mu\text{g/g}$ of OMP, glutamic decarboxylase activity was reduced to 73 per cent of the normal value, as shown in Table I. This coincides closely with the results reported by Namba (1) in the rat. The ABA-KGA transaminase activity, on the other hand, was 96 per cent as shown in Table II. Matsuo (4) reported that in *in vitro* experiments, phosphate suppressed glutamic decarboxylase but no significant inhibition was noted against ABA-KGA transaminase. The inhibition was noted against ABA-KGA transaminase. The inhibitory effect of OMP on glutamic decarboxylase is therefore seen both *in vitro* and *in vivo*.

TABLE I
Brain Glutamic Decarboxylase

Treatment	CO ₂ evolved (μL)	Average	Relative activity ^a
Normal	56.2, 56.0, 54.2, 53.5, 48.4, 48.4	52.8	100
200 $\mu\text{g/g}$ OMP	40.4, 40.3, 39.4, 32.9	38.3	73
200 $\mu\text{g/g}$ OMP + 100 $\mu\text{g/g}$ pyridoxine, twice	54.2, 51.5, 51.4, 48.7	51.4	97
1500 $\mu\text{g/g}$ MAAMP, after 60 min	52.9, 48.4, 45.2, 45.0	47.9	91
1500 $\mu\text{g/g}$ MAAMP, after 120 min	51.4, 50.8, 44.7, 44.3	47.8	91
1500 $\mu\text{g/g}$ MAAMP, after 200 min	57.8, 52.9, 52.8, 47.1	52.6	100
200 $\mu\text{g/g}$ OMP + 1500 $\mu\text{g/g}$ AMP, 60 min before	50.4, 48.4, 43.8, 43.1	46.4	89
13 $\mu\text{g/g}$ thiosemicarbazide	43.8, 41.6, 39.1, 38.3	40.7	77
13 $\mu\text{g/g}$ thiosemicarbazide + 100 $\mu\text{g/g}$ pyridoxine, thrice	54.3, 52.7, 47.2, 42.2	49.1	93

^a Normal is taken as 100. Also the same in Table II.

TABLE II
Brain γ -Aminobutyric- α -Ketoglutaric Transaminase

Treatment	Succinic semialdehyde produced (μmoles)	Average	Relative activity
Normal	2.66, 2.46, 2.45, 2.35, 2.35, 2.33	2.45	100
200 $\mu\text{g/g}$ OMP	2.64, 2.39, 2.08	2.35	96
200 $\mu\text{g/g}$ OMP + 100 $\mu\text{g/g}$ pyridoxine, twice	2.59, 2.57, 2.48	2.55	104
1500 $\mu\text{g/g}$ MAAMP, after 60 min	1.64, 1.52, 1.23, 1.23	1.43	58
1500 $\mu\text{g/g}$ MAAMP, after 120 min	2.15, 2.03, 1.99, 1.96	2.03	83
1500 $\mu\text{g/g}$ MAAMP, after 200 min	2.33, 2.27, 2.21	2.27	93
200 $\mu\text{g/g}$ OMP + 1500 $\mu\text{g/g}$ MAAMP, before 60 min	2.28, 2.24, 2.19	2.23	91
13 $\mu\text{g/g}$ thiosemicarbazide	2.28, 2.10, 2.07	2.15	87
13 $\mu\text{g/g}$ thiosemicarbazide + 100 $\mu\text{g/g}$ pyridoxine, thrice	2.42, 2.38, 2.26	2.35	95

Mandokoro (12) found that brain glutamate is reduced during the induced

running fit. This finding together with the finding that glutamic decarboxylase is inhibited, suggests the reduction of brain γ -amino butyrate during the running fit.

2. Enzymic Activity after Suppressing the Running Fit Induced by OMP by Pyridoxine

The effect of pyridoxine alone on glutamic decarboxylase and ABA-KGA transaminase was first examined. Pyridoxine was injected in doses of 2, 5 and 50 $\mu\text{g/g}$ respectively and after 60 minutes, the glutamic decarboxylase activity was measured. As shown in Table III, there was no increase in activity after injection of 2 $\mu\text{g/g}$, but an increase took place with more than

TABLE III
Effect of Pyridoxine on Brain Glutamic Decarboxylase

	CO ₂ evolved (μl)					Average
Normal	66.8,	60.8,	53.9,	51.9,	57.8	58.1
2 $\mu\text{g/g}$ pyridoxine	67.6,	62.7,	60.1,*	55.7,	55.4	59.9
5 $\mu\text{g/g}$ pyridoxine	69.8,	65.3,	63.9,	62.0,	58.7	63.9
50 $\mu\text{g/g}$ pyridoxine	70.0,	66.9,	64.6,	62.0		65.9

5 $\mu\text{g/g}$. This is in agreement with the results of *in vitro* experiments performed by Matsuo (4) and Roberts (6, 31).

On the contrary, there was no increase in ABA-KGA transaminase activity even after injection of as much as 50 $\mu\text{g/g}$ of pyridoxine as can be seen in Table IV. The results coincide with the *in vitro* experiments of Konishi

TABLE IV
Effect of Pyridoxine on Brain γ -Aminobutyric- α -Ketoglutaric Transaminase

	Succinic semialdehyde produced (μmoles)					Average
Normal	2.35,	2.21,	2.17,	2.16,	2.14	2.20
50 $\mu\text{g/g}$ pyridoxine	2.31,	2.19,	2.18,	2.16,	2.12	2.19

(3) and Bessman (7). These findings clearly show that in the normal mouse, pyridoxine enhances glutamic decarboxylase activity, but not ABA-KGA transaminase activity.

The effect of suppressing the OMP-induced running fit by pyridoxine was then examined. Pyridoxine was injected in a dose of 100 $\mu\text{g/g}$ and after 60 minutes, both 200 $\mu\text{g/g}$ OMP and 100 $\mu\text{g/g}$ pyridoxine were injected together. The animals remained quiet without occurrence of running fit. Measurements were carried out about 2.5 hours after injection of OMP. As shown in Tables I and II, the activities of glutamic decarboxylase and ABA-KGA transaminase were 97 and 104 per cent respectively; compared to the normal, there was no inhibition. Matsuo (4) showed that *in vitro* inhibition of glutamic decarbox-

ylase by OMP-phosphate is restored by pyridoxal phosphate and in the present experiment it was found that the inhibition of glutamic decarboxylase by OMP is also restored by administering pyridoxine *in vivo*. Since ABA-KGA transaminase activity, is neither inhibited by OMP nor increased by pyridoxine, it is only natural that the enzymic activity is unaffected after prevention of running fit by pyridoxine. Brain glutamate was also measured and as shown in Table V, a close to normal level was maintained. From the results, it can be assumed that little alteration in the brain aminobutyrate content takes place when the OMP running fit is prevented.

TABLE V
Brain Glutamic Acid Level
(expressed as mg per 100 g)

- I. Normal
- II. 200 μ g OMP + 100 μ g/g pyridoxine was injected twice, *i.e.*, 60 min before and simultaneous.
- III. 200 μ g OMP + 1500 μ g MAAMP injected 60 min before.

	I	II	III
	193.6	192.1	163.1
	193.9	184.4	162.2
	179.7	163.1	158.0
	179.2		158.0
	168.6		156.0
	167.4		156.0
	158.0		152.0
	143.2		
Average	172.9	179.9	157.9

3. Effect of MAAMP Alone

Based on the results on Hayashi (14), 1500 μ g/g of MAAMP was injected and the enzymic activities followed successively. Glutamic decarboxylase activity was found to be 91 per cent of the normal after 60 and 120 minutes and it returned to the normal value after 200 minutes. In other words, there was only about a 10 per cent inhibition. ABK-KGA transaminase activity, on the other hand, dropped to 58 per cent after 60 minutes, 83 per cent after 120 minutes and 90 per cent after 200 minutes. It can thus be seen that glutamic decarboxylase activity decreased by only 10 per cent after 60 and 120 minutes and returned to normal after 200 minutes, whereas ABK-KGA transaminase activity was markedly inhibited and even after 200 minutes some inhibition remained. These findings agree with the results of Moriya that ABK-KGA transaminase was most strongly inhibited by MAAMP *in vitro*.

4. Suppression of OMP-Running Fit by MAAMP

Healthy male mice, weighing 14–15 g, were used and each group contained 10 animals. OMP was injected subcutaneously in a dose of 200 μ g/g and 1500 μ g/g of MAAMP was injected intraperitoneally.

Injection of MAAMP after OMP—Twenty minutes after injecting 200 μ g/g of OMP, 1500 μ g/g of MAAMP was injected. All the ten animals of

the control group showed the running fit and died, while in the MAAMP-injected group, some effect was noted in 6 of the 10 animals but the difference was not marked.

Injection of MAAMP before OMP—MAAMP was injected 120 and 60 minutes respectively before injection of OMP in doses of $750\text{ }\mu\text{g/g}$. The running fit occurred in all the animals of the control group, whereas in the MAAMP-group, 4 of the 10 animals showed the running fit. Next, the total quantity of MAAMP ($1500\text{ }\mu\text{g/g}$) was once injected 60 or 120 minutes before OMP injection. Six of the 10 control animals showed the running fit leading to death. When MAAMP was given 60 minutes before OMP, one of the ten animals showed the running fit leading to death, and when MAAMP was given 120 minutes before OMP, two of the ten animals showed the same results.

Comparing these findings with those of the previous experiment, MAAMP is found to be more effective for prevention when it is given prior to OMP.

5. Enzymic Activity after Suppressing the Running Fit by MAAMP

$1500\text{ }\mu\text{g/g}$ of MAAMP was injected one hour before injection of $200\text{ }\mu\text{g/g}$ of OMP and the enzymic activity measured. The animals were killed by decapitation 2.5 hours after injection of OMP. None of the animals showed the running fit till the time of killing. As shown in Tables I and II, the glutamic decarboxylase activity was 89 per cent and the ABK-KGA transaminase activity was 91 per cent. From the results obtained in the experiment with MAAMP alone, it can be understood that there is no marked inhibition of ABA-KGA transaminase activity, since 3.5 hours had elapsed after injection of MAAMP. An inhibition of about 10 per cent was found in the glutamic decarboxylase but as a restoration was found in the case of administering MAAMP alone after 150 minutes, this inhibition must be attributed to OMP. The degree of inhibition, however, is less marked than that observed with OMP alone. Matsuo (4) has shown that OMP inhibits glutamic decarboxylase after conversion to OMP phosphate but it was not clarified whether the enzyme involved in its phosphorylation in the brain is the same as pyridoxal kinase. According to Hurwitz (15, 16), of the compounds with a structure similar to vitamin B₆, those with no substitutable group at the 5 position are phosphorylated by pyridoxal kinase. OMP possesses a substitutable group, satisfying the demand. Therefore, if OMP is phosphorylated by pyridoxal kinase in the brain, the above decrease in inhibition can be readily explained considering the fact that MAAMP inhibits pyridoxal kinase, as Moriya (5) found. In other words, MAAMP inhibits the phosphorylation of OMP by pyridoxal kinase and as a result, inhibition of glutamic decarboxylase will be decreased.

If, however, there is another phosphorylating enzyme for OMP, it must be assumed that MAAMP has an inhibitory action against this enzyme. At any rate, inhibition of OMP-phosphate production by MAAMP must be considered for the reduction in the above inhibition. The greater preventative effect which was observed after injecting MAAMP before OMP in the running fit prevention experiment, can be understood as being due to this fact. The glutamic acid level was also measured but as can be seen in Table V,

there was no marked decrease.

From the above results, it may be assumed that when the OMP-running fit is inhibited by MAAMP, inhibition of glutamic decarboxylase is slight and, on the other hand, there is a marked inhibition of ABA-KGA transaminase, so that the reduction in brain aminobutyrate is also suppressed.

6. Enzymic Activity in Thiosemicarbazide-Induced Running Fit

Kumagai (17) has shown that various carbonyl reagents possess a running fit-inducing action and the seizure is prevented by vitamin B₆ except that induced by isonicotinic hydrazide and benzoic hydrazide. In the present study, the enzymic activity was examined with one of these carbonyl reagents, thiosemicarbazide. Based on the results of Kumagai (17), 13 $\mu\text{g/g}$ of thiosemicarbazide was injected subcutaneously and 100 $\mu\text{g/g}$ of pyridoxine injected thrice, *i.e.*, 60 minutes before, 30 minutes before, and simultaneously with, thiosemicarbazide. The running fit occurs 60–90 minutes after injection of 13 $\mu\text{g/g}$ of thiosemicarbazide. The animals were killed by decapitation immediately after the start of the seizure and the enzymic activity measured. The animals receiving pyridoxine fail to show the running fit. They were also killed by decapitation after 60–90 minutes. Tables I and II show the results. Glutamic decarboxylase activity is reduced to 77 per cent in the thiosemicarbazide-induced running fit. These results coincide with those of Yamanaka (2) using rats. The activity of ABA-KGA transaminase was 81 per cent. The enzymic activities of glutamic decarboxylase and of ABA-KGA transaminase after inhibiting the seizure by pyridoxine was 93 and 95 per cent respectively, *i.e.*, they remained close to the normal value. The findings on enzymic activities are in agreement with the results obtained by Kumagai (17) in the toxicity experiments and it is assumed that the decrease in brain γ -aminobutyrate is slighter, in the case of thiosemicarbazide, too, when the running fit is suppressed than when the seizure is allowed to take place.

SUMMARY

1. Brain glutamic decarboxylase is inhibited in the OMP-induced running fit, but γ -aminobutyric- α -ketoglutaric transaminase is not inhibited.
2. Both enzymes show normal activities when the seizure is prevented by pyridoxine.
3. MAAMP inhibits γ -aminobutyric- α -ketoglutaric transaminase to a greater extent than glutamic decarboxylase.
4. In preventing the OMP running fit with MAAMP, it is more effective to give it before OMP.
5. The inhibition of glutamic decarboxylase by OMP is slighter when the running fit is prevented by MAAMP.
6. Both glutamic decarboxylase and γ -aminobutyric- α -ketoglutaric transaminase are inhibited in the thiosemicarbazide-induced running fit, but when the seizure is prevented by pyridoxine, there is no inhibition.
7. From these results, it is assumed that brain aminobutyric acid is decreased when the running fit occurs but is not reduced when the running fit

is prevented by pyridoxine.

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