

AN INVESTIGATION OF SOME ASPECTS OF THE CENTRAL NERVOUS SYSTEM
ACTIONS OF GAMMA-AMINOBUTYRIC ACID AND RELATED COMPOUNDS AS
ASSESSED BY THE USE OF SYSTEMIC AND INTRACEREBRAL ROUTES OF
ADMINISTRATION IN MICE

by

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An Investigation of Some Aspects of the Central Nervous System

Actions of Gamma-Aminobutyric Acid and Related Compounds as

Assessed by the Use of Systemic and Intracerebral Routes of

Administration in Mice.

This Thesis for the M.S. degree by

Om Datt Gulati

The effects of intracerebrally administered gamma-aminobutyric acid (GABA) and related compounds and the anticonvulsant activity of systemically and intracerebrally administered GABA were studied in mice. Systemic administration of GABA in doses of 1 g/kg or less did not induce neurotoxicity or exhibit anticonvulsant activity. Intracerebrally administered GABA, glycine, β -alanine and 5-amino-n-valeric acid elicited clonic convulsions followed by depression which increased in intensity as the dose of the compounds were increased when loss of righting reflex was observed. GABA and β -alanine were of equimolar potency and were more active than glycine and 5-amino-n-valeric acid which were equipotent. Intracerebrally administered 6-amino-n-caproic acid and 8-amino-n-caprylic acid induced clonus followed by tonic extensor seizures. The number of animals exhibiting tonic extensor seizures increased as the dose of the amino acids increased. 8-amino-n-caprylic acid was approximately ten times as potent as 6-amino-n-caproic acid on a molar basis. Clonus due to pentylentetrazol (0.5% IV and 87.5 mg/kg SC) and convulsions due to minimal electroshock were prevented in low doses (20-60 μ g/20 g) and potentiated in higher doses (80-160 μ g/20 g) of intracerebrally administered GABA. Tonic extensor seizures and death induced by pentylentetrazol (0.5% IV) were prevented by intracerebrally adminis-

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An Investigation of Some Aspects of the Central Nervous System (V) were

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kg SC Assessed by the Use of Systemic and Intracerebral Routes of were

not Administration in Mice. by administered GABA.

Thesis directed by Hubert C. Stanton, Instructor in Pharmacology

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I recommend its publication.

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tered GABA. Clonus and tonic extensor seizures induced by caffeine (1% IV) and tonic convulsions induced by ammonium acetate (5% IV) were potentiated by intracerebrally administered GABA. Strychnine (1.25 mg/kg SC) convulsions or those induced by supramaximal electroshock were not altered by intracerebrally administered GABA.

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This abstract of about 220 words is approved as to form and content. I recommend its publication.

to Dr. Hubert C. Stanton under whose direction this research was
Signed Hubert C. Stanton
conducted. Thanks are expressed for the assistance and encouragement of Dr. Stanton, Instructor in charge of dissertation

encouragement, understanding and constructive criticism while this investigation was being carried out.

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Mephenein, Tridione and Dilantin sodium were generously supplied by E. R. Squibb and Sons, Abbott Laboratories, and Parke Davis and Co., respectively.

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INTRODUCTION

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INTRODUCTION

Hawkins and Sarett (34) reported that orally administered gamma-aminobutyric acid (GABA) exhibits anticonvulsant activity against convulsions induced by methylethylglutarimide or pentylenetetrazol (ED_{50} 200 mg/kg and 400 mg/kg) in mice. Florey and McLennan (28) reported that the subcutaneous injection of purified brain extracts which may contain GABA as an active constituent (6, 7) protected mice against strychnine induced convulsions. A similar observation was made for GABA itself by McLennan (58). Conversely, other investigators could not demonstrate any anticonvulsant action for GABA (12, 88). Furthermore, the observations of Purpura et al. (69) and van Gelder and Elliott (94) that systemically administered GABA does not cross the blood brain barrier tend to preclude a significant anticonvulsant effect for the systemically administered compound. In view of the controversies in the literature concerning the anticonvulsant actions of systemically administered GABA, the following study was undertaken.

The purpose of this study was threefold:

- 1) To investigate the anticonvulsant action of systemically administered GABA against convulsions produced by several procedures.
- 2) To study the anticonvulsant action of the compound when administered intracerebrally;
- 3) To investigate the central nervous system actions of related compounds and compare their activity with that of GABA.

REVIEW OF LITERATURE

It is not surprising that the pioneer observations of Ackerman (2) on the production of gamma-aminobutyric acid (GABA) from glutamic acid by the action of putrefying material and the failure to confirm this observation by Abderbalden et al. (1) two years later aroused no interest among scientists for some time since instances of this kind are not new and are not difficult to find in the scientific literature.

The demonstration of the presence and/or production of GABA in various bacteria (29, 97) led to its identification in a number of living systems such as yeast extract (72), Bramby's seedling apples (41), fresh beet roots (95), potato tubers (87) and barley roots (8). Roberts et al. (79) and Awapara et al. (4) simultaneously and independently observed that the brain tissue of various mammalian species contained relatively large amounts of GABA in a predominantly free form. Udenfriend (93) positively identified the substance in brain by the use of isotope tracer techniques.

Relationship Between Factor I and GABA:

Florey (23, 24) observed that certain extracts of mammalian central nervous system exerted inhibitory and excitatory effects upon the discharge of the crayfish stretch receptor neuron. The portion of the extract responsible for the inhibitory action was designated as Factor I and extracts containing it were called "Factor I extracts". The method utilizing the crayfish has been simplified and standardized and has been used for the assay of Factor I activity (19, 22). With the aid of this assay procedure, Bazemore et al. (6, 7) concentrated

Factor I activity from a large quantity of beef brain. This material was crystallized and identified as GABA by infra red spectroscopy and paper chromatography. Estimates of the GABA content of the brain by Factor I assay agreed reasonably well with those obtained by the chemical methods. Synthetic GABA also showed the same activity as the isolated material using this assay procedure. It seems likely, therefore, that the GABA content of the brain could account for virtually all the Factor I activity. It is of interest to note that the physiological properties of Factor I and GABA were studied independently before it was recognized that a relationship existed between Factor I and GABA.

Recently, McLennan (58) observed that GABA did not duplicate the actions of Factor I on the squid rectum and the monosynaptic reflex in the cat. McLennan (59) has also reported that GABA could not be detected chromatographically in a partially purified brain extract which showed a good deal of Factor I activity. McLennan, therefore, has suggested that such extracts contain at least one other active principle which possesses those physiological actions not exhibited by GABA or a precursor of GABA which is active on the stretch receptor. Elliot and Jasper (21) have suggested that other inhibitory factors which are not detected by the use of crayfish stretch receptors may be present. Further, a number of substances and preparations from brain have been found to exert inhibitory actions.

The evidence in favor of the suggestion that the activity of Factor I is due to its GABA content is not conclusive, but is strong and therefore in what follows the two will be discussed together.

Distribution of GABA and Factor I in Animal Tissues:

Tallan et al. (92) estimated the GABA content of various tissues in a cat and based on wet tissue weight, found 23.4 mg/100 g in the brain and less than 1 mg/100 g or ml in liver, pancreas, urinary bladder, kidneys, gastrocnemius muscle, urine, and plasma. Chromatograms of the saliva (61) and gastric juice of normal fasting individuals (30) always showed the presence of small amounts of GABA. GABA has also been demonstrated in the retina and choroid of the dog and ox, but not in the iris and ciliary body (50). Although GABA is not present in mammalian peripheral nerve (79) small amounts of GABA have been detected in the sciatic nerve of hens (65). Logothetis (55) detected 0.4 µg/ml GABA in the cerebrospinal fluid of man and dog, and Knauff (49) showed the presence of GABA in the cerebrospinal fluid of normal human subjects and others suffering from various neurological and non-neurological diseases. However, van Gelder and Elliott (94) could not detect Factor I activity in the cerebrospinal fluid drawn from human subjects suffering from a variety of conditions.

No Factor I activity has been detected in extracts of liver, spleen, cardiac muscle, mammalian sciatic nerve or sympathetic ganglia (27) although small amounts of Factor I activity have been found in peripheral nerves of crustaceans (24).

Factor I or GABA activity has been found in the central nervous system of many non-mammalian and mammalian species (24, 73). Florey and Florey (26) demonstrated that the grey matter of extrapyramidal centers, superior cerebral peduncle, optic tract and crus cerebri of beef brain have pronounced Factor I activity.

Though GABA has been found in unhydrolysed brain extracts (4, 76, 92), most of the Factor I activity in the brain is present in an 'occult' condition from which it can be readily released by heat, by suspension of the tissue in hypotonic medium, or by treatment with mild acid or alkali (19). It has been suggested (21) that GABA in the brain occurs in three forms: a minimal amount present in cell-free media, a portion which is readily released on homogenization and a larger amount which is strongly occluded.

Formation and Breakdown of GABA:

Most strains of *E. coli*, *Proteus vulgaris*, *B. subtilis* and *B. mesenteroides* and some strains of *Clostridia* produce l-glutamic acid decarboxylase which requires pyridoxal phosphate as a coenzyme (29). Schales et al. (82) have also demonstrated the presence of this pyridoxal phosphate-dependant enzyme in plants.

Roberts et al. (79) and Awapara et al. (4) in their experiments on the isolation of GABA from the mammalian brain tissue suggested the presence of a new type of enzyme capable of decarboxylating l-glutamic acid. However, the actual demonstration of such an enzyme system did not come until somewhat later (77, 78, 96). The enzyme was found to be dependent upon pyridoxal phosphate as a co-enzyme, was inhibited by semicarbazide and hydroxylamine, had an optimum pH of 6.4-6.5 and was specific for l-glutamic acid. Though an equilibrium constant for l-glutamic decarboxylase has been demonstrated (70 at 23-27° C) by Koppelman et al. (51), earlier work suggests that the reaction goes virtually to completion. An extremely rapid production of GABA from l-glutamic acid has been demonstrated by a radioisotope tracer

technique (81). A wide species variation in the l-glutamic decarboxylase activity and also variation in different areas of the brain has been demonstrated (56). In general, the l-glutamic decarboxylase activity was greater in the granular layer than in the molecular layer of the brain and the activity in the grey matter was much higher than that in the white matter. This confirms the earlier observations of Roberts et al. (80) that l-glutamic acid decarboxylase is present chiefly in the grey matter of the central nervous system. Roberts et al. (80) presented evidence which suggested that glutamic decarboxylase activity increased in the embryo as maturation progressed. Other tissues do not show as much enzyme activity as the brain.

A system transaminating GABA and alpha-ketoglutaric acid to yield glutamic acid and succinic semialdehyde has been demonstrated in rat, rabbit and calf brain homogenates and to a lesser extent in rat liver "particles". No enzyme for catalyzing this reaction could be demonstrated in rat kidney "particles". This GABA-alpha-ketoglutarate transamination reaction is reversible and, in the brain, the entire enzymatic activity is found in the insoluble "particles" (10). In a similar system brain and liver residues and acetone powders of mouse brain, both GABA and B-alanine have been shown to transaminate with alpha-ketoglutarate (75). The transaminase, like the decarboxylase, is dependent upon pyridoxal phosphate as a coenzyme. However, the apoenzyme of l-glutamic acid decarboxylase seems to have a lower affinity for the coenzyme than that of GABA transaminase (cited from Roberts, et al. 81).

Baxter and Roberts (5) reported that grey matter of beef brain is a far richer source of GABA transaminase than white matter. The pH optimum of the enzyme is 8.2 and substrate specificity has been shown.

Alpha-ketoglutarate seems to be the only effective amino group acceptor but specificity with regard to amino group donor is less clear since several omega-amino acids other than GABA transaminate with alpha-ketoglutarate. A free sulphydryl(-SH) group may be necessary for the functioning of the enzyme since it is inhibited by iodoacetamide, p-chloromercuribenzoate and N-ethyl-maleimide. Alpha-ketoglutarate and pyridoxal phosphate protect the enzyme from heat inactivation.

Albers and Salvador (3) have demonstrated in the brain a soluble DPN dependent dehydrogenase which catalyses the oxidation of succinic semialdehyde. A similar enzyme, more active with TPN than with DPN, has been obtained from a strain of *Pseudomonas* (83). It has, therefore, been suggested (73, 81) that in the brain a shunt operates around the alpha-ketoglutarate oxidase system of the tricarboxylic acid cycle. In this shunt alpha-ketoglutaric acid could be withdrawn through the formation of glutamic acid by transamination with GABA and the carbon chain of GABA could enter the cycle at the succinate level. Pisano et al. (64) have demonstrated that enzymes in the kidney and liver catalyse the transamidation between GABA and arginine to yield gamma-guanidinobutyric acid which has been isolated from brain and kidney (42). Rabbit brain "particles" have been shown (84) to convert GABA to gamma-amino-B-hydroxy butyric acid in a reaction for which ATP, FAD were absolute requirements and Mg ions accelerated the reaction, while methylene blue and nitrophenol inhibited the reaction.

Possible Role of GABA as an Inhibitory Transmitter Substance in the Brain:

When substances are identified in the central nervous system, it exists of an inhibitory factor in the brain which seems to differ

is natural to inquire into their function and thus they may become candidates for various roles, including that of potential neurohumoral transmitters. This is now the case with GABA which has been suggested as a possible inhibitory transmitter in the central nervous system. The evidence for the chemical transmission at the neuromuscular junction is based upon the fulfillment of the following criteria (63): The presynaptic neuron should 1) synthesize the transmitter and release it in a pharmacologically identifiable form. 2) The action of the transmitter on the postsynaptic cell should reproduce the specific events of normal transmission, and should be antagonized by competitive blocking agents. 3) There should exist an enzyme capable of destroying the transmitter. With regard to the possible role of GABA as an inhibitory transmitter, the available literature will be evaluated in the light of these criteria.

The evidence that GABA is synthesized in the presynaptic neuron in the central nervous system is indirect, but is suggested by the following observations: 1) differential localization of Factor I in the central nervous system (24, 26), 2) concomitant occurrence of l-glutamic acid decarboxylase with GABA in large amounts in the grey matter of the brain (80), 3) the embryological appearance of l-glutamic acid-decarboxylase and GABA with maturation of the brain (80), 4) the inability of GABA to cross the blood barrier (69, 94).

Although it could be assumed that GABA is released by the presynaptic neuron, Hayashi and Nagai (36) and Hayashi (35) believe that gamma-amino-B-hydroxy-butyric acid is more important as an inhibitory transmitter. Lissak and Endroczi (54) have presented evidence for the existence of an inhibitory factor in the brain which seems to differ

somewhat from Factor I, while Purpura et al. (68) have suggested that a number of vitamins and amino acids may be of physiological and pathological significance as agents affecting the central synapse. Furthermore, the experimental proof for the release of GABA by neurons in amounts adequate to block transmission of impulses in the central nervous system is not yet available.

Florey (25) has shown that GABA mimics the stimulation of the inhibitory cardiac fibers in the lobster heart as well as in crayfish stretch receptor. The effects of GABA on certain membrane phenomena are similar to those of the naturally occurring inhibitory transmitter (52). Purpura et al. (66) have demonstrated that the local application of GABA to the surface of the cortex inhibited the excitatory synapses rapidly and reversibly. In a more extensive report, Iwama and Jasper (43) have confirmed these findings. Purpura et al. (67) investigated a number of omega-amino acids applied topically to the surface of the cortex and presented evidence for the presence of inhibitory synapses in the superficial layers of the cortex. While comparing GABA with the acetylcholine as a transmitter substance in the central nervous system, Killam (46) has suggested that GABA might be elaborated by inhibitory brain cells.

Semicarbazides and related compounds have a convulsive action (45, 47) and these convulsions develop only after a latent period of one hour. They are associated with a reduction of GABA content of brain, probably due to inhibition of the pyridoxine-dependant enzyme l-glutamic decarboxylase and this decrease of intrinsic GABA level is also associated with an increase in the size of the surface negative potential (48). The effect of semicarbazide was reversed by the administration

of vitamin B₆ intravenously. Das Gupta et al. (17) were able to antagonize electrically induced seizures in the hippocampus and on the cortex by the topical application of GABA and to increase seizures by blockade of GABA synthesis. Thus, alterations of cortical responses were produced by the manipulation of exogenous and endogenous brain levels of GABA. It is apparent, therefore, that the postulated ability of GABA to reproduce the specific events of normal transmission, is based on deductions derived from 'in vitro' experiments or from results obtained following the local application of GABA to the cerebral cortical surfaces.

As yet no competitive blocking agents for GABA are known. GABA transaminase can inactivate GABA (10, 75) but the enzyme is less specific in this regard (5), and it remains an open question as to whether this reaction occurs in the inactivation of GABA in the intact cell. Furthermore, no drug is known that can selectively inhibit the transaminase, but if such a drug is discovered, intrinsic levels of GABA could be increased in the brain and more critical information about the role of GABA in the brain could be derived.

The most decisive evidence for chemical transmission such as the collection and identification of the transmitter after stimulating the presynaptic neuron and the demonstration that the transmitter applied artificially can reproduce the characteristic post-synaptic events, has not yet been obtained for any specific central synapse.

Peripheral Actions:

Takahashi et al. (90) reported that intravenous injections of GABA into anesthetized rabbits caused a transient reduction in blood pressure, and brain extracts blocked synaptic transmission in the inferior

bradycardia and stimulation of respiration. Similarly, a depression of blood pressure was previously reported for Factor I by Florey and McLennan, (27). In a subsequent study, Takahashi et al. (91) postulated depressor points in the medulla as the site of depressor action of GABA. Stanton and Woodhouse (86) observed respiratory stimulation, pressor followed by depressor responses and bradycardia with doses of 0.02-0.32 μ mole/kg of GABA. On the basis of their data they suggest carotid sinus and aortic chemoreceptor stimulation as the mechanism of action of pressor and respiratory responses and peripheral ganglionic blockade as the mechanism for the depressor response. Elliott and Hobbiger (20) have reported considerable species variability, such as a fall in blood pressure with transitory bradycardia, respiratory depression preceded by a single gasp in dogs, hypotension only in rabbits, no effects in unanesthetized rats with midbrain sections, but a transitory rise in a similarly prepared monkey, and transitory gasping, various paresthesias and transitory bradycardia and hypotension with low doses in man.

Florey (24) observed that Factor I antagonized the action of acetylcholine on the guinea pig ileum and Hobbiger (38, 39) confirmed this for GABA and also demonstrated antagonistic effects to submaximal doses of nicotine, histamine and 5-hydroxytryptamine on the same organ. Species differences between guinea pig, rat and rabbit were found. GABA alone produced stimulation and/or relaxation, the stimulating effects being abolished by atropine.

Although effects of GABA on peripheral ganglionic transmission have not been studied thoroughly, Florey and McLennan (27) found that purified brain extracts blocked synaptic transmission in the inferior

mesenteric and stellate ganglia of the cat. However, excitatory effects were observed on the hypoglossal nucleus of the same animal.

It is unlikely that GABA (52) or Factor I (27) have effects on nerve conduction or neuromuscular transmission.

Other Active Substances Related to Factor I and GABA:

Bazemore et al. (7) investigated a large number of amino acids utilizing the crayfish stretch receptor preparation and observed inhibitory activity, like that of GABA, with: 1) DL, gamma-amino-B-hydroxy-butyric acid which was about one-half as active as GABA. 2) B-alanine and 5-amino-n-valeric acid which were $\frac{1}{20}$ as active as GABA. 3) Alpha-gamma-diamino butyric acid which was $\frac{1}{60}$ as active. Gamma-guanidino butyric, which has been isolated from brains of dogs, cattle, and humans (42) also has some activity on the crayfish stretch receptor (52). Hayashi and Nagai (36), Hayashi and Shuhara (37) and Hayashi (35) have found that convulsions induced in dogs by electrical stimulation of the motor areas of the cortex or by cortical topical application or by intracarotid or intraventricular injection of metrazol or glutamate could be inhibited by intracarotid or intraventricular injections of a number of omega-amino-acids. GABA and higher omega-amino-acids were usually inhibitory but sometimes showed excitatory activity. The inhibitory activity of L, gamma-amino-B-Hydroxybutyric acid was approximately ten times that of GABA. Hayashi (35) believes that L-gamma-amino-B-hydroxybutyric acid can be derived from GABA, and is more significant as an inhibitory substance.

On the basis of evidence presented by Lissak and Endroczi (54) it would seem that extracts of neural tissue contain at least one other

principle which would distinguish it from Factor I.

Hosein (40) has isolated gamma-butyrobetaine from brains removed from rats and cats suffering convulsions induced by a variety of convulsant drugs. Intracerebral injection of small amounts of this substance produced violent and fatal convulsions in unanesthetized animals. Gamma-amino-butyryl choline has been isolated chromatographically from dog brain by Kuriaki et al. (53) who observed that this substance exerts a variety of peculiar effects like muscarine, nicotine, atropine and antihistamines, and therefore suggest that it might be an inhibitory neurotransmitter.

The procedure used for the intracerebral injections was essentially as described by Shaper and McCormick (54). The test agents were dissolved in water-soluble solutions containing sodium bicarbonate. The volume of solution for each drug was 0.01 ml. Control animals received 0.01 ml of 0.9% saline solution. The pH of the resultant solution was adjusted to 7.4. With taurine, which was not soluble in water, it was necessary to add sodium bicarbonate to obtain a solution. The animals were allowed to recover before being compared with animals receiving saline. The animals were then sacrificed and the brains removed and analyzed for the presence of the test agent. The following anticonvulsant tests were employed throughout the investigation.

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MATERIALS AND METHODS

Fifteen hundred adult male albino mice (Webster Strain, National Laboratory Animal Company) varying in weight from 20-25 g were used in this investigation. The animals were maintained on Purina Laboratory Chow and were allowed free access to food and water at all times. The following compounds were tested: glycine, B-alanine, GABA, 5-amino-n-valeric acid, 6-amino-n-caproic acid and 8-amino-n-caprylic acid. Each of the compounds was administered either systemically or intracerebrally. In the case of systemic administrations, the agents were dissolved in normal saline and were administered in the appropriate doses either intravenously into the lateral tail vein, subcutaneously or orally by means of intubation. The injection volume used with these systemic routes never exceeded 0.38 ml.

The procedure used for the intracerebral injections was essentially that described by Haley and McCormick (33). The test agents were dissolved in Locke-Ringer's solution containing sodium bicarbonate. The injection volume for each dose of each drug was 0.01 ml. Control animals received 0.01 ml of Locke-Ringer's solution. The pH of the resultant solutions of amino acids varied from 7.0-7.4. With taurine, which was quite acid, it was necessary to add more sodium bicarbonate to obtain a pH 7.0. Control mice which were to be compared with animals receiving taurine received Locke-Ringer's solution containing the additional quantity of sodium bicarbonate which had been needed to neutralize the taurine solution.

The following anticonvulsant tests were employed throughout the investigation:

1. Subcutaneous pentylenetetrazol test, (13). In this procedure mice were administered 87.5 mg/kg pentylenetetrazol subcutaneously and were observed for the appearance of clonic movements of the head and face lasting for at least 7 seconds. Animals not exhibiting clonus were considered protected.

2. Subcutaneous strychnine test. In this procedure each animal received 1.25 mg/kg strychnine nitrate subcutaneously. Abolition of the tonic extensor phase of the convulsion was considered as protection.

3. Timed intravenous infusions of pentylenetetrazol 0.5%, strychnine nitrate 0.01%, ammonium acetate 5% and caffeine 1% (62, 14).

The amount of the intravenously administered convulsant required to produce first signs of tonic extension of the hind limbs and respiratory arrest was noted. In the case of pentylenetetrazol and caffeine infusions, the amount required to produce first signs of clonic seizures was also recorded. In all cases the convulsant was infused at the rate of 0.05 ml/10 seconds. The total injection volume never exceeded 1.0 ml per mouse; the animal was considered to be protected from the extensor component of the seizure when tonic extension of the hind limbs did not occur after 1.0 ml of the convulsant had been delivered.

4. Minimal electroshock seizure threshold test (MET) as described by Swinyard et al. (89).

5. Supramaximal electroshock seizure pattern test (MES) as described by Swinyard et al. (89).

The amino acids used in this investigation were obtained from California Foundation for Biochemical Research.

The statistical connotation and procedures used to evaluate the data obtained in this study are those outlined by Bliss (11) and

Snedecor (85).

RESULTS

Systemically Administered GABA

Tables I and II summarize the results obtained with various convulsant test procedures following the systemic administration of GABA. The first convulsant test employed was the timed intravenous infusion test of Orloff et al. (62) in which pentylenetetrazol (0.5%) was the convulsant agent studied. As can be seen in Tables I and II, no significant protection against pentylenetetrazol-induced clonus or tonic extensor convulsions and death was observed regardless of the dose of GABA administered or the time interval between the administration of GABA and the start of the intravenous infusion of pentylenetetrazol. In another experiment, GABA was administered in a dose of 400 mg/kg (orally) and studied after two hours for anticonvulsant activity against the clonus induced by pentylenetetrazol (100 mg/kg SC). Again no protection was observed utilizing this convulsant test at two hours following the oral administration of the GABA dose. As can be seen in Table I and II, 1 g/kg doses of GABA administered either subcutaneously or intravenously induced no significant protection against strychnine nitrate (SC), ammonium acetate (IV infusion), caffeine (IV infusion), minimal electroshock or supramaximal electroshock. Although 20% protection was observed when strychnine (SC) was administered immediately after the 1 g/kg (IV) dose of GABA, this degree of protection was not considered of significance since the dose of GABA employed was very large and since no protection had been observed previously when GABA 400 mg/kg (SC) had been studied at various time intervals for its anticonvulsant activity against strychnine nitrate given by the timed intravenous infusion technique. The validity of several of the con-

TABLE I

EFFECT OF SYSTEMICALLY ADMINISTERED GABA UPON CONVULSIONS INDUCED BY PENTYLENETETRAZOL, STRYCHNINE
NITRATE, AMMONIUM ACETATE AND CAFFEINE (TIMED INTRAVENOUS INFUSION).

Convulsant	Dose of GABA and route of administration	Number of Mice	Time of Injec- tion of the con- vulsant after the administra- tion of GABA	Mean amt. of the convulsant in ml. required to produce clonus and 95% Confidence Intervals	Mean amt. of the convulsant in ml. required to produce T.E.* and 95% Confidence Intervals
Pentylene- tetrazol 0.5%	Control	21	-	0.21	0.33
	0.2 g/kg SC	7	1/2 hour	0.21	0.29
	" " "	6	1 hour	0.22	0.35
	" " "	7	2 hours	0.19	0.30
	" " "	4	3 hours	0.18	0.32
	Control	7	-	0.21	0.39
	0.4 g/kg SC	6	1/4 hour	0.18	0.35
	" " "	7	1/2 hour	0.18	0.36
	" " "	6	1 hour	0.21	0.37
	Control	8	-	0.20	0.44
Strychnine Nitrate 0.01%	1 g/kg SC	8	1/2 hour	0.17	0.41
	" " "	10	1 hour	0.23	0.52
	" " "	6	1-1/2 hour	0.20	0.46
	Control	7	-	-	0.25
	0.4 g/kg SC	6	1/2 hour	-	0.23
	" " "	7	1 hour	-	0.28
	Control	7	-	-	0.23
	0.4 g/kg SC	6	1/2 hour	-	0.19
	" " "	7	1 hour	-	0.24
	Control	7	-	-	0.23

TABLE I (CONT'D.)

Convulsant	Dose of GABA and route of administration	Number of Mice	Time of Injection of the convulsant after administration of GABA	Mean amt. of the convulsant in ml. required to produce clonus	95% Confidence Intervals and	Mean amt. of the convulsant in ml. required to produce T.E.*	95% Confidence Intervals and
Ammonium Acetate 5%	Control	4	-	-	-	0.40	(0.30 - 0.50)
	1 g/kg SC	4	1/4 hour	-	-	0.41	(0.33 - 0.49)
	" " "	3	1 hour	-	-	0.45	(0.40 - 0.52)
Caffeine 1%	Control	9	-	-	-	0.42	(0.39 - 0.45)
	1 g/kg SC	5	1/4 hour	-	-	0.42	(0.37 - 0.47)
	" " "	5	1/2 hour	-	-	0.40	(0.32 - 0.48)

* T. E. = Tonic Extensor

TABLE II

EFFECT OF SYSTEMICALLY ADMINISTERED GABA UPON CONVULSIONS INDUCED BY PENTYLENETETRAZOL (SC)
 STRYCHNINE NITRATE (SC), MINIMAL ELECTROSHOCK AND SUPRAMAXIMAL ELECTROSHOCK

Convulsive Procedure	Dose of GABA	Route of Administration	Time of Application of Convulsive Procedure After the Administration of GABA	# Mice Convulsing	
				Total # Mice Used	
Pentylentetrazol 87.5 mg/kg SC	Control	-	-	10/10	
	1 g/kg	Intravenous	Immediately	10/10	
	1 g/kg	Subcutaneous	1/2 hour	10/10	
	1 g/kg	Subcutaneous	1 hour	10/10	
Pentylentetrazol 100 mg/kg SC	1 g/kg	Subcutaneous	2 hours	9/10	
	Control	-	-	9/10	
	0.4 g/kg	Oral	2 hours	10/10	
Strychnine Nitrate 1.25 mg/kg SC	Control	-	-	10/10	
	1 g/kg	Intravenous	Immediately	4/5	
	1 g/kg	Subcutaneous	1/2 hour	9/10	
	1 g/kg	Subcutaneous	1 hour	10/10	
Minimal Electroshock	Control	-	-	5/5	
	1 g/kg	Intravenous	Immediately	6/6	
	1 g/kg	Subcutaneous	1/2 hour	10/10	
Supramaximal electroshock (50 MA & 0.2 sec. duration)	Control	-	-	5/5	
	1 g/kg	Subcutaneous	1/2 hour	5/5	

vulsant tests utilized was assessed by the use of reference anticonvulsants. The results of these tests are summarized in Table III. It should be noted that the results compare favorably with those obtained by other investigators and therefore the anticonvulsant testing procedures were considered to be valid.

GABA administered by the oral, subcutaneous or intravenous route (in doses of 1 g/kg or less) did not induce discernible evidence of neurotoxicity when the criteria for this response as outlined by Swinyard et al. (89) were utilized. Systemic GABA in doses of 1 g/kg or less did not prolong the latency period required for the onset of convulsive patterns elicited by strychnine nitrate (SC), pentylenetetrazol (SC), minimal electroshock or supramaximal electroshock.

Effects of Intracerebral Administration of GABA and Some Related Amino Acids

In contrast to the results obtained following the systemic administration of GABA, injection of GABA intracerebrally induced reproducible and significant evidence of neurotoxicity. Doses of GABA from 10-160 μ g/20 g body weight administered intracerebrally induced a biphasic response pattern. Within 6 to 42 seconds following the administration of GABA intracerebrally, the animal showed distinct clonic-type movements such as facial and front leg clonus. At this time the animal was hyperexcitable to touch and external stimuli. The intensity of excitement did not seem to increase as the dose of the compound was increased. The duration of this initial clonus varied widely from 12 seconds to 2.5 minutes and was not regularly influenced by the size of the dose of GABA administered. The period of hyperexcitability was then followed

TABLE III

COMPARISON OF ED₅₀'S OF TRIMETHADIONE, DIPHENYLHYDANTOIN SODIUM AND MEPHENESIN OBTAINED IN THE PRESENT STUDY WITH THOSE REPORTED PREVIOUSLY IN THE LITERATURE

Anticonvulsant and Route of Ad- ministration	Test Employed	Time After Anti- convulsant Admin- istration that Test was Employed	No. of Mice Utilized to Obtain ED ₅₀	ED ₅₀ * ± SE	Previously Re- ported ED ₅₀ ± SE from Literature	Reference No.
Trimethadione (SC)	Pentylene- tetrazol 87.5 mg/kg (SC)	1 hour	28	287.0±38.2	375.0±35.0	13
Diphenylhydantoin Sodium (SC)	Supramaximal Electroshock	3 hours	15	21.9±4.45	9.1± 0.7	13
Mephenesin (SC)	Strychnine Nitrate 1.25 mg/kg (SC)	10 minutes	28	80.5±27.6	80.0**	9

* ED₅₀ calculated by the method of Miller and Tainter (60).

** Calculated by Berger (9) as the ED₅₀ (of mephenesin IV) protecting against strychnine sulfate (1.29 mg/kg IV). This concentration of strychnine sulfate was calculated as 3 times the M.L.D. of the convulsant.

by depression, the intensity of depression varying with the dose of GABA employed. In lower doses (10-60 $\mu\text{g}/20\text{ g}$) the animals were simply depressed as exemplified by their inability to walk the rim of a cage, loss of exploratory activity, drowsiness and slowing of respiratory rate. However, as the dose was increased, loss of righting reflex occurred in certain animals; the number of animals showing loss of righting reflex increasing as the dose of the amino acid administered was increased. Apnea was observed in many mice at doses of from 80-160 $\mu\text{g}/20\text{ g}$ and in some of these animals death resulted from respiratory arrest. However, there did not seem to be a regular relationship between the dose of GABA causing loss of righting reflex and that causing death, and the major portion of the animals exhibiting loss of righting reflex recovered within 2.8-8.3 minutes following GABA administration. A number of the animals exhibited hyperexcitability and mild front limb and facial clonus following the depression phase. In some cases, the same volume of Locke-Ringer's solution, injected intracerebrally in controls, elicited a 1-5 second period of stunning, but only a small number of animals showed signs of neurotoxicity and damage following this stunning period.

Similar responses were observed following the intracerebral administration of glycine, B-alanine and 5-amino-n-valeric acid. However, it seemed that the initial clonic phase was greatest in intensity with 5-amino-n-valeric acid and least with glycine. Taurine induced loss of righting reflex and death in some animals in doses of 320-640 $\mu\text{g}/20\text{ g}$. However, no constant dose-effect relationship could be demonstrated at these doses. Dose-effect relationships were obtained from data obtained on the loss of righting reflex induced by the other compounds. A

summary of these experiments with appropriate statistical analyses is presented in Part A of Table IV. It should be noted that the dose response curves for all the compounds are linear as shown by X_L^2 values for each curve. Table V (Part A) summarizes the comparison of the molar potency of each compound as compared to GABA (1.00). It should be noted from X_p^2 in this table that the curve for GABA was parallel in each case with the curve for the compound with which it was being compared. Examination of the 95% confidence intervals for ED_{50} 's derived for each of the compounds revealed that GABA and B-alanine were essentially of the same potency in eliciting the loss of righting reflex. Glycine and 5-amino-n-valeric acid were essentially equipotent but less active than GABA and B-alanine.

Omega-amino acids of chain lengths greater than five carbons produced no depressant effects; only excitatory effects were seen. Intracerebral administration of 6-amino-n-caproic acid and 8-amino-n-caprylic acid elicited primary facial and forelimb clonus which was followed in certain animals by tonic extensor convulsions. The number of animals exhibiting the tonic extensor effect increased as the dose of the particular compound increased. Table IV (Part B) summarizes the data obtained with these excitatory amino acids. It should again be noted that a linear relationship between dose and effect occurred as indicated by the X_L^2 values listed in the Table. Table V (Part B) contains the potency comparison between 6-amino-n-caproic and 8-amino-n-caprylic acid. Again these curves were found to be parallel as shown by the X_p^2 value. 6-amino-n-caproic acid was found to be 0.106 times as potent as the 8-amino compound in eliciting tonic extensor convulsions.

TABLE IV

SUMMARY OF THE STATISTICS UTILIZED TO CALCULATE ED_{50} ($\mu\text{g}/20 \text{ g}$) AND POTENCY OF OMEGA AMINO ACIDS

Response	Compound	Dose $\mu\text{g}/20\text{g}$	No. of Mice	% Mice Respond- ing	a^*	b^+	X_L^{2**}	Proba- bility	ED_{50} and 95% Confi-
Part A Loss of Righting Reflex	Glycine	160.0	7	28.6	-16.730	9.610	0.5420	0.50-0.30	168.3 (147.6-191.9)
		180.0	7	42.9					
		200.0	7	71.4					
	B-alanine	80.0	7	40.0	-0.983	3.010	0.0025	0.98-0.95	96.6 (61.24-154.3)
		120.0	5	60.0					
		160.0	5	71.4					
	GABA	80.0	7	28.6	-4.965	4.950	0.6460	0.50-0.30	103.3 (80.17-133.0)
		100.0	7	42.9					
		120.0	7	71.4					
Part B Tonic Extensor Convul- sion	5-amino- n-valeric acid	240.0	7	28.6	-16.396	8.755	0.1672	0.70-0.50	275.4 (254.1-298.5)
		280.0	7	55.6					
		320.0	7	71.4					
	6-amino n-caproic acid	340.0	5	20.0	-15.755	7.871	0.1077	0.80-0.70	432.5 (363.9-514.0)
		400.0	5	40.0					
		460.0	5	60.0					
	8-amino- n-capry- lic acid	40.0	5	20.0	-4.950	5.695	0.036	0.90-0.80	55.9 (44.4-70.4)
		50.0	5	40.0					
		60.0	5	60.0					

$a^* = 0$ intercept $b^+ =$ regression coefficient (slope) $X_L^{2**} = X^2$ test for heterogeneity of the data.

TABLE V

CHEMICAL STRUCTURE, ED_{50} 'S (μ moles/20 g) AND POTENCY IN TERMS OF LOSS OF RIGHTING REFLEX AND TONIC EXTENSOR CONVULSION OF DIFFERENT OMEGA AMINO ACIDS

Response	Compound	Structure	ED_{50} + and 95% Confidence μ moles/20 Intervals	χ^2_p **	Probability	Potency
Part A Loss of Righting Reflex	Glycine	NH_2CH_2COOH	2.240 (1.97-2.55)	0.39	0.70-0.50	0.453
	B-alanine	$NH_2CH(CH_3)COOH$	1.085 (0.69-1.73)	0.21	0.70-0.50	0.890
	GABA*	$NH_2CH_2CH_2COOH$	1.001 (0.78-1.29)	-	-	1.000
	5-amino-n-valeric acid	$NH_2CH_2(CH_2)_3COOH$	2.354 (2.17-2.55)	0.36	0.70-0.50	0.423
Part B Tonic Extensor Convulsion	6-amino-n-caproic acid	$NH_2CH_2(CH_2)_4COOH$	3.300 (2.78-3.92)	1.42	0.30-0.20	0.106
	8-amino-n-caprylic acid*	$NH_2CH_2(CH_2)_6COOH$	0.351 (0.28-0.44)	-	-	1.000

* Reference compounds for loss of righting reflex and tonic extensor convulsion potency respectively.

+ Calculated from ED_{50} (μ g/20 g) Table IV.

** $\chi^2_p = \chi^2$ for parallelism between regression lines used in the potency assay. With compounds inducing loss of righting reflex, GABA was compared to each of the others. With compounds inducing tonic extensor convulsions, 6-amino-n-caproic and 8-amino-n-caprylic were compared.

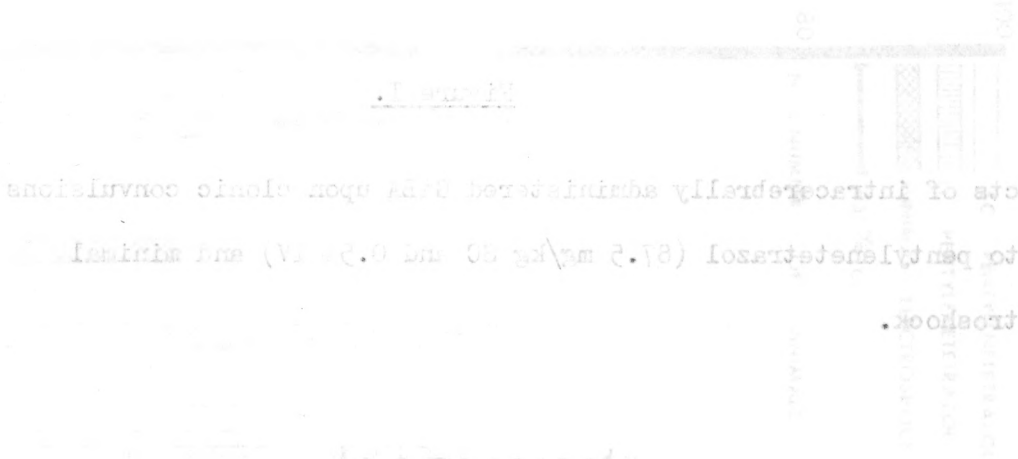
Effect of Intracerebrally Administered GABA Upon Convulsive Seizures

Unless otherwise stated, the convulsive test procedures to be discussed were employed within 1 minute following the intracerebral administration of GABA.

Fig. 1 summarizes the effects of various doses of intracerebrally administered GABA upon clonic convulsive activity induced by pentylenetetrazol (87.5 mg/kg SC), pentylenetetrazol (IV infusion 0.5%) and minimal electroshock seizure threshold test. A biphasic response was observed as the dose of GABA was increased. Protection against clonus induced by pentylenetetrazol (IV) was observed in doses of 40, 60, 80 $\mu\text{g}/20\text{ g}$ GABA. This protection was statistically significant ($P < 0.05$) from the Locke-Ringer's controls when 80 $\mu\text{g}/20\text{ g}$ GABA had been administered. A similar trend was observed with pentylenetetrazol (SC) and minimal electroshock when doses of GABA of 60 and 80 $\mu\text{g}/20\text{ g}$ and 10, 20, 40 and 80 $\mu\text{g}/20\text{ g}$ respectively were administered. When doses of GABA greater than 80 $\mu\text{g}/20\text{ g}$ were administered, the clonic convulsant activity of pentylenetetrazol increased and when 160 $\mu\text{g}/20\text{ g}$ of GABA was administered, less pentylenetetrazol was required to induce clonus than had been previously required for the Locke-Ringer controls ($P < 0.05$). The number of animals showing clonus with pentylenetetrazol (SC) was greater following GABA (160 $\mu\text{g}/20\text{ g}$) than following the injection of Locke-Ringer's solution. Similarly, less protection against minimal electroshock was observed following 120 $\mu\text{g}/20\text{ g}$ GABA than following 20, 40, and 80 $\mu\text{g}/20\text{ g}$.

The effects of intracerebrally administered Locke-Ringer's solution and GABA upon tonic extensor convulsions and/or death induced by pentylenetetrazol (IV infusion, 0.5%) and strychnine nitrate (1.25 mg/kg, SC

2.5 mg/kg (0.5 mg/kg) 2.5 mg/kg
 0.5 mg/kg 2.5 mg/kg



Effects of intracerebrally administered GABA upon electroconvulsions due to pentyltetrazolol (87.5 mg/kg 30 and 60 sec IV) and animals electroconvulsed.

Electroconvulsions were induced by pentyltetrazolol (87.5 mg/kg 30 and 60 sec IV) and animals electroconvulsed.

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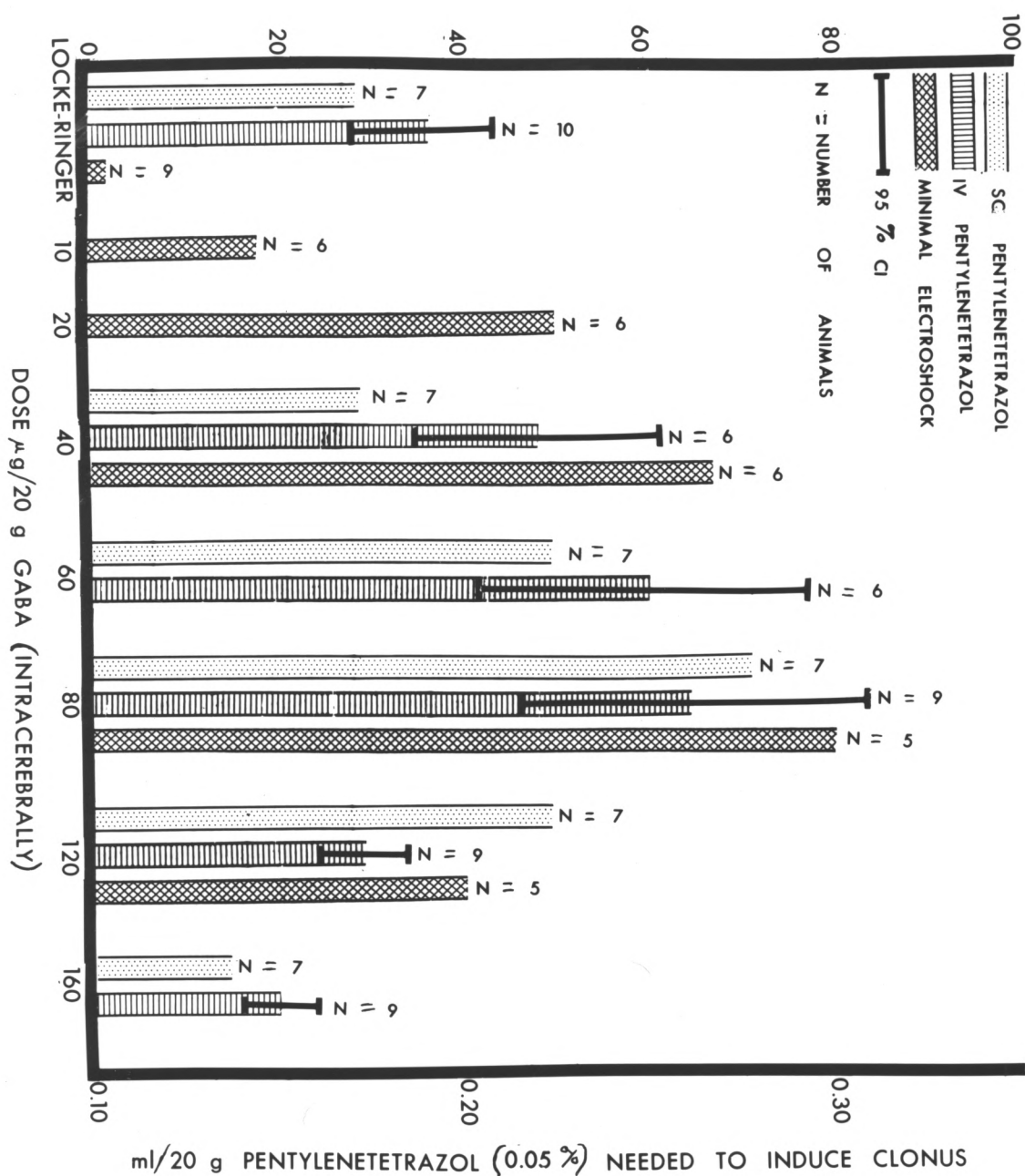
Electroconvulsions were induced by pentyltetrazolol (87.5 mg/kg 30 and 60 sec IV) and animals electroconvulsed.

Electroconvulsions were induced by pentyltetrazolol (87.5 mg/kg 30 and 60 sec IV) and animals electroconvulsed.

Figure I.

Effects of intracerebrally administered GABA upon clonic convulsions due to pentylenetetrazol (87.5 mg/kg SC and 0.5% IV) and minimal electroshock.

PROTECTION AGAINST SC PENTYLENETETRAZOL (87.5 mg/kg) &
MINIMAL ELECTROSHOCK (20%) ABOVE THRESHOLD



and IV infusion 0.01%) are summarized in Fig. II. GABA (20-160 μ g/20 g), administered intracerebrally, did not protect the animals against tonic extensor convulsions induced by strychnine nitrate (SC or IV infusion). However, in doses of 120 and 160 μ g/20 g, GABA protected against tonic extensor convulsions and death induced by the intravenous infusion of pentylenetetrazol. It should also be noted that as the dose of GABA was increased many of the mice receiving the intravenous infusion of pentylenetetrazol died without exhibiting tonic extension of the hind limbs. GABA, administered in doses of 20, 40 and 80 μ g/20 g did not protect against tonic extensor convulsions induced by supramaximal electroshock (50 mA, 0.2 sec. duration). The latency period required for the onset of tonic extensor convulsions induced by strychnine nitrate (SC) or supramaximal electroshock, was not altered by GABA regardless of the dose administered.

Caffeine and ammonium acetate were also utilized as convulsants in this investigation. The influence of intracerebrally administered GABA upon the convulsant activity of these agents was investigated and the results of these studies are summarized in Fig. III. The clonic convulsive and tonic extensor convulsive activity, induced by the intravenous infusion of caffeine 1%, was increased by the prior administration of GABA (5-80 μ g/20 g). A statistically significant ($P < 0.05$) reduction in the amount of caffeine required to induce clonus was observed following GABA (20, 40 and 80 μ g/20 g). Less caffeine was required to induce tonic extensor convulsions following 10, 20, 40 and 80 μ g/20 g of GABA than was required to induce tonic extensor convulsions in the controls receiving Locke-Ringer's solution only ($P < 0.05$). In the case of ammonium acetate, significantly less convulsant ($P < 0.05$) was

Figure II.

Effects of anticholinergically administered ACh upon tonic extensor
 reflexes due to atropine nitrate (1.25 mg/kg and 0.01% IV) and
 pentylentetrazol (0.5% IV).

Figure II.

Effects of intracerebrally administered GABA upon tonic extensor seizures due to strychnine nitrate (1.25 mg/kg SC and 0.01% IV) and pentylenetetrazol (0.5% IV).

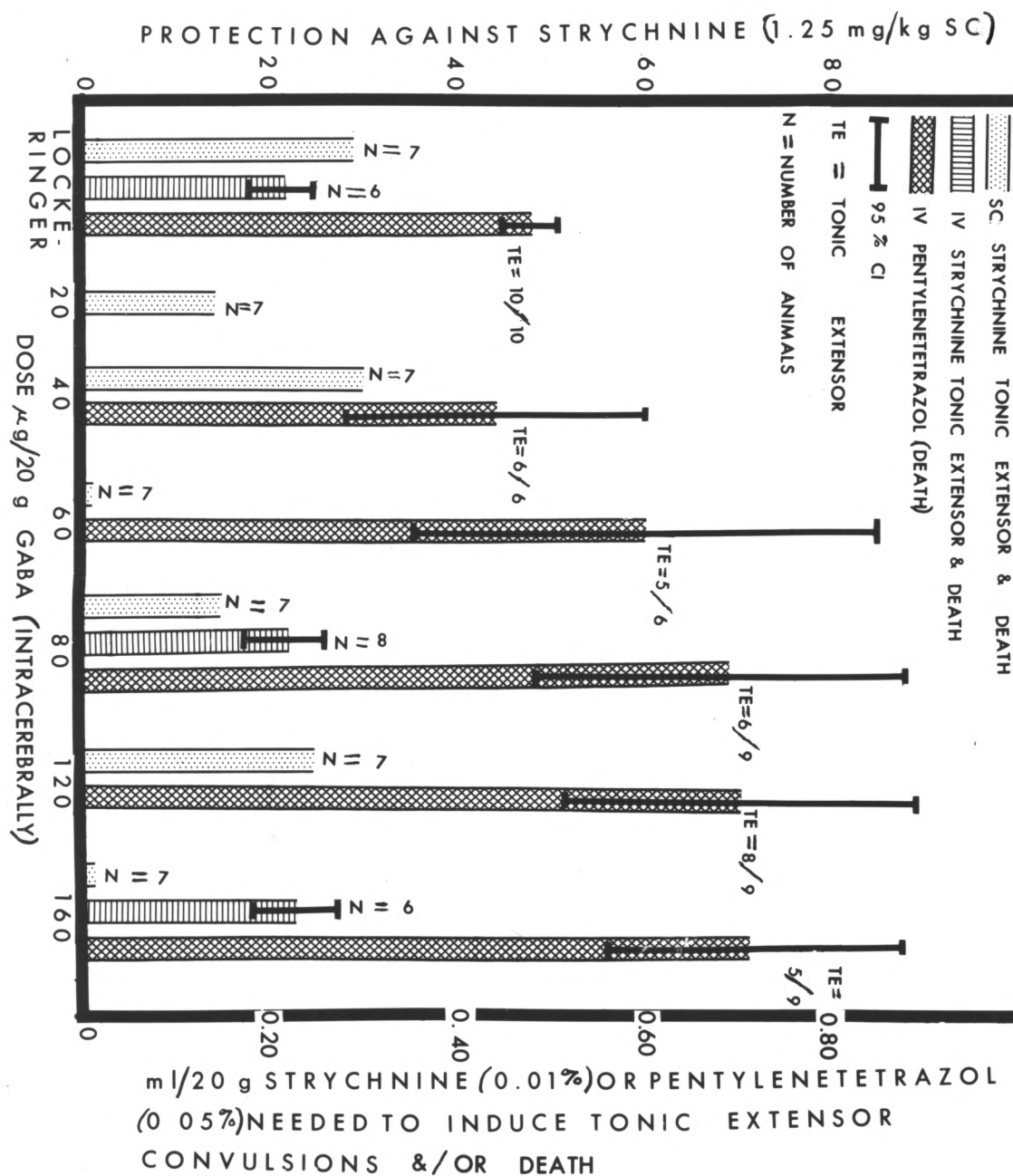


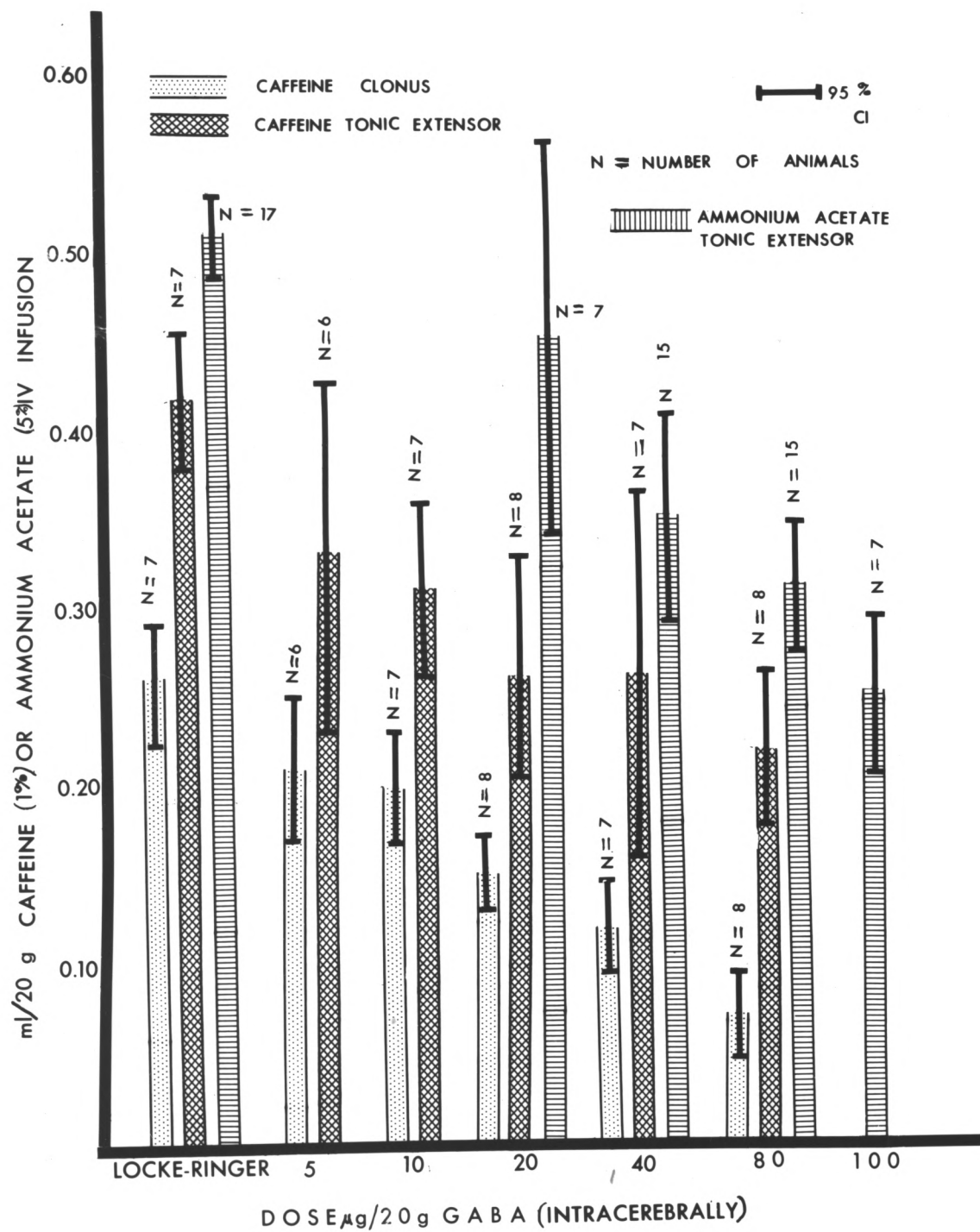
Figure III

Effects of intracerebrally administered GABA upon clonic convulsions due to caffeine (1% IV) and tonic extensor seizures due to caffeine (1% IV) and ammonium acetate (1% IV).



Figure III.

Effects of intracerebrally administered GABA upon clonic convulsions due to caffeine (1% IV) and tonic extensor seizures due to caffeine (1% IV) and ammonium acetate (5% IV).



required to induce tonic extensor convulsions in the animals receiving GABA 40, 80 and 100 $\mu\text{g}/20\text{ g}$ than was required for those receiving Locke-Ringer's solution only ($P < 0.05$).

The duration of action of intracerebrally administered GABA was assessed in other studies by the use of pentylenetetrazol (IV infusion, 0.5%) and ammonium acetate (IV infusion, 5%) following GABA (80 $\mu\text{g}/20\text{ g}$). In the case of pentylenetetrazol, protection was observed with GABA for 1-5 minutes following the injection; while with ammonium acetate, potentiation of convulsant activity was observed for 1-2 minutes following the administration of the amino acid.

DISCUSSION

The results obtained in the present investigation do not support the claims of certain investigators that systemically administered GABA will protect against certain convulsive seizures induced in mice by chemical means. Hawkins and Sarett (34) reported that orally administered GABA afforded protection against convulsive seizures induced in mice by subcutaneously administered pentylenetetrazol or methylethylglutarimide. However, in the present study, it was not possible to demonstrate a protective action for orally administered GABA (400 mg/kg) against pentylenetetrazol (100 mg/kg SC). This 400 mg/kg dose of GABA is the protective dose 50 reported by Hawkins and Sarett (34), for orally administered GABA tested against pentylenetetrazol (100 mg/kg SC). Other experiments summarized in Table I of the present study do not support the suggestion that systemic GABA possesses anti-convulsant activity against pentylenetetrazol induced convulsions. GABA, administered in doses of 1 g/kg (SC) did not afford protection against the clonic or tonic extensor seizures induced by the timed intravenous infusion of pentylenetetrazol (0.5%).

McLennan (58) reported that subcutaneously administered GABA (100 mg/kg) afforded protection against tonic extensor convulsions induced in mice by strychnine (1.5 mg/kg I.P.). Elliott and Hobbiger (20) reported that GABA (SC) elicited a slight but variable protection against strychnine induced convulsion in mice. However, the degree of protection observed was not statistically significant. Conversely, Brockman and Burson (12) could not demonstrate protection in mice when GABA (10-20 mg/total dose) was administered intraperitoneally

and was followed five minutes later by strychnine 40 μ g (total dose IP). The results obtained in the present investigation did not indicate that systemically administered GABA protects against strychnine nitrate induced convulsions. In one series of anticonvulsant tests, GABA (1 gm/kg IV) seemed to afford some protection against strychnine nitrate induced convulsions, but this protection can hardly be considered of significance since the dose of GABA was very large and the amount of protection observed was small (20%). Furthermore, GABA (400 mg/kg SC) did not protect against tonic extensor seizures induced by the timed intravenous infusion of strychnine nitrate (0.01%) regardless of the time elapsing between GABA administration and the start of the strychnine infusion.

GABA, when injected systemically, did not alter the convulsant pattern elicited by other convulsant procedures such as minimal electroshock, supramaximal electroshock, caffeine infusion or ammonium acetate infusion. Furthermore, the latency period required for the onset of convulsions induced by pentylenetetrazol (SC), strychnine nitrate (SC), minimal electroshock or supramaximal electroshock was not altered by the prior systemic administration of GABA.

Systemically administered GABA not only lacks anticonvulsant activity, but it also lacks significant neurotoxicity when administered intravenously in doses of 1 g/kg. This lack of central nervous system activity could be explained in at least three ways: 1) The compound does not readily cross the blood brain barrier; 2) The compound is rapidly destroyed in the peripheral tissues and adequate concentrations do not reach the brain; 3) The compound does not possess significant central nervous system activity which can be demonstrated by whole

animal behavioral studies. The last possibility seems unlikely in view of the results obtained following the intracerebral administration of the compound in this study. In regard to the other two possibilities, some support exists for the first. Purpura et al. (69) reported that destruction of portions of the blood-brain barrier in cats significantly increased the neurophysiological activity of systemically administered GABA. Marrazzi et al. (57) observed that GABA abolished transcallosal conduction when the compound was given into the carotid circulation, but not when it was administered by the intravenous route. This observation can be interpreted as indicating that adequate concentrations of the compound do not reach the blood brain barrier following intravenous administration. Whether this is simply due to excessive dilution of the compound in the vascular system, or whether the compound is also rapidly destroyed peripherally by its passage through the liver circulation has not been determined. Experiments elucidating the rate of peripheral destruction of GABA would therefore seem in order.

One study indicates that rapid peripheral destruction of GABA may not be a significant factor in explaining the lack of central activity of GABA following the systemic administration of the compound. van Gelder and Elliott (94) observed that Factor I activity, as determined by means of crayfish stretch receptor assay, increased significantly in various organs following the intraperitoneal injection of GABA. However, no increase of Factor I activity in the brain was observed. The Factor I activity of liver remained elevated for as long as 80 minutes following the GABA injection.

The effects of intracerebral administration of compounds to unanesthetized mice were first reported by Haley and McCormick (33).

Before a study of GABA and related amino acids was undertaken, a certain number of mice were administered acetylcholine and epinephrine intracerebrally and responses reported by Haley and McCormick '33) were qualitatively reproduced. In the present study a quantitation of effects produced by the intracerebral administration of drugs in mice has been attempted. It was also observed in preliminary experiments that the intracerebral injection of Locke-Ringer's solution produced less initial "stunning" effect than the injection of a similar volume of normal saline. Therefore, all the compounds tested in the present study were dissolved in Locke-Ringer's solution and this solution was used to obtain controls for each procedure.

According to the original authors of the technique for intracerebral injection into mice, the site of injection and the length and gauge of the needle employed are critical factors. Hence, if the injection is performed too far rostrally, penetration into the ventricles will not be accomplished. Long needles tend to increase movements during the injection, thus resulting in excessive brain damage. These variables were, therefore, rigidly controlled in the present study. In addition, the fluid volume and the pH of the solutions injected were kept constant. When all these factors were considered and kept constant, less than 5% of the injected mice showed neurological symptoms which persisted for longer than 5 seconds following the injection of Locke-Ringer's solution. It was felt, therefore, that although the intracerebral injection technique is rather crude and one cannot be certain about the exact site of injection, the method has merit inasmuch as a large number of unanesthetized animals can be studied, and as indicated in the results of this study, the effects elicited by certain active compounds

can be quantitated and dose-response relationship can be obtained.

As indicated in the results, the intracerebral administration of GABA to mice resulted in a biphasic response. The animal first exhibited clonic movements, which were then followed by depression. In addition, low doses of GABA protected against clonus induced by pentylenetetrazol and minimal electroshock. However, larger doses of GABA tended to increase the incidence of experimentally induced clonus. It would seem, therefore, that GABA is not an anticonvulsant or depressant of the classical type (i.e., barbiturates). It is interesting to note that Hayashi (35) reported that GABA induced both excitatory and inhibitory effects when applied to the cortex of anesthetized dogs. He has postulated that GABA is a precursor of two compounds in the central nervous system, a methylated derivative which is excitatory and gamma-amino-B-hydroxybutyric acid which is inhibitory only. It is rather difficult to explain the results of this study in the light of Hayashi's postulate. However, if the formation of the methylated derivative preceded the formation of the oxy derivative, one would expect to see a primary stimulation. This stimulation might then be followed by depression provided that the threshold for the hydroxy compound is lower and the equilibrium is in favor of the formation of this compound. An explanation for why higher doses of GABA potentiate pentylenetetrazol and minimal electroshock induced clonus is not apparent, however, on the basis of this excitatory and inhibitory compound theory.

Chen et al. (13) suggested that compounds which antagonize the clonic seizures induced by pentylenetetrazol (SC or IV) or by a current of low intensity do so by the same neurophysiological mechanism. In the present study, GABA (intracerebrally) yielded qualitatively similar

results when studied against the clonus induced by pentylenetetrazol (SC or IV infusion) or minimal electroshock. This essentially supports Chen's hypothesis. However, Chen and Bohner (15) and Goodman et al. (31) have also suggested that the tonic extensor convulsions induced by supramaximal electroshock and pentylenetetrazol (IV) occur by similar mechanisms. In the present study, intracerebrally administered GABA protected against the tonic extensor convulsions and death induced by pentylenetetrazol (IV infusion), but did not alter the tonic extensor seizure pattern elicited by supramaximal electroshock. These results suggest a different mechanism of action for the induction of tonic extensor seizures by the two procedures and therefore are not in agreement with the hypothesis that the tonic extensor seizures are induced by a similar neurophysiological mechanism.

It would be of interest to speculate concerning the mechanism of action of the omega amino acids in terms of the classical pharmacological receptor theory. Two types of receptors can be postulated as occurring at certain central postsynaptic endings. One receptor could be designated as Type E and could occur at the "excitatory" postsynaptic endings. Activation of Type E receptors would then give rise to excitatory postsynaptic potentials (EPSP) and general excitation, such as convulsions, would occur if enough of these receptors were activated. Conversely, another receptor designated as Type I could be present in the postsynaptic region of the "inhibitory" synapses and activation of these receptors would tend to reduce the activity of the excitatory synapses, and an inhibitory postsynaptic potential (IPSP) would be observed. The overall synaptic activity of the central nervous system would then be the algebraic sum of the activities of the excitatory

and inhibitory synapses. The concept of excitatory and inhibitory synapses in the central nervous system has been postulated and in certain areas their presence has been demonstrated electrophysiologically by Eccles, (18) and Grundfest (32). Furthermore, Purpura and Grundfest (71) have demonstrated that certain compounds such as pentylenetetrazol stimulate excitatory synapses, hence intensifying EPSP's recorded in cerebral and cerebellar cortical regions. The nature of the normal postsynaptic neurohumoral transmitters for the excitatory and inhibiting synapses has not as yet been elucidated.

As has been observed by Purpura et al. (70) omega-amino acids do not seem to activate the axodendritic endings but instead seem to block EPSP's recorded from the excitatory postsynaptic areas and the IPSP's recorded from the inhibitory postsynaptic areas. One must, therefore, think of these agents in terms of their ability to induce a block of Type E and Type I receptors in the respective postsynaptic regions. On the basis of the data obtained in the present study and that obtained by Purpura et al. (70), it would seem appropriate to suggest that omega-amino acids with normal carbon chains of from C_2 - C_5 block predominating Type E receptors as evidenced by depression or reduction of the EPSP's. The optimal configuration for blockade of these receptors would seem to be: 1) a 3-4 normal carbon chain; 2) a carboxyl group in the 1 position; 3) and a terminal amino group in the 3 position for B-alanine or the 4 position for GABA. It may be seen from Table VI that the potency comparison of different omega-amino acids obtained in the present study agree closely with those obtained by Purpura et al. (70).

If the carbon chain length is reduced or lengthened (glycine or 5-amino-n-valeric acid) the affinity for the Type E receptor would be

reduced. As the carbon chain length is increased above five carbons as represented by 6-amino-n-caproic acid and 8-amino-n-caprylic, affinity for the Type I receptors is increased and affinity for the Type E receptors is reduced. The overall effect observed might therefore manifest itself as excitation in terms of convulsive activity and blockade of recorded IPSP's. The results obtained in the present study indicate however, that this blockade of the Type E receptor at least is not entirely specific. With all of the compounds exhibiting loss of righting reflex, a period of clonus preceded the period of depression. The intensity of this clonus observed with the depressant omega-amino acids seemed to be least with glycine and greatest with 5-amino-n-valeric acid. This could be interpreted as indicating that the depressant omega-amino acids possess some affinity for Type I receptors as well as for Type E, and that the affinity for Type I receptors increases as the chain length increases. This is substantiated in part by Purpura et al. (70) who obtained some indication of a dual site of action of C_3 - C_5 omega-amino acids. This dual action was manifest as a transient augmentation of cerebral dendritic negativity after the amino acids had been washed away from the cortical areas. In another report, Purpura et al. (67) observed that although GABA itself blocked excitatory synapses, its application to the cortex already treated with C_6 or C_8 omega-amino acids augmented their ability to block IPSP's. Again this might be interpreted as evidence for a dual site of action for GABA.

Other factors besides steric configuration may be necessary for optimal receptor blockade. The variable depressant activity observed with taurine, as compared with the consistent depressant effect induced by B-alanine, suggests that the carboxyl group is essential for activity

TABLE VI

COMPARISON OF POTENCY (FOR STIMULATION OR DEPRESSION OF THE CENTRAL NERVOUS SYSTEM) OBTAINED IN THE
PRESENT STUDY WITH THAT REPORTED IN THE LITERATURE

Omega Amino Acid	Potency (ED ₅₀ in μ mole/20 g needed to induce a response)		Central Synaptic Blocking Activity As Measured by Purpura, et al. (70)	
	Loss of Right- ing Reflex	Tonic Extensor Convulsions	EPSP	IPSP
Glycine (C ₂)	2.240		+	
B-alanine (C ₃)	1.085		+	
GABA (C ₄)	1.001		+	
5-amino-n-valeric acid (C ₅)	2.354		+	
6-amino-n-caproic acid (C ₆)		3.30		+
8-amino-n-caprylic acid (C ₈)		0.35		+
Taurine	variable		0	0

EPSP = Excitatory post-synaptic potential

IPSP = Inhibitory post-synaptic potential

In addition, the amino group must be on the terminal carbon since a few preliminary experiments showed that alpha-amino-butyric acid was not active in doses of 640 μ g/20 g and the responses induced by B-amino-butyric acid in doses of 320-640 μ g/20 g, though of the GABA-type, were not consistent and reproducible. It would seem of interest also to study the secondary and tertiary amines of GABA in order to assess the effects of increasing the size of the terminal group and altering the electrical charge on the nitrogen. If one postulates that attachment to the receptor sites occurs at the two ends of the compound (i.e., carboxyl and amino group) the alteration of the electrical configuration of the molecule by altering the electrical charge of either group should in turn reduce the affinity of the molecule for the receptor and hence its activity.

In one respect, the observation in the present study, that GABA did not antagonize strychnine induced convulsions is surprising in view of the suggestion of Eccles (18) that strychnine blocks inhibitory synapses in the central nervous system. If the convulsant activity of strychnine is due to a blockade of inhibitory synapses, then it would be suggestive that these convulsions are due to hyperactivity of excitatory synapses. If GABA blocks excitatory synapses (70), then one might expect an antagonism of the strychnine-induced effects by this omega-amino acid. Jasper et al. (44) could not demonstrate antagonism between GABA and strychnine in cats, utilizing the dendritic response elicited by local cortical stimulation. Purpura et al. (66) noted that strychnine merely eliminated the surface positivity by blocking the inhibitory synapses that remain after GABA has blocked the excitatory synapses. This can hardly be interpreted as true antagonism. In the present study, at least two explanations for the observation that GABA does not alter strychnine-induced convulsions

can be given. Purpura et al. (70) reported that GABA blocks only axo-dendritic endings in the cerebral and cerebellar cortex and does not block excitatory axo-somatic synapses in the deeper areas of the central nervous system. Since, in the case of strychnine convulsions, one might expect the involvement of all central nervous system synapses (i. e., excitatory axo-somatic as well as axo-dendritic synapses would become hyperactive) it is not surprising that GABA does not significantly alter the convulsions induced by strychnine. In addition, if strychnine tonic extensor convulsions are initiated predominantly from spinal cord regions, it is conceivable that GABA, injected into the ventricles, does not diffuse in sufficient concentration into the lower central nervous system areas to prevent convulsions from these areas.

However, GABA might be expected to antagonize pentylenetetrazol convulsions since Purpura and Grundfest (71) suggest that pentylene-tetrazol induces convulsions by stimulating excitatory synapses in the brain. Furthermore, as stated previously, it has been demonstrated that GABA blocks excitatory axo-dendritic synapses. If the contribution of these excitatory axo-dendritic synapses to the convulsive seizure induced by pentylenetetrazol is great, then one would expect antagonism of pentylenetetrazol induced convulsions as noted with smaller doses of GABA in the present study. Why potentiation of the pentylenetetrazol induced clonus occurs with higher doses of GABA is not obvious on the basis of present knowledge concerning the neurophysiological actions of GABA.

Why GABA potentiates the convulsant activity of ammonium acetate and caffeine is not apparent. However, Chen et al. (16) noted that reserpine tended to potentiate convulsions induced by maximal electro-

shock, pentylenetetrazol and caffeine but did not potentiate convulsions induced by strychnine. It would seem, therefore, that reserpine and GABA could be used to advantage in the further elucidation of the mechanisms by which convulsions are induced by certain procedures.

SUMMARY

1. The effects of intracerebrally administered gamma-amino-butyric acid (GABA) and related omega-amino acids and the anticonvulsant activity of GABA administered both systemically and intracerebrally were investigated in mice.
2. No evidence of neurotoxicity was observed with systemically administered GABA in doses of 1 g/kg. Further, in doses of 1 g/kg or less, GABA did not protect the animals against the clonus due to pentylenetetrazol (0.5% IV and 87.5 mg/kg SC) or minimal electroshock or tonic extensor seizures due to pentylenetetrazol (0.5% IV) strychnine nitrate (1.25 mg/kg SC), caffeine (1% IV), ammonium acetate (5% IV) or supramaximal electroshock. However it was possible to quantitate the anticonvulsant activity of three reference compounds against the appropriate convulsant test procedures.
3. The technique of intracerebral administration of compounds to mice has been modified slightly and the responses obtained after the intracerebral administration of several compounds have been quantitated. Mice receiving GABA, glycine, B-alanine, and 5-amino-n-valeric acid showed biphasic responses, i.e. clonic movements followed by depression; the intensity of the depression was related to the dose of the compound administered. Intensity of the depression was quantitated in terms of loss of the righting reflex. GABA and B-alanine were of equimolar potency and were more active than glycine and 5-amino-n-valeric acid which were also equipotent. Alpha-amino-n-butyric acid was inactive in doses of 640 µg/20 g or less. B-amino-n-butyric acid and taurine elicited GABA-type

responses but these were not reproducible.

6-amino-n-caproic acid and 8-amino-n-caprylic acid produced clonus followed by tonic extensor seizures and death. The number of animals exhibiting tonic extensor seizures increased as the dose was increased. 8-amino-n-caprylic was approximately ten times as potent as 6-amino-caproic acid.

4. Intracerebrally administered GABA (20-60 μ g/20 g) protected against clonus due to pentylenetetrazol (0.5% IV and 87.5 mg/kg SC) and minimal electroshock; but higher doses (80-160 μ g/20 g) potentiated the clonus induced by the three procedures. Tonic extensor seizures induced by strychnine nitrate (1.25 mg/kg SC) or supramaximal electroshock were not affected by intracerebrally administered GABA. Protection from tonic extensor seizures and death due to pentylenetetrazol (0.5% IV) was observed. A potentiation of the clonus and tonic extensor activity induced by caffeine (1% IV) and tonic extensor activity due to ammonium acetate (5% IV) was observed with intracerebrally administered GABA.
5. Possible reasons for the lack of neurotoxicity and anticonvulsant activity of systemically administered GABA have been given. The biphasic effects of intracerebrally administered GABA have been discussed. A structure-activity-relationship between the different omega-amino acids has been proposed, and on the basis of the present study and literature reports, two types of receptors designated as type E and type I have been speculated to exist at certain central nervous system synapses.

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