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THE NEUROMODULATORY ACTION OF TAURINE IN A GENETIC EPILEPSY

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THE NEUROMODULATORY ACTION OF TAURINE
IN A GENETIC EPILEPSY

BY

Douglas William Bonhaus

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PHARMACOLOGY
In Partial fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

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As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Douglas William Bonhaus
entitled THE NEUROMODULATORY ACTION OF TAURINE IN A GENETIC EPILEPSY

and recommend that it be accepted as fulfilling the dissertation requirement
for the Degree of Doctor of Philosophy.

Ryan J Huxtable

September 26, 1983

Date

John E. Smith

Sep. 19, 1983

Date

Maus Zoude

Sep 19, 1983

Date

S. Lynn Sipes

Sept 19, 1983

Date

David A. Kunkin

Sept. 19, 1983

Date

Final approval and acceptance of this dissertation is contingent upon the
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Ryan J Huxtable

Dissertation Director

September 26, 1983

Date

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DEDICATION

This dissertation is dedicated to my parents, Raymond and Jean, and to my wife, Sue, in appreciation of all their support, guidance and patience for me throughout my graduate studies.

PREFACE

"Epilepsy is the term given to chronic disorders of the central nervous system which produce sudden and transitory episodes of abnormal, excessive neuronal activity." This definition, while accurate, does not adequately describe the diversity and complexity of epilepsy. Epilepsy is not a single syndrome of similar signs. It is a collection of disorders which may manifest themselves as episodes of involuntary and abnormal motor activity, inappropriate autonomic responses or sensory perceptions or changes in affect.

These episodes of abnormal behavior in epileptics have resulted in an association of this disorder with magic, demons and possession. The word "epilepsy" comes from the Greek word "epilepsia" meaning "to come upon, to be grabbed hold of or thrown down, to attack and seize hold of." The association of epilepsy with the supernatural is reflected today by the social stigma attached to epilepsy.

For all the confusion, mysticism and fear surrounding epilepsy, it is nevertheless a common affliction. Between 5 and 10 of every 1000 people have epilepsy. More people have epilepsy than all the cases of cancer, Parkinsonism, Huntingtons chorea, muscular dystrophy, multiple sclerosis and cerebral palsy combined.

Epilepsy is a debilitating disorder which handicaps people as much by the fear of seizures as by the seizures themselves. Epilepsy is a disorder which kills people. Nearly one-half of all epileptics die as the result of their affliction, either directly by the seizures

or indirectly as a result of accident or suicide. The life expectancy of an epileptic may be as much as 10 years less than that of a non-epileptic.

The current treatments for epilepsy are inadequate. Fifty percent of all treated human epileptics have an occasional seizure. This occasional seizure, as infrequent as once every two years, can prevent the epileptic from receiving a driver's license and bars access to many types of employment. As many as 20% of all treated epileptics routinely have seizures. These people frequently have degeneration of cognitive functions and require constant care.

The lack of success in preventing or treating many forms of epilepsy may in part, be due to a lack of understanding of the biochemical, physiological or anatomical bases of epileptogenesis. Therefore, the work presented in this dissertation was undertaken in an attempt to gain insight into how the brain acts to regulate its own "excitability" and how defects in these regulatory processes may contribute to seizure-susceptibility. As epilepsy is not a single disorder, it is unlikely that the underlying basis of the seizures are identical in all epilepsies. However, alterations in the concentration or metabolism of neuroactive amino acids in the central nervous system are frequently associated with seizure disorders. Furthermore, the metabolism of amino acids in the brain may play a key role in the regulation of "excitability". Therefore, my research has focused upon the relationship between altered central nervous system handling of amino acids and seizure-susceptibility.

The work presented in this dissertation could not have been accomplished without the guidance of my research adviser, Ryan J. Huxtable, and the other members of my doctoral committee, Klaus Brendel, David Kreulen, Hugh E. Laird III, and I. Glenn Sipes. Shirley E. Lippincott must also be recognized for her immense help in the quantitative determination of amino acids in body tissues. I thank these people for their assistance in not only making this project possible but also for making it fruitful and rewarding.

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ABSTRACT

Taurine (2-aminoethane sulfonic acid) is one of the most abundant inhibitory amino acids in the mammalian central nervous system (CNS). Substantial evidence exists to suggest that this amino acid is a physiological modulator of neuronal excitability. Taurine is also a potent anticonvulsant in a variety of animal epilepsies and in certain human epileptics. The mechanisms of these neuromodulatory and anticonvulsant actions of taurine are not known.

I have investigated a proposed relationship between altered amino acid metabolism, seizure-susceptibility and the anticonvulsant action of taurine.

The findings of the work presented in this dissertation indicate that in the genetically seizure-susceptible rat there are alterations in the subcellular concentration and transport of taurine. Furthermore, the data presented here indicate that these alterations in the CNS handling of taurine are not a consequence of seizure activity but rather may be contributing to the seizure-susceptibility. This supports the hypothesis that taurine is a physiological modulator of neuronal excitability and that defects in this neuromodulatory process may contribute to seizure-susceptibility.

The action of taurine was found to not be mediated by a redistribution of glutamate in the brain but instead may be by increasing the conversion of glutamate to GABA.

INTRODUCTION

Epilepsy is the term given to chronic disorders of the brain that produce sudden and transitory episodes of abnormal, excessive, neuronal firing in the central nervous system (CNS). These episodes of excessive neuronal firing, called seizures, may be restricted to a single region of the brain or may spread from the source, or focus, to encompass the entire CNS. When the excessive neuronal activity of a seizure enters areas of the brain important for interpreting sensory information the person having the seizure may perceive this seizure activity as a form of sensory stimulation such as a taste, smell or somatic sensation (Niedermeyer, 1974a). When the seizure activity involves areas of the brain related to the control of motor movement the seizure manifests itself as a convulsion (White, 1981).

A brain which is predisposed to having seizures may be triggered into seizure activity by forms and intensities of stimulation which would be innocuous to the normal brain. Examples of such an inappropriate response to environmental stimulus include sound induced seizures in audiogenic rats and mice (Hall, 1947; Newmark and Penry, 1980); seizures induced by shaking or tossing in the vestibular sensitive mouse (Kurokawa et al., 1966) and light stimulated seizures in the photosensitive baboon (Newmark and Penry, 1979). Certain human epileptics are sound- and photo-sensitive. These people may be triggered into having a seizure by light stimulation such as that of a

rapidly flashing strobe light or even the flickering light of a television receiver (Newmark and Penry, 1979) or sound stimulation such as the tapping of a pencil (Neidermeyer, 1974b).

The degree to which neuronal firing in one region of the brain, such as that generated by chemical, electrical or sensory stimulation, produces a synchronized firing of large populations of neurons and is transmitted to other regions of the CNS is determined by the gross excitability of the CNS. In the healthy brain homeostatic regulation of excitability is achieved by the balanced action of excitatory and inhibitory mechanisms. This regulation of excitability prevents the inappropriate, excessive, spread of neuronal firing from a site of stimulation (Eccles, 1969; Morselli et al., 1981).

Neuroactive Amino Acids and Excitability

Many different systems and mechanisms have been proposed to be involved in the regulation of excitability and hence seizure-susceptibility (Eccles, 1969; Morselli et al., 1981). However, one of the most important determinants of gross excitability is the balance of action of excitatory and inhibitory amino acids (Iversen et al., 1975). Excitatory amino acids are defined as those which increase the activity of neurons, while inhibitory amino acids are those which decrease neuronal activity. Excitatory and inhibitory amino acids are present in high concentrations throughout the CNS (Meldrum, 1980). Some of these neuro-active amino acids are shown in figure 1. Many of these amino acids have specific mechanisms for release and inactivation

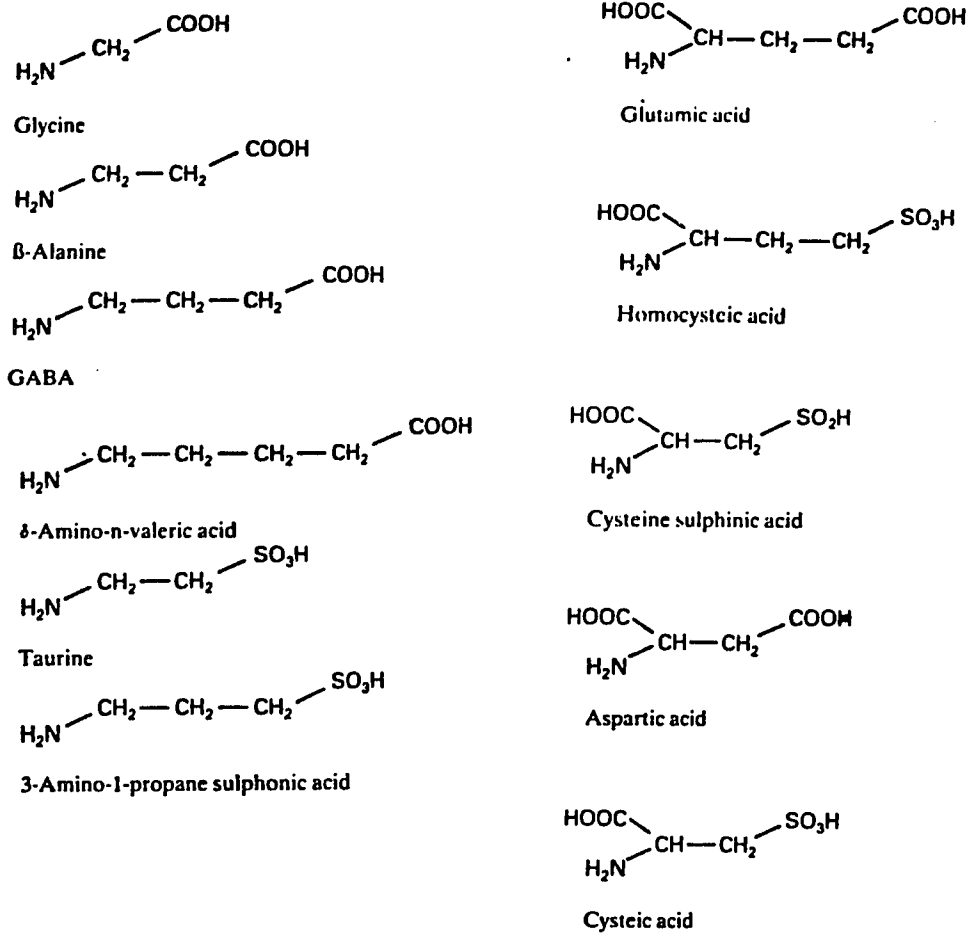


Figure 1. Neuroactive amino acids.

Molecular formula of inhibitory and excitatory amino acids.

Cooper et al., 1978; Fagg and Lane, 1979; Iversen et al., 1975 and Logan and Snyder, 1972).

Alterations in the concentration, transport, or metabolism of these neuro-active amino acids have been associated with seizure disorders (Emson, 1978; Huxtable et al., 1983; Perry and Hanson, 1981; Bakay and Harris, 1981; Airaksinen, 1979; Goodman and Connolly, 1980; Van Gelder and Courtois, 1972; Van Gelder, 1982).

Taurine, Glutamate and Epilepsy

The two most abundant neuroactive amino acids present in the brain are taurine and glutamate. Alterations in the concentrations of these two amino acids are among the most frequently reported biochemical anomalies in seizure disorders (Van Gelder, 1980 and 1982). The metabolic relationships between taurine, glutamate, their precursors and their metabolites are shown in figure 2. The acidic amino acids glutamate, cysteic acid and cysteine sulfinic acid are excitatory, whereas, their neutral metabolic products gamma aminobutyric acid (GABA), hypotaurine, and taurine are inhibitory.

The physiological importance of the carboxylic acid portion of this scheme in regulating neuronal excitability is demonstrated by the convulsant action of inhibitors of the conversion of glutamate to GABA and the anticonvulsant action of activators of this process. Compounds such as allylglycine and the pyridoxal phosphate antagonists which inhibit the decarboxylases responsible for the conversion of glutamate to GABA (glutamic acid decarboxylase, GAD) increase concentrations of glutamate, decrease concentrations of GABA and produce seizures (Killam

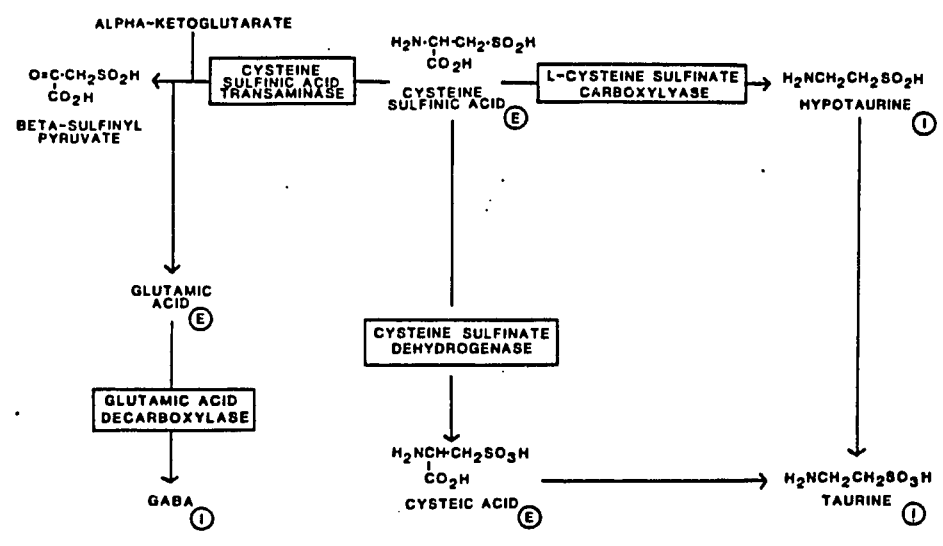


Figure 2. Neuro-active amino acid metabolism.

Carboxylic and sulfur amino acid metabolism. E= excitatory amino acids, I= inhibitory amino acids (From Huxtable and Pasantes-Morales, 1981).

and Bain, 1957; Holtz and Palm, 1964; Meldrum, 1980; Gale and Iadarola, 1980; Tapai, 1978; Loscher, 1981a). An example of a compound which raises seizure threshold by altering glutamic acid metabolism is valproic acid. This anticonvulsant, which is efficacious in certain human epilepsies, acts by increasing the conversion of glutamate to GABA and by decreasing the degradation of GABA (Loscher 1981b and 1981c).

The lack of specific inhibitors of the enzymatic conversion of excitatory sulfur amino acids to inhibitory metabolites has prevented a similar verification of the role of sulfur amino acid metabolism in regulating seizure threshold. However, intracerebralventricular (ICV) injection of the excitatory sulfur amino acids produces seizures, whereas, ICV injection of inhibitory amino acids or analogs of inhibitory amino acids raises seizure threshold (Kontro et al., 1983 Iversen et al., 1975). Furthermore, while a relationship between sulfur amino acid synthesis and seizure-susceptibility has not been established, alterations in the second means of regulating concentrations of sulfur amino acids in the brain, namely transport, have been associated with seizure disorders. Goodman et al. (1980 and 1982) have shown that human epileptics tend to be disproportionately high renal reabsorbers of taurine. Airaksinen (1979) has reported that human epileptics with progressive myoclonus epilepsy have a decreased rate of taurine transport into platelets. Fabisiak and Schwiak (1980) have shown that in the P2 fractions derived from the cerebellum of rats made epileptic by amygdaloid kindling there is an increased active transport of taurine.

Thus, substantial evidence exists to indicate that regulation of the concentrations, metabolism and transport of both the carboxylic and the sulfur amino acids in the brain is important to the regulation of neuronal excitability. Therefore, a rational approach to determining the underlying biochemical basis of seizure-susceptibility in seizure disorders of unknown cause could begin with an investigation of the CNS handling of these neuro-active amino acids. Furthermore a better understanding of the relationship between amino acid metabolism and excitability would allow the development and rational use of new types of anticonvulsants. As pointed out above, at least one anticonvulsant currently used to treat human epilepsy acts by modifying the metabolism of carboxylic amino acids in the brain. However, the potential benefit of altering sulfur amino acid metabolism has not yet been determined even though one of the most abundant inhibitory amino acids in the brain is a sulfur amino acid.

Taurine: A Modulator of Excitability

Taurine (2-aminoethane sulfonic acid) is the most abundant inhibitory amino acid in many regions of the mammalian CNS. The inhibitory action of taurine upon neuronal tissue was first demonstrated in electrophysiological experiments by Curtis and Watkins (1960 and 1965). Since then, taurine has been shown to decrease the spontaneous firing of neurons in the spinal cord, brain stem and cerebral cortex (Haas and Hoshi, 1973; Curtis and Crawford, 1969; Krnjevic and Puil, 1976). An inhibitory action of taurine has also been demonstrated in behavioral

experiments. Taurine decreases temperature, eating and locomotor activity (Kerwin and Pyock, 1979; Hruska et al., 1976).

Taurine is anticonvulsant in a large variety of genetic and experimental epilepsies. The genetic models of epilepsy in which taurine has been shown to be effective include the genetically seizure-susceptible rat (Huxtable and Laird, 1978), the photosensitive baboon (Wada et al., 1975) and the vestibularly sensitive mouse (Iwata et al., 1979). Experimental models of epilepsy in which taurine is anticonvulsant include ouabain induced seizures (Barbeau and Donaldson, 1974), penicillin induced seizures (Durelli et al., 1976), cobalt induced seizures (Van Gelder, 1972), the hypoxic rat (Sanberg and Willow, 1980) and pentylenetetrazol induced seizures (Izumi et al., 1974). Taurine has also reduced seizure frequency in some human epileptics resistant to other forms of treatment (Huxtable, 1981, for review).

As taurine is present in the brain in high concentrations, has an inhibitory effect on behavior and neuronal firing and is anticonvulsant, it has been proposed that this amino acid is key physiological modulator of neuronal excitability. However, the mechanisms of the neuro-inhibitory actions of taurine are not known. A difficulty in determining the mechanisms of the physiological, or pharmacological, actions of taurine has not been due to an inability to find effects of taurine in neuronal tissue but rather in determining as to which of these effects are related to the inhibitory actions.

Taurine: A Neurotransmitter?

One hypothesis regarding the mechanism of action of taurine holds that taurine is an inhibitory neurotransmitter. This hypothesis is based upon the finding that there is a pool of taurine which is present in a unique population of synaptosomes and that this synaptic pool of taurine can be released by electrical or chemical depolarizing stimulus (Davison and Kaczmarek, 1971; Kaczmarek and Davison, 1962; Collins and Topilawa 1974). A neurotransmitter role for taurine is further supported by the selective action of a putative taurine antagonist, 6-aminomethyl-3-methyl-4H,1,2,4-benzothiadiazine-1,1-dioxide, (Yarbrough, 1981; Martin et al., 1981; Curtis et al., 1982) upon the firing of Purkinje cells following electrical stimulation of stellate neurons (Okamoto et al., 1983).

Arguing against a neurotransmitter role for taurine are the observations that most of the taurine present in the brain is not localized in the synaptic terminals, that no calcium-dependent release mechanism for taurine has been established for most regions of the brain and no unique taurine receptor has yet been characterized (Lopez-Colome and Pasantes-Morales, 1981). Thus the issue of whether taurine has a neurotransmitter function has not been resolved. However, it is apparent that the bulk of the taurine present in the brain is not fulfilling a neurotransmitter role.

Taurine: A Neuromodulator?

Given the conflicting data regarding the role of taurine as a neurotransmitter, it is possible that the more important physiological

function of taurine is that of modulation of neurotransmission rather than neurotransmission per se. Biochemical experiments, conducted with sub-cellular fractions of brain tissue, and electrophysiological experiments, conducted on invertebrate neurons, suggest that taurine may modulate neurotransmission by both pre- and post-synaptic mechanisms.

Taurine alters the conductance of certain ions across axonal membranes. Gruener and Bryant (1975) and Gruener et al., (1976) have demonstrated that taurine increases the membrane permeability to potassium and chloride ions when applied to giant axons of squid. This action of taurine on ionic conductance, if it occurs in mammalian neurons, may contribute to the inhibitory action of taurine as it would hyperpolarize postsynaptic membranes and thus decrease excitability.

Modification of ionic conductances presynaptically may also be involved in the inhibitory action of taurine. Taurine reduces the temperature-sensitive uptake of calcium into synaptosomes when the concentration of calcium in the incubation media is similar to the extracellular concentration of calcium (Pasante-Morales and Gamboa, 1980; Pasantes-Morales et al., 1982). As calcium uptake into the presynaptic terminal is apparently essential for neurotransmitter release and as synaptosomes are probably sealed off presynaptic terminals it is possible that this inhibition of calcium transport, in synaptosomes, reflects an in vivo mechanism of the neuro-inhibitory action of taurine. In support of such a hypothesis is the observation that taurine inhibits the calcium dependent release of neurotransmitters from brain slices and synaptosomes (Kuriyama et al., 1978).

One problem with these proposed mechanisms of action of taurine is that they would affect excitatory and inhibitory neurotransmission in an identical manner. Taurine, for example, inhibits the calcium dependent release of inhibitory neurotransmitters such as GABA and norepinephrine as well as the excitatory neurotransmitters from brain slices and synaptosomes (Kuriyama et al., 1978). Furthermore, under certain conditions an appropriate dose of taurine will increase seizure threshold without altering other parameters of behavior such as locomotor activity (Huxtable and Laird, 1978). Thus, it is unlikely that the anticonvulsant action of taurine is the result of a non-specific action on ionic conductances. A more likely hypothesis is that taurine is anticonvulsant by a specific effect on those mechanisms responsible for regulating excitability.

Taurine: A Regulator of Glutamate Metabolism?

One proposed mechanism for the anticonvulsant action of taurine, which does involve a specific effect on mechanisms regulating excitability, involves an action of taurine on the metabolism and distribution of glutamate (VanGelder, 1976 and 1978). As stated above, alterations in glutamate concentration are frequently associated with seizures disorders. This hypothesis holds that these alterations in glutamate concentration reflect defects in the CNS handling of this amino acid which contribute to the seizure-susceptibility. The anticonvulsant action of taurine then would be by a rectification of these alterations in glutamate concentration or distribution. It has been proposed that taurine alters either the cellular distribution of glutamate or the

fraction of total (glutamate + glutamine) which is in the form of glutamate (Van Gelder, 1976, 1978a, 1978b and 1982). If taurine were to decrease the glutamate present in excitatory neurons that release glutamate and thus decrease the amount of glutamate released, an inhibitory action would result. However, the hypothesis as advanced by Van Gelder suggests that taurine increases the intra-neuronal pool of glutamate. Increasing the neuronal concentration of glutamate, could result in an inhibitory action if the increase in glutamate resulted in an increased formation of the inhibitory neurotransmitter GABA. The rate limiting step in the release of GABA is the synthesis of GABA from glutamate (Roberts, 1974) (figure 3). This hypothesis of an action of taurine on glutamate metabolism is supported by the observation that administration of taurine to animals made epileptic by the administration of colbalt rectifies the abnormally low concentrations of glutamate in the epileptic foci of these animals (Van Gelder, 1976; Van Gelder, 1978).

The observation of a high statistical correlation between concentrations of taurine and glutamate in the brain (Van Gelder and Courtois, 1972; Van Gelder, 1981; Rassin, 1981) has lead to a more general hypothesis, that the physiological action of taurine is by regulation of the cellular distribution of glutamate.

These hypotheses, of a direct metabolic relationship between taurine and glutamate is appealing as it suggests an action of taurine which would specifically raise seizure threshold rather than just having a non-specific action on all types of neuronal firing.

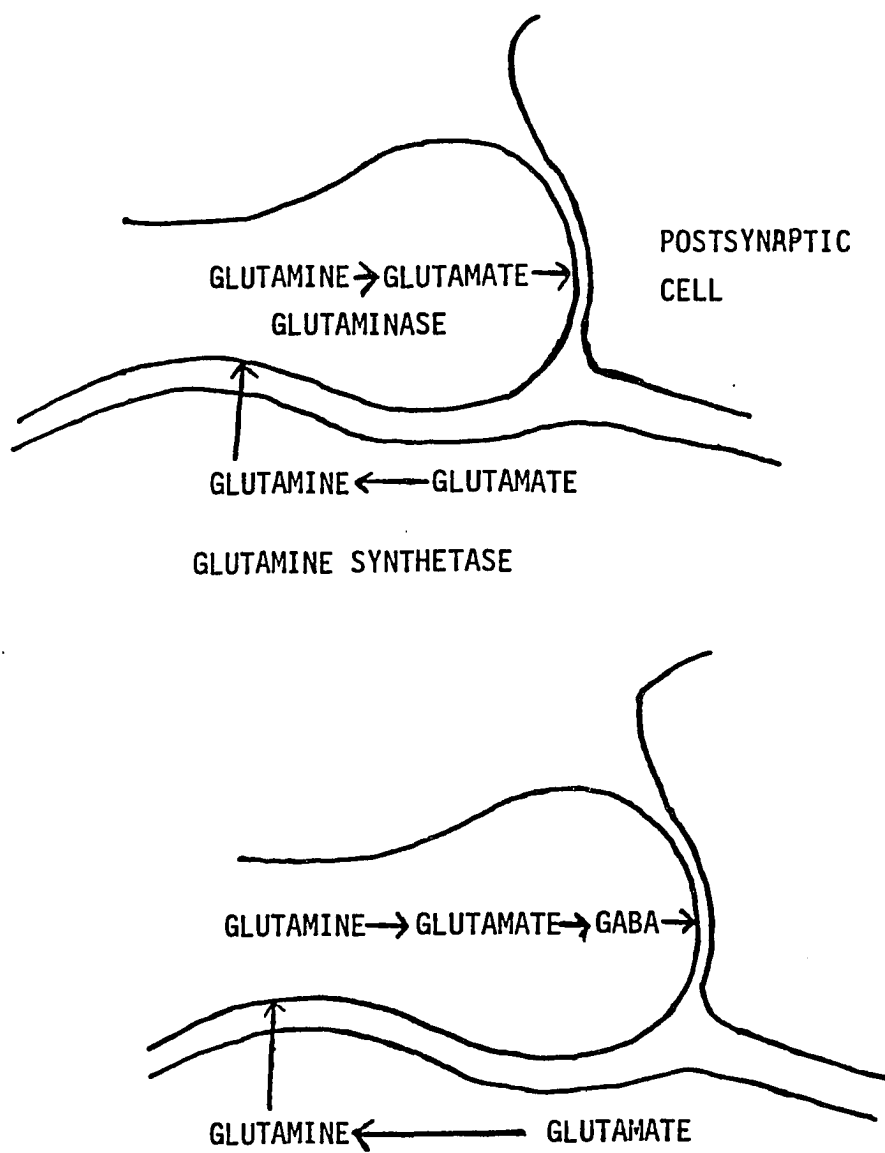


Figure 3. Glutamate in excitatory and inhibitory neurotransmission.

Purpose

An understanding of the relationship between altered amino acid metabolism, seizure-susceptibility and the anticonvulsant action of taurine, if such a relationship exists, would not only provide insight into the physiological and pharmacological actions of taurine but would also provide insight into the mechanisms by which sulfur amino acids in general regulate neuronal excitability. An understanding of the mechanisms by which sulfur amino acids regulate excitability might then pave the way for the development of novel anticonvulsants.

Therefore, the purpose of the work presented in this dissertation focused on determining the mechanism of action of taurine in the brain and specifically to investigate the proposed relationship between altered amino acid metabolism and the anticonvulsant action of taurine.

Approach

The action of taurine in the brain was investigated by increasing or decreasing the concentration of taurine in the brain and then examining the brains for biochemical changes which may be related to the anticonvulsant action of taurine. The action of taurine was also investigated by examining the relationship between altered taurine metabolism in the genetically epileptic rat and the seizure-susceptibility of these animals.

Amino Acid Metabolism in the Epileptic Rat

The CNS handling of taurine, glutamate and other neuroactive amino acids in the genetically seizure-susceptible rat was compared to that in the seizure resistant rat.

Most experimental investigations of epilepsy have utilized animals made epileptic by the application of noxious chemicals such as cobalt, penicillin or ouabain to the brain. The disadvantages of these models of epilepsy include the fact that seizures in these animals occur spontaneously in changing frequencies and intensities. Another disadvantage of these focal epilepsies is that there may be pathological changes in the brains of these animals unrelated to the genesis of the seizure-susceptibility.

The seizure-susceptible strain of rats, on the other hand, are an advantageous model of epilepsy in that the seizure response to a given stimulus is constant and reproducible. Furthermore, these animals rarely if ever have spontaneous seizures, but can be easily provoked into having a seizure. This allows a control of the frequency of convulsion which in turn permits the separation of conditions associated with seizure-susceptibility from those which are a consequence of seizure activity. Taurine is a potent and long acting anticonvulsant in this model of epilepsy (Huxtable and Laird, 1978). If the anticonvulsant action of taurine does indeed involve rectification of abnormalities in amino acid distribution or metabolism, then these abnormalities should be present in the epileptic rat.

Biochemical Actions of Taurine in the Brain

If the anticonvulsant action of taurine does involve an effect on glutamate metabolism or distribution, then it would be expected that administration of taurine to seizure-susceptible rats would modify the CNS handling of glutamate. To test this hypothesis taurine was

injected into brains of seizure-susceptible rats and then these brains were examined for alterations in glutamate concentration or distribution which would be associated with the anticonvulsant action of taurine.

Biochemical Actions of a Taurine antagonist

The converse experiments to those in which taurine is injected into the brain would be those in which taurine is depleted from the brain. This was accomplished by administration of a taurine transport antagonist, guanidinoethane sulfonate (GES) to rats. The effect of chronic administration of GES upon taurine and glutamate concentration and cellular distribution was examined. The effect of chronic exposure to GES upon chemoshock threshold also was determined. As taurine concentrations are greatest in the neonatal rat (Davis and Himwich, 1973; and Agrawal and Himwich, 1970) and as taurine may have a physiological role during development (Rassin et al., 1978; , Sturman, 1979; Sturman et al., 1978; Huxtable and Lippincott, 1982) the GES was administered to rats during prenatal development and thereafter.

Thus, in summary, the proposed relationship between altered amino acid metabolism, seizure-susceptibility and the anticonvulsant action of taurine was examined by both the addition to and depletion of taurine in the brain. This proposed relationship also was examined by a comparison of the biochemistry of seizure-susceptible and seizure resistant brains.

METHODS AND MATERIALS

Chemicals

[¹⁴C]Glutamic acid (2.10 TBq/mole) and [¹⁴C]GABA (9.25 TBq/mole) were purchased from New England Nuclear, Boston, Massachusetts.

[³H]Taurine was prepared by tritium exchange by New England Nuclear and had been purified by ion exchange chromatography (Hruska et al., 1977). The final specific activity of the taurine was 0.31 TBq/mole.

[³⁵S]Cysteine (2.78 TBq/mole) was obtained from Amersham, Arlington Heights IL. GES was synthesized and purified from taurine as described by Huxtable et al. (1979). Other reagents were obtained from The Sigma Chemical Company, St Louis, Missouri.

Animals

Control, seizure-resistant Sprague-Dawley rats were obtained either from Hilltop Lab Animals, Chatsworth, California or from the Division of Animal Resources at the University of Arizona. These animals are designated as SR rats. Genetically produced seizure-susceptible Sprague-Dawley rats with the official designation of UAz;AGS(SD)(Consroe et al., 1981) were from the University of Arizona colony. In this dissertation these animals were designated as SS rats. Unless otherwise specified, SS rats were screened by sound stimulus on one occasion only to verify seizure-susceptibility. Rats were rated for seizure intensity as described by Jobe et al. (1973) and all rats designated as SS were maximal responders. Non-susceptible progeny

from SS rats were designated as NSP rats. NSP rats, which are less than 5% of the total progeny of SS rats, show no seizure response to the sound stimulus used to determine audiogenic seizure-susceptibility.

ICV Cannulations

Cannulas were placed into the left lateral ventricle of 220-300 g rats at least 24 h prior to the ICV administration of any drug. To place the cannulas the animals were anesthetized by intraperitoneal administration of Ketamine (2 ml/kg). Prior to the surgery a lack of response to painful stimuli was verified by a vigorous tail pinch. Cannulas, constructed of polyethelene tubing (Clay Adams-PE20) were implanted 2 mm posterior to bregma and 2 mm left of the sagittal suture. The beveled tip of the cannulas were sunk to a depth of 5 mm from the outer edge of the skull. The cannulas were immobilized by the application of dental acrylic to the skull. The cannulas were then filled with distilled water to prevent clogging. After completion of the experiment, when possible, the proper placement of the cannula was verified by dissection of the brain.

Brain Fractionation

Subcellular fractions of brain tissue were prepared by a method similar to that described by Gray and Whittaker (1962) except that a fixed angle rotor was used. Rats were killed by cervical dislocation and the brains were immediately placed in 9 volumes of ice cold 0.32 M sucrose. The brains were homogenized in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate, H, was then

centrifuged at 1000 X G for 10 min at 4°C. The supernatant fluid, designated S1, was removed from the pellet, P1, and centrifuged at 18000 X G for 20 min. The supernatant fluid, S2, was removed and the pellet, P2, was resuspended. If the P2 fraction was to be used for transport experiments it was resuspended in a Krebs-phosphate buffer. If the P2 fraction was to be further fractionated it was resuspended in 0.32 M sucrose. Resuspended P2 (4 ml) was layered onto 7.0 ml of 0.8 M sucrose and centrifuged at 40,000 X G for 30 min. The material at the 0.32-0.8 M sucrose gradient interface was collected and designated the A fraction. The pellet of the 40,000 X g centrifugation was resuspended in 5.0 ml of 0.8 M sucrose, then layered onto 7.0 ml of 1.2 M sucrose and centrifuged at 40,000 X G for 30 min. The material at the 0.8-1.2 M sucrose interface was collected and designated the B fraction. The pellet below the 1.2 M sucrose layer was designated the C fraction.

The nomenclature used here to designate fractions is identical to that used by Gray and Whittaker (1962). The identifiable cellular constituents which correspond to these letter designations are: P1-myelin, nuclei and undisrupted cell fragments. P2-myelin, glial cell membranes, synaptosomes and mitochondria. S2-microsomes and soluble cytoplasmic constituents. A-myelin. B-synaptosomes. C-mitochondria.

Platelet-Rich-Plasma Preparation

Rats were anesthetized with ether. The peritoneum was opened and 6.0 ml of blood was gently drawn from the aorta into a plastic syringe containing 0.6 ml of 3.8% sodium citrate. The blood and sodium citrate were mixed and then centrifuged for 5 min at 150 X G in plastic tubes. The platelet rich plasma was then decanted from the red blood cell pellet.

Kidney Slices

Rats were killed by cervical dislocation and the kidneys were removed. The kidneys were stripped of their capsule, rinsed and placed in an ice cold Krebs phosphate buffer. Kidney slices, less than 0.5 mm thick, were prepared from kidney cortex. The outermost slices were discarded and the remainder were transferred to an oxygenated Krebs-phosphate buffer for determination of taurine transport.

Amino Acid Transport in P2

In P2 and B fractions of SR, SS and NSP rat brains the temperature sensitive, sodium dependent, transport of taurine, glutamate and GABA was determined. Taurine uptake was quantified by incubation of approximately 0.5 mg P2 or B fraction protein per ml with 4.0 μ M [3 H] taurine in a Krebs-phosphate buffer at 37°C for 5 min. The incubations were terminated by the addition of 5 volumes of ice cold buffer or by centrifugation at 10,000 X G for 5 min.

The uptake of glutamate and GABA into P2 fractions of SS and SR rat brains was determined in an identical manner except that the incubations were carried out at 27°C and the concentration of substrate was

10 μM . Incubations with glutamate and GABA were terminated after 1 and 2 min, respectively. These are the conditions used by Roskoski (1978).

Taurine efflux from P2 fractions of SS and SR rats was determined by incubating the P2 fractions in a taurine-free buffer, that was otherwise identical to the buffer used for the uptake experiments. The change with time in concentration of endogenous taurine in the P2 fractions was determined.

Taurine Transport in Platelets

Taurine transport into platelets of SS and SR rats was determined by incubation of platelet-rich-plasma in a Krebs-Henseleit bicarbonate buffer with 10 μM [^3H]taurine. Incubations of platelet-rich-plasma, approximately 0.25 mg protein per ml were at performed 37°C for 60 min. The reactions were terminated and the platelets were collected by centrifugation at 10,000 X G for 5 min.

Taurine Transport in Kidney Slices

Cortical slices, 5-15 mg dry weight, from kidneys of SS and SR rats were incubated for 40 min at 37°C with 4 μM [^3H]taurine in 6.0 ml of a continuously oxygenated Krebs-phosphate buffer. The uptake was terminated by removal of the slices from the incubation medium. The slices were immediately placed on a gauze blotter for several seconds and then transferred to glass vials for drying. Slices were dried at 80°C for 6 h, weighed and then solubilized in Protosol prior to scintillation counting.

Taurine Uptake and Turnover In Vivo

In vivo uptake of taurine into brains of SR and SS rats was determined by measuring the amount of radioactivity in the brains 4 h after intraperitoneal administration of [³H]taurine (0.72 MBq/100 g body weight). Uptake was expressed as the ratio of radioactivity per gram brain tissue to the radioactivity per ml platelet free plasma.

The rates of taurine efflux from heart, brain, kidney and the entire animal were estimated after first equilibrating body stores of taurine with [³H]taurine. Equilibration was accomplished by placing the SS and SR rats on a taurine free diet and giving drinking water containing 5 mM [³H]taurine for two weeks (Huxtable and Lippincott, 1982). Groups of animals were killed 0, 3, 6 and 9 days after being returned to taurine free water and diet. The amount of radioactivity remaining in the organs was then determined after precipitation of the protein with 3.5% sulfosalicylic acid.

Taurine Biosynthesis

Taurine biosynthesis in homogenates of brain and liver of SS and SR rats was determined by incubation with 2 mM [³⁵S]taurine in 50 mM Tris-HCl buffer, pH 7.4. Reactions were terminated by addition of an equal volume of a saturated aqueous picric acid solution. [³⁵S] Taurine was separated from [³⁵S]cysteine and other sulfur metabolites of cysteine by dual-bed anion and cation exchange chromatography. The specificity of the method was checked by analyzing the collected radioactive fraction on a 15-cm column of Beckman AA 15 resin (Huxtable

and Bressler, 1976). Greater than 98% of the activity eluted at the retention time of authentic taurine.

Effect of Taurine on Glutamate Transport

Taurine, in concentrations ranging from 0 to 500 μ M was added to P2 fractions of SR rat brains being incubated with 10 μ M glutamate. The conditions of glutamate transport were identical to those previously described.

Effect of Taurine on the Subcellular Distribution of Glutamate

Taurine, 10 μ l of 0.5 M, was administered to SS and SR rats by ICV injection. The animals were killed 24 h later and the brains were fractionated as described above. Control animals received 10 μ l of 0.3 M NaCl.

Effect of Taurine on Glutamic Acid Decarboxylase In Vitro

Brains of SS and SR rats were homogenized in 0.2% Triton-X100. Aliquots of homogenate were incubated in a solution containing the following concentrations: [1- 14 C]glutamic acid 1 mM; potassium phosphate (pH 6.5) 100 mM; dithiothreitol 0.5 mM; pyridoxal phosphate 0.5 mM and, where appropriate, taurine 10 mM. The reaction volume was 1 ml. Reactions were stopped by the addition of 0.2 ml 3 M sulfuric acid. [14 C]Carbon dioxide released during the reaction was trapped in hyamine hydroxide, and this was subsequently counted to give an estimate of the decarboxylase activity.

Effect of Taurine on Glutamate Metabolism In Vivo

[1-¹⁴]Glutamic acid was administered by ICV injection to SS and SR rats in the presence or absence of an ICV injection of taurine. The amount of radioactivity remaining in the brain 15 min after injection of the glutamate was then determined. The duration of the effect of taurine on glutamate metabolism was estimated by administering the taurine 0, 0.25 and 24 h prior to the injection of the glutamate.

Effect of Prenatal Exposure to GES Upon Taurine Concentration, Development and Seizure Threshold.

Adult female Sprague-Dawley rats were obtained from the Division of Animal Resources at the University of Arizona and maintained one to a cage. Dams were placed on drinking water containing 1% GES for the duration of the experiment and allowed food ad lib. The rat diet was a custom formulated taurine free, casein based, diet obtained from Bioserve inc. Frenchtown, New Jersey (product # 0998).

The female rats were mated two weeks after being placed on 1% GES in drinking water and taurine-free diet. Upon birth of the pups, litter sizes were reduced to 10 pups. Litters containing less than six pups were terminated. Twenty one days after birth the pups were weaned onto diet and water identical to the dams from which they were weaned. Control dams and pups were maintained on the identical taurine free diet and untreated water.

Pups were killed by decapitation at birth and at regular intervals during development. Taurine and GES concentrations in the tested organs of the dams and pups were determined as previously described

(Huxtable and Lippincott, 1982). Immediately after suckling, pups were killed and the stomach contents were assayed for GES and taurine to give an estimate of the concentrations of these compounds in the milk.

Taurine transport and synthesis in tissue fractions of brains of GES treated neonatal rats was determined as previously described (Huxtable and Lippincott, 1981).

Chemoshock threshold was determined in 50 day old control and GES treated pups by intraperitoneal administration of pentylenetetrazol. To determine the convulsant dose (CD50) pentylenetetrazol was administered in doses which ranged from 0 to 50 mg/kg body weight. No animal was injected more than once. The CD50 and the 95% confidence limits of the CD50 were determined as described by Thakur and Fezio (1981).

RESULTS

I investigated the brains of SS rats for alterations in the concentration and metabolism of neuro-active amino acids which could account for the seizure-susceptibility. I also investigated the effects of taurine on the CNS handling of other neuro-active amino acids as a possible mechanism of anticonvulsant action.

SubCellular Distribution of Amino Acids In brains Of SS And SR rats

There was no difference between SS and SR rat brains in the sub-cellular distribution of glutamate, alanine, GABA or glycine. However, in the B fraction of brains of SS rats there was a decreased content of taurine (table 1). In a separate experiment, using rats of the seizure susceptible strain but which had never been audiogenically stimulated into having a seizure, taurine concentrations were found to be decreased in P2, B and C fractions (table 2). When the B fractions were examined for alterations in other amino acids only taurine and phosphoethanolamine were found to be different (table 3).

Amino Acid Transport In Vitro

The rate of glutamate uptake into P2 fractions was constant for 2 min (Figure 4) and was proportional to the protein concentration up to 0.24 mg/ml. Transport did not occur in incubations carried out in ice water baths. The rate of uptake of glutamate into P2 fractions from SR and SS rats was 2.23 ± 0.59 and 2.15 ± 0.77 nmole/mg/min

Table 1

Subcellular Distribution of Amino acids in SS and SR Rat Brains.

umole/Brain

Frac. strain	taurine	glutamate	glutamine	glycine	alanine	GABA
H.						
SR	11.57±1.55	21.55±1.55	1.13±0.33	2.67±0.63	0.90±0.08	5.52±0.49
SS	9.95±1.25	18.89±3.74	0.88±0.41	2.34±0.52	0.77±0.20	5.04±0.77
S1						
SR	8.42±1.18	17.69±0.39	4.92±0.55	1.90±0.31	0.83±0.06	3.75±0.19
SS	8.11±1.25	15.40±3.12	3.30±1.29	1.68±0.38	0.75±0.10	3.60±0.44
S2						
SR	6.59±1.54	10.09±2.72	3.18±1.67	1.52±0.48	0.55±0.10	3.10±0.31
SS	7.30±1.52	12.00±2.72	4.25±2.23	1.34±0.10	0.52±0.10	3.15±0.11
P1						
SR	3.42±0.08	4.82±0.75	0.12±0.02	0.75±0.19	0.21±0.06	1.34±0.13
SS	3.38±1.39	3.83±1.52	0.06±1.02	0.58±0.24	0.13±0.10	0.98±0.33
P2						
SR	1.44±0.30	2.29±0.20	0.05±0.01	0.25±0.03	0.13±0.02	0.70±0.12
SS	1.49±0.29	2.18±0.11	0.04 —	0.25±0.04	0.11±0.02	0.67±0.04
A						
SR	0.29±0.02	0.24±0.02	0.04±0.01	0.02±0.01	0.01 —	0.07±0.01
SS	0.34±0.07	0.28±0.03	0.04±0.02	0.03±0.01	0.01 —	0.07±0.01
B						
SR	0.19±0.03	0.26±0.04	0.01 —	0.01 —	0.01 —	0.08±0.02
SS	0.07±0.02*	0.18±0.04	-----	0.01 —	-----	0.05±0.01
C						
SR	0.02±0.01	0.05±0.01	0.01 —	0.01 —	-----	0.01 —
SS	0.03±0.02	0.05±0.02	0.01 —	0.01 —	-----	0.01 —

Values represent the mean and standard deviation for four animals per group. — Indicates that values were not determinable.

* Indicates a statistically significant difference (p<0.05).

Table 2

Subcellular Distribution of Taurine in brains of SR Rats
and Rats of SS Stock Which Had Never Had a Seizure.

nmole/mg protein

Fraction	SR	SS
P2	82.40 ± 8.61	57.91 ± 6.15 *
A	38.02 ± 7.16	35.10 ± 10.54
B	62.09 ± 4.87	43.81 ± 6.51 *
C	29.52 ± 3.57	18.65 ± 5.52 *

Values represent the mean and standard deviation for four animals per group. * Indicates a statistically significant difference (p<0.05) between SS and SR rats.

Table 3

Concentration of Amino Acids in the B Fraction of SR Rats and Rats of SS Stock Which Have Never Had a Seizure.

nmole/mg protein

amino acid	SR	SS	
PEA	10.77 ± 0.61	9.07 ± 0.34	*
TAU	50.64 ± 2.10	37.24 ± 2.58	*
ASP	38.23 ± 1.66	37.61 ± 2.12	
THR	2.46 ± 1.01	2.96 ± 0.54	
SER	7.57 ± 1.79	7.44 ± 1.11	
GLN	3.79 ± 1.67	3.93 ± 1.01	
GLU	70.10 ± 5.10	64.06 ± 5.98	
GLY	10.20 ± 1.47	9.19 ± 0.39	
ALA	9.02 ± 0.03	8.93 ± 1.24	
MET	1.84 ± 0.15	2.45 ± 0.68	
ILEU	1.22 ± 0.02	1.18 ± 0.34	
LEU	2.55 ± 2.04	4.50 ± 0.36	
PHA	3.95 ± 0.20	4.02 ± 0.19	
GABA	32.65 ± 1.20	33.77 ± 0.90	

Values represent the mean and standard deviation for three animals per group. * Indicates a statistically significant difference (p<0.05) between SR and SS rats.

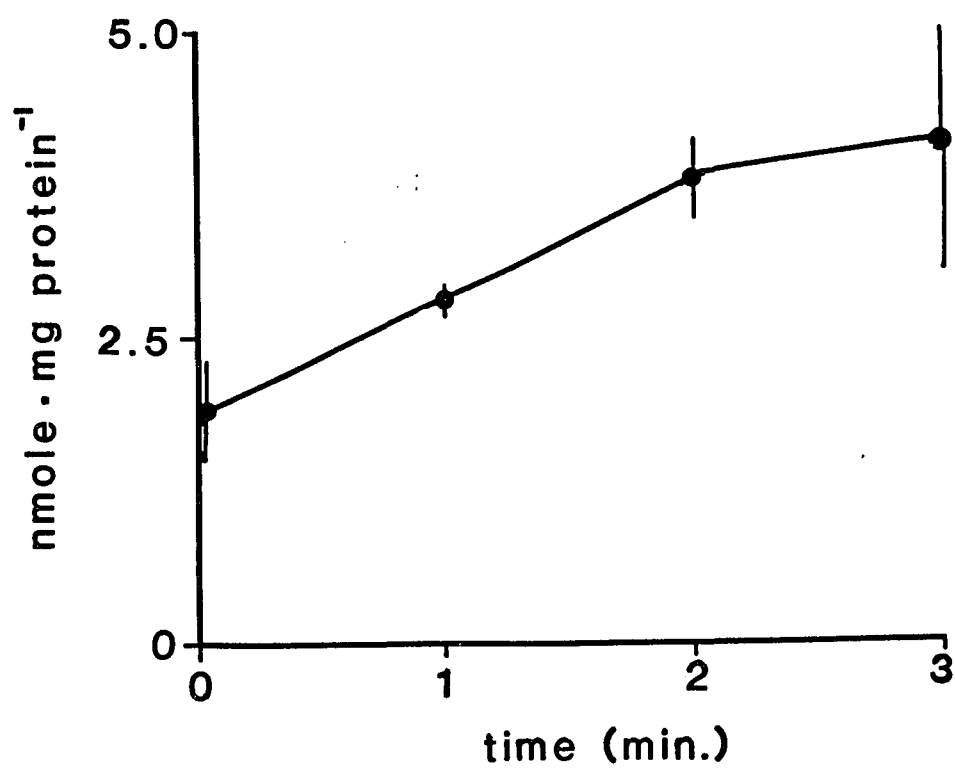


Figure 4. Glutamate uptake into P2 fractions from SR rats; linearity with time.

Values are the mean \pm standard deviation of the uptake into P2 fractions derived from 3 SR rats.

respectively. These values are the mean and standard deviation of 4 animals per group. There was no statistically significant difference ($P < 0.05$).

The rate of GABA uptake into P2 fractions of brain homogenates was constant for at least 3 min and was proportional to protein concentration up to at least 0.24 mg protein/ml. Uptake did not occur in incubations carried out in ice water baths (figure 5). There was no difference in the rate of uptake of GABA into P2 fractions of brains of SR, NSP or SS rats (figure 6).

The rate of taurine uptake into P2 fractions of brain homogenates was constant for at least 10 min and was proportional to protein concentrations up to 1.2 mg/ml. Transport did not occur in incubations carried out in ice water baths or in the absence of sodium ions (figure 7). Taurine uptake into P2 fractions of SS rats was 50% of that in SR rats. There was no statistical difference between rates of taurine transport in SR and NSP rats (figure 8).

Taurine transport into B fractions of brain homogenates was constant for 10 min and was proportional to protein concentration up to 11 mg/ml. There was no difference in the rate of taurine uptake into B fractions of SS and SR brains (Table 4).

During incubations, there was no detectable efflux of taurine from P2 fractions of SS and SR rats. At the beginning of the incubation, taurine concentrations in P2 fractions of SS and SR rats were 11.7 ± 1.8 and 16.0 ± 2.9 nmole per mg protein, respectively. After 10 min of incubation at 37°C, the concentration of endogenous taurine in

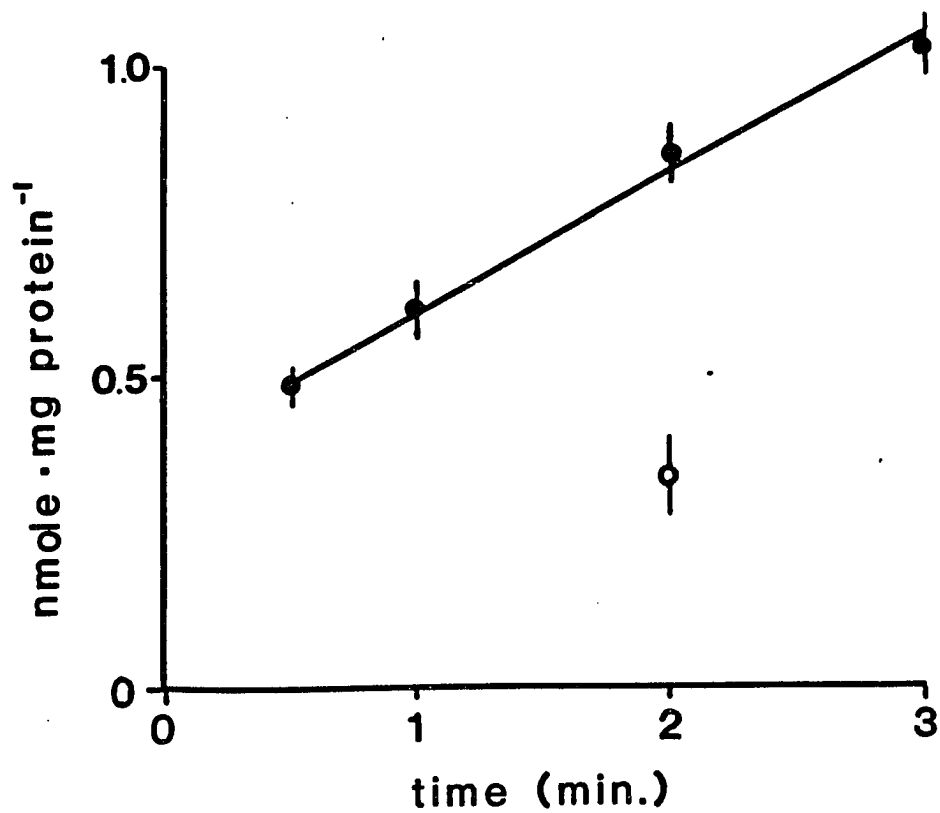


Figure 5. GABA uptake into P2 fractions of SR rat brains; linearity with time.

Values are the mean \pm standard deviation for 3 preparations at each time tested. ●-Uptake at 37°C.

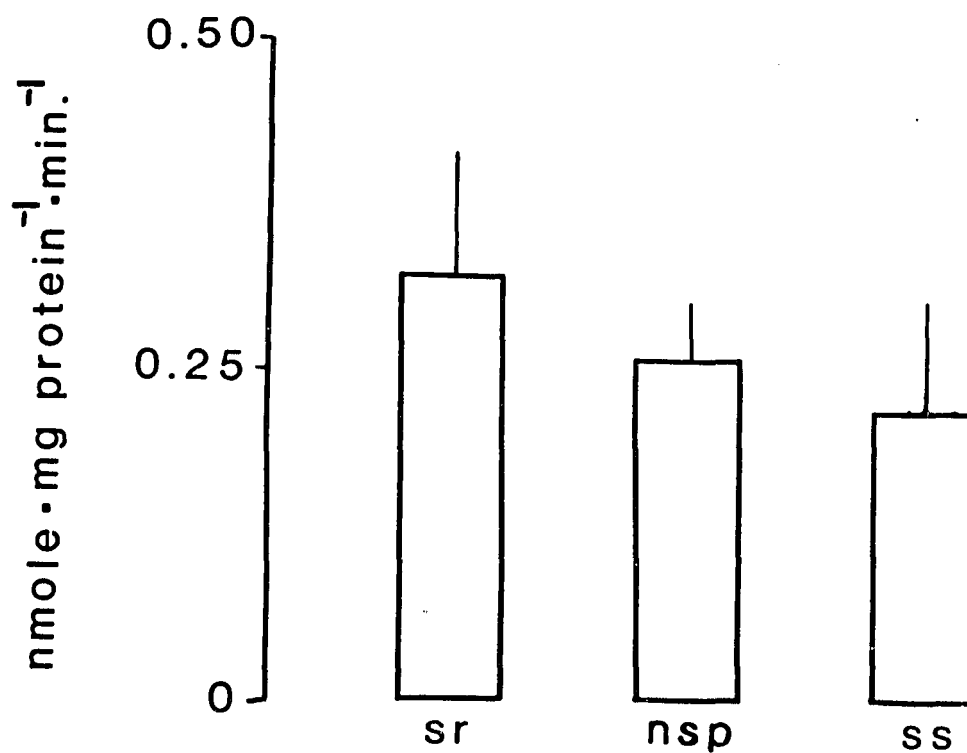


Figure 6. Uptake of GABA into P2 fractions of rat brains.

The net temperature-sensitive uptake of GABA in P2 fractions of SR, SS and NSP rats. Values are the mean \pm standard deviation of at least 4 animals in each group. Uptakes of GABA into P2 fractions of SR, NSP and SS rats were 0.32 ± 0.10 , 0.26 ± 0.04 and 0.22 ± 0.09 pmole/mg protein/min, respectively. There were no statistically significant differences between groups.

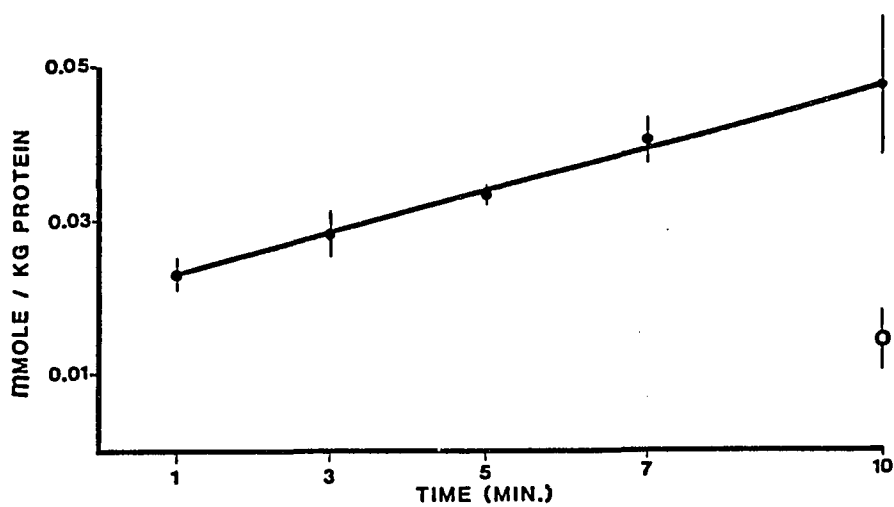


Figure 7. Taurine uptake into P2 fractions of SR rat brains; linearity with time.

Values represent the mean \pm standard deviation of 3 determinations at each time tested. ●-uptake at 37°C; ○-uptake in an ice water bath.

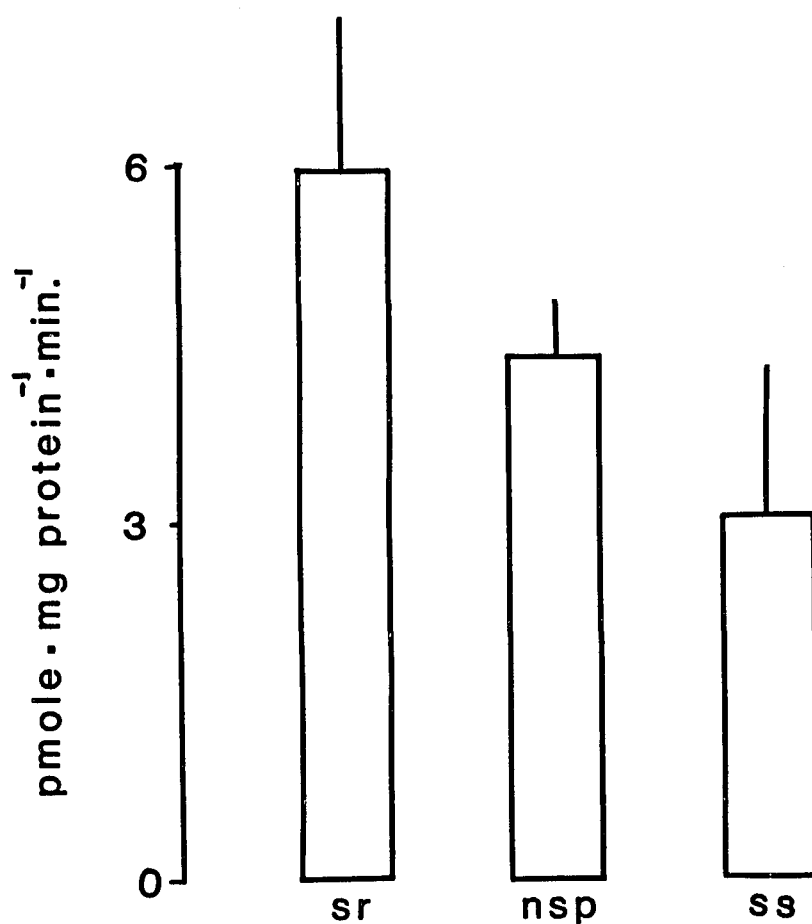


Figure 8. Uptake of taurine into P2 fractions of rat brains.

The net temperature-sensitive uptake of taurine into P2 fractions of SR, SS and NSP rats. Values are shown as mean \pm standard deviation for at least 4 animals in each group. Rates of uptake into SR, SS and NSP rat P2 fractions were 5.99 ± 2.10 , 3.05 ± 1.50 and 4.36 ± 0.39 pmole/mg protein/min, respectively. Uptake in SS rat P2 fractions was statistically less than that of SR rats ($P < 0.05$), (Dunnet, 1955).

Table 4

Taurine Transport in Brain Tissue
Preparations of SS and SR Rats.

nmole/mg protein/h

Fraction	SR	SS	
P2	0.39 ± 0.10	0.13 ± 0.05	*
B	1.36 ± 0.36	1.22 ± 0.46	

Values are the mean and standard deviation for at least 5 animals per group. P2 Contains mitochondria, synaptosomes and glial membranes. B Fraction is a synaptosome enriched fraction derived from the P2 fraction. * Indicates a statistically significant difference ($p < 0.05$) between SS and SR rats.

the P2 fractions of SR and SS rat brains was 14.0 ± 2.9 and 15.6 ± 3.4 nmole per mg protein, respectively (figure 9).

Taurine Transport into Kidney Slices

The rate of taurine uptake into kidney cortex slices was constant for at least 40 min, and uptake did not occur in incubations carried out in ice water baths (Figure 10). The rate of taurine uptake into kidney slices from SS rats was 115% of that in slices from SR rat kidneys (Figure 11).

Taurine Transport into platelets

The rate of taurine transport into platelets was constant for at least 75 min and was proportional to protein concentration up to 0.7 mg/ml. Transport did not occur in the absence of sodium ions or in incubations carried out in ice water baths (figure 12). The rate of taurine uptake into platelets of SS rats was 75% of the rate in platelets of SR rats (figure 13).

Taurine transport In Vivo

The in vivo uptake of [^3H]taurine into brains of SS rats was less than that in SR rats. Regional differences in the uptake of taurine into brains of SS and SR rats are shown (table 5).

The rate of loss of [^3H]taurine from organs of SS and SR rats equilibrated with [^3H] taurine was determined (figure 14) . In brains of SR and SS rats the calculated half-lives for the net elimination of radioactivity were 3.1 and 4.1 days, respectively. These values were not statistically different. There also was no difference in the rate

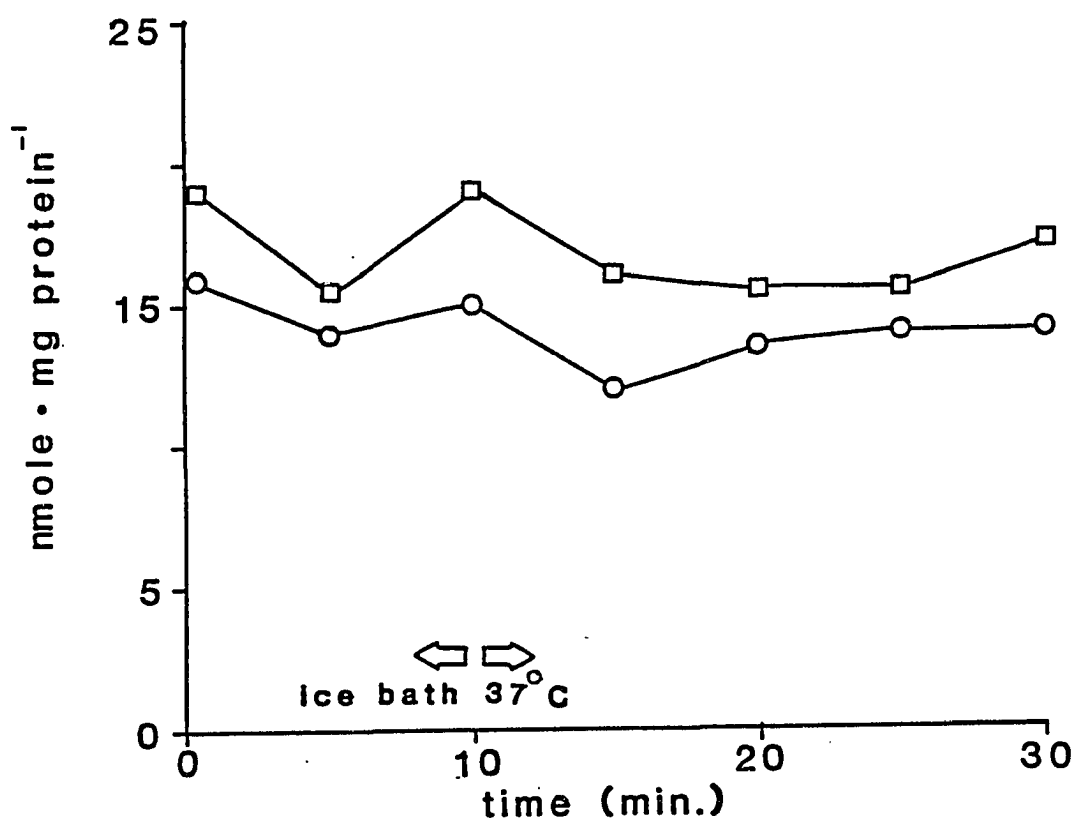


Figure 9. Efflux of taurine from P2 fractions of rat brains.

Values are the mean \pm standard deviation of three determinations at each point. Time 0 represents the moment of resolubilization of the P2 pellet in buffer. O-SR rats; \square -SS rats. There was no difference between SS and SR rats at any point tested. There was no difference between any value and the respective time 0.

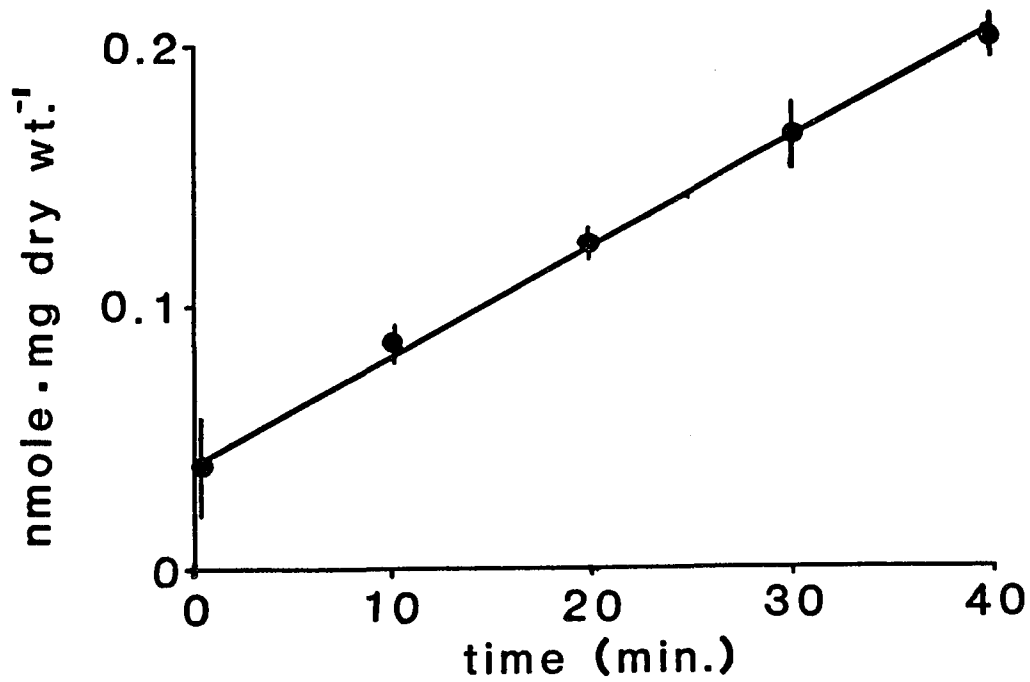


Figure 10. Taurine uptake into kidney slices of SR rats; linearity with time.

Each value represents the mean \pm standard deviation of 3 determinations at each time.

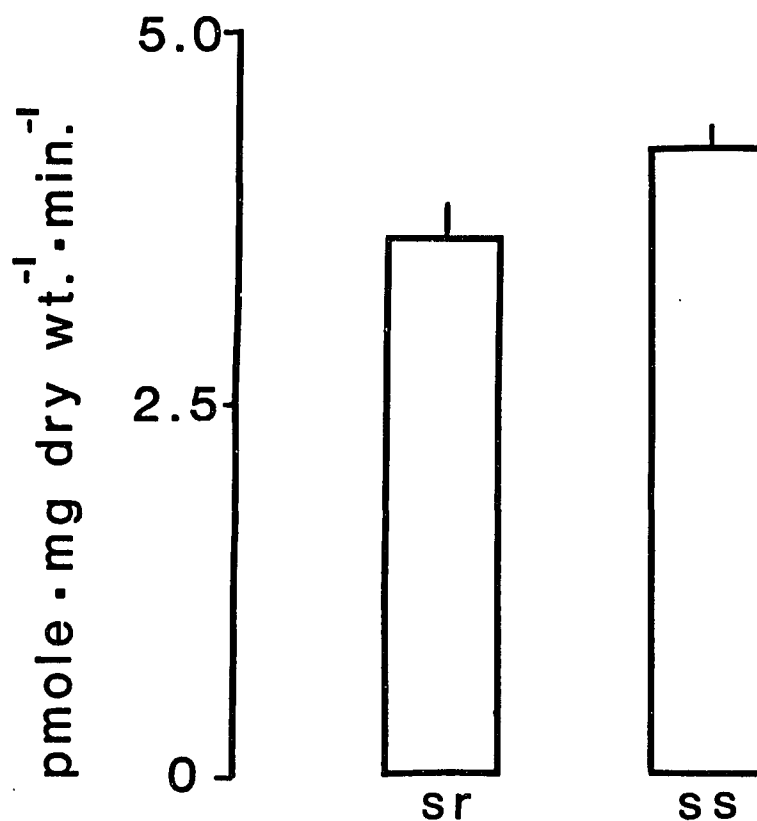


Figure 11. Taurine uptake into kidney slices of SS and SR rats.

Net temperature sensitive uptake of taurine into kidney slices of SR and SS rats. Values represent the mean \pm the standard deviation of at least 5 animals per group. Uptakes in SS and SR rat kidney slices were 4.19 ± 0.21 and 3.66 ± 0.34 pmole/mg protein/min, respectively. the difference was statistically significant ($p < 0.05$).

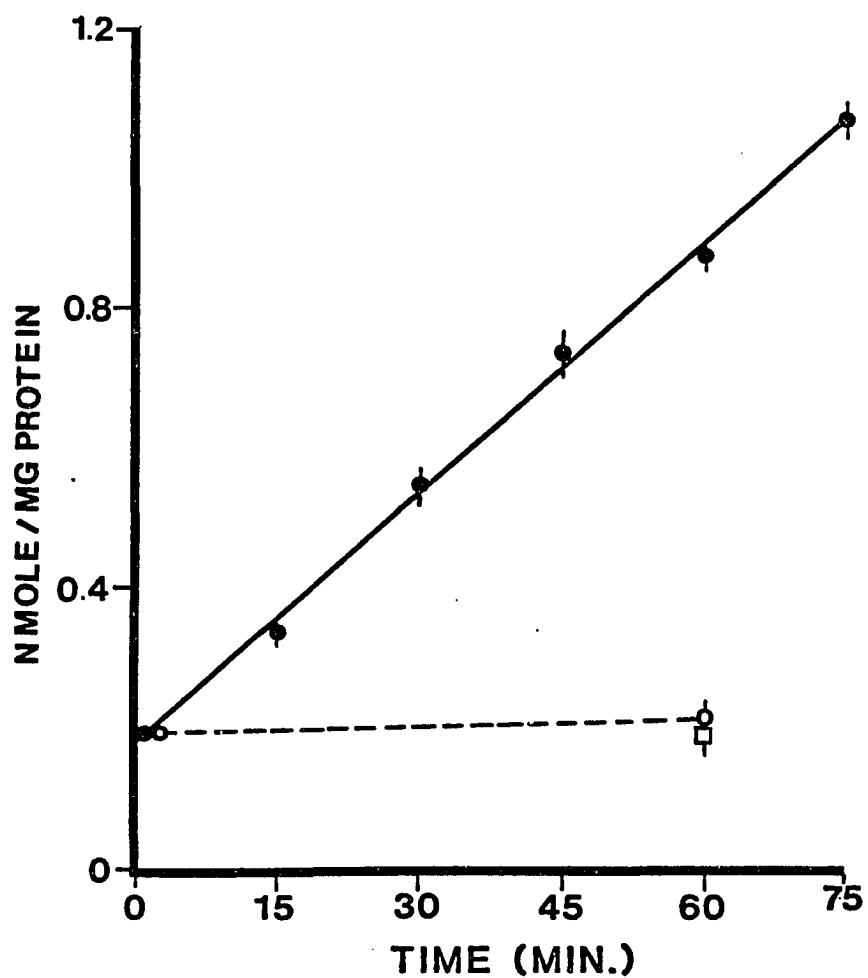


Figure 12. Taurine uptake into platelets of SR rats; linearity with time.

Values are the mean \pm standard deviation of at least 3 determinations at each point tested. ●-Uptake at 37°C; ○-uptake at 37°C in the absence of sodium ions; ◻-uptake in incubations carried out in ice water baths.

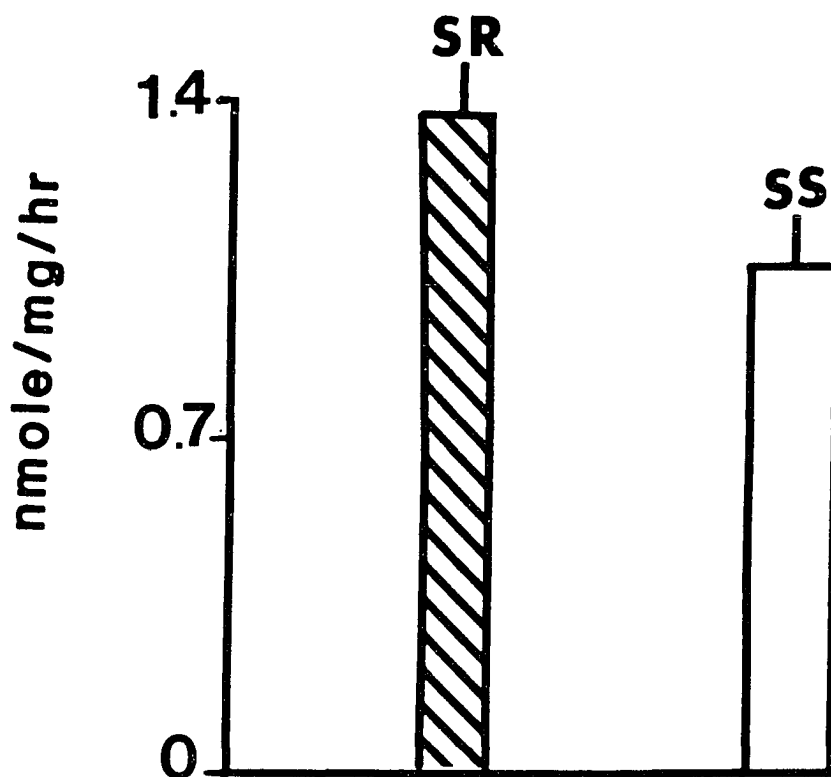


Figure 13. Taurine uptake into platelets of SS and SR rats.

The rate of taurine uptake into platelets of SR and SS rats was 1.38 ± 0.05 and 1.04 ± 0.09 nmole/mg protein/h respectively. Values are the mean and standard deviation of 5 animals per group. *The difference was statistically significant ($p < 0.05$).

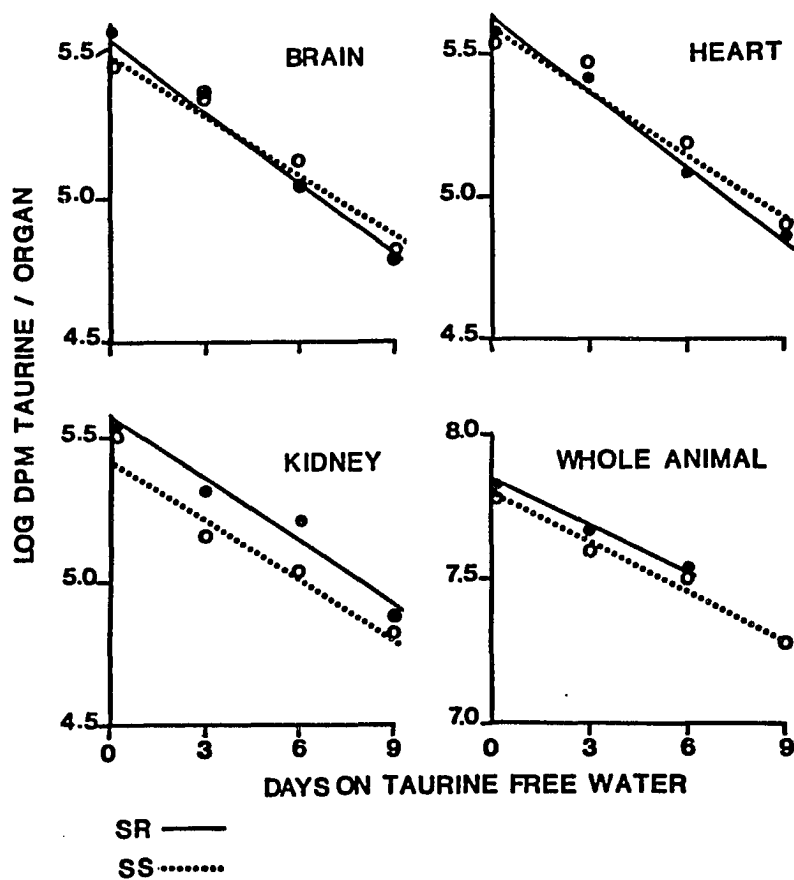


Figure 14. Taurine efflux from organs of SR and SS rats.

Brain content of $[^3\text{H}]$ taurine. Day zero represents the day the animals were placed on taurine-free drinking water. Values are the mean of the log of the total dpm per brain. 0-SS brains; ●-SR brains. There was no difference in the rate of net elimination of $[^3\text{H}]$ taurine between groups.

Table 5

Transport of Taurine Into the Central Nervous System
in SS and SR Rats.

Brain area	SR	SS	% Control
Ventral Cochlear Nuc.	3.64 ± 0.87	4.29 ± 2.64	118
Hypothalamus	5.66 ± 3.67	5.36 ± 1.73	95
Midbrain	3.74 ± 1.75	2.61 ± 0.65	70
Cerebellum	4.32 ± 0.19	2.97 ± 0.88	69 *
Medial Cort.	2.87 ± 0.65	1.92 ± 0.69	67
spinal Cord	5.49 ± 5.30	3.57 ± 1.74	65
Frontal Cort.	4.24 ± 0.85	2.60 ± 0.13	61 *
Reticular Form.	3.86 ± 0.36	2.20 ± 1.10	57 *
Inferior Collic.	7.70 ± 1.46	3.00 ± 0.59	29 *
Anterior Cort.	10.34 ± 6.86	2.58 ± 1.21	25

Values are the mean and standard deviation for 3 animals per group. Values expressed are the ratio of dpm/g wet weight of tissue to dpm/ml plasma. * Indicates a statistically significant difference (p<0.05) between SS and SR rats.

of elimination of taurine from any other organ tested or the entire carcass of SS and SR rats (Table 6).

Taurine Transport in Neonatal Rats

Both the in vivo uptake and the in vitro net temperature sensitive transport of taurine in P2 fractions of neonatal (14-18 day old) SS rats were less than that in the age matched SR rats (Figure 15).

Taurine Biosynthesis

The rate of taurine biosynthesis from cysteine was constant for at least 15 min in brain homogenates and 30 min in liver homogenates (figure 16). The rate of biosynthesis was proportional to protein concentration up to at least 35 mg/ml. There was no measurable biosynthesis in boiled homogenates or in incubations carried out in ice water baths. There was no difference between SR and SS rats in the rate of taurine biosynthesis in whole brain or liver preparations (table 7). In a separate experiment it was found that, with the exception of the cerebral cortex, there were also no differences between SS and SR rats in the rate of biosynthesis of taurine in homogenates derived from different regions of the brain (table 8).

Effect of GES on Neonatal Rat Development

Pretreatment of dams with GES had no effect on the number of dams delivering litters, litter size or pup weight at birth. Of the 18 females in each group 13 of the controls and 12 of the GES treated dams delivered litters. Litter size at birth in the control and GES groups

Table 6

Half-life of Elimination of [^3H]Taurine From Rat Tissues.

Organ	<u>SR</u>		<u>SS</u>	
	correlation coefficient	half-life (days)	correlation coefficient	half-life (days)
Brain	0.91	3.1	0.98	4.1
Heart	0.98	3.5	0.84	3.3
Kidney	0.97	4.4	0.88	5.3
Carcass	0.97	7.0	0.95	6.1
Whole animal	0.97	6.8	0.95	6.1

These data were obtained from plots such as that in Figure 15. None of the pairs of half-lives differ significantly.

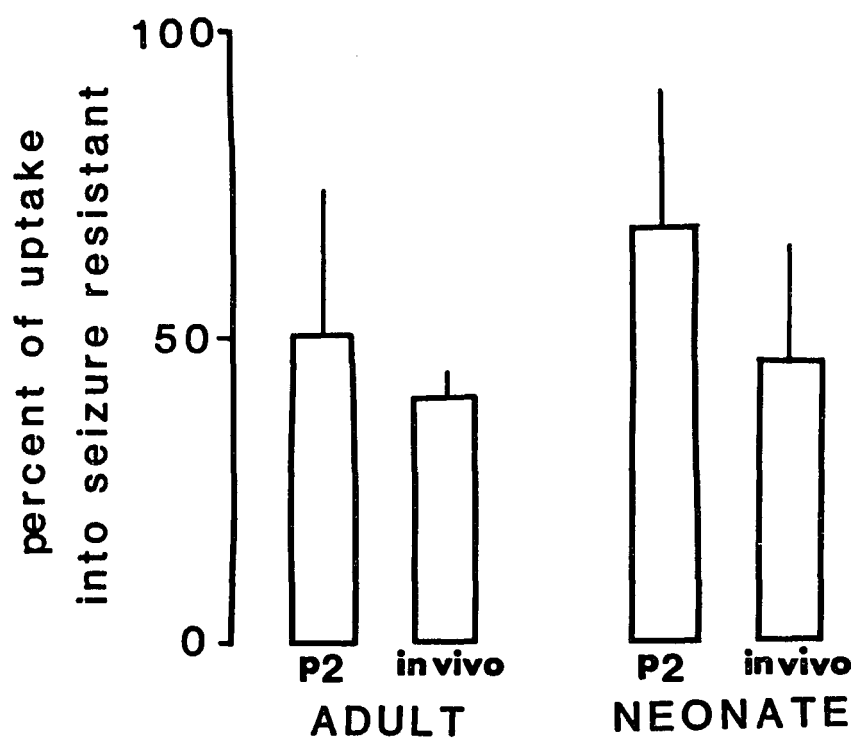


Figure 15. Taurine transport in neonatal rats.

Taurine uptake into P2 fractions and whole brains of adult and neonate SS rats expressed as a percent of uptake into SR rats. Values are the mean \pm standard deviation of the difference between SR and SS rats for at least four animals in each group. In all cases the uptake into SS rats was less than that in SR rats ($p < 0.05$).

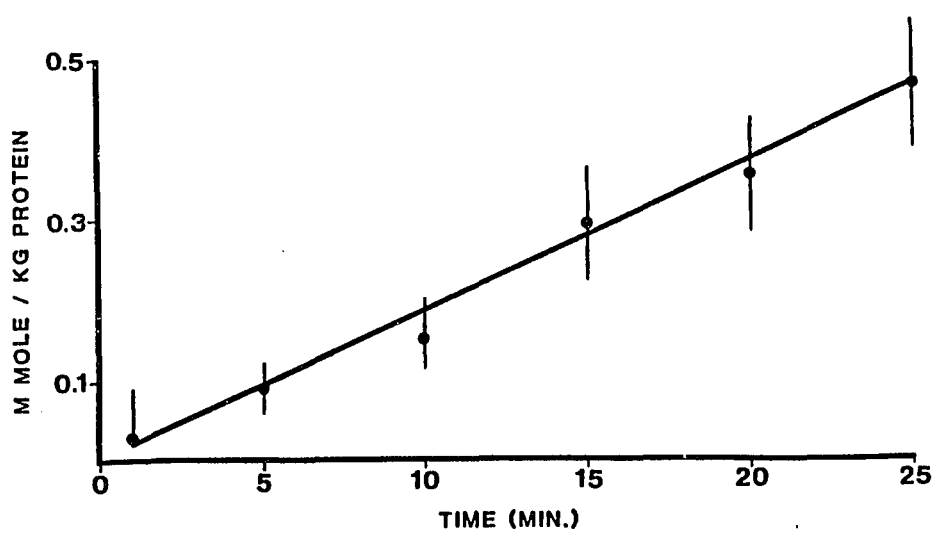


Figure 16. Taurine biosynthesis in rat brain; linearity with time.

Taurine biosynthesis in homogenates of SR rat brains. Values are standard deviation of three determinations at each point.

Table 7

Taurine Biosynthesis in Brain and Liver
Homogenates From SS and SR Rats.

nmole/mg protein/h

Organ	SS	SR
Brain	0.98 ± 0.19	0.78 ± 0.19
Liver	3.42 ± 1.10	4.59 ± 1.13

Values are the mean and standard deviation for at least 4 animal per group. There was no difference in the rates of synthesis between preparations from SS and SR rats.

Table 8

Taurine Biosynthesis in SS and SR Rat Brain Homogenates

nmole/mg protein/h

Brain Area	SR	SS
Cerebral Hem.	1.07 ± 0.26	1.43 ± 0.09 *
Cerebellum	0.92 ± 0.31	1.34 ± 0.31
Pons Medulla	1.43 ± 0.31	1.15 ± 0.24
Midbrain	0.85 ± 0.31	0.68 ± 0.10
Inferior Col.	1.72 ± 1.16	1.03 ± 0.08

Values are the mean and standard deviation for 3 animals per group.
* Indicates a significant difference ($p > 0.05$) between preparations from SS and SR rats.

was 10 ± 4 and 9 ± 6 pups, respectively. Birth weights and organ to body weight ratios of GES and control pups were not different (table 9).

Effect of GES on Taurine Concentration in the Neonatal Rat

At birth all organs of GES-treated pups, with the exception of the lung, were depleted of taurine. However, taurine concentrations in brain, heart, liver and kidney of 5 day old pups exposed to GES were not different from those of respective organs of control pups. With continued exposure to GES, all organs tested were depleted of taurine by 25 days of age. However, concentrations of taurine in the brains of 25 day old GES-treated pups were still 80% of control values. At 40 days of age, taurine concentrations in the brains of the GES treated pups were reduced to 60% of those in the control rats (table 10).

GES Concentrations in Milk and Organs of Pups Exposed to GES

GES was taken up into prenatal rats. GES levels in organs of one day old pups from dams treated with GES ranged from 4.4 $\mu\text{mole/g}$ wet weight in the liver to 11.4 $\mu\text{mole/g}$ wet weight in the brain. GES concentrations decreased to approximately one half of those values at birth by 5 days of age and then slowly increased (table 11).

Milk from dams on GES contained GES and was depleted of taurine to approximately one half the concentration found in the milk of control dams (table 12).

Table 9

Body Weight and Organ to Body Weight Ratios of
one day old rat pups control and prenatally exposed to GES.

(mg/g)

Organ	Control	GES-treated
Brain	35.2 ± 5.7	36.9 ± 4.7
Eyes	5.6 ± 0.7	5.7 ± 0.6
Heart	5.2 ± 0.7	5.2 ± 0.6
Kidneys	11.5 ± 2.1	10.2 ± 0.7
Liver	29.5 ± 3.9	38.6 ± 6.2
Lung	23.9 ± 4.9	22.9 ± 3.8
Spleen	2.2 ± 0.6	1.8 ± 0.4
Body weight (g)	6.8 ± 0.5	7.5 ± 0.8

There was no difference between SS and SR rats in body weight or organ to body weight ratio for any for any organ tested. There were three animals per group.

Table 10

Taurine Concentrations in Control Pups and Pups Exposed to GES.

umole/g

	At Birth		5 Days of AGE	
	Con.	GES	Con	GES
Brain	18.2 ± 1.6	11.8 ± 2.0 *	11.4 ± 0.5	11.5 ± 1.3
Eye	9.0 ± 0.8	5.3 ± 0.4 *	-----	-----
Heart	16.9 ± 1.7	10.1 ± 0.9 *	10.4 ± 0.8	10.5 ± 0.2
Kidney	10.6 ± 0.6	6.6 ± 0.9 *	6.9 ± 0.8	6.3 ± 0.3
Liver	7.2 ± 2.9	4.4 ± 0.6 *	7.0 ± 0.5	6.1 ± 0.3
Lung	7.3 ± 2.2	6.1 ± 0.8	10.9 ± 0.3	7.0 ± 0.5 *
Carcass	8.1 ± 0.6	5.3 ± 0.2 *	5.6 ± 0.3	4.1 ± 0.4 *

	25 Days of Age		40 Days of Age	
	Con.	GES	Con.	GES
Brain	6.9 ± 0.4	5.6 ± 0.3	6.4 ± 0.3	3.7 ± 0.1 *
Heart	18.9 ± 1.6	9.0 ± 0.8 *	20.2 ± 0.4	4.4 ± 0.9 *
Kidney	6.5 ± 0.3	3.0 ± 0.4 *	7.9 ± 0.4	2.7 ± 0.3 *
Liver	8.1 ± 2.3	2.5 ± 1.6 *	2.5 ± 0.8	0.5 ± 0.1 *
Lung	9.5 ± 0.6	4.4 ± 1.2 *	9.6 ± 2.4	2.9 ± 0.6 *
Carcas	7.5 ± 1.0	3.7 ± 0.3 *	7.9 ± 0.4	1.9 ± 0.3 *

Values are the mean and standard deviation for 4 animals per group. * Indicates a statistically significant difference ($p < 0.05$) between concentrations in control and GES treated pups.

Table 11

GES Concentrations in Pups Treated with GES.

umole/g

Organ	Days of AGE			
	0	5	25	40
Brain	2.1 ± 0.2	0.5 ± 0.2	1.0 ± 0.8	1.4 ± 0.1
Heart	6.4 ± 0.1	3.2 ± 1.9	15.5 ± 1.3	15.4 ± 1.2
Kidneys	3.2 ± 0.2	1.5 ± 0.8	7.1 ± 0.6	8.1 ± 0.4
Liver	4.6 ± 0.4	4.3 ± 2.0	17.3 ± 1.6	17.4 ± 1.6
Lung	2.5 ± 0.1	1.6 ± 1.0	6.0 ± 0.5	9.7 ± 0.5
Carcass	4.3 ± 0.3	2.3 ± 1.5	8.5 ± 0.3	9.6 ± 0.5

Values are the mean and standard deviation for 4 animals in each group.

Table 12
GES and Taurine Concentrations in
Milk from Control Dams and Dams Treated With GES.

umole/g

Treatment	Taurine	GES
Control	0.86 ± 0.03	0.06 ± 0.03
GES	0.39 ± 0.19 *	0.49 ± 0.16 *

The values represent the mean and standard deviation for 3 animals per group. * Indicates a statistically significant difference (p<0.05) between control and GES treated dams milk.

Effect of GES on Taurine Transport and Biosynthetic Capability

Pre- and postnatal exposure to GES did not alter the high affinity P2 transport of taurine in brain preparations of one or 45 day old rats (table 13).

Chronic exposure to GES did not affect the ability of the brain or liver to biosynthesize taurine (table 14).

When GES was added to incubations of taurine in P2 fractions of 5 day old rat brains, the GES did inhibit taurine transport (figure 17).

Effect of GES on Chemoshock Threshold

The pentylenetetrazol chemoshock threshold was determined in 50 day old control and GES-treated pups. The convulsant dose (CD50) was found to be 39 ± 3 and 46 ± 1 mg/kg in control and GES treated pups respectively. The difference was statistically significant ($p < 0.05$) (figure 18).

Effect of ICV Taurine on Amino Acid Concentration and Distribution

Injection of an anticonvulsant dose of taurine (6.0 umole), ICV, had no effect on concentration or subcellular distribution of glutamate, glycine or GABA (table 15).

Effect of ICV Taurine on Glutamate Metabolism

During the first 30 min after IVC injection of [^{14}C]glutamate, the rate of loss of radioactivity was approximately linear (figure 19).

Taurine increased the rate of removal of radioactivity from brains of SS and SR rats following ICV injection of [^{14}C]glutamate only if the taurine was injected concomitantly with the glutamate or

Table 13

Taurine uptake into P2 fractions from pups pre- and postnatally exposed to GES

pmole/mg protein/ min

Treatment	1 day old pups	45 day old pups
Control	12.9 ± 1.9	9.9 ± 0.7
GES	14.2 ± 3.4	9.8 ± 0.6

Values represent the mean and standard deviation of 4 animals per group at one day of age and 3 animals per group at 45 days of age. There was no statistically significant difference in the rate of transport between control and GES treated pups.

Table 14

Taurine Biosynthesis in Brain and Liver Homogenates
of Control and GES Treated 45 Day old Rats.

pmole/mg protein/min

Treatment	Brain	Liver
Control	15.0 ± 1.2	20.5 ± 3.7
GES	13.2 ± 1.2	21.3 ± 0.8

Values represent the mean and standard deviation of 4 animals per group. There was no statistically significant difference between the respective organs of control and GES treated pups in the rate of taurine synthesis.

Table 15

Amino Acid Content in Brain fractions Derived From Control SS Rats
and SS Rats Treated With Taurine.

nmole/mg protein

Fraction	TAU	GLU	GLN	GABA	GLY
Treatment					
H Con	45.8 ± 5.5	81.9 ± 9.2	16.6 ± 2.0	13.6 ± 0.6	37.5 ± 2.9
H Tau	69.2 ± 3.4*	82.1 ± 6.9	16.1 ± 2.0	12.5 ± 1.7	35.3 ± 1.7
P2 Con	24.7 ± 4.7	26.7 ± 8.5	5.8 ± 1.0	5.1 ± 1.2	16.7 ± 4.6
P2 Tau	31.2 ± 1.5*	29.3 ± 3.3	6.8 ± 1.1	5.4 ± 1.1	17.6 ± 2.0
B Con	36.8 ± 11.3				
B Tau	39.4 ± 15.1				

Values represent the mean and standard deviation of 3 animals in the control group and 4 animals in the taurine treated group. * Indicates a significant difference ($p < 0.05$) between control and taurine treated groups.

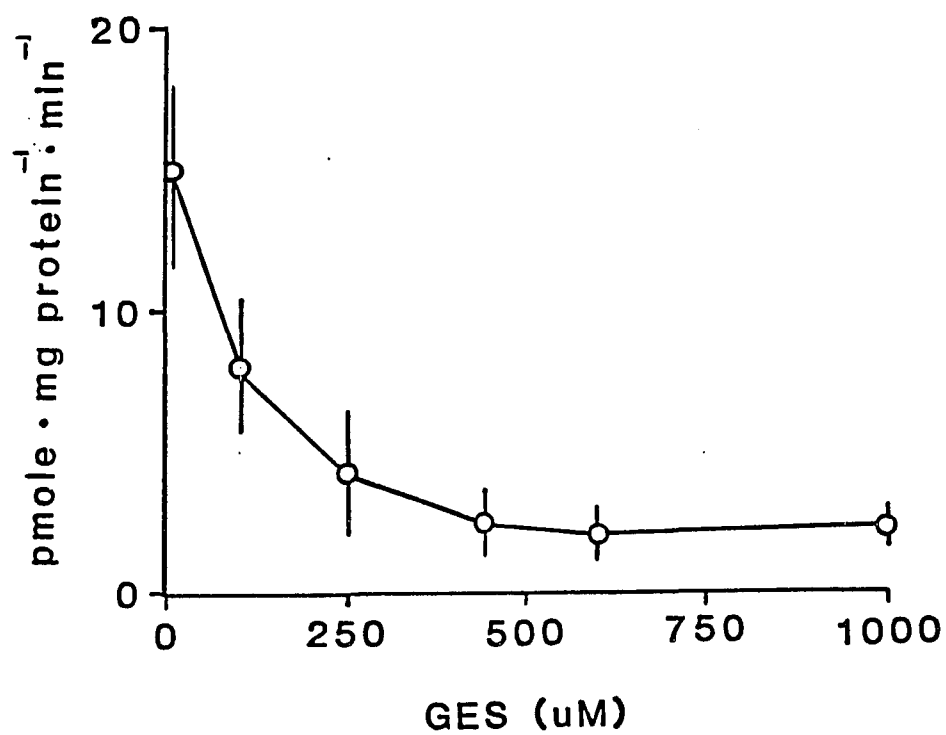


Figure 17. Effect of GES on taurine transport into P2 fractions.

Values are the mean and standard deviation of the rate of taurine transport into P2 fractions derived from the brains of 5 day old rats. N=4.

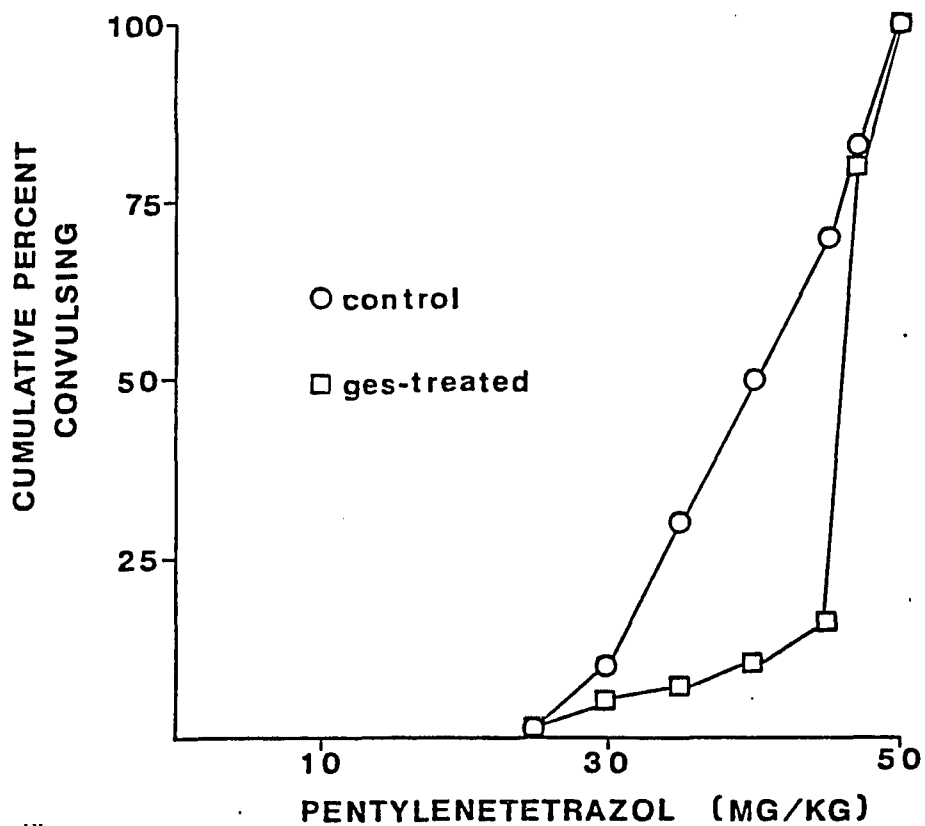


Figure 18. Effect of GES on seizure threshold.

Values represent the cumulative fraction of control (O) and GES-treated (□) rats which had had a seizure at the given dose of PTZ. (Reed-Muench, 1938). The CD50 of control and GES-treated rats was 39.4 ± 2.9 and 46.0 ± 1.3 respectively. The difference was statistically significant ($p < 0.05$) (Pizzi, 1950).

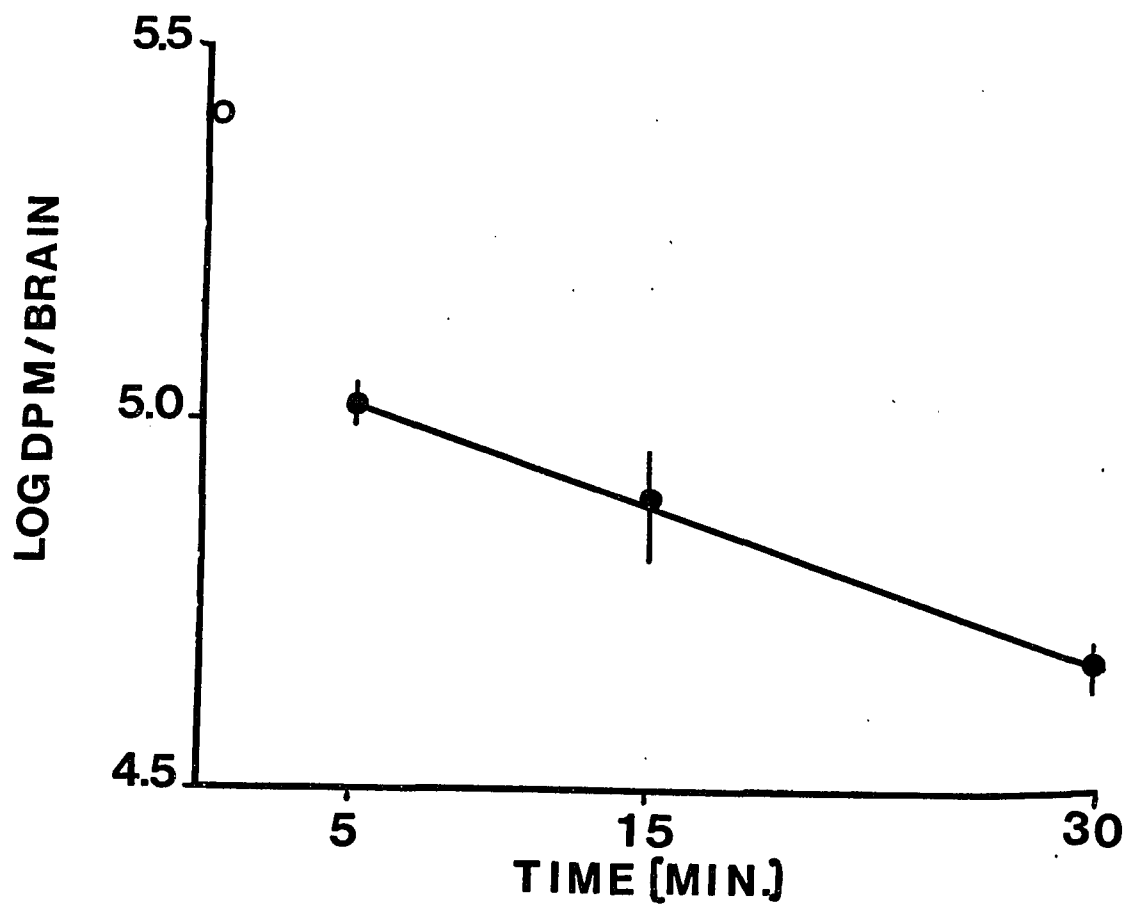


Figure 19. Elimination of [1-¹⁴C]glutamate from the brain.

The elimination of [1-¹⁴C]glutamate from the brain after ICV injection; relationship with time. ○-Value calculated for the time of injection; ●-experimentally determined values.

15 min prior to the injection of the glutamate in the case of the SS rats. When the taurine was administered 24 h prior to administration of the glutamate, it had no effect on the rate of removal of radioactivity (Table 16).

Effect of Taurine on Glutamate transport in P2 Fractions

Under the conditions of glutamate transport previously described, taurine at concentrations up to 500 μ M had no effect on the rate of glutamate transport (figure 20).

Effect of Taurine on Glutamate Metabolism In Vitro

There was no difference in GAD activity between SS and SR rats, measured either in the presence or the absence of exogenous pyridoxal phosphate. However, addition of 10 mM taurine to incubations of brain homogenates derived from SS rats, carried out in the presence of exogenous pyridoxal phosphate, resulted in a 20% increase in the rate of glutamate decarboxylation. Taurine had no effect on the rate of decarboxylation in homogenates derived from SR rat brains (table 17).

Table 16

Effect of Taurine on Glutamate
Metabolism in Brain

% Of Injected Dose of Glutamate
Remaining in the Brain

Treatment	SR	SS
Control (10 ul 0.3 M NaCl).	33.3 ± 2.4 (25)	41.4 ± 3.9 (7)
Taurine concomitant with the glutamate.	21.1 ± 1.5 (24) *	25.3 ± 2.8 (4) *
Taurine 15 min prior to the glutamate.	29.0 ± 3.1 (4)	26.3 ± 1.0 (3) *
taurine 24 h prior to the glutamate.	-----	39.7 ± 1.7 (3)

Values are the mean and standard error. The number of animals per group is given in parentheses. * Indicates a statistically significant difference from the respective control value ($p < 0.05$), (Dunnets t test), (Dunnet, 1955). There was no difference between the control groups of the two strains in the rate of loss of radioactivity from the brain.

Table 17

Decarboxylation of Glutamate in Brain Homogenates
From Brains of SR and SS Rats.

nmole/mg protein/h

Strain	No Taurine Added	+ 10 mM Taurine
SR	111 ± 23	96 ± 19
SS	99 ± 23	133 ± 28 *

Values are the mean and standard deviation for at least four animals per group. * Indicates a statistically significant difference ($p < 0.05$) from both the rate of decarboxylation in the SR rat in the presence of taurine and from the rate of decarboxylation in the SS rat in the absence of taurine.

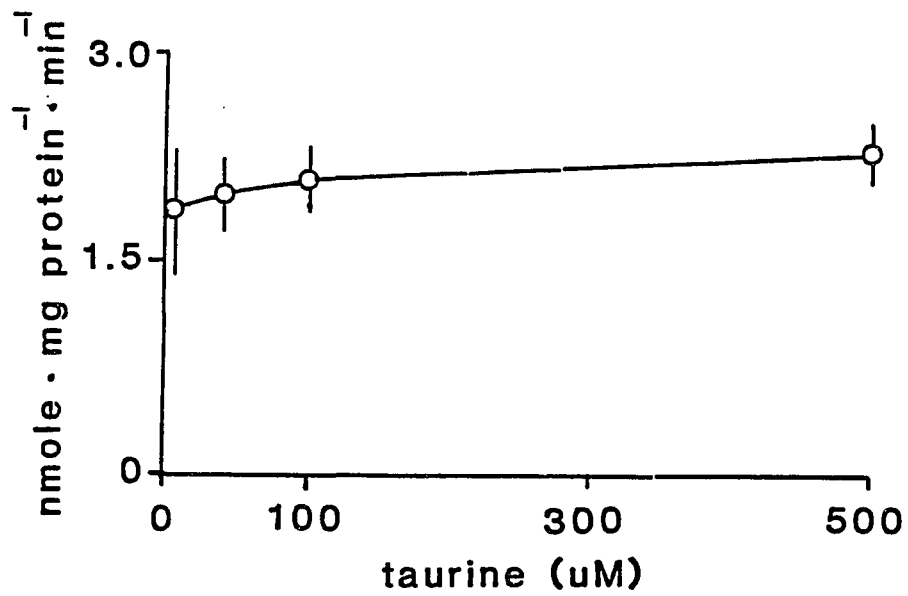


Figure 20. Effect of taurine on glutamate transport in P2 fractions.

The effect of taurine upon glutamate uptake into P2 fractions of SR rats was determined. Values are the mean \pm standard deviation of at least 3 determinations at each dose tested. At no dose of taurine was there any alteration in glutamate transport.

DISCUSSION

There must be alterations in the brains of SS rats that correlate with their seizure-susceptibility. I investigated a number of aspects of amino acid metabolism in brains of SS rats for alterations which could account for the seizure-susceptibility. I also investigated the effects of taurine on the CNS handling of amino-acids as a possible mechanism of anticonvulsant action.

Amino Acid Distribution in SS and SR Rat Brains

Various and conflicting alterations in the concentration or distribution of neuro-active amino acids have frequently been reported with seizure disorders (Van Gelder and Courtois, 1972; Emson, 1978; Huxtable, 1981; Bakay and Harris, 1981; Perry and Hanson, 1981; Van Gelder, 1982; Huxtable et al., 1983). Some investigators have suggested that these alterations are an underlying basis of seizure-susceptibility. However, alterations in gross tissue concentrations of amino acids are not essential for seizure-susceptibility. Huxtable and Laird (1978b) have shown that there is no measurable difference in the concentrations of free amino acids in brains of SS rats when compared to SR rats. At that time it was also shown that there were no alterations in the ratios of concentrations of these amino acids.

I report here that there is also no measurable difference between SS and SR rats in the cellular or subcellular distribution of the

neuro-active amino acids glutamate, glutamine, GABA, alanine or glycine. However, in the B or synaptic fraction of brains of SS rats there was a decreased amount of taurine (table 1). This decreased taurine content was also found in rats of seizure-susceptible stock which had never been stimulated into having a seizure. In these animals the decrease was found in the B and C fractions of the P2 pellet (table 2). When the B fraction was analyzed for other amino acids it was found that only taurine and phosphoethanolamine were altered (table 3). However, phosphoethanolamine eluted at a time near that of taurine and there was some overlap of the eluting peaks, so it was possible that the decrease in phosphoethanolamine was actually reflecting the altered taurine content. The converse proposition is not likely as the taurine content was much greater than the phosphoethanolamine content. From these experiments it can be concluded that in epileptic animals where alterations in the concentration of neuroactive amino acids may not be detectable upon analysis of whole brains, subtle alterations on a sub-cellular basis may nevertheless be present.

The relationship between the altered taurine content in the synaptic and mitochondrial fractions and the seizure-susceptibility of SS rats was not determined. However, the decreased taurine content is not likely the consequence of seizure activity as the data presented in tables 2 and 3 were collected from seizure-susceptible rats which had never been stimulated into having a seizure. The decreased taurine content may reflect a decreased taurine concentration in the synaptic terminals, a decreased number of terminals containing taurine or a

decreased stability of the synaptic pool of taurine. The decreased taurine content in the mitochondrial fraction may reflect an actual decrease in taurine content in mitochondria or may be the result of cross contamination of synaptosomes into the mitochondrial fraction.

Amino acid Transport in SS and SR Rats

There was a decreased rate of taurine transport into platelets and P2 fractions of SS rat brains (figures 9 and 14). The decreased transport was not the result of a difference in the rate of spontaneous efflux (figure 10) and was not a general phenomenon affecting all amino acids (figures 4 and 6). The decreased taurine transport was not present in kidney slices or in the synaptosomal (B) fraction of SS rat brains and thus was not a global phenomenon affecting all taurine transport mechanisms (table 2 and figure 11).

In certain human epileptics alterations in taurine transport similar to those found in the SS rat are apparently present (Airaksinen, 1979; Goodman, 1980; Goodman and Connolly, 1982). Due to the presence of anticonvulsant drugs, poor seizure control and the limitations of human subject experimentation, the relationship between the altered taurine transport and the seizure-susceptibility in these human epileptics could not be established. However, genetically seizure-susceptible rats only rarely have spontaneous seizures and are not exposed to anticonvulsant drugs. Therefore, it was possible to use the SS rat to investigate the relationship between altered taurine transport and seizure-susceptibility.

The possible relationships between altered taurine transport and seizure-susceptibility are: That there is no relationship, cause or effect between the altered transport and the seizure-susceptibility; that the altered taurine transport is a consequence of seizure activity even though these SS rats have had only one seizure to verify seizure-susceptibility; that the decreased taurine transport is a response, perhaps compensatory, to the seizure-susceptibility; that the decreased taurine transport reflects a biochemical defect in the CNS handling of taurine which may be contributing to the seizure-susceptibility. The results of the experiments presented here suggest that this last hypothesis, that the decreased taurine transport is a defect which is contributing to the seizure-susceptibility, is probably valid.

The fact that taurine transport is not decreased in NSP rats (figure 9) suggests that, even within the colony of rats of seizure-susceptible parentage, the presence of a decreased taurine transport is correlated with the actual audiogenic seizure-susceptibility; at least, those animals which show audiogenic susceptibility appear to have the greatest deficit in taurine transport.

The decreased taurine transport, which is present in neonatal SS rat brains (figure 16), cannot be a consequence of seizure activity as these neonatal rats have never had a convulsion and do not have convulsions when exposed to the sound stimulus which would cause an adult SS rat to have a seizure. In unrestrained SS rats, electrically stimulated seizure activity was always accompanied by convulsions (Hugh

E. Laird personal communications). Therefore it is unlikely that these neonatal rats have ever had a seizure.

The SS rat takes taurine up into the brain, in vivo, slower than the SR rat. This suggests that the decreased transport in vitro is related to the decreased uptake of taurine in vivo. This, in turn, suggests that there is a decreased turnover of taurine in the SS rat brain.

Taurine Turnover in the SS Rat Brain

Both biosynthesis and transport are involved in the regulation of taurine in the rat brain. With the exception of the cerebral cortex, no differences in taurine biosynthetic rates in in vitro preparations from SS and SR brains was found (table 5). However, these results only indicate a lack of difference in the two strains in the maximum biosynthetic capability, as the concentration of cysteine in the incubation was greater than that found in vivo. The in vitro rate of synthesis corresponds to approximately 4 umoles per day per brain in an organ which contains a total of approximately 5 umoles taurine, with an estimated half-life of 3-4 days. Nevertheless a decreased rate of taurine influx with no difference in apparent rates of synthesis is consistent with a decreased turnover of taurine.

In a steady-state system, where the concentration of a substance is constant, the rate of turnover of that substance can be determined either by measuring the rate of input to the system via synthesis and uptake or by measuring the rate of removal of the substance from the system. The estimation of the relative differences in turnover of

taurine in brains of SS and SR rats by measuring the rates of taurine input by synthesis and uptake clearly showed a decreased taurine turnover in the SS rat brain. However, estimation of turnover by measuring the half-life of taurine in brains of SS and SR rats, calculated from the rate of loss of radiolabeled taurine from the brain showed no statistically significant difference (figure 15). This apparent contradiction can be explained by the observation that the whole body half-life of taurine was similar to the apparent half-life of taurine in the brain. Back-flux of taurine from the peripheral regions of the animal into the brain, therefore, prevented an accurate determination of taurine half-life in the brain by this method. Given that the rate of [³H]taurine movement from the periphery of the animal into the brain in the SS rat was about one-half that in the SR rat (table 3), a lack of difference in efflux rates actually is consistent with a decreased turnover of taurine in the SS rat brain.

The decreased turnover of taurine in vivo, the decreased transport of taurine into P2 preparations and the decreased taurine content in the synaptic and mitochondrial fractions in SS rats, taken together, indicate that in the brains of SS rats there is an alteration in the handling of taurine which is probably a defect contributing to the seizure-susceptibility. The alterations in the CNS handling of taurine by SS rats cannot be a consequence of the seizure process as the alterations are present in animals which are seizure-susceptible but have probably not yet had a seizure.

It is interesting to note that there was no difference between SS and SR rats in the rate of uptake of taurine into B fractions. This

suggests that the site of the defect in taurine transport is not the presynaptic terminal but may be at the mitochondrial or glial membrane. Of that taurine which is not in the soluble fraction of whole brain homogenates, only 20 percent is in the synaptic fraction, whereas 70 percent is in mitochondria. Both mitochondria and glial cells have the capability to transport taurine (Borg et al., 1976, 1980; Schousboe et al., 1976). However, as previously noted, there is some cross contamination of mitochondria and synaptosomes into the B and C fractions. The alterations in taurine transport, like the alterations in taurine content, can not be definitively associated with either the mitochondria or the synaptosomes. An alternate explanation for the lack of an altered taurine transport in the purified B fraction of SS rat brains is that some unknown factor which is modifying transport differentially in SS and SR rat brains is lost during the isolation of the B fraction from the P2 fraction.

An interesting observation in the apparent association between altered taurine transport and seizure-susceptibility comes from the finding that in the P2 fractions of cerebellum of rats made seizure susceptible by amygdaloid kindling, the net temperature sensitive transport of taurine is increased (Fabisiak and Schwark, 1982). One could argue that the increased taurine transport in the genetically normal but experimentally epileptic rat is a compensatory response to the kindling process or the subsequent seizures, whereas the decreased taurine transport in the genetically epileptic rat is a defect contributing to the seizure-susceptibility. If this reasoning is valid, then

this in turn would suggest that the inhibitory action of taurine is the result of an intracellular process or a process which requires the transport of taurine across membranes. Baba et al.,(1983) have demonstrated that the inhibitory effect of taurine on the formation of cyclic AMP is a transport-dependent process.

Effect of GES on the Neonatal Rat

In an attempt to learn more about the relationship between taurine transport and the neuromodulatory action of taurine, the competitive blocker of taurine transport, guanidinoethane sulfonate (GES) was administered to pregnant dams in an attempt to expose prenatal rats to GES. The reason for choosing this treatment was that several investigators have suggested that the most important functions of taurine in the brain are expressed during neuronal development (Rassin et al., 1978; Sturman, 1979; Sturman et al., 1978; Huxtable and Lippincott, 1982).

Rats prenatally exposed to GES had no apparent anomalies in fetal development (table 7). However, Pasantes-Morales examined the retinas of these rats at 30 days of age and found morphological changes identical to those previously reported for the GES-treated rat (Pasantes-Morales et al., 1983).

Fetal rats of dams on GES took up GES into all organs tested. This uptake was associated with a depletion of taurine in most organs of the newborn rat. By 5 days of age however, taurine concentrations had returned to normal (tables 9 and 10). These results indicate that the fetal uptake of GES and the depletion of taurine in the dam are

sufficient to deplete taurine concentrations in the prenatal rat. The lack of taurine depletion in the neonatal (5 day old) rat exposed to GES was not because of an insensitivity of the transport system to GES (figure 18) but rather because the small amount of GES delivered to the pups in the milk (0.1 ug/ml) was insufficient to block taurine transport (table 10). It is not surprising that the 50 % reduction in taurine concentration in the milk of dams on GES did not affect taurine concentrations in the neonatal rat as Huxtable and Lippincott (1982) have demonstrated that the neonatal rat has the capability to synthesize taurine and that much of the taurine present in the neonatal rat has been biosynthesized in the pup.

In fifty day old GES-treated rats, depleted of 50% of their normal content of taurine, the biosynthetic and uptake processes for taurine in the brain and liver do not appear to be altered (tables 11 and 12). Thus, the depletion of taurine by GES does not appear to be by modification of taurine transport or biosynthetic capabilities. However, this interpretation may just reflect the limitations of in vitro transport and synthesis experiments to predict actual in vivo biosynthetic and transport rates. Huxtable and Lippincott (1982) have shown that GES depletes taurine by mechanisms other than merely a competitive inhibition of transport.

In fifty day old pups treated with GES the pentylenetetrazol, chemoshock threshold was slightly but statistically significantly greater than that in the control pups. This small increase in seizure threshold is contrary to the action of ICV and intracisternal administration of GES (Huxtable, 1981; Mori et al., 1981). The

relationship between this small increase in seizure threshold and the chronic exposure to GES was not determined. However, as the direct administration of GES to the brain produces seizures, it is unlikely that GES is functionally replacing taurine. The variances in the reported action of GES upon seizure threshold may be a consequence of the differences in the route and manner of administration or may reflect a differential effect of GES upon different forms of seizure-inducing stimulus.

The results of these experiments indicate that taurine transport processes in the neonatal rat can be blocked with GES and that prenatal exposure to GES will deplete taurine concentrations in the newborn pup. However, this treatment regimen does not maintain these lowered taurine concentrations and with the exception of the retinal degeneration no abnormalities in the developing pups are produced. The finding that chronic exposure to GES did deplete much of the taurine in the brains of newborn rats indicates that GES can be used to probe the function of taurine in the developing rat. However, it is apparent that the usefulness of GES for probing the function of taurine in the brain will be limited until the pharmacology of GES is better defined.

The Action of Taurine in the Brain

The persistent anticonvulsant action of taurine in the SS rat, following a single injection of taurine (Huxtable and Laird, 1978a), must be accompanied by changes in brain biochemistry capable of altering seizure threshold. It is possible that the physiological, neuromodulatory actions of taurine are by similar mechanisms.

One hypothesis regarding the actions of taurine held that the physiological action of taurine was by modification of the subcellular distribution of glutamate and that the anticonvulsant action of taurine was by rectification of abnormalities in glutamate concentration or distribution (Van Gelder, 1976, 1978a, 1978b, 1982). However, taurine is an efficacious anticonvulsant in the genetically epileptic rat and Huxtable and Laird (1978b) have shown that there are no alterations in glutamate concentrations in the brains of these animals. Furthermore I show here that there are no detectable alterations in the subcellular distribution of glutamate, glutamine or GABA (tables 1-3). Thus, the anticonvulsant action of taurine in these animals cannot be due to rectification of abnormalities in glutamate concentration or distribution. However, these observations do not in and of themselves preclude an interaction of taurine and glutamate.

The high statistical correlation between concentrations of taurine and glutamate in the brain has been cited in support of a direct metabolic relationship between taurine and glutamate (Van Gelder, 1978 and 1982). But, Huxtable et al., (1982) have shown that there is nothing unique about the statistical correlation between concentrations of taurine and glutamate. The concentrations of many amino acids, even those with no metabolic relationship, have a high statistical correlation in the brain. Furthermore, I have shown that ICV injection of taurine does not affect glutamate transport in vitro (figure 20) or subcellular concentration in vivo (table 13). Thus, it is unlikely that the anticonvulsant or neuromodulatory actions of

taurine are by an alteration in the concentration or sub-cellular distribution of glutamate.

Taurine increases the rate of decarboxylation of glutamate in brain homogenates from SS rats. This action of taurine on the decarboxylation of glutamate (table 17) may be a mechanism of the anticonvulsant action of taurine as the decarboxylation of glutamate is the rate limiting step in the biosynthesis of the inhibitory amino acid GABA (Roberts, 1974).

The differential effect of taurine on GAD in brain homogenates of the SS and SR strains suggests that there may be strain differences in GAD or factors which modify GAD activity. This observation is intriguing, as Huxtable and Laird (1978a) have shown that taurine increases the intracerebral electroshock threshold in the SS rat, but not in SR rats. Thus the action of taurine on GAD in these preparations is positively correlated with the action of taurine on intracerebral electroshock threshold. Although the mechanism of the interaction of taurine with GAD was not determined, it could involve the enzyme, the cofactor, pyridoxal phosphate, or some other cause. There are a number of enzymes exhibiting GAD activity, including cysteine sulfinic acid decarboxylase. The stimulatory effect of taurine may be for one of these enzymes.

The increased rate of removal of [^{14}C]glutamate from brains of SS and SR rats after ICV injection of taurine (table 14) may reflect an increased decarboxylation in vivo or may reflect an effect of taurine on the removal of glutamate from the brain. However, as taurine has no

effect on glutamate distribution or transport, the former explanation appears more likely.

The effect of taurine on the in vivo metabolism of glutamate in the experiments presented here did not last as long as the anticonvulsant action of taurine as reported in the experiments of Laird and Huxtable (1978a). This would seem to suggest that the effect of taurine on glutamate decarboxylation, if it does indeed occur in vivo, is not the sole explanation of the anticonvulsant action of taurine. However, differences in the design of these two experiments may also account for the difference. For example, in the experiments conducted by Laird and Huxtable (1978) the taurine was injected directly into the structure where intracerebral electroshock threshold was being determined whereas in my experiments the taurine was administered by ICV injection.

Summary

In summary, the work presented in this dissertation was undertaken to investigate the proposed relationship between seizure-susceptibility, altered amino acid transport or concentration and the anticonvulsant action of taurine. From this investigation it was also hoped that something could be learned of the physiological function of taurine in the brain.

I have found that alterations in the subcellular concentration or distribution of glutamate need not be present for taurine to be an effective anticonvulsant. I have also found that administration of

taurine does not alter the distribution or concentration of neuro-active amino acids. Thus it is unlikely that the action of taurine is by a modification of glutamate distribution. Taurine did increase GAD activity in preparations derived from the brains of SS rats. There is some evidence that this activation occurs in vivo, but it is not clear if this activation of GAD is sufficient to be the sole mechanism by which taurine acts as an anticonvulsant.

Although no alteration in amino acid concentration is detectable in whole brains of SS rats (Huxtable and Laird 1978), there is a decreased content of taurine in specific sub-cellular fractions of SS rat brains. There is also a decreased transport of taurine in SS rats. I suggest that these alterations in taurine content and transport are not a consequence of seizure activity but rather may be contributing to the seizure susceptibility.

As the neuromodulatory action of taurine may be impaired in SS rats and as this impaired function may be associated with a decreased transport and subcellular content it is possible that the neuromodulatory action of taurine is by an intracellular or transport related process.

Thus, future investigations into the action of taurine might best be centered around the intracellular and transport-related processes. The compound GES, a competitive blocker of taurine transport may be a useful tool in such an undertaking. However, the pharmacology of GES in the brain must first be defined.

A continued investigation into the actions of taurine in the brain would be worthwhile as such an endeavor would not only

give insight into the action of the most abundant neuro-inhibitory compound in the brain but might also provide the information necessary for the development of novel anticonvulsants.

APPENDIX A

A LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
Bq	Becquerel (1 Curie = 3.7×10^{10} Bq)
CNS	Central nervous system
CD50	Convulsant dose-50% response
GABA	Gamma-aminobutyrate
GAD	Glutamic acid decarboxylase
GES	Guanidinoethane sulfonate
ICV	Intracerebral ventricular
NSP	Non susceptible progeny
P2	Crude mitochondrial fraction of brain homogenate
A	Myelin fraction of P2
B	Synaptosomal fraction of P2
C	Mitochondrial fraction of P2
SR	Seizure resistant
SS	Seizure susceptible

REFERENCES

- Agrawal, H.C. and Himwich, W.A.: Amino acids, proteins and monoamines of developing brain. In *Developmental Neurobiology*. ed. by W.A. Himwich, pp. 287-310, C.C Thomas Springfield, IL, 1970.
- Ahtee, Lisa, Boullin, D.J. and Paasonen, M.K.: Transport of taurine by normal human blood platelets. *Br. J. Pharmacol.* Vol 52: 245-251, 1974.
- Airaksinen, Eila, M.: Uptake of taurine, GABA, 5HT and dopamine by blood platelets in progressive myoclonus epilepsy. *Epilepsia*. Vol 20: 503-510, 1979.
- Baba, Akemichi, Lee, Eibai, Tatsumo, Tohree and Iwata, Heitaroh: Cysteine sulfinic acid in the central nervous system: Antagonistic effect of taurine on cysteine sulfinic acid-stimulated formation of cyclic AMP in guinea pig hippocampal slices. *J. of Neurochem.* Vol 38: 1280-1285, 1982.
- Baba, Akemichi, Tatsumo, Tohree, Kumagae, Yoshihiro and Iwata, Heitaroh: Inhibitory effect of taurine on the formation of cyclic AMP in guinea pig hippocampus. In *Sulfur Amin Acids: Biochemical And Clinical Aspects*, ed. by K. Kuriyama, R.J. Huxtable, H. Iwata, pp. 161-168, Alan R. Liss, Inc New York, 1983.
- Bakay, Roy, A.E. and Harris, Basil, A.: Neurotransmitter and biochemical changes in monkey cortical epileptic foci. *Brain Res.* Vol 206: 387-404, 1981.
- Barbeau, A. and Donaldson, J.: Zinc, taurine and epilepsy. *Arch. Neurol.* Vol 30: 52-58, 1974.
- Borg, Jacques, Balcar, Vladimir, J. and Mandel, Paul: High affinity uptake of taurine in neuronal and glial cells. *Brain Res.* Vol 118: 514-516, 1976.
- Collins, G.G.S. and Topiwala, S.H.: The release of [¹⁴C]taurine from slices of rat cerebral cortex and spinal cord evoked by electrical stimulation and high potassium concentration. *Br. J. Pharmacol.* Vol 50: 451p-452p, 1974.
- Consroe, P., Picchioni, A., Laird, A. and Chin, L.: The audiogenic seizure susceptible rat. *Inst. Lab. Animal Resources (ILAR) News* Vol XXIV (2+3): 27, 1981.

- Cooper, Jack, R., Bloom, Floyd, E. and Roth, Robert, H. eds:
Y-Aminobutyric acid, glycine, glutamic acid and taurine. in *The Biochemical Basis of Neuropharmacology*, 3rd edition pp 223-256, Oxford Press, 1978.
- Curtis, D.R. and Crawford, J.M.: Central synaptic transmission-Microelectrophoretic studies. *Annu. Rev. Pharmacol.* Vol 9, 209-240, 1969.
- Curtis, D.R. and Watkins, J.C.: The excitation and depression of spinal neurons by structurally related amino acids. *J. Neurochem* Vol 6: 117-141, 1960.
- Curtis, D.R., Leah, J.D. and Peet, M.J.: Lack of specificity of a "taurine antagonist". *Brain Research.* Vol 244: 198-199, 1982.
- Davis, J.M. and Himwich, W.A.: Amino acids and proteins of developing mammalian brain. In *Biochemistry Of The Developing Brain.* ed. by Whitmore Marcel Dekken, pp. 55-99, 1973.
- Davison, A.N. and Kaczmarek, L.K.: Taurine-A possible neurotransmitter. *Nature.* Vol. 234: 107-108, 1971.
- Dunnett, C.W.: A multiple comparison procedure for comparing several treatments with a control. *J. Am. Statist. Assoc* Vol 50: 1096-1121, 1955.
- Durelli, L., Mutani, R., Delsedime, M., Quattrocchio, G., Buffa, C., Mazzarino, M. and Fumero, S.: Electroencephalographic and biochemical study of the antiepileptic action of taurine administered by cortical superfusion. *Exp. Neurol.* Vol 52: 30-39, 1976.
- Eccles, J.C.: Excitatory and inhibitory mechanisms in brain. In *Basic Mechanisms Of The Epilepsies.* ed by H.H. Jasper, A.A Ward and A. Pope, 229-252, Little, Brown and Company, Boston, 1969.
- Emson, P.C.: Biochemical and metabolic changes in epilepsy. in *Taurine And Neurological Disorders.* ed. by A. Barbearu and R.J. Huxtable, 319-338, Raven Press, New York, 1978.
- Fabisiak J.P. and Schwark W.S.: Cerebral free amino acids in the amygdaloid kindling model of epilepsy. *Neuropharm.* Vol: 21, 179-181, 1982.
- Fagg, C.E. and Lane, J.D.: The uptake and release of putative amino acid neurotransmitters. *Neuroscience* Vol 4: 1015-1036, 1979.
- Gale, Karen and Iadarola, Michael, J.: Seizure protection and increased nerve-terminal GABA: Delayed effects of GABA transaminase inhibition. *Science.* Vol 208: 288-291, 1980.

- Goodman, Harold, O. and Connolly, Brian, M.: Taurine transport alleles: Dissecting a polygenic complex. in Genetic Basis Of The Epilepsies. Ed. by V.E. Anderson, W.A. Hauser, J.K. Penry and C.F. Sling, Raven Press, New York, 1982.
- Goodman, Harold, O., Connolly, Brian, M., McLean, William and Resnick, Martin: Taurine transport in epilepsy. Clinical Chemistry Vol 26: 414-419, 1980.
- Gray, E.G. and Whittaker, V.P.: The isolation of nerve endings from brain: An electron-microscopic study of cell fragments derived by homogenization and centrifugation. J. of Anatomy. Vol 96: 79-88, 1962.
- Gruener, Raphael and Bryant, Howard, J.: Excitability modulation by taurine: Action on axon membrane permeabilities. J. Pharmacol. Exp. Ther. Vol 194: 514-521, 1975.
- Haas, H.L., Hosli, L.: The depression of brain stem neurons by taurine and its interaction with strychnine and bicuculline. Brain Res. Vol 52: 399-402, 1973.
- Hall, C.: Genetic differences in fatal audiogenic seizures between two inbred strains of house mice. J. Hered. Vol 38: 3-6, 1947.
- Holtz, P. and Palm, P.: Pharmacological aspects of vitamin B6. Pharmacol. Rev. Vol 16: 113-178, 1964.
- Hruska, Robert, E., Huxtable, Ryan, J. and Yamamura, Henry, I.: High-affinity, temperature-sensitive, and sodium-dependent transport of taurine in rat brain. In Taurine And Neurological Disorders. ed by A. Barbeau and R.J. Huxtable, pp 109-117, Raven Press, New York, 1978.
- Hruska, Robert, E., Padjen, Ante, Bressler, Rubin and Yamamura, Henry, I.: Taurine: Sodium-dependent, high-affinity transport into rat brain synaptosomes. Molecular Pharm. Vol 14: 77-85, 1978.
- Huxtable, Ryan, J.: Insights on Function: Metabolism and pharmacology of taurine in the brain. In The Role Of Peptides And Amino Acids As Neurotransmitters. ed by J.B. Lombardini and A. Kenny, pp. 53-97, Alan R. Liss inc. New York, 1981.
- Huxtable, R. and Bressler, R.: The metabolism of cysteamine to taurine. in Taurine. ed. by R. Huxtable and A. Barbeau, pp. 45-57, Raven Press New York, 1976.
- Huxtable, R. and Laird, H.E.: The prolonged anticonvulsant action of taurine on genetically-determined seizure-susceptibility. Can. J. Neurol. Sci. Vol 5: 205-221, 1978a.

- Huxtable, R. and Laird, H.E.: Are amino acid levels necessarily abnormal in epileptic brains? Studies on the genetically seizure-susceptible rat. *Neurosci. Letts.* Vol 10: 341-345, 1978b.
- Huxtable Ryan, J., Laird, Hugh, Bonhaus, Douglas and Thiese, Cole, A.: Correlations between amino acid concentrations in brains of seizure-susceptible and seizure-resistant rats. *Neurochem. Int.* Vol 4: 73-78, 1982.
- Huxtable, Ryan, J., Laird, Hugh, E. and Lippincott, Shirley, E.: The transport of taurine in the heart and the rapid depletion of tissue taurine content by guanidinoethyl sulfonate. *J. Pharmacol and Exp. Ther.* Vol 211: 465-471, 1979.
- Huxtable, R.J., Laird, H., Lippincott, S.E. and Walson, P.: Epilepsy and the concentrations of plasma amino acids in humans. *Neurochem. Int.* Vol 5: 125-135, 1983.
- Huxtable, Ryan, J. and Lippincott, Shirley, E.: Comparative metabolism and taurine-depleting effects of guanidinoethane sulfonate in cats, mice, and guinea pigs. *Archives of Biochemistry and Biophysics.* Vol 210: 698-709, 1981.
- Huxtable, Ryan, J. and Lippincott, Shirley, E.: Sources and turnover rates of taurine in newborn, weanling and mature rats. In *Taurine In Nutrition And Neurology.* ed by Ryan J. Huxtable and Herminia Pasantes-Morales. pp 23-45, Plenum Publishing, New York, 1982.
- Iversen, Leslie, L., Iversen, Susan, D. and Snyder, Solomon, H. eds.: Amino acid neurotransmitters in *Handbook Of Psychopharmacology Vol 4* Plenum Press, New York 1975.
- Iwata, H., Yamagami, S., Lee, E., Matsuda, T. and Baba, A.: Increase of brain taurine contents of El mice by physiological stimulation. *Japan J. of Pharmacol.* Vol 29: 503-507, 1979.
- Kaczmarek, L.K. and Davison, A.N.: Uptake and release of taurine from rat brain slices. *J. Neurochem.* Vol 19: 2355-2362.
- Kerwin, R.W. and PycocK, C.J.: Role of taurine as a possible transmitter in the thermoregulatory pathways of the rat. *J. Pharm. Pharmacol.* Vol 31: 466-470, 1979
- Killam, K.F. and Bain, J.A.; Convulsant hydrazids. 1. in vitro and in vivo inhibition of vitamin B6-enzymes by convulsant hydrazides. *J. Pharmacol. Exp. Ther.* Vol 119: 255-262, 1957
- Kontro, Pirjo, Linden, I.-B. and Oja, S.S.: Novel anticonvulsant taurine derivatives. in *Sulfur Amino Acids: Biochemical and Clinical Aspects.* ed. by K. Kuriyama, R.J. Huxtable and H. Iwata. pp 211-220, Alan R. Liss, New York, 1983.

- Krnjevic, K. and Puil, E.: Electrophysiological studies on actions of taurine. in Taurine. ed. by R.H. Huxtable and A. Barbeau. pp 179-190, Raven Press, New York, 1976.
- Kuriyama, K., Muramatsu, M., Nakagawa, K. and Kakita, K.: Modulating role of taurine on release of neurotransmitters and calcium transport in excitable tissues. In Taurine And Neurological Disorders. ed by A. Barbeau and R.J. Huxtable. pp 201-216, Raven Press, New York, 1978.
- Kurokawa, M., Nauruse, H. and Kato, M.: Metabolic studies on ep mouse: A special strain with convulsive predisposition. In Progress In Brain Research Vol 21A, Correlative Neurosciences Part A-Fundamental Mechanisms. ed by T. Tokizane and J.P. Schade. pp 112-130, 1966.
- Laird, Hugh, E. and Huxtable, Ryan, J.: Taurine and audiogenic epilepsy. In Taurine And Neurological Disorders. ed by A. Barbeau and R.J. Huxtable. pp 339-357, Raven Press, New York, 1978.
- Logan William, J. and Snyder, Solomon, H.: High affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous tissues. Brain Res. Vol 42: 413-431, 1972.
- Loscher, W.: Biochemical pharmacology of inhibitors of GABA metabolism and valproic acid. In Neurotransmitter, Seizures And Epilepsy. ed by P.L. Morselli, W. Loscher, Lloyd, and Reynolds. pp. 93-105 Raven Press, 1981a.
- Loscher, W.: Effects of inhibitors of GABA aminotransferase on the metabolism of GABA in brain and synaptosomal fractions. J of Neurochem. Vol 36: 1521-1527, 1981b.
- Loscher, W.: Correlations between alterations in brain GABA metabolism and seizure excitability following administration of GABA aminotransferase inhibitors and valproic acid a re-evaluation. Neurochem. Int. Vol 3: 397-404, 1981c.
- Martin, G.E., Bendsky, R.J. and Williams, M.: Further evidence for selective antagonism of taurine by 6-aminomethyl-3-methyl-4H-1,2,4-benzothiadiazide-1,1-dioxide. Brain Res. Vol 299: 530-535, 1981.
- Meldrum, B.B.: Mode of action of anticonvulsant drugs: Biochemical effects. In The Treatment Of Epilepsy. ed by J.H. Tyler. pp 29-59, J.B. Lippincott Co, Philadelphia, 1980.

- Mori, A., Katayama, Y., Yokoi, I. and Matsumoto, M. Inhibition of taurocyamine (guanidinotaurine)-induced seizures by taurine. In *The Effects Of Taurine On Excitable Tissues*. ed by S.W. Schaffer, S.I. Baskin and I.J. Kocsis. pp 41-48 Spectrum Publications, New York, 1981.
- Morselli, P.L., Loscher, W., Lloyd, K.G., Meldrum, B. and Reynolds, E.H. eds.: *Neurotransmitters, Seizures, And Epilepsy*. Raven Press, New York, 1981.
- Mutani, R., Monaco, F., Durelli, L. and Delsedine, M.: Levels of free amino acids in serum and cerebrospinal fluid after administration of taurine to epileptic and normal subjects. *Epilepsia*, Vol 16: 765-769, 1979.
- Newmark, Michael, E. and Penry, Kiffin, J. eds.: *Photosensitivity and Epilepsy: A Review*. Raven Press, New York, 1979.
- Newmark, Michael, E. and Penry, Kiffin, J. eds.: *Genetics Of Epilepsy: A Review*. pp 6-10, Raven Press, New York, 1980.
- Niedermeyer, E.: Special types of epilepsy according to the site of the focus. In *Compendium Of The Epilepsies*. Ed by E. Niedermeyer. pp 106-114, Charles C. Thomas, Springfield, Illinois, 1974.
- Niedermeyer, E.: Rare special forms of epilepsy (unusual triggering mechanisms). In *Compendium Of The Epilepsies*. Ed by E. Niedermeyer. pp 145-151, Charles C. Thomas, Springfield, Illinois, 1974.
- Okamoto, Koichi, Kimuro, Hideo and Sakai, Yutaka: Antagonistic action of 6-Aminomethyl-3-methyl-4H,1,2,4-Benzothiadiazine-1,-1-dioxide (TAG), and evidence for a transmitter role of taurine in stellate interneurons in the cerebellum. In *Sulfur Amino Acids: Biochemical and Clinical Aspects*. Ed by K. Kuriyama, R.J. Huxtable and H. Iwata. pp 151-160, Alan R. Liss, New York, 1983
- Pasantes-Morales, H., Arzate, N.E. and Cruz, C.: The role of taurine in nervous tissue: Its effects on ionic fluxes.. in *Taurine In Nutrition And Neurology*. Ed by R.J. Huxtable and H. Pasantes-Morales. pp 273-292, Plenum Press, New York, 1982.
- Pasantes-Morales, H., Quesada, O., Carabez, A. and Huxtable R.J.: Effects of the taurine transport antagonist, guanidinoethane sulfonate, and b-alanine on the morphology of the rat retina. *J. Neurosci. Res.* Vol 9: 135-143, 1983.
- Perry, T.L. and Hansen, S.: Amino acid abnormalities in epileptogenic foci. *Neurology*. Vol 31: 872-876, 1981.

- Rassin, David, K., Sturman, John, A. and Gaul, Gerald, E.: Sulfur amino acid metabolism in the developing rhesus monkey brain: Subcellular studies of taurine, cysteine-sulfinic acid decarboxylase, γ -aminobutyric acid, and Glutamic acid decarboxylase. *J. of Neurochem.* Vol 37: 740-748, 1981.
- Roberts, E.: Gamma-Aminobutyric and nervous system functions-A perspective. *Biochem. Int.* Vol 3: 2637-2649.
- Roskoski, Robert, Jr.: Net uptake of L-glutamate and GABA by high affinity synaptosomal transport systems. *J. Neurochem.* Vol 31: 493-498, 1978.
- Sanberg, Paul, R. and Willow, Max: Dose-dependent effects of taurine on convulsions induced by hypoxia in the rat. *Neuroscience Letters.* Vol 16: 297-300, 1980
- Tapia, Ricardo: Biochemical pharmacology of GABA in the CNS. in *Handbook Of Psychopharmacology. Vol 4: Amino Acid Neurotransmitters.* Ed by L.L Iversen, S.D. Iversen and S.H. Snyder. pp 1-58, Plenum Press, New York, 1978.
- Thakur, Ajit, K. and Fezio, Lane, W.: A computer program for estimating LD 50 and its confidence limits using modified Behrens-Reed-Muench cumulant method. *Drug and Chemical Toxicology.* Vol 4: 297-305, 1981.
- Yarbrough, George, G., Singh, Dilip, K. and Taylor, David, A.: Neuropharmacological characterization of a taurine antagonist. *J. Pharmacol. and Exp. Ther.* Vol 219: 604-613, 1981.
- Van Gelder, Nico, M.: Rectification of abnormal glutamate acid levels by taurine; In *Taurine.* Ed by R. Huxtable and A. Barbeau. pp 293-302, Raven Press, New York, 1976.
- Van Gelder, Nico, M.: Taurine, the compartmentalized metabolism of glutamic acid and the epilepsies. *Can. J. Physiol. Pharmacol.* Vol 56: 362-374, 1978a.
- Van Gelder, Nico, M.: Glutamic acid and epilepsy: The action of taurine. in *Taurine And Neurological Disorders.* Ed by A. Barbeau and R.J. Huxtable. pp 387-402, Raven Press, New York, 1978b.
- Van Gelder, N.M.: Glutamic acid metabolism and epilepsy. *Japan J. NRA* 6 (Supp), 163-176, 1980.
- Van Gelder, Nico, M.: Changed taurine-glutamic acid content and altered nervous tissue cytoarchitecture. in *Taurine In Nutrition And Neurology.* Ed by R.J. Huxtable and H. Pasantes-Morales. pp 239-256, Plenum Press, New York and London, 1982.

- Van Gelder, N.M. and Courtois, A. : Close correlations between changing content of specific amino acids in epileptogenic cortex of cats and severity of epilepsy. *Brain Res.* Vol 43: 477-484, 1972.
- Wada, J.A., Osawa, T., Wake, A. and Corcoran, M.E.: Effects of taurine on kindled amygdaloid seizures in rats, cats and photosensitive baboons. *Epilepsia.* Vol 16 1975.
- White, Lowell, E. Jr.: Anticonvulsants: a clinical overview of epilepsy. In *Neuropharmacology Of Central Nervous System And Behavioral Disorders.* Ed by G.C. Palmer. pp 393-406, Academic Press, New York, 1981.