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MULTIPLE EFFECTS OF REPEATED ADMINISTRATION OF γ-ACETYLENIC GABA ON RAT BRAIN METABOLISM

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Abstract—When 4-amino-hex-5-ynoic acid, the acetylenic analogue of GABA, is given chronically to rats (50 mg/kg) over a period of 8 days, several biochemical modifications are found in the brain. The following enzymatic activities are severely affected: GABA-T, GAD, Ala-T, and to a lesser extent GOT and CSA-T. The drug does not appear to interfere with pyridoxal phosphate synthesis. Increases in cerebral GABA, glutamate, alanine, phenylalanine, ornithine and taurine levels were observed. There were no changes in the levels of GAD and GABA-T apoenzymes despite a chronic increase in the brain GABA level. These biochemical alterations are accompanied by behavioural changes including 'grand mal' like seizures and a modification of feeding activity. Considering the multiple biochemical effects of the drug, these behavioral changes are probably not related to a specific alteration in GABA metabolism.

Inhibition of GABA* transaminase and increases in the cerebral content of GABA, an inhibitory neurotransmitter, have often been correlated with depression of brain excitability and anticonvulsant action [1–2].

GABA-transaminase inhibitors can be classified into 3 groups (for review, see [3]): (1) carbonyl trapping reagents (which link to the cofactor) and substrate coenzyme analogues; (2) GABA analogues which act mainly by competition with the substrates; (3) K cat inhibitors or suicide compounds which react with the target enzyme, causing its irreversible inhibition. Three derivatives of this type were recently proposed either as pharmacological tools or as therapeutic agents: γ-acetylenic GABA [4], γ-vinyl GABA [5] and GABAculine [6]. However, in the mammalian brain several enzymes do not possess a very high specificity toward their substrates. For instance, substrates for GABA-T include β-alanine, *d,l*-β-aminoisobutyric acid and β-aminovaleric acid [7], substrates for cysteine sulfinic acid transaminase (CSA-T; cysteine sulphinate 2-oxoglutarate transaminase) include aspartate, and cysteic acid [8], and substrates for cysteine sulphinic acid decarboxylase (L-cysteine sulphinate decarboxylase EC 4.1.1.29) include glutamate [9].

It has been reported that γ-acetylenic GABA increases brain GABA levels by several-fold in rats and mice [4]. During such increases in GABA, an anticonvulsant effect has been observed on several types of seizures, including electroshock seizures, audiogenic seizures [10], photosensitive epilepsy [11], and convulsions produced by administration of thiosemicarbazide, isoniazid, strychnine [10] and hyperbaric oxygen [12]. The proposed mechanism of action of γ-acetylenic GABA involves initial Schiff base formation with pyridoxal phosphate attached to the enzyme and subsequent irreversible reaction of the acetylenic group to a nucleophile near or at the active site [13]. It is thus possible that other pyridoxal phosphate dependent enzymes which metabolize amino-acids may be inhibited in a similar fashion.

The percentage increase in brain GABA level required to obtain 50 per cent protection against audiogenic seizure with γ-acetylenic GABA was rather high; about 320 per cent for DBA mice [10]. Also, γ-acetylenic GABA, like aminooxyacetic acid, hydroxylamine, and some other drugs, interferes with glutamate decarboxylase (L-glutamate 1 carboxylase EC 4.1.1.15—GAD), the activity of which exerts direct control over the GABA pool involved in neurotransmission [10]. The anti-convulsant effect of γ-acetylenic GABA has usually been investigated after a single day of treatment. The few reports concerning chronic injections of γ-acetylenic GABA dealt especially with its action on the GABA system [14, 15], although it is essential to investigate its possible action on other systems. This seems particularly important as an increased GABA level has been observed simultaneously with intermittent convulsions [16]. However, intraperitoneal injections of GABA can elevate GABA levels in the brain without producing seizures [17–18]. Moreover, convulsions are not observed with all GABA-T inhibitors that produce increases in brain GABA level [19, 20].

We also became interested in the effects of chronic administration of γ-acetylenic GABA in view of the possibilities that either the inactivation of GABA-T or the chronic increase of GABA would induce changes in the rate of synthesis of GABA-T or GAD

*Abbreviations: GABA: 4-aminobutyric acid; PLP: pyridoxal 5 phosphate; GABA-T: 4-aminobutyrate 2 oxoglutarate aminotransferase; GOT: glutamate oxaloacetate transaminase; CSA-T: cystein sulphinate transaminase; Ala-T: alanine aminotransferase; PPK: pyridoxal phosphokinase; GAD: glutamate decarboxylase.

apoenzyme. The effects of inhibition of GAD, which are apparently without any consequence in an acute treatment [10], may be significant after repeated administration of this drug. The effects of chronic injections of γ-acetylenic GABA on some parameters of rat behavior and cerebral amino acid metabolism are presented in this report.

MATERIALS AND METHODS

Materials

γ-Acetylenic GABA (4-amino-hex-5-ynoic-acid) was a gift of Merrell Laboratories, Strasbourg, France. Lactate dehydrogenase (L-lactate oxidoreductase EC 1.1.1.17) prepared from porcine muscle was obtained from Boehringer, Mannheim, West Germany. All radioactive compounds were obtained from the Radiochemical Centre, Amersham. Tyrosine apoenzyme decarboxylase was from Sigma (St Louis). All other reagents were of analytical grade.

Methods

Animals. Male Wistar rats (150–200 g), housed singly, were used. During the first two days of experimentation, the animals received i.p. two injections of 50 mg/kg γ-acetylenic GABA (one at 9 a.m., the second at 5 p.m.) in order to rapidly achieve a large inhibition of GABA-T. Preliminary experiments showed that one injection per day produced only about 50 per cent inhibition of GABA-T after two days, whereas two daily injections induced 70 per cent inhibition. This inhibition was maintained for the following 6 days with a single injection per day (9 a.m., 50 mg/kg). In fact, two daily injections during 8 consecutive days caused the death of about 70 per cent of the animals. Control rats were injected in parallel with saline. Food (aliments composés complets—UAR—A04) and tapwater were given *ad lib.* A 12 hr light–dark cycle (7.00–19.00 hr light) was employed.

Measurement of body weight. During the 8 days of treatment, the animals were weighed every day at 9 a.m. before receiving injections. The amount of food consumed by each animal was also measured.

Electroencephalographic studies. Five rats were used. Four screw electrodes were fixed into the skull over the frontal (2 symmetric electrodes) and parietal cortex (2 symmetric electrodes) under pentobarbital anesthesia. Animals were allowed to recover for 1 week, and then injected with saline; recordings were made every day for 5 days at 8 a.m. (just before injections) to establish the control EEG record. Then, the animals were injected with 50 mg/kg of γ-acetylenic GABA, according to the protocol previously described and recordings were made every day at 8 a.m. for 1 hr, before the drug injection. Some recordings were made 5 hr after drug injection when the GABA level had reached its maximum [4].

Tissue homogenization for enzymatic studies. For measurements of GAD, GABA-T, glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1.) and cysteine sulfinate transaminase (CSA-T, cysteine sulfinate: 2-oxoglutarate transaminase) activities, and the levels of GAD and GABA-T apoenzymes, the animals were sacrificed by decapitation, and the brains rapidly removed then homogenized at 0° in 10 vol. (w/v) of 2 mM phosphate buffer (pH 7.2) containing 0.5 mM pyridoxal phosphate, 1 mM aminoethylisothiouronium bromide hydrobromide, 0.5% Triton X-100 (v/v) and 0.1 mM EDTA. Enzyme activities were immediately measured. For radioimmunoassay of GAD and GABA-T, homogenates were kept frozen at −25°, and used after one week. For determinations of pyridoxal kinase (PPK, ATP: pyridoxal-5′-phosphotransferase, EC 2.7.1.35) and alanine aminotransferase (EC. 2.6.1.2.—Ala-T) activities, brains were homogenized in the medium described above, except that pyridoxal phosphate was omitted. Homogenates were then centrifuged at 80,000 *g* for 30 min. using a Beckman R-30 Rotor. Determinations of the enzymatic activities in the supermatants were carried out immediately.

Enzyme assays. GABA-T activity was determined by measuring the rate of formation of deaminated products from [^{3}H(U)]GABA, using a previously described method [21]. GAD activity was measured by the $^{14}CO_2$ trapping method under the conditions described by Blindermann *et al.* [22], except that the final concentration of glutamate in the medium was 10 mM. Under the conditions used, this method gives the same results as those based on [^{14}C (U)]GABA determination formed from [^{14}C (U)]glutamate.

Measurement of the levels of GABA-T and GAD apoenzymes were carried out by radioimmunoassays described respectively by Ossola *et al.* [21] and Blindermann *et al.* [22] using antibodies obtained by injection of pure GABA-T or pure GAD into rabbits. The specificities of these antibodies have already been demonstrated [21, 22].

Inhibition tests of the fixation by specific antibodies of pure [^{125}I]GAD and of pure [^{125}I]GABA-T by unlabelled antigen or by rat brain homogenate have been carried out [21, 22]. Preliminary experiments have shown that the same curves can be obtained if unlabelled antigen (i.e., pure GAD or GABA-T) or rat brain homogenate is substituted for the product of the reaction *in vitro* of γ-acetylenic GABA with the antigen or with rat brain homogenate. These results qualify the two radioimmunoassays for determination of GAD or GABA-T in the presence of γ-acetylenic GABA.

GOT and CSA-T activities were determined as previously described by Recasens *et al.* [23].

PPK Activities were measured according to the method of Karawya and Fonda [24] in which [^{3}H]pyridoxine phosphate formed from [^{3}H]pyridoxine is isolated with DEAE-cellulose discs. The assay mixture and incubation conditions are the same as described by the authors.

Ala-T was measured according to Bergmeyer and Berndt [25] with a volume of 60 μl supernatant in a total volume of 660 μl of Tris–HCL buffer (pH 7.5; 124 mM) containing, at final concentrations: L-alanine 170 mM, 2-oxoglutarate 16.9 mM, NADH 0.267 mM, EDTA 8 mM, and lactate dehydrogenase from porcine muscle 4.73 units/ml. Disappearance of absorption at 366 nm was followed for 6 min at 37°. Blanks were run in parallel without L-alanine.

Purification of GABA-T, GAD, GOT and CSA-T from rat brain. These four pure enzymes were

Table 1. Period of inactivation of purified enzymes of rat brain in presence of γ-acetylenic GABA. All kinetics determined are of first order rate

Enzyme	Concentration of acetylenic GABA 10^{-3}M	Period of inactivation (min)
GABA-T	0.5	24 ± 3
GAD	0.5	60 ± 5
GOT	1	36 ± 5
CSA-T	1	149 ± 10

obtained from rat brain as previously described for GABA-T [26], GAD [27], GOT and CSA-T [28]. The actions of γ-acetylenic GABA on these enzymes were determined by pre-incubation of each enzyme with the drug at 37° in the buffers used for determination of enzymatic activities. At various time intervals, aliquots of enzyme were taken to determine the remaining enzymatic activities by the different assays described (Table 1).

Determination of the free amino acid content. The free amino acid content of the brains of injected and control rats were determined each day for one week. Rats were injected daily at 9 a.m. with 50 mg/kg of γ-acetylenic GABA; control rats received saline. Each day, 5 hr after the injection, when the GABA level was at its maximum [4], 4 rats were killed in a microwave oven. Irradiation of the head of the animal never exceeded 7 sec. The brains were homogenized in 10 vol. (w/v) ethanol 80%, centrifuged at 4,000 g for 30 min, and re-extracted with 5 vol. of the same medium. The supernatants were pooled, ethanol was evaporated at 60°, and the remaining water was removed by lyophilization. Each extract

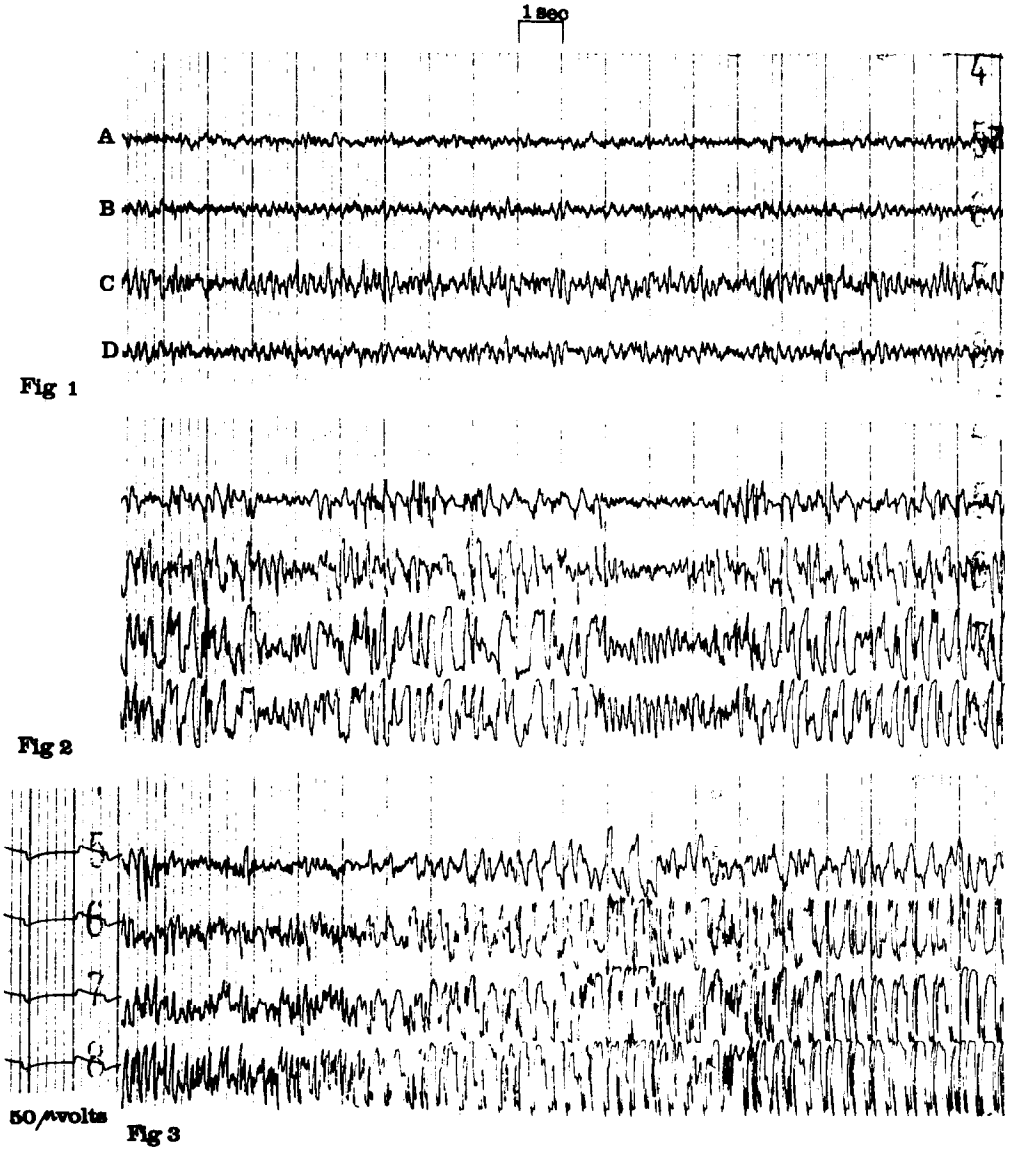

Fig 1

Fig 2

Fig 3

Figs. 1–3. Rat EEG. Fig. 1 (upper Record): control EEG; Fig. 2 (middle Record): 5 h after 50 mg/kg γ-acetylenic GABA-slow waves with periods of synchronisation; Fig. 3 (lower Record): after 4 injections of 50 mg/kg γ-acetylenic GABA during 2 days, the first observed seizure appears during the morning of the third day. Leads: A = fronto-frontal cortex; B = Parieto-parietal cortex; C = Fronto-parietal cortex (right); D = Fronto-parietal cortex (left).

was dissolved in 500 μl of HCl (N/100) and the amino acids were measured using a Technicon amino-acid analyser.

Taking into account that all experiments were performed on rats that were killed 16 hr after the last injection, and as the biological half life of γ-acetylenic-GABA in brain is less than 2 hr [4], we never demonstrated the existence of free γ-acetylenic-GABA on chromatograms of free amino acids at the time of sacrifice.

Under the conditions of amino-acid chromatography used, γ-acetylenic-GABA, used as a marker, was clearly separated from other amino-acids.

Pyridoxal 5-phosphate assay. The procedure of Bayoumi and Smith was used [29], which consists of measuring the enzymatic decarboxylation of [^{14}C (U)]tyrosine by apotyrosine decarboxylase in the presence of suitably diluted brain extract. However, in our procedure, we used the $^{14}CO_2$ trapping method instead of determining the tyramine formed. Other conditions were identical to the method described.

Protein determinations were carried out by the method of Lowry *et al.* [30]. Statistical analysis of data was carried out using Student's *t*-test.

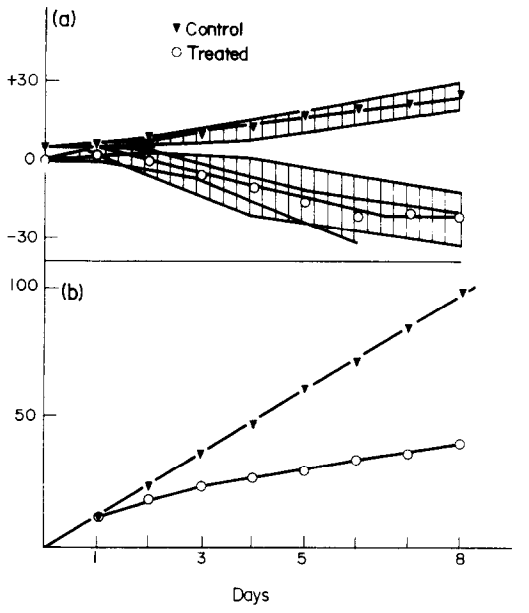

Fig. 4. Variation of body weight as per cent of initial weight (a). The shaded area represents the range value of body weights between rats treated in the same way, food intake as per cent of the initial total amount of food given to the animals at the start of the treatment (b). Animals received the drug as described in the text.

RESULTS AND DISCUSSION

EEG and behavioural studies

It has been reported that five hours after the first injection of γ-acetylenic GABA, the cerebral GABA level reaches its maximum [4]. At this time, as compared with records made under the same conditions in control rats (Fig. 1), we observed a lower frequency and higher voltage with periods of synchronization (Fig. 2) After the rats had received two daily injections for two days, EEG recordings on the third day (16 hr after the last injection) usually revealed the first clusters of spikes and the rats exhibited their first partial seizures with myoclonic–tonic extension (Fig. 3). During the following five days, while rats received one injection per day, they displayed interative 'grand mal' seizures and about 30 per cent of the rats died, therefore, experiments were not conducted for a longer period of time.

Table 2. Some biochemical parameters of whole rat brain from animals that had received four injections of γ-acetylenic GABA (50 mg/kg—2 injections per day). At this time, animals begin to exhibit 'grand mal' seizures

Enzymes	Control rats	Treated rats	Percentage of change
		10^{-9} moles·min^{-1} mg^{-1}	
GABA-T	6.35 ± 0.80	1.91 ± 0.20	70
GAD	3.70 ± 0.12	1.90 ± 0.29	49
GOT	595 ± 10	490 ± 20	16.8
CSA-T	1620 ± 20	1380 ± 40	14.8
PPK	0.90 ± 0.06	1.12 ± 0.03	unchanged
Ala-T	100.6 ± 0.42	37.1 ± 3.68	63.2
Amino-acids		10^{-9} moles·mg^{-1}	
GABA	11.53 ± 2.5	80.4 ± 20.7	597
Glutamate	28.16 ± 1.17	59.16 ± 6.90	110
Phenylalanine	0.32 ± 0.01	0.78 ± 0.15	143
Alanine	2.42 ± 0.5	3.30 ± 0.46	36
Ornithine	0.12 ± 0.01	0.46 ± 0.16	283
Taurine	3.82 ± 0.35	5.77 ± 1.04	51
(Aspartate, threonine, serine, glycine, valine, leucine, isoleucine, tyrosine, lysine, arginine, histidine and methionine did not differ significantly from control.)			
		10^{-9} g·mg^{-1}	
PLP	24.08 ± 1.91	23.74 ± 2.60	unchanged

All results are expressed as means ± SEM (4 treated vs 4 control rats). All percentage differences between treated and control rats are significant ($P < 0.05$).

We also observed, after a latency of one day, a modification of feeding activity in treated rats. After one day of treatment, food ingestion began to decrease, and after one week it represented about 1/3 of the food ingested by control rats. As a consequence, the body weight of treated rats decreased regularly, and after 8 days it was only about 40% that of control rats (Fig. 4). This difference may be attributed to the sedation that occurs when the GABA level reaches its maximum (about 5–6 hr after injection) but might also be due to the appearance and repetition of partial and total seizures which modify feeding behavior. Moreover, several reports have indicated that GABA may control appetite mechanisms and food intake by an action on the rat hypothalamus. Kelly *et al.* [31] reported that a GABA antagonist, bicuculline, injected into the anterolateral hypothalamus increased food intake. Cooper *et al.* [32] have demonstrated the anorexic effect of ethanolamine-*O*-sulfate (an irreversible GABA-T inhibitor) when given intracisternally to rats. During this latter treatment, GABA-T was inhibited by 60 per cent and recovery of GABA-T activity coincided with a return of body weight to control values.

It is noteworthy that several behavioral and physiological alterations were described after γ-acetylenic GABA treatment. Acute doses induced, in addition to piloerection, lacrimation, hypothermia, a certain degree of sedation [10]. The same authors [16] have also reported the existence of intermittent excitation or convulsions following administration of an acute dose of this drug in mice. The same effects have been reported for GABA-T inhibitors which are well known to be non specific for GABA-T (e.g., aminooxyacetic acid) [33–34]. In contrast, increases in cerebral GABA produced by intraperitoneal injection of GABA in young chicks or by GABA-T inhibition with other inhibitors (ethanolamine-*O*-sulfate, di-n-propylacetate) never induced convulsions despite a large GABA elevation [19–20]. EEG recordings carried out in the present study showed that the intermittent excitation or convulsions previously observed are indeed epileptic type seizures.

Biochemical studies

All the biochemical studies were conducted on rats killed 16 hr after their last injection. At this time, no sedation was evident. The rats showing an epileptic seizure within one hour of the time chosen for sacrifice were not used for biochemical investigations. We examined several biochemical parameters of the treated rat brain under two experimental conditions: (1) when seizures appeared for the first time, i.e., in the morning of the third day, and (2) after 8 days of treatment, i.e., in the morning of the ninth day.

After two days of treatment, when seizures appeared, we examined: GABA-T, GAD, GOT, CSA-T, PPK and Ala-T activities, free amino-acid content and pyridoxal phosphate level in brain homogenates. Results are summarized in Table 2 and compared to results obtained with control rats.

As previously reported [4], both GABA-T and GAD activities were markedly reduced in treated rats, and GABA levels were strikingly increased. However, the activities of other transaminases (e.g., GOT, CSA-T and especially Ala-T) were significantly reduced. The elevation of glutamate levels (110%) may be related to inhibition of GOT, Ala-T and GAD in treated rats via the following mechanisms: (1) inhibition of transaminases using glutamate as a substrate thus increasing the level of this amino acid in the metabolic pool [35]; (2) inhibition of GAD which reduces the formation of GABA in the presynaptic pool with a resultant increase in glutamate at this level. These modifications could also induce an alteration in the glial pool of glutamate. In view of the much larger degree of GABA-T inactivation by the drug ($660 \cdot 10^{-9}$ moles min^{-1} for a rat brain of 1.5 g) as compared to the amount of GAD inactivation in the same rat brain ($277 \cdot 10^{-9}$ moles min^{-1}), the partial inhibition of both enzymes still induces GABA accumulation. Alanine, which increases by about 36 per cent, can be correlated with the 63 per cent decrease in activity of Ala-T. The decrease of GAD activity, is believed to parallel a decrease in cysteine sulfinate decarboxylase activity [9]. In addition, the inhibition of CSA-T and the increase in taurine (51%) shows that the drug also interferes with taurine metabolism. Inhibition of CSA-T might increase the level of CSA available for taurine synthesis and could explain the increase in this amino acid. The alterations in phenylalanine and ornithine levels may be attributed to inhibition of other enzymes. Indeed, inhibition by γ-acetylenic GABA of ornithine transaminase purified from rat liver or from a rat brain homogenate and of pure GOT from pig heart has been reported [36, 37].

Pyridoxal phosphokinase activities, and pyridoxal phosphate levels remained unchanged in the brains of treated animals. These two parameters were investigated in order to verify whether significant increases in GABA inhibit PPK and diminish PLP synthesis in brain. Indeed, Kwok and Churchich [38] have reported that purified PPK of pig brain is competitively inhibited by GABA ($K_i = 2$ mM), and Ebadi and Govitrapong [39] have demonstrated a 25 per cent decrease in PLP formation by 5×10^{-4}M GABA. However considering that PPK inhibition by GABA is competitive *vis à vis* ATP, and the level of ATP is high in synaptosomes (and in brain tissue in general) and that the K_m of PPK for ATP is about 10^{-5}M [40], it seems unlikely that a GABA-induced inhibition of PPK activity is of significance *in vivo*. Actually, PPK activity and the PLP level were unchanged after drug treatment.

Several hypotheses may be considered in order to explain the appearance of seizures despite the high brain GABA level. First, the high amount of GABA accumulated may be predominately in the glial or metabolic pool but not in the presynaptic pool where GAD is located [41]. Indeed it seems reasonable to attribute the anticonvulsant effect to an increased GABA level in the presynaptic pool. Moreover, if GABA accumulates in the metabolic pool, the total energy produced for neuronal function may be significantly affected. This phenomenon may contribute to seizures activity. Two other amino acids, glutamate and taurine which are putative neurotransmitters and have been implicated in the modulation of

Table 3. Some biochemical parameters of whole rat brain from animals which had received 8 days treatment

Enzymes	Control rats	Treated rats	Percentage of change
Enzymes		10^{-9}moles·min^{-1}·mg^{-1}	
GABA-T	5.87 ± 0.45	1.95 ± 0.05	67
GAD	3.58 ± 0.10	1.43 ± 0.3	60
GOT	550 ± 50	415 ± 20	24.5
CSA-T	1660 ± 100	1190 ± 20	28.3
PPK	1.11 ± 0.11	1.09 ± 0.05	unchanged
Ala-T	99.96 ± 2.56	29.52 ± 1.7	70.5
Apoenzymes		10^{-12}moles·mg^{-1}	
GABA-T	22.4 ± 0.88	25.0 ± 3.2	unchanged
GAD	3.65 ± 0.14	3.73 ± 0.28	unchanged
Amino-acids		10^{-9}moles·mg^{-1}	
GABA	11.53 ± 2.5	62.2 ± 8.2	439
Glutamate	28.16 ± 1.17	52.94 ± 1.95	88
Phenylalanine	0.32 ± 0.01	0.61 ± 0.11	90.6
Alanine	2.42 ± 0.5	3.72 ± 0.82	53.7
Ornithine	0.12 ± 0.01	0.20 ± 0.02	66.6
Taurine	3.82 ± 0.35	4.98 ± 1.21	30.3
		10^{-9}g·mg^{-1}	
PLP	21.63 ± 0.68	22.20 ± 0.52	unchanged

All results are expressed as means ± SEM (4 treated vs 4 control rats). All percentage differences between treated and control rats are significant ($P < 0.05$).

CNS excitability, appear to be markedly modified by the drug treatment. Changes in their levels may contribute to the appearance of seizures [42]. This appears possible especially for glutamate, which produces excitation at a number of synapses [35]. It seems possible that the changes in amino acid levels observed are due to a direct alteration of some enzymatic pathways rather than to the high GABA level maintained for 2 days. This change in GABA level could explain some behavioural effects previously reported [10] but it is unlikely that the increase in GABA induces seizures and the multiple enzymatic alterations observed.

Table 3 shows the same parameters as Table 2, but after 8 days of treatment. GABA-T inhibition (67%) was quite similar to that obtained after two days, but GAD inhibition increased to 60 per cent. This increase of GAD inhibition after 8 days is significant ($P < 0.02$) as compared with 2 days of treatment. Since levels of GABA and glutamate remained very high for 8 days (see Fig. 5), it was of

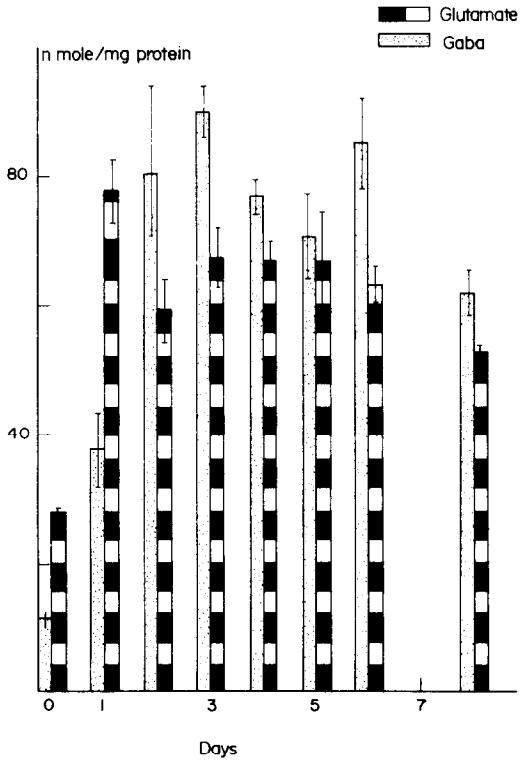

Fig. 5. Variation of GABA and glutamate levels from rat brain during a 8 days treatment with γ-acetylenic GABA (50 mg/kg/day). Each value is the mean of 4 rats ± S.E.M.

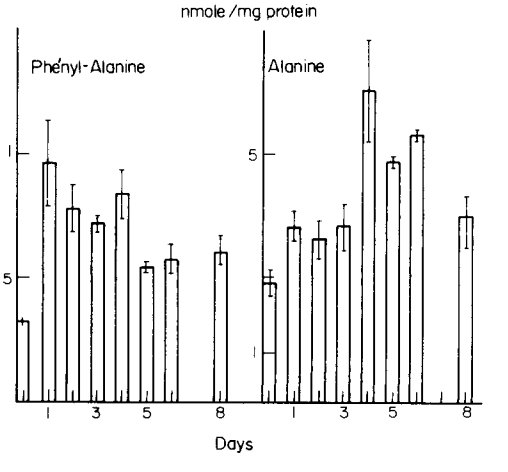

Fig. 6. Same measurements as Fig. 5 for phenylalanine and alanine.

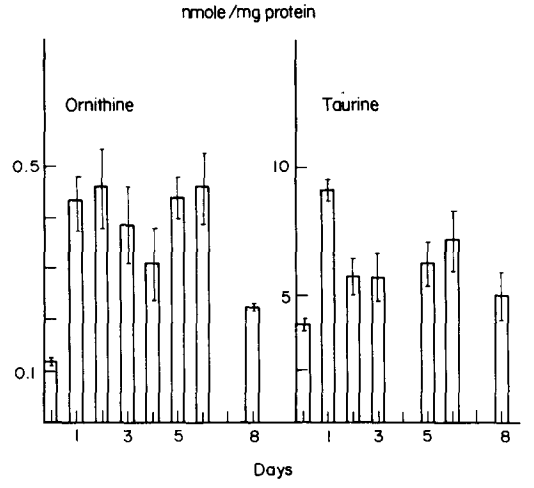

Fig. 7. Results for ornithine and taurine. Same conditions as for Fig. 5.

interest to determine whether the levels of GAD and GABA-T apoenzymes had been altered. However, as indicated in Table 3, the levels of these two proteins were unchanged by chronic drug treatment. Obviously, we cannot exclude a modification in the turnover rate of these two proteins induced by high amount of GABA or glutamate or by the inactivation of an important part of the activities supported by these proteins. This suggests that γ-acetylenic GABA inactivated enzymes were still measured by the radioimmunoassays.

GOT, CSA-T and Ala-T activities were more affected after 8 days of treatment, than after two days of treatment ($P < 0.05$).

The levels of amino acids after two days of treatment remained altered (see Figs 5, 6 and 7) while the other major free amino acids of the brain (aspartate, serine, glycine, valine, leucine, isoleucine, tyrosine, lysine, arginine, histidine, and methionine) were not significantly affected.

In conclusion, it appears that while γ-acetylenic GABA, administered in acute doses, produces more inhibition of GABA-T than of GAD and an essentially anticonvulsant effect [10], repeated administration of this drug induced modifications in the metabolism of several other cerebral amino acids. In addition, animal behavior was severely affected: an electrophysiological profile of seizure appeared after 3 days of treatment, and latter repeated seizures as well as modification of feeding behavior and sedation were observed. All of these changes cannot be related to a single modification of brain metabolism because of the multiplicity of drug action. Thus, the behavioral alterations induced by administration of γ-acetylenic GABA must be examined by considering alterations other than those related to GABA metabolism.

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