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# A NEUROCHEMICAL STUDY OF THIOSEMICARBAZIDE SEIZURES AND THEIR INHIBITION BY AMINO-OXYACETIC ACID*

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**Abstract**—Effects of intravenous thiosemicarbazide were studied in dogs by recording cortical electrical activity and by analyzing cerebral tissue frozen *in situ*. Free amino acids and related substances were measured. Generalized seizures were accompanied by a decrease in γ-aminobutyric acid and increases in alanine, ammonia, lactic acid, and tyrosine. In another group of dogs, intravenous amino-oxyacetic acid induced a decrease in brain aspartic acid and increases in alanine, γ-aminobutyric acid, ammonia, glutamine, lactic acid, lysine, and tyrosine. The changes in aspartate, alanine, glutamine, and lactate may be secondary to the high brain ammonia, which in turn can be attributed at least in part to ammonia appearing in the blood. These chemical changes were usually accompanied by varying degrees of electrographic depression, but occasionally by signs of cerebral excitation and in one instance by a generalized seizure (the excitatory effects being more prominent at higher dose levels). Amino-oxyacetic acid had an inhibitory effect on thiosemicarbazide seizures. The inhibition was overcome by increasing the dose of thiosemicarbazide, although γ-aminobutyric acid in the brain remained above normal. The combined effects of the two drugs on the chemical pattern were essentially the same as the effect of amino-oxyacetic acid alone. Amino-oxyacetic acid raised the convulsive threshold to pentylenetetrazol but showed little antagonism to picrotoxin. The inhibitory effects of amino-oxyacetic acid may be due to the increase in brain of γ-aminobutyric acid. The excitatory effects of hydrazides and of amino-oxyacetic acid, which are carbonyl-trapping agents, may involve the same (unknown) basic mechanism, with a decrease in γ-aminobutyric acid potentiating excitation by hydrazides and an increase opposing excitation by amino-oxyacetic acid.

RECENT efforts to elucidate the biochemical mechanisms underlying the excitatory action of the convulsant hydrazides have been discussed extensively in reviews by Roberts and Eidelberg[2] and by Williams and Bain.[3] Attention has been centered on the cerebral metabolism of γ-aminobutyric acid (γ-ABA), an intermediate that appears to have an inhibitory action on neuronal activity. γ-ABA is formed by the action of L-glutamic acid decarboxylase (GAD), and is further metabolized by the action of γ-ABA-α-ketoglutarate transaminase (γ-ABA-T). These two reactions are among those requiring pyridoxal phosphate as a coenzyme. The seizures induced by hydrazides are thought to bear a special resemblance to those occurring in vitamin $B_6$ deficiency,

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being antagonized by various forms of vitamin $B_6$, whereas those induced by other well-known convulsants are not. Convulsant hydrazides are known to reduce the level of $\gamma$-ABA in the brain *in vivo* by decreasing the activity of GAD while the $\gamma$-ABA-T remains unaffected. Hydroxylamine, on the other hand, raises the $\gamma$-ABA level by preferentially inhibiting the activity of $\gamma$-ABA-T, and tends to reduce the cerebral excitability. The hydrazides and hydroxylamine are carbonyl-trapping agents which react with the aldehyde group of pyridoxal phosphate. However, $\gamma$-ABA-T binds the coenzyme more tightly than does GAD, and hydroxylamine may react with the bound form. A further complication arises from the fact that some of the hydrazides form pyridoxal hydrazones which inhibit the phosphorylation of pyridoxal.

The use of hydrazides and hydroxylamine to alter the $\gamma$-ABA content of the brain has indicated an inverse relationship between the $\gamma$-ABA level and the excitatory responses to electrical stimulation[2, 4] and to pentylenetetrazol.[4] However, it has become clear that hydrazide seizures cannot be attributed solely to the decrease in brain $\gamma$-ABA, since thiosemicarbazide induces seizures while $\gamma$-ABA remains above normal in animals treated with hydroxylamine.

Wallach[5] introduced another agent, amino-oxyacetic acid (AOAA), which has an action similar to that of hydroxylamine. AOAA is more potent than hydroxylamine and induces a more prolonged increase in the brain $\gamma$-ABA content.[6] It inhibits $\gamma$-ABA-T both *in vitro* and *in vivo*.[5–7] It is also a potent inhibitor of GAD *in vitro*, but does not affect this enzyme in the brain *in vivo*.[6, 7] DaVanzo *et al.*[8] found that AOAA has an anticonvulsant action against thiosemicarbazide in cats, rats, and mice and also protects cats against methionine sulfoximine seizures.

In most studies of the actions of convulsant hydrazides and their antagonists, cerebral constituents other than $\gamma$-ABA have received scant attention. The $\gamma$-ABA concentrations which have been reported are generally higher than the true *in-vivo* levels, since it has recently been found that $\gamma$-ABA increases in the brain during the first few minutes post mortem.[9, 10] The present report describes experiments in which many free amino acids and related substances were measured in cerebral tissue frozen *in situ*. Chemical and electrographic changes induced by thiosemicarbazide, by AOAA, and by combinations of these agents were observed. The combined effects of AOAA and pentylenetetrazol were studied likewise, and the effect of AOAA on the responsiveness to picrotoxin was tested.

## METHODS

Adult male dogs were used. Food was withheld for 18 to 20 hr preceding the experiment. The animal was given morphine sulfate (5 mg/kg s.c.), and transient anesthesia was induced 30 to 40 min later with thiopental sodium (about 12 mg/kg i.v., with subsequent small doses if needed). The cranium was exposed and opened, and most of the calvarium was removed, the dura mater remaining intact. Preparation was made for electrographic recording from three cortical areas and for freezing the brain *in situ* with liquid air. Polyethylene cannulas were inserted into a femoral artery for blood pressure recording and into a femoral vein for injections. An endotracheal tube was inserted for the administration of artificial respiration when necessary. After completion of these procedures, a period of 40 min or more was allowed for disappearance of the thiopental effects. The rationale for selection of these conditions and more complete details have been published elsewhere.[10]

Thiosemicarbazide, AOAA, and pentylenetetrazol were dissolved in physiological saline for injection. Doses of AOAA are stated in milligrams of the hemihydrochloride.

The brain was frozen at the chosen time, and the exposed parts of the cortex were quickly removed to a depth of about 1 cm. The tissue was analyzed for the following constituents: N-acetylaspartic acid, alanine, γ-ABA, ammonia, arginine, aspartic acid, citric acid, glutamic acid, glutamine, glutathione, glycerophosphoethanolamine, glycine, histidine, lactic acid, leucine, lysine, the sum of methionine and cystathionine, phenylalanine, phosphoethanolamine, serine, taurine, threonine, tyrosine, urea, and valine. The amino acids were separated by ion exchange chromatography. The analytical procedures have been described.[10]

## RESULTS

### *Thiosemicarbazide seizures*

Half an hour or more after injection of thiosemicarbazide (20 mg/kg i.v.) the electrocorticogram began to show abnormalities consisting of occasional spikes and slow waves. The spikes appeared singly or in groups, at first unaccompanied by visible twitching. A gradual build-up occurred, with the appearance of twitching often associated with spikes synchronized in all leads. This culminated in a severe clonic or tonic–clonic convulsion 43 to 87 min after injection. The electrographic records showed typical epileptiform activity, closely resembling the cortical records obtained by Killam *et al.*[11] from cats given doses of the same magnitude. Arterial blood pressure and heart rate were increased during the seizure.

Artificial respiration was started at the beginning of the seizure in most instances, and the freezing of the brain was started after the seizure had been in progress for about 20 sec. The analyses showed significant changes from control levels in five cerebral constituents (Table 1). The γ-ABA level showed a mean decrease of 34%.

TABLE 1. CHEMICAL CHANGES IN THE BRAIN DURING SEIZURES INDUCED BY THIOSEMICARBAZIDE*

| Constituent | Control exps. Mean ± S.D. | Seizures Mean ± S.D. |
|---|---|---|
| Alanine | 0·14 ± 0·04 (9) | 0·19 ± 0·04 (4)† |
| γ-ABA | 0·83 ± 0·08 (9) | 0·55 ± 0·06 (4)‡ |
| Ammonia | 0·26 ± 0·07 (13) | 0·39 ± 0·01 (3)§ |
| Glutamic acid | 7·81 ± 0·65 (19) | 8·42 ± 0·57 (4) |
| Lactic acid | 1·04 ± 0·26 (17) | 3·61 ± 0·36 (4)‡ |
| Tyrosine | 0·029 ± 0·006 (9) | 0·045 ± 0·008 (3)‡ |

* Twenty mg/kg. Values are in micromoles per gram. Number of animals in parentheses.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.02$.

The mean value of its precursor, glutamic acid, was slightly above normal, but the increase was not statistically significant. Alanine, ammonia, lactic acid, and tyrosine were increased. The other constituents measured did not show significant differences from control values reported earlier.[10]

In one experiment (not included in Table 1) the dose of thiosemicarbazide was

increased to 60 mg/kg. The seizure occurred 57 min after injection. The levels of cerebral constituents at this time were similar to those found with the lower dosage. In another instance the brain was frozen before a seizure occurred. A period of 53 min had elapsed since the injection of thiosemicarbazide (20 mg/kg), and minimal electrographic changes were evident. The $\gamma$-ABA had decreased to a level equal to the highest value observed during a seizure (0·63 $\mu$moles/g). Tyrosine was 0·036 $\mu$moles/g, a value higher than the mean control level and equal to the lowest value observed during a seizure, although exceeded in one control experiment. The other measured constituents were all within normal limits.

*Effects of amino-oxyacetic acid*

In dogs subjected to no other procedures, intravenous injection of AOAA in doses of 5, 10, or 20 mg/kg induced a state of profound depression and flaccidity in 15 to 25 min, usually preceded by vomiting. The depressed condition differed from anesthesia, since the animal could be aroused even when almost completely paralyzed. Recovery occurred after a period of several hours, the duration of the depression varying with the dosage, and there were no apparent after effects. Wallach[5] has made similar observations on dogs, cats, rats, and mice.

After surgical preparation of the animal, AOAA (20 mg/kg) induced a gradually developing depression in the electrocorticogram, manifested by diminishing amplitude of the normal fast-frequency waves and developing slow wave activity. The changes were usually minimal or undiscernible 1 hr after injection, becoming quite apparent by 2 hr and showing further development at 3 hr.

Wallach[5, 12] mentioned a convulsant effect of large doses of AOAA in dogs and other animals but did not give the dose levels required for this response. In our experiments convulsions never occurred at 20 mg/kg, although slow spikes occasionally appeared. In one instance 30 mg/kg induced a seizure with typical epileptiform cortical activity at 115 min. This was preceded by only minimal depression and the appearance of slow spikes. However, doses of 30 to 40 mg/kg in five instances induced moderate to profound cortical depression at 2 hr. In one of these (40 mg/kg) a tonic seizure of 1 min duration occurred at 171 min, while the cortex remained profoundly depressed. This apparently was a seizure of subcortical origin which failed to invade the cortex.

AOAA induced changes in eight of the cerebral constituents measured. A rough indication of the progression of these effects was obtained from a series of seven dogs in which each was given 20 mg/kg, and the brain was frozen 1 to 4 hr later (Table 2). $\gamma$-ABA showed a marked increase which appeared to continue throughout the 4-hr period. (Wallach[5] found that this change reached its maximum in 6 to 8 hr.) Ammonia showed a large increase which reached its maximum at about 2 hr, with a subsequent decline almost to the normal level within 4 hr. Glutamine also showed a large increase, reaching its maximum possibly a little later than did the ammonia; the course of recovery of the glutamine level remains uncertain, since only one value (the last) indicated recovery. Tyrosine showed a marked increase which roughly paralleled the change in glutamine. Lysine increased more slowly, reaching a peak at about 3 hr. Lactic acid tended to be high during the first 3 hr but returned quickly to normal thereafter. Alanine also in some instances showed high levels during the first 3 hr, but this change was not entirely consistent. It has been noted previously[10] that alanine

TABLE 2. CHEMICAL CHANGES IN THE BRAIN INDUCED BY AMINO-OXYACETIC ACID*

| Constituent | Control expts. Mean ± S.D. | Minutes after AOAA injection | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 63 | 93 | 120 | 145 | 181 | 210 | 233 |
| Alanine | 0·14 ±0·04 (9) | 0·19 | 0·17 | 0·15 | 0·28 | 0·09 | 0·17 | 0·14 |
| γ-ABA | 0·83 ±0·08 (9) | 1·49 | 2·25 | 2·07 | 3·41 | 3·52 | 2·60 | 4·10 |
| Ammonia | 0·26 ±0·07 (13) | 0·69 | 0·72 | 1·23 | 1·03 | 0·85 | 0·70 | 0·32 |
| Aspartic acid | 2·45 ±0·22 (19) | 1·85 | 1·68 | 1·75 | 1·69 | 3·48 | 2·39 | 2·42 |
| Glutamine | 5·6 ±1·7 (13) | 9·0 | 8·3 | 8·8 | 14·0 | 9·3 | 13·1 | 4·5 |
| Lactic acid | 1·04 ±0·26 (17) | 1·60 | 2·50 | 1·37 | 2·22 | 1·09 | 1·23 | 1·09 |
| Lysine | 0·15 ±0·03 (9) | 0·14 | 0·17 | 0·19 | 0·21 | 0·30 | 0·22 | 0·16 |
| Tyrosine | 0·029±0·006 (9) | 0·186 | 0·171 | 0·143 | 0·256 | 0·130 | 0·148 | 0·039 |

* Twenty mg/kg. Values are in micromoles per gram. Number of control animals in parentheses.

and lactic acid tend to change in parallel fashion, probably because each is closely related to pyruvic acid. Leucine and phenylalanine occasionally showed increases, but these changes were not consistent and are not included in Table 2. Aspartic acid was the only constituent to show a decrease, reaching a minimum at about 2 hr and returning to normal within 4 hr. An anomalous high value appeared at 3 hr.

Larger doses of AOAA induced similar changes, of greater magnitude in some instances (Table 3). In the single instance of a cortical epileptiform seizure induced by AOAA, the γ-ABA level was above normal but was considerably lower than in other comparable experiments. The ammonia was not unusually high. Lactic acid showed a greater increase than was seen in other animals given AOAA, undoubtedly as a result of the convulsive activity. Lysine was not elevated in this experiment.

TABLE 3. CHEMICAL CHANGES IN THE BRAIN INDUCED BY AMINO-OXYACETIC ACID*

| Constituent | Minutes after injection of: 30 mg/kg | | | 40 mg/kg |
|---|---|---|---|---|
| | 88 | 115† | 157 | 210 |
| Alanine | 0·16 | 0·18 | 0·17 | 0·33 |
| γ-ABA | 3·14 | 1·99 | 5·59 | 4·24 |
| Ammonia | 0·93 | 0·87 | 0·74 | 2·14 |
| Aspartic acid | 1·54 | 1·59 | 2·30 | 1·02 |
| Glutamine | 9·8 | 8·0 | 5·6 | 16·0 |
| Lactic acid | 2·42 | 4·93 | 2·07 | 3·66 |
| Lysine | 0·20 | 0·14 | 0·19 | 0·28 |
| Tyrosine | 0·225 | 0·161 | 0·098 | 0·362 |

* Thirty or forty mg/kg. Values are in micromoles per gram. Control values are given in Table 2.

† Brain frozen during seizure.

In several experiments ammonia was measured in the blood at various times after injection of AOAA, and was found to be greatly increased (Table 4). It was ascertained that AOAA itself does not break down to form ammonia under the conditions of the analysis.

TABLE 4. EFFECT OF AMINO-OXYACETIC ACID ON BLOOD AMMONIA*

| Time† | Dog 1 | Dog 2 | Dog 3 | Dog 4 | Dog 5 |
|---|---|---|---|---|---|
| 0 | 0·12 | | 0·12 | 0·14 | 0·11 |
| 7 | | 0·38 | | | |
| 60 | | | 0·84 | 0·70 | |
| 75 | 1·25 | 1·69 | | | |
| 120 | | | 1·19 | 1·36 | |
| 180 | | | | | 0·65 |

* Values are in micromoles per milliliter.
† Minutes after injection of AOAA (20 mg/kg).

*Effects of AOAA on thiosemicarbazide seizures*

Whereas severe generalized seizures invariably occurred after injection of thiosemicarbazide alone in doses of 20 mg/kg, the excitatory response was greatly reduced or prevented by 20 mg AOAA/kg (Table 5). In three experiments spikes and slow waves appeared, but without associated convulsive movements. In one of these a convulsive pattern of moderate amplitude subsequently appeared in two cortical

TABLE 5. COMBINED EFFECTS OF AMINO-OXYACETIC ACID AND THIOSEMICARBAZIDE ON CEREBRAL CONSTITUENTS*

| | Seizures inhibited | | | | Seizures | | |
|---|---|---|---|---|---|---|---|
| Dose of TSC (mg/kg) | 20 | 20 | 20† | 30‡ | 60 | 60 | 60 |
| Time of freezing: | | | | | | | |
| Min after AOAA§ | 150 | 150 | 180 | 180 | 120 | 120 | 123 |
| Min after TSC | 90 | 150 | 90 | 120 | 70 | 60 | 63 |
| Alanine | 0·15 | 0·36 | 0·32 | 0·14 | 0·43 | 0·17 | 0·22 |
| γ-ABA | 1·07 | 3·22 | 2·15 | 4·16 | 2·40 | 1·60 | 1·97 |
| Ammonia | 0·71 | 2·26 | 1·25 | 0·58 | 2·28 | | 0·99 |
| Aspartic acid | 1·86 | 1·26 | 1·46 | 2·64 | 1·32 | 2·90 | 1·79 |
| Glutamine | 11·2 | 14·2 | 12·8 | 9·8 | 11·1 | | 9·8 |
| Lactic acid | 2·10 | 6·23 | 5·10 | 1·49 | 8·90 | 2·18 | 3·72 |
| Lysine | 0·15 | 0·30 | 0·20 | | 0·22 | 0·22 | 0·21 |
| Tyrosine | 0·132 | 0·210 | 0·248 | 0·174 | 0·256 | 0·102 | 0·196 |

* Analytical values are in micromoles per gram. Control values are given in Table 2.
† A 5-min period of restricted epileptiform activity occurred. Brain frozen 12 min later.
‡ Complete suppression of excitatory effects of thiosemicarbazide.
§ AOAA dosage was 20 mg/kg in each instance.

leads and persisted for nearly 5 min, but failed to spread to the third lead. Twitching occurred briefly just before the epileptiform waves disappeared. In a fourth experiment the excitatory effects of 30 mg thiosemicarbazide/kg were completely suppressed. These results supplement the similar findings of DaVanzo *et al.*[8] in other species.

However, increasing the dose of thiosemicarbazide to 60 mg/kg induced typical generalized seizures in three animals despite prior administration of AOAA (Table 5). In each case the brain was frozen while a seizure was in progress.

The chemical findings obtained with combinations of AOAA and thiosemicarbazide were quite similar to those with AOAA alone. The γ-ABA levels were always high,

even during convulsions. Ammonia, lactic acid and alanine were sometimes higher than with AOAA alone.

*Effects of AOAA on pentylenetetrazol seizures*

The effect of AOAA on the convulsive threshold to pentylenetetrazol was tested by giving the treated animal small intravenous doses of the convulsant at 30-sec intervals until a generalized seizure occurred. The threshold in control animals (after the usual surgical preparation) was found to be 10 to 15 mg/kg. Tests were made 1 to 3 hr after injection of 20 mg AOAA/kg (six experiments) or 30 mg/kg (one experiment). The threshold was raised in every instance, but there was a wide variation in the magnitude of this effect. The lowest threshold was found to be 15 to 20 mg pentylenetetrazol/kg and the highest 85 mg/kg, with an average of 50 mg/kg. The thresholds were not correlated with the time allowed after AOAA injection, nor with the brain $\gamma$-ABA levels.

Six brains in this series were frozen during a seizure and analyzed. The chemical pattern was essentially the same as that found with AOAA alone, except that the lactic acid tended to be higher (reflecting the usual rise resulting from convulsive activity). A convulsant dose of pentylenetetrazol alone induces a slight increase in alanine, a moderate increase in ammonia, a slight decrease in glutamic acid, and no significant changes in the other nitrogenous constituents measured.[10]

*Response to picrotoxin after treatment with AOAA*

A dose of 1 mg picrotoxin/kg i.v. is adequate to induce a seizure in the dog under the conditions of these experiments. This dose may be considerably above the convulsive threshold; the latter is not easily ascertained because of the relatively slow onset of picrotoxin seizures.[13] In two experiments, picrotoxin at the dose level of 1 mg/kg was given 2 hr after injection of AOAA (20 mg/kg). The seizures were not suppressed by AOAA. The appearance of spikes and of the subsequent initial twitching appeared to be delayed by a few minutes, but the build-up to a generalized seizure occurred thereafter, and the pattern of the seizure was similar in all respects to that seen in animals not given AOAA.[13]

## DISCUSSION

*Chemical changes induced by thiosemicarbazide*

The decrease in brain $\gamma$-ABA induced by convulsant hydrazides, first noted by Killam and Bain[14] in rats given semicarbazide, has been confirmed in several laboratories.[15–20] Thiosemicarbazide has been shown to have this effect in cats,[20] rats,[15, 16] and mice.[19, 20] A significant increase in brain alanine has been noted in rats convulsed with semicarbazide,[18] whereas other nitrogenous constituents measured were in the normal ranges (aspartic and glutamic acids,[14, 18] glutathione,[18] phosphoethanolamine,[18] and taurine[14]). In rats given thiosemicarbazide, Baxter and Roberts[7] found that paper chromatograms of the brain amino acids showed essentially normal patterns except for the decrease in $\gamma$-ABA. In two cats given thiosemicarbazide, Tower[20] found low initial levels of glutamic acid and glutamine in cortical slices prepared for metabolic studies. In mice, Balzer *et al.*[17] found that some hydrazides induced decreases in both $\gamma$-ABA and glutamic acid in the brain, but thiosemicarbazide

failed to show these effects. Lovell and Elliott[21] also found that thiosemicarbazide did not significantly decrease the γ-ABA level in rat brain frozen *in situ*.

It is known that hydrazides added *in vitro* inhibit several pyridoxal-dependent enzymes, including brain GAD, brain glutamic–aspartic transaminase, and liver L-cysteine desulfhydrase,[14] but not brain γ-ABA-T.[2] GAD is the only one of these that is measurably inhibited *in vivo* by convulsant doses of hydrazides, but there remains the possibility of such an action on other enzymes. The observed increase in tyrosine suggests inhibition of an enzyme such as tyrosine-α-ketoglutaric transaminase, which is known to be present in brain tissue.[22, 23] However, the tyrosine increment could come from protein in the brain or from tyrosine production elsewhere in the body, since it is known that this amino acid rapidly penetrates the blood–brain barrier in the rat.[24]

The chemical pattern in thiosemicarbazide seizures shows two characteristics, namely the decrease in γ-ABA and the increase in tyrosine, which do not appear in seizures induced by other agents so far studied in this laboratory (picrotoxin,[10] pentylenetetrazol,[10] and fluoro-fatty acid esters[25]). Lactic acid is increased by all these convulsants, and alanine by all except picrotoxin. Ammonia is increased greatly by fluoro-fatty acids, moderately by pentylenetetrazol, and insignificantly by picrotoxin; in this respect the action of thiosemicarbazide most closely resembles that of pentylenetetrazol. However, pentylenetetrazol decreases the glutamic acid level slightly, and picrotoxin has a similar effect on aspartic acid. The fluoro-fatty acids, which are known to block the tricarboxylic acid cycle at the stage of citrate oxidation, decrease both aspartic and glutamic acids and induce increases in leucine and serine as well as in citrate. These changes do not appear in thiosemicarbazide-induced seizures.

*Chemical effects of amino-oxyacetic acid*

The increase in brain γ-ABA after administration of AOAA has previously been observed in several species.[5–7, 21] Baxter and Roberts,[7, 26] using paper chromatography, found no other detectable changes in the brain amino acid pattern with the possible exception of an increase in β-alanine. However, Wallach (quoted by DaVanzo *et al.*[8]) observed the increase in glutamine. This change is probably a consequence of the high concentration of ammonia in the brain. The increases in alanine and lactic acid and the decrease in aspartic acid also may be secondary to the rise in ammonia, since all these changes occur upon intravenous infusion of buffered ammonium chloride.[10]

The rise in brain ammonia may be due in whole or in part to the increase of this constituent in the blood, and hence presumably to a metabolic effect of AOAA on the liver. Wallach[5] stated that a large dose of AOAA alters the free amino acid profile of the dog liver, but further details were not given. Hopper and Segal[27] observed that AOAA inhibits the purified glutamic–pyruvic transaminase of rat liver. Baxter and Roberts[6, 7] found an increase in β-alanine, but no other significant changes, in the liver of the rat given AOAA.

The possibility must be considered that AOAA inhibits a variety of pyridoxal-dependent enzymes. Inhibition of glutamic–oxaloacetic transaminase could be involved in the observed decrease in brain aspartic acid, and inhibition of tyrosine-α-ketoglutaric transaminase might account for the increase in tyrosine. The effect of

AOAA on the tyrosine level is greater than the similar effect of thiosemicarbazide, but may involve the same mechanism since both drugs are carbonyl-trapping agents.

*Convulsive activity*

Although the convulsant action of hydrazides cannot be adequately explained at this time, it is reasonable to regard the γ-ABA level in the brain as one of the factors regulating neuronal excitability. Many investigations have shown that this substance has inhibitory effects.[11, 28–30] It is probable that the depressant action of AOAA is due at least in part to the associated increase in endogenous γ-ABA. The inhibitory effect is relatively weak, inasmuch as it is easily overcome by increasing the dose of thiosemicarbazide or of pentylenetetrazol and is scarcely discernible upon injection of a convulsant dose of picrotoxin. AOAA shows definite antagonism to methionine sulfoximine seizures,[8] and inhibits those induced by 1 mg methyl fluoroacetate/kg but is not completely effective against the same dose of methyl fluorobutyrate.[25] DaVanzo *et al.*[8] found that in rats the protective action against thiosemicarbazide is maximal during the first 3 hr after administration of AOAA, whereas the γ-ABA levels continue to rise for about 8 hr. This suggests that the anticonvulsant action may be unrelated to the γ-ABA level, but an alternative possibility is that an accommodation process occurs, allowing recovery of excitability in the presence of excess γ-ABA.

It is evident that AOAA also has another action tending in the opposite direction, toward increased excitability, inasmuch as large doses are known to induce seizures. The excitatory effects appeared infrequently in the experiments reported here. Wallach[12] has stated that these seizures, like those induced by hydrazides, are inhibited by pyridoxal phosphate. It is possible that the excitatory effects of the hydrazides and of AOAA involve the same basic mechanism, with a decrease in γ-ABA potentiating excitation by hydrazides and an increase opposing excitation by AOAA. Other agents influencing the γ-ABA level may also exhibit such dual or multiple pharmacologic properties; on this basis the lack of correlation between γ-ABA levels and neuronal excitability in response to these agents is understandable. For example, hydroxylamine induces a rise in γ-ABA and an associated decrease in neuronal excitability,[4, 31] but convulsions may occur during a brief period preceding the rise in γ-ABA.[31] L-Glutamic acid-γ-hydrazide also increases γ-ABA, and has depressing effects at low doses but induces seizures at higher doses.[32] Hydrazine[19] and L-2,4-diaminobutyric acid[20] raise the γ-ABA level and sometimes induce seizures. Vitamin $B_6$ antimetabolites (3-deoxypyridoxine phosphate,[19] 4-methoxymethyl pyridoxine,[33] and methoxypyridoxine[34]) are known to bring about a decrease in γ-ABA in association with seizures, but the convulsant potencies of three such compounds (toxopyrimidine, 3-deoxypyridoxine, and ω-methylpyridoxine) showed no correlation with their inhibitory effects on brain GAD *in vivo*.[35] Variations in intracellular distribution of γ-ABA ('free' and 'occult' forms[36, 37]) might also be expected to influence the complex pattern of response to these diverse agents.

The seizures induced by thiosemicarbazide clearly are not of a cholinergic type, since they are not antagonized by large doses of atropine[38] or hyoscine.[39] In this respect they resemble those induced by pentylenetetrazol, picrotoxin, and the fluorofatty acids.[13] It has been suggested that the convulsant effects of agents which block GAD or γ-ABA-T are a consequence of a reduced rate of metabolism through the γ-ABA pathway, [12, 17, 20] a route that appears to have a unique importance in nervous

tissue. The data reported here are compatible with this view. Another possibility is evident from the finding of Dixon and Williams[40] that the pyridoxal and pyridoxal phosphate complexes of thiosemicarbazide and of several other hydrazides are powerful convulsants when injected intracerebrally. The presence of such hydrazones in the brain after injection of hydrazides has been demonstrated.[41]

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