c.1

ALTERATIONS IN BRAIN DIPEPTIDE AND AMINO ACID CONTENT IN NEUROLOGICAL AND PSYCHIATRIC DISORDERS

by

STEPHEN JOHN KISH

M.Sc. The University of Southampton, 1973

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

Department of Pharmacology, Faculty of Medicine

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

July 1980

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Pharmacology

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date August 12, 1980

ABSTRACT

My thesis is divided into 4 major sections. The first section is devoted in part to a description of the biochemical abnormalities in the metabolism of homocarnosine (γ-aminobutyryl-L-histidine, HCarn) occurring in a patient with homocarnosinosis. The patient studied and two of her siblings have a progressive neurological disorder with grossly elevated concentrations of HCarn in their CSF. HCarn content was four times higher in a biopsy from the patient's frontal cortex than in biopsied cortex from a large group of control subjects. Using new techniques for the measurement of the HCarn synthesizing and catabolizing enzymes, it was found that the activity of HCarn-Carn synthetase was not increased in the patient's biopsy whereas homocarnosinase activity was undetectable. It is concluded that the elevated HCarn in brain and CSF in the homocarnosinosis patient is due to a deficiency of brain homocarnosinase activity.

The first description of the regional distribution of the two HCarn metabolizing enzymes in human brain was also obtained. The remainder of the first section deals with a description of the neuropharmacological properties of HCarn. Intraventricular injection of HCarn in the rat produced hyperexcitability and in high doses, convulsions, whereas unilateral intrastriatal injection of HCarn resulted in contralateral myoclonus. The results of these experiments are consistent with the possibility that HCarn may be involved in the neuronal excitability of brain.

The second section describes experiments which test the hypothesis that the content of the inhibitory neurotransmitter GABA is altered in the autopsied brains of some patients dying with schizophrenia. The mean content of GABA was reduced by 20-25% in nucleus accumbens, caudate nucleus, frontal cortex and thalamus of the schizophrenic patients as compared to a

control group. However, the differences were found to be statistically significant for only the caudate and thalamus. Extraneous factors such as age of patient at death and prolonged drug treatment did not readily explain the observed reduction in GABA content. The results of the investigation suggest an association between a deficiency of GABAergic function in certain brain areas with some forms of schizophrenia.

The third section describes experiments which test the hypothesis that a deficiency of aspartate found in the cerebellar cortex of some patients with dominantly inherited cerebellar disorders might be due to reduced activity of two enzymes involved in the synthesis of aspartate, namely, aspartate aminotransferase and pyruvate carboxylase. No deficiency of either enzyme was observed in the cerebellar specimens studied. The results of this investigation suggest that the aspartate deficiency in cerebellar cortex found in some dominantly inherited cerebellar disorders does not result from a deficiency of either of these two brain enzymes.

In the final section, experiments are described which study the effects of chronic administration of γ-vinyl GABA and of hydrazine on the contents of GABA and other amino compounds in rat brain. Both of these compounds are presently under consideration for use in clinical trials on patients with disorders involving a brain GABA deficiency. Chronic administration of either γ-vinyl GABA or of hydrazine markedly increased brain GABA content in the rat. Prolonged treatment with γ-vinyl GABA, but not hydrazine, produced a decrease in the activity of glutamic acid decarboxylase (GAD) in rat brain. Since GAD is localized to a large extent in nerve endings, the possibility exists that γ-vinyl GABA might reduce the amount of GABA available for release at synapses, a potentially undesirable effect. The contents of many brain amino compounds other than GABA were

markedly altered by both drugs. Since the potential harmful effects of these unexpected biochemical alterations in brain are unknown, the non-specific effects of γ-vinyl GABA and hydrazine are disturbing.

TABLE OF CONTENTS

page

SECTION B - BRAIN GABA AND PSYCHIATRIC DISEASE

page

List of Tables

List of Figures

Acknowledgements

I wish to thank my supervisor, Dr. Thomas Perry for his guidance and encouragement throughout the course of this work.

I am grateful to my friends and colleagues in research, Shirley Hansen and Maureen Murphy who generously helped me in so many ways.

I am particularly thankful to Dr. David Godin, Dr. Ernest Puil, Dr. David Quastel, Dr. Michael Walker, and Dr. James Wright for their valuable and constructive comments and criticism.

I also wish to thank Dr. H. McLennan and Dr. David Albert for their invaluable help and for the use of their facilities.

As part of Dr. Perry's research team, I was involved in the brain amino acid analyses and human brain dissections described in the following pages, whereas I was solely responsible for all of the enzymatic analyses. I was solely responsible for all of the pharmacological experiments contained in Section A with the exception of the microiontophoretic study, which was carried out in collaboration with Dr. H. McLennan. The histological analyses described in Section D were performed by Dr. H. Dunn. Since the present work is part of an ongoing investigation in Dr. Perry's laboratory, I have also taken advantage of a large accumulation of his brain amino acid data for control and patient groups which appear in several tables of my thesis.

This study was supported by a grant from the Medical Research Council, as well as a Huntington Society of Canada Studentship.

SECTION A - STUDIES OF HOMOCARNOSINE IN BRAIN

I. ABSTRACT

The enzymatic synthesis and degradation of homocarnosine (γ-aminobutyryl-L-histidine, HCarn) was measured for the first time in human brain. HCarn-carnosine synthetase enzyme activity in biopsy specimens of human frontal and temporal cortex was approximately 10 times greater than has been reported for rat cerebral cortex. This enzyme was found to synthesize carnosine (β-alanyl-L-histidine, Carn) 3-5 times as rapidly as HCarn. Homocarnosinase activity was determined in human brain in the presence of Co^{2+} ions using a newly developed radiometric procedure. HCarn content varied over a 6-fold range in different regions of autopsied human brain, being highest in the dentate nucleus and inferior olive. Differences in the regional distribution of HCarn-Carn synthetase and homocarnosinase activities, as well as regional differences in GABA content in human brain did not readily account for the regional differences in HCarn content.

Biochemical studies were also carried out on a brain biopsy obtained from a 40-year-old woman with homocarnosinosis. This patient and two of her siblings have a progressive neurological disorder characterized by spastic paraplegia, dementia, and retinal pigmentation. HCarn concentrations in their CSF, as well as in the CSF of their neurologically normal mother, were elevated 20-40-fold. HCarn content was 4 times higher in the patient's frontal cortex than in biopsied cortex from a large group of control subjects. The activity of the synthetic enzyme, HCarn-Carn synthetase, was not increased. Homocarnosinase activity, however, was undetectable in the patient's brain biopsy, while it could be readily measured in comparable biopsies from control subjects. It is concluded that the elevated HCarn in brain and CSF in the homocarnosinosis family was the result of a deficiency of brain homocarnosinase. The fact that the mother in the

homocarnosinosis family has as high a concentration of HCarn in her CSF as those of her three neurologically affected children, and continues to present no evidence of any neurological abnormalities suggests that the enzymatic deficiency is unlikely of itself to have caused the neurological syndrome affecting the three siblings.

In additional experiments, pharmacological studies employing HCarn were undertaken to seek clues as to the possible physiological role of HCarn in the CNS. The results of these experiments are consistent with the possibility that HCarn may be involved in the excitation of neurons in the brain. Intraventricular injection of HCarn in the rat produced hyperexcitability, and in higher doses, convulsions. Unilateral intrastriatal injection of HCarn resulted in contralateral forepaw myoclonus, a syndrome which could be blocked by the potent GABA-mimetic muscimol. Since this syndrome can also be produced by intrastriatal injection of picrotoxin, an action which has been ascribed to antagonism of GABA-mediated function in the striatum, the ability of HCarn to antagonize the depressant effects of GABA in two invertebrate and one mammalian preparation was then examined. HCarn, however, did not act as a GABA antagonist in any of the three preparations studied. Thus, further experiments will be required to determine the mechanism of the dyskinetic and convulsant actions of this dipeptide.

II. HISTORICAL BACKGROUND

Homocarnosine (HCarn)

The dipeptide homocarnosine (γ-aminobutyryl-L-histidine, HCarn) was first identified by Pisano et al. (1961) as a compound present in bovine brain. Later, Abraham and co-workers (1962) and others (Anastasi and Erspamer, 1967; Kanazawa and Sano, 1967) reported that HCarn was present in the central nervous system (CNS) of all mammalian species studied as well as the chick, duck and frog. To date, no trace of this dipeptide has been found in any mammalian organ outside the CNS.

The concentration of HCarn in brain varies over a 1000-fold range among different species studied. HCarn is found in relatively large amounts (.2-1.2 μmol/g tissue) in brains of human (Perry et al., 1971) monkey (Enwonwu and Worthington, 1973) baboon (Hansen et al., 1973) and frog (Anastasi and Erspamer, 1964) and in relatively lower amounts (.03-.18 μmol/g tissue) in the brain of other species such as the rat, mouse, rabbit and guinea pig (Kanazawa and Sano, 1967). Surprisingly, a much lower brain content of HCarn (.002-.004 μmol/g tissue) has been reported for the pig, dog and cat (Anastasi and Erspamer, 1964).

Preliminary data on the regional distribution of HCarn in autopsied human brain were reported by Kanazawa and Sano (1967) and later by Perry et al. (1971). The concentration of this dipeptide was found to be relatively high in hypothalamus and thalamus and in cerebellar grey and white matter. Relatively lower levels of HCarn were observed in frontal and temporal cerebral cortical grey and white matter and in the corpus callosum.

Synthesis of HCarn

Kalyankar and Meister (1959) first reported the details of the enzymatic synthesis of HCarn in vitro from GABA and histidine using crude chick

pectoral muscle homocarnosine-carnosine synthetase. This enzyme was found to be capable of catalyzing the synthesis of a large number of dipeptides, including carnosine (β-alanyl-L-histidine, Carn), anserine and ophidine. Later, Abraham and co-workers (1961) demonstrated the in vivo synthesis of HCarn in skeletal muscle, kidney and liver of rats fed a diet containing GABA. Additional studies of the in vivo synthesis of HCarn in brain were conducted by Yockey and Marshall (1969) who demonstrated the incorporation of ^{14}C-histidine into ^{14}C-HCarn in frog brain following intracerebral injection of the radiolabelled histidine. In further experiments these and other investigators have detected HCarn-Carn synthetase activity in rat, mouse, chick and rabbit brain (Ng and Marshall, 1976a) and have described its purification from rat (Skaper et al., 1973) and mouse (Horinishi et al., 1978) brain. In addition, the regional and subcellular distribution of this enzyme has been described in the rabbit and rat CNS (Ng et al., 1977; Ng and Marshall, 1978).

Catabolism of HCarn

Much less is known about the enzyme which is responsible for the degradation of HCarn in brain. Abraham and co-workers (1964) found that HCarn was not hydrolyzed by rat brain slices during a three hour incubation in Krebs-Ringer buffer. Other investigators were also unable to detect any hydrolysis of HCarn in either rat (Turnbull and Slater, 1970) or mouse (van Balgooy and Roberts, 1975) brain.

Lenney et al. (1977) have recently isolated and purified a cobalt-activated enzyme, homocarnosinase, from hog kidney. It is the first enzyme known that has the capacity to cleave HCarn. Surprisingly, although Lenney et al. found homocarnosinase activity in the rat kidney, uterus, lung and liver, they could detect no activity in brain, the only organ of the rat

which contains HCarn. In contrast to the findings of Lenney et al., Ng and co-workers reported the enzymatic hydrolysis of HCarn in homogenates of both rat (Ng and Marshall, 1978) and rabbit (Ng et al., 1977) brain. However, unlike Lenney et al., these investigators did not use any activating metal ions in their degradative enzyme assays and their incubation mixtures contained dithioerythritol, which would probably have chelated any Co^{+2} ions naturally present in the brain extracts they used. There is therefore some doubt as to whether they measured the activity of the same dipeptidase isolated by Lenney et al.

Pharmacological properties of HCarn

Very little information is available concerning the pharmacological properties of HCarn, and consequently the physiological role of this dipeptide is unknown. In the earliest reported study, Hayashi (1965) demonstrated that introduction of 100 µmol HCarn into the CSF of dogs produced seizures. However, the same dose of HCarn was also found to be effective in antagonizing seizures resulting from either the injection of carnitine into the CSF or electrical stimulation of the cerebral cortex. Behavioural excitation following administration of HCarn was also observed by Turnbull et al. (1972), who demonstrated that intraventricular injection of 1 µmol of this dipeptide in the rat produced hyperexcitability, which was sometimes accompanied by a brief period of convulsions. In addition, these investigators found that intraventricular injection of HCarn reduced the median dose of pentylenetetrazol required to produce tonic convulsions, and shortened the pentobarbital sleeping time. This "analeptic" action of HCarn was also observed by Bissette and associates (1978) who demonstrated that HCarn shortened pentobarbital sleeping time in mice following either intraperitoneal or intracisternal injection.

In other experiments, Slater and Turnbull (1969) showed that intraventricular injection of HCarn, but not of its constituent amino acids GABA and histidine, produced hypothermia in the rat. Intravenous administration of this dipeptide was found to have no effect on heart rate, blood pressure, or respiration in the rabbit, or on the monosynaptic spinal reflex in the chloralose anaesthetized cat (Turnbull et al., 1972).

The limited number of studies dealing with the electropharmacological actions of HCarn has not yet provided any insight into the possible physiological role of the dipeptide in the CNS. In the first such study Turnbull and co-workers (1972), using an extracellular recording technique, found that iontophoretically applied HCarn had no significant effect on the spontaneous activity of neurons in the pons and medulla of the cat. Nor did this dipeptide block the inhibitory or excitatory actions of GABA and glutamate, respectively, on these neurons. On the other hand, a weak inhibitory action of HCarn on the frequency of action potentials of rat olfactory bulb mitral cells has recently been reported (MacLeod, 1978). A similar weak inhibitory action of HCarn was recently observed in the isolated neurohypophyseal-infundibular preparation in the rat, in which the dipeptide was found to have 4% of the activity of GABA in reducing the amplitude of the antidromically conducted compound action potential (Mathison and Dreifuss, 1980).

In recent years, the analysis of drug-receptor interactions has been extended to a study of the binding characteristics of radiolabelled ligands to isolated membrane fractions. Although these binding studies offer the advantage of simplicity and rapidity of operation, the data arising from these experiments must be interpreted with caution, since these binding methods simply measure the concentration of ligand bound to fragments of membranes and do not indicate the relative efficacies of different drugs.

Although the binding characteristics of HCarn itself to CNS membrane fragments have not yet been studied, two groups of investigators have examined the ability of HCarn to inhibit the specific binding of GABA in the mammalian CNS. "Specific binding" is obtained by subtracting from the total bound radiolabelled GABA the amount not displaced by a high (1 mM) concentration of unlabelled GABA. In these two studies, HCarn was found to inhibit the specific, Na^+-independent binding of ^3H-GABA to membrane fractions derived from whole rat brain (Enna and Snyder, 1976) and from human cerebellar cortex (Lloyd and Dreksler, 1978) with IC50's of 17 and 43 μM respectively. The results of these binding studies suggest that HCarn might interact with the mammalian GABA receptor. However, no information is available from such studies indicating whether such interaction might be to mimic, block or otherwise modulate the effects of GABA.

HCarn in altered nutritional states

Several interesting behavioural disturbances associated with changes in brain HCarn content have been observed following altered dietary protein or amino acid intake in mammals. However, it must be emphasized, that since HCarn was not independently manipulated in these studies one cannot directly attribute the observed behavioural changes solely to an alteration in brain HCarn. In one such study, Enwonwu and Worthington (1973) found that monkeys fed a low protein (2% casein) diet for about three months exhibited profound tremors of the head and limbs which were associated with a large increase in the whole brain content of HCarn (+185%) and one of its constituent amino acids, histidine (+230%). Smaller increases in the contents of other brain amino acids, as well as a 25% decrease in the concentration of glutamate, were also noted. Quinn and Fisher (1977) reported the effects of a dietary deficiency of histidine on brain HCarn content and

behaviour in the rat. These investigators observed that rats deprived of dietary histidine for 2-16 weeks showed a 50-60% decrease in the whole brain content of HCarn which was associated with increased irritability and sensitivity of the animals to loud disruptive noises.

A genetically inherited metabolic disorder: homocarnosinosis

Another approach to the elucidation of the possible role of oligopeptides and amino acids in the mammmalian central nervous system has been to study patients with inherited metabolic disorders involving these compounds. Such studies have stimulated interest in the possible physiological role of several amino compounds in brain including GABA (Perry et al., 1973b), glycine (Perry et al., 1975a) and glutathione (Marstein et al., 1976).

Gjessing and Sjaastad (1974) have described a metabolic disorder involving HCarn occurring in a Norwegian family. Three adult siblings in this family are afflicted with progressive spastic paraplegia, mental deterioration and retinal pigmentation. The concentration of HCarn in their CSF is elevated to levels 20-40 times greater than those found in the CSF of control subjects. The neurologically normal mother of these three patients also has a greatly elevated concentration of HCarn in her CSF, while the other tested family members have normal CSF HCarn levels.

III. HYPOTHESES TO BE TESTED AND EXPERIMENTAL OUTLINE

In the following pages I will describe the biochemical abnormalities in HCarn metabolism found in a brain biopsy obtained from one of the neurologically affected homocarnosinosis patients (Gjessing and Sjaastad, 1974). The major objective of this phase of the study was to determine the cause of the elevated concentration of HCarn in the CSF of this patient. The

specific hypothesis to be tested was that the elevated CSF HCarn in the homocarnosinosis patient is associated with an altered metabolism of the dipeptide in brain. Furthermore, since a basic knowledge of the metabolism of HCarn is lacking for human brain, a considerable portion of this study was devoted to a description of the biochemical properties of the HCarn synthesizing and catabolizing enzymes, as well as the regional distribution of HCarn and its metabolizing enzymes in human brain.

The remainder of this investigation was concerned with the neuropharmacological properties of HCarn. The overall aim of this phase of the study was to provide clues as to the possible role of this dipeptide in the human CNS. The major hypothesis that I have tested is that HCarn has the properties of a GABA antagonist. My rationale for testing this hypothesis was based primarily on the results obtained from two types of studies. First, in vitro binding studies had shown that HCarn in μmolar concentrations displaces ^{3}H-GABA from binding sites in both human and rat brain membrane fragments. Since a good (although not perfect) correlation exists between the order of potency of compounds in GABA binding experiments and in in vivo neurophysiological studies (Greenlee et al., 1978; Arnt, et al., 1979; Roberts et al., 1979), the ability of HCarn to bind to GABA "receptors" (recognition sites?) in vitro suggests that HCarn may have a direct action on the mammalian GABA receptor. Secondly, several pharmacological studies have shown that direct administration of HCarn into the central nervous system will produce convulsions, a property shared (although not exclusively) by compounds which block many of the effects of GABA, such as picrotoxin and bicuculline. As a first step towards strengthening or altering this hypothesis, I have examined the action of HCarn in three preparations which are sensitive to GABA antagonists and agonists: alterations in behaviour following intrastriatal drug injection in the rat, the crayfish claw

preparation, and the crayfish stretch receptor preparation. In addition, in an exploratory study, I have examined the effects of iontophoretic application of HCarn on rat CNS neurons using an extracellular recording technique.

III. METHODOLOGY

Chemicals

For sources of chemicals see Kish et al., 1979 and Perry et al., 1979d.

Control subjects

One or more brain biopsies of frontal or temporal cortex were obtained (with informed consent) from 38 adult patients in Vancouver and were frozen in liquid nitrogen within 10 seconds of surgical removal. Single specimens were obtained from 13 non-epileptic patients during neurosurgical exploration for deep-seated tumours which required removal of apparently normal superficial cortex. 42 additional biopsy specimens were epileptogenic foci removed from 25 different patients with focal epilepsy who had failed to respond to treatment with anticonvulsant drugs. No evidence of any biochemical disorder involving the metabolism of HCarn or its constituent amino acids, histidine and GABA, was found in any of these 38 patients. Brain biopsies were stored at -80°C for periods varying from a few days to three years before biochemical studies were carried out.

Specimens were dissected from a number of regions of brain obtained at autopsy from 29 adult patients who died without evidence of neurological or mental disease. In addition, autopsied brain specimens were used for enzyme studies from three patients who died with inherited olivopontocerebellar atrophy because the intervals between death and freezing of brain were unusually short in these individuals. No evidence of any disorder in the metabolism of HCarn could be detected in these patients. Specimens of

brain obtained at autopsy were kept frozen at -80°C for periods up to 5 years until biochemical studies were carried out.

Amino acid analyses

Brain specimens obtained at biopsy or autopsy were homogenized and deproteinized with 0.4 M perchloric acid as previously described (Perry et al., 1971), except that homogenizations were carried out with motor-driven Teflon pestles in glass tissue grinders. Chromatography was performed on 130 x 0.6 cm columns of Chromobeads, Type B, on a Technicon automatic amino acid analyzer, using the lithium citrate buffer elution system of Perry et al. (1968).

Identification of HCarn

Since HCarn and Carn (β-alanyl-L-histidine) could not be reliably separated on the amino acid analyzer using this procedure (Perry et al., 1968), the presumed HCarn from brain was first isolated, and identified by its electrophoretic behaviour and by its hydrolysis products. HCarn was isolated from brain and CSF in three different ways. In some instances, HCarn (together with other basic amino acids) was separated from deproteinized brain homogenates on Dowex 50 columns, using the procedure of Harding and Margolis (1976), as described later for homocarnosinase assays. In other cases, the presumed HCarn was isolated, without any contamination by other basic amino compounds, by using the amino acid analyzer as a preparative instrument. Finally, when it was desirable to isolate HCarn from large amounts of brain or CSF, peptides were first separated from the much larger amounts of α-amino acids on columns of copper Sephadex, as described by Perry et al. (1977a). The presumed HCarn was then purified by preparative chromatography on the amino acid analyzer. Some samples of the

dipeptide isolated from brain or CSF were subjected to high voltage paper electrophoresis in 0.1 M borate buffer (pH 10), which effectively separates Carn and HCarn from each other. The isolated peptide was also hydrolyzed in 6 M HCl at 110°C for 16 h, and the relative amounts of histidine, GABA and β-alanine produced were then quantitated on the amino acid analyzer.

HCarn-Carn synthetase enzyme assay

This enzyme was assayed using a modification of the methods of Ng et al. (1977) and Harding and Margolis (1976) employing histidine and either GABA or β-alanine as co-substrate. Brain tissue was homogenized at 4°C in a glass tissue grinder with a motor-driven Teflon pestle in 6-9 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 30 mM glucose, 50 mM $NaHCO_3$, and 5 mM dithiothreitol. The homogenate was then centrifuged at 12,000 x g for 30 min at 4°C, and the supernatant was retained. The incubation mixture contained 300 µl brain supernatant, 100 µl Tris-HCl buffer (250 mM, pH 7.4), 20 µl ATP (100 mM), 5 µl $MgCl_2$ (400 mM), 5 µl NAD (20 mM), 5 µl dithiothreitol (200 mM), 20 µl $NaHCO_3$ (750 mM), 12.5 µl glucose (800 mM), 10 µl GABA or β-alanine (500 mM) and 1 µCi of L-[ring-2-^{14}C] histidine (55.4 µCi/µmol) in a final volume of 0.5 ml. The reaction mixtures were incubated in a Dubnoff metabolic shaker for 1 hour at 37°C. Enzyme activity was found to be linear with time for at least 100 min, and linear with respect to amount of tissue up to 50 mg wet wt. The reaction was stopped by addition of 1.5 ml ethanol, and samples were centrifuged at 20,000 x g for 10 min.

Following the addition of 1 µmol of histidine and 1 µmol of HCarn or Carn in ethanolic solution, each incubation supernatant was applied to a 5 x 0.75 cm column of Dowex 50 x 8, 200-400 mesh, in the α-picoline form, as described by Harding and Margolis (1976). The unreacted radioactive

histidine was washed from the column with 60 ml 0.1 M α-picoline, after which the radioactive HCarn or Carn, together with cold carrier dipeptide, was eluted from the column with 10 ml 1.0 M NH_4OH. The eluate was divided into two approximately equal aliquots, to each of which 12.5 ml of Aquasol were added, and the samples were counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer.

Preliminary experiments showed that radioactive histidine was eluted from the Dowex 50 columns by the α-picoline wash, and that radioactive HCarn or Carn was retained on the columns until eluted with NH_4OH. In addition, when ammoniacal eluates were taken to dryness and subjected to high voltage paper electrophoresis, only one spot of radioactivity was observed on electrophoretograms which co-migrated with authentic HCarn or Carn when GABA or β-alanine respectively, had been used as substrates. Synthesis of both HCarn and Carn was measured in each brain specimen, and assays were performed in duplicate.

Homocarnosinase enzyme assay

This enzyme was assayed using a modification of the method of Lenney et al. (1977). Brain was homogenized at 4°C in 15-19 volumes of 5 mM N-ethylmorpholine buffer, pH 7.2, containing 0.1 mM $CoSO_4$. The homogenate was centrifuged at 12,000 x g for 30 min at 4°C, and the supernatant was retained for the enzyme assays. The incubation medium contained 0.6 ml of brain supernatant, 0.2 ml of $CoSO_4$ (5 mM) in N-ethylmorpholine buffer (0.6 M, pH 7.2), and 0.5 μmol of HCarn in a final volume of 0.9 ml. The 0.5 μmol of added HCarn included 1.7 μCi of L-[γ-aminobutyryl-1-^{14}C] homocarnosine. The endogenous HCarn in each brain specimen (previously estimated in a portion of the specimen by amino acid analysis), together with the added HCarn, gave a final concentration of HCarn in each assay

mixture of from 0.56 to 0.61 mM, or approximately 80% of the saturating concentration for the enzyme. This subsaturating concentration was employed, since addition of further unlabelled HCarn to the incubation medium yielded less precise determinations of enzyme activity as a result of the reduced specific radioactivity of the substrate. In the homocarnosinase assays reported here, the endogenous HCarn in each incubation medium constituted 1-9% of the total substrate, depending upon the brain region being assayed.

Reaction mixtures were incubated for 25 min at 30°C. Enzyme activity was found to be linear with time for at least 35 min, and linear with respect to amount of tissue up to 60 mg wet wt. The reaction was stopped by placing the tubes in a boiling water bath for 3 min, and samples were then centrifuged at 20,000 x g for 10 min.

Radioactive GABA released by the hydrolytic enzyme was then separated from the residual radioactive HCarn substrate by applying the supernantant to Dowex 50 columns as described for the HCarn-Carn synthetase assay (Harding and Margolis, 1976). In this case, the [^{14}C] GABA was eluted from the column by the first 11 ml of 0.1 M α-picoline, while the [^{14}C] HCarn was retained on the cation exchange resin. The α-picoline eluate was collected in two approximately equally aliquots, mixed with Aquasol, and counted for radioactivity as described above. Homocarnosinase assays were carried out in duplicate on each brain specimen.

Protein determination

Protein concentrations were determined in brain supernatants by the method of Lowry et al. (1951), using bovine serum albumin as standard. It was found, however, that supernatants from homogenized brain biopsy specimens contained 2-3 times more protein per g of original tissue than did

supernatants from autopsied brain specimens, presumably due to the presence in living brain of some soluble protein(s) which rapidly disappears after brain death. Accordingly, enzyme activities for both homocarnosine-carnosine synthetase and homocarnosinase have been expressed in term of grams wet weight of brain. To have expressed homocarnosinase activities on a g protein basis would have resulted in higher enzyme activities in autopsied than in biopsied brain, which does not seem reasonable.

Homocarnosinosis patients and family:

The three neurologically affected homocarnosinosis siblings are now aged 42, 40 and 35, and their illnesses have been described in detail (Sjaastad et al., 1976).

Case 1. L.S. is a 42-year-old woman. During her early years, her development seemed normal mentally and physically. At age 5-6 years, her gait became increasingly slow and clumsy and spastic paraplegia was diagnosed. As an adult she was employed as a hairdresser. However, because of increasing mental deficiency, spastic paraplegia and involvement of the upper extremities, she had to discontinue her work as a hairdresser at the age of 28. When she was later admitted to hospital a psychological investigation revealed a subnormal I.Q. (Wechsler, average IQ=60). Ophthalmoscopy revealed a bilateral diffuse pigmentation of the choroid. Pronounced spasticity was present in the lower extremities with clonic myotonic reflexes and extensor plantar responses.

Cases 2 and 3. (aged 35 and 40) are less severely affected brothers of Case 1. Both brothers appeared to have normal neurological and psychological development up to the age of 28-29. At that age the brothers began to

develop moderate spasticity in the lower limbs, extensor plantar responses and exaggerated reflexes in the upper extremities. Mild to moderate reduction in mental capacity as well as chorioretinal pigmentation were also observed. All three patients revealed a normal EEG, electromyography and electroretinography.

The father, mother, unaffected daughter and two sisters of the mother were also examined and all appeared to have at least normal intelligence.

HCarn levels in the CSF of the three neurologically affected patients ranged between 50 and 57 µmol/l. These compare with published mean values for HCarn in CSF of control adult subjects of 1.8 ± 1.8 µmol/l (Perry et al., 1975b), and 2.7 ± 2.5 µmol/l (Gjessing et al., 1972). The mother of these three patients, a neurologically normal woman, was found at the age of 65 years to have a HCarn level in her CSF of 75 µmol/l. On a recent examination at the age of 69 years, she still showed no neurological abnormalities and again had a greatly elevated HCarn concentration in her CSF (40 µmol/l). The father of the three neurologically affected siblings, their normal sister, and two maternal aunts all have normal levels of HCarn in their CSF.

After informed consent had been obtained, a brain biopsy was removed from the non-dominant frontal cortex of the oldest and most severely affected of the three siblings (Case 1) when she was 40 years of age. Part of the biopsy was used for histological studies in Norway while the remaining portion was instantly immersed in liquid nitrogen. CSF was also collected from the subarachnoid space overlying the biopsy site immediately before removal of the surgical specimen and was frozen at -80°C. CSF was also obtained by lumbar puncture from the three neurologically affected siblings and the mother. The brain biopsy and CSF were later shipped on

dry ice from Oslo, Norway to Vancouver, Canada where the biochemical studies were performed.

Intraventricular and intrastriatal injections and behaviour measurement

Male Long-Evans hooded rats weighing 275-325 grams were obtained from the Canadian Breeding Farms and Laboratories (St. Constant, P.Q.) and were housed in group cages prior to surgery and in individual cages thereafter. Under sodium pentobarbital anaesthesia (50 mg/kg) the rats were mounted in a stereotaxic frame. The incisor bar was positioned 5 mm above the intra-aural line. An infusion cannula made from 23 gauge stainless steel tubing was implanted into the right rostral striatum using the coordinates 2.4 mm anterior to bregma, 3.0 mm lateral to the midline suture, and 3.6 mm below the dura mater. The coordinates for the intraventricular injection were 1.5 mm lateral and 3.3 mm below dura. Four support screws were fixed to the skull adjacent to the hole and dental cement was placed around the screws and cannula until the screws were covered and the cannula held firmly in place. Immediately following surgery cannula plugs were placed in the cannulae. Injection needles were made from 30 gauge tubing and extended 1 mm beyond the end of the cannula.

Behavioural testing was conducted 7 days postoperatively. The cannula plugs were removed and an injection needle connected by 1 meter of polyethylene tubing to a Hamilton glass syringe was inserted without anaesthetizing the animal. The injection needle was held in the cannula by a polyethylene sleeve at the junction of the injection needle and the top of the cannula.

The animal was then placed in an open box measuring 40 x 30 x 25 cm. The rat was allowed to explore the test box for 5 min before testing

began. A 4 µl (lateral cerebral ventricle) or 1 µl (striatum) drug solution was then delivered over a 3 minute period. This infusion rate was controlled by a syringe pump (Sage Instruments). Ten minutes following injection, the injection needle was removed and was replaced by the cannula plug. The dyskinetic effects of the drugs were evaluated by recording the time to onset, frequency, duration and intensity of dyskinesias. The intensity of the dyskinesias was quantitated using a 5 point scale in which the animals were scored as to the maximum distance moved by the head or paw of the rat during the abnormal movement (see below). All drugs were administered blind and in random order.

Score	Maximum Distance Moved by Rat During Myoclonus
1	1/4" - 1/2"
2	1/2" - 1
3	1 - 1 1/2"
4	1 1/2" - 2"
5	2 - 3"

At the conclusion of the experiments, the animals were killed by cervical dislocation. Their brains were removed and placed in 10% formol-saline. Frozen sections, 30 µm thick, were made on a cryostat. An examination of the site of the cannula was conducted using the atlas of Pellegrino and Cushman (1967) as a guide.

Crayfish claw preparation

The claw of the crayfish Procambarus clarkii was prepared in a fashion similar to that employed by Robbins and van der Kloot (1958). Immediately after removal from the body, the claw was perfused through a rubber tube,

slipped over the cut end of the propodite so that perfusion fluid (modified van Harreveld's solution, van der Kloot, 1960) flowed directly over the opener muscle. It was found that the optimal rate of perfusion was 2-3 ml/min. The temperature of the perfusion medium was maintained at 13-15°C. The external skeleton of the ischiopodite was removed and a pair of platinum electrodes was inserted into the muscle mass containing the excitatory nerve. The nerve was stimulated with a train of 0.5 msec 6 mvolt pulses at 90/sec and 40 msec duration. This stimulation was repeated once every 50 sec. Isometric recordings of the contraction of the dactylopodite were made using a Grass transducer and polygraph. Drugs were administered either by continuous perfusion or by a 0.5 ml bolus injection made into a rubber tube attached to the tip of the propodite. All drugs were administered in van Harreveld's solution as modified by van der Kloot (1960) at pH 7.5.

Crayfish stretch receptor preparation

Large crayfish of the same species were used in this study. The procedure for recording impulses from the stretch receptor sensory axon has been described by Elliott and Florey (1956). Drug solutions in modified van Harreveld's solution, pH 7.5 (see above) were applied by pipette to the basin naturally formed by the exoskeleton which contained the stretch receptor. The stretch receptor organ was exposed to the drug for 15 seconds and then excess fluid in the shell of the crayfish was sucked away. This procedure was performed a total of three times for each drug solution. After the final wash the effect of the drug on the firing rate of the stretch receptor was observed. When the effects of drug antagonists were to be determined, the stretch receptor was bathed in the solution containing the blocking agent for a period of two minutes, unless otherwise stated.

Electropharmacology of HCarn in the rat CNS

An exploratory study was performed using extracellular recording techniques on one male Wistar rat weighing 350 g. The experiment was performed under urethane anaesthesia. The animal was placed in a stereotaxic frame and the calvarium and dura were removed. Electrodes were introduced stereotaxically into the frontal cortex and thalamus.

The drugs were administered electrophoretically from seven barrel micropipettes. The recording barrel contained 4 M NaCl. Amplified signals were fed into an oscilloscope and a ratemeter for recording the spike frequency. The majority of neurons tested did not fire spontaneously and therefore, were driven with a continuous minimal application of DL-homocysteate. In experiments designed to test the effect of potential antagonists of GABA on the spike frequency, a constant time interval was maintained between drug administration.

The solutions used to fill the electrode barrels were the following: sodium DL-homocysteate (0.2 M, pH 8), HCarn sulfate (0.5 M, pH 4), carnosine (0.5 M, pH 4), GABA (0.5 M, pH 4) bicuculline (5 mM in 165 mM NaCl, pH 4) and NaCl (center barrel, 4 M NaCl). HCarn was tested on 8 cortical neurons (2 mm lateral, 2 mm posterior to bregma, 150-1500 µm deep) as well as on 4 thalamic neurons. These neurons were selected on the basis of their stable firing rate and a consistent response to GABA.

V. RESULTS

Identification of HCarn in control human brain and CSF

Since HCarn and Carn (β-alanyl-L-histidine) are not reliably separated with the analytic method used (Perry et al., 1968) the identification of HCarn was first verified in individual specimens of autopsied human frontal and occipital cortex (n=5). The presumed HCarn was isolated by collecting

the fraction of the amino acid analyzer run which corresponded to authentic HCarn. The amino acid content in the acid hydrolyzate of this fraction was then quantitated on the amino acid analyzer. Only GABA and histidine were produced by acid hydrolysis of the isolated compound. In addition, samples of the unhydrolyzed HCarn zone were subjected to high voltage paper electrophoresis in 0.1 M borate buffer (pH 10) followed by spraying with diazotized sulfanilic acid. This procedure separates Carn and HCarn from each other. Only one spot on the electrophoretogram was detected which co-migrated with authentic HCarn, thus confirming the identification of HCarn and excluding the presence of contaminating Carn.

In a second type of experiment, pooled specimens of human putamen-globus pallidus (from 8 brains), inferior olive (from 11 brains) and olfactory bulb (from 6 brains), were collected. HCarn, Carn, and other basic amino acids and oligopeptides were isolated from each of the three pools on Dowex 50 columns using the technique of Harding and Margolis (1976). Each of the three pools was subjected to amino acid analysis before and after acid hydrolysis. Based on the amount of β-alanine and GABA released by acid hydrolysis, less than 1.8 % of the presumed HCarn in putamen-globus pallidus and less than 0.5% of the presumed HCarn in the inferior olive could have been Carn. Likewise, a maximum of 19 % of the presumed HCarn in the olfactory bulb pool could have been Carn. The actual amounts of Carn present in these three pools may have been less than indicated by this technique, since the procedure could also have collected uracil and unidentified β-alanine-containing peptides, which then would have yielded β-alanine on acid hydrolysis. In an additional experiment, the unhydrolyzed human olfactory bulb pool was subjected to high voltage paper electrophoresis. Two spots were observed which co-migrated respectively with authentic

HCarn and Carn, thus confirming the presence of both these dipeptides in human olfactory bulb.

In contrast, when this procedure was applied to pools of dog (3 brains), cat (4 brains) and mouse (40 brains) olfactory bulb, Carn was found to be the predominant dipeptide. Based on the products of hydrolysis, not more than 2.6% (dog), 3.5 % (cat) and 4.4 % (mouse) of the HCarn-Carn fraction could have been HCarn. In addition, when unhydrolyzed fractions of dog, cat and mouse olfactory bulb pools were subjected to high voltage paper electrophoresis, only one spot was observed, which co-migrated with authentic Carn.

In further experiments, the total presumed HCarn in 100 ml of CSF pooled from 20 patients was collected using the copper Sephadex procedure of Perry et al. (1977a) and subjected to acid hydrolysis. No β-alanine was produced, and the equimolar amounts of GABA and histidine formed indicated that only HCarn was present in this pool of CSF.

HCarn-Carn synthetase activity in control human cerebral cortex

Preliminary experiments showed that no synthesis of HCarn or Carn could be detected in crude homogenates of brain, and all synthetase enzyme assays were therefore carried out in the supernatants from 12,000 x g centrifugations of crude homogenates.

HCarn-Carn synthetase activity was greatest in rapidly-frozen biopsy specimens of cerebral cortex and in autopsied cortex which had been frozen soon after death (Table 1). Activity was undetectable in autopsied cortex that had been frozen more than 6 hours after death. Carn was found to be synthesized at a rate 2-5 times greater than was HCarn when 10 mM concentrations of β-alanine and GABA were employed as substrates in the enzyme assay.

Table 1. Effect of interval between death and freezing on HCarn-Carn synthetase activity in human frontal and temporal cortex.

Tissue sample	HCarn synthesis	Carn synthesis
Biopsy (12)	1.22 - 9.93 6.01 ± 0.87*	5.09 - 40.43 23.09 ± 3.11*
Autopsy, 2 h** (5)	0 - 6.47 3.24 ± 1.15*	1.17 - 20.50 13.38 ± 3.70*
Autopsy, 2-6 h** (2)	0.27 - 1.23	1.17 - 7.00
Autopsy, >6 h** (3)	0	0

Ranges of dipeptide synthesis are expressed in nmol/h/g wet wt. Figures in brackets indicate number of brains assayed.
* Mean ± S.E.M.
** Interval between patient's death and freezing of brain at -80°C.

In a specimen of autopsied temporal cortex that was frozen shortly after the patient's death, an apparent K_m of 1.8 mM was obtained for β-alanine, with a V_{max} of 31 nmol/h/g (determined from Eadie-Hofstee plot; for complete details see Kish et al., 1979). In the same tissue specimen, the apparent K_m for GABA was 8.8 mM, with a V_{max} of 18 nmol/h/g. These results indicate that, at least under the conditions of the in vitro assay, β-alanine is the preferred substrate for HCarn-Carn synthetase.

Homocarnosinase activity in control human cerebral cortex

Homocarnosinase activity was readily detectable in crude human brain homogenates, as well as in the supernatants of such homogenates after centrifugation at 12,000 x g for 30 min. As shown in Table 2, the enzyme was markedly activated by Co^{2+}, and to a lesser degree by Mn^{2+}. Addition of dithiothreitol (2 mM), which one would expect to chelate metal ions such as Co^{2+} or Mn^{2+}, reduced homocarnosinase activity to 9% of the control.

Table 3 compares homocarnosinase activity in biopsies of frontal or temporal cortex, and in autopsy specimens of frontal cortex frozen at varying intervals after death. The mean values for biopsied and freshly autopsied cortex did not differ significantly, and homocarnosinase activity appeared to be stable in unhomogenized autopsied brain for at least 10 hours under the usual morgue conditions. Enzyme activity was linear with time for at least 35 min when incubations were carried out at 30°C, but for only 10 min when incubated at 37°C, suggesting that the enzyme is rather rapidly inactivated in homogenized brain.

Table 2. Effects of various conditions on homocarnosinase activity of brain supernatant.

Assay system*	Homocarnosinase activity	Per cent of activity in complete system
Complete	232	100
Complete, with added 2 mM dithiothreitol	20	9
Complete, but Co^{2+} omitted	17	7
Complete, Co^{2+} omitted, and 4 mM Mn^{2+} added	107	46

Homocarnosinase activity was measured in a single specimen of autopsied frontal cortex under various conditions, and hydrolysis of HCarn is expressed in nmol/25 min/g wet wt.

* The complete assay system contained Co^{2+} (1.2 mM) in N-ethylmorpholine buffer (137 mM, pH 7.2).

Table 3. Homocarnosinase activity in frontal and temporal cortex.

Tissue sample	Homocarnosinase activity	
	Range	Mean ± S.E.M.
Biopsy (15)	69–287	149 ± 18
Autopsy, <2 h* (5)	92–316	190 ± 47
Autopsy, 5–10 h* (8)	68–233	136 ± 23
Autopsy, 11–25 h* (4)	46–108	73 ± 16

Hydrolysis of HCarn is expressed in nmol/25 min/g wet wt. Figures in brackets indicate number of brains assayed.
* Interval between patient's death and freezing of brain at -80°C.

Regional distribution of HCarn and its metabolizing enzymes in autopsied human brain

Table 4 shows the mean HCarn content of 15 different regions of human brain obtained at autopsy within 24 hours of death, as well as the mean HCarn content of frontal and temporal cortical biopsies. Our earlier results were published in Kish et al. (1979) and Table 4 brings the results from our human brain data up to date. The cerebral and cerebellar cortical regions studied consisted largely or entirely of grey matter, but no attempt was made to separate different cortical layers. The entire substantia nigra was selected for biochemical study. HCarn content was found to be highest in the dentate nucleus and the inferior olivary nucleus, and relatively high in the substantia nigra, globus pallidus and cerebellar cortex.

Table 5 shows the regional distribution of HCarn-Carn synthetase activity in the brains of three patients with dominantly inherited cerebellar disorders, where brain tissue was frozen at -80°C within 60 min of the patients' deaths. Brain from neurologically normal individuals could not be obtained at autopsy within such a short interval after death, and it was impractical to explore regional variations in enzyme activity in autopsied material that was less fresh, because of the rapid inactivation of the synthetic enzyme after death. However, we have no evidence that any abnormality in HCarn metabolism was present in these cerebellar ataxia patients.

The only consistent finding from the three brains studied was the relatively low activity of the synthesizing enzyme which was found in the cerebellar cortex.

Table 6 shows the regional distribution of homocarnosinase activity in autopsied human brain. Based on the limited number of brains examined,

Table 4. HCarn content of different regions of adult human brain.

Brain region	HCarn content (μmol/g) wet wt	
Olfactory tubercle	0.29 ± 0.04	(6)
Olfactory bulb	0.31 ± 0.01	(3)
Frontal cortex	0.28 ± 0.04	(25)
Frontal cortex	0.34 ± 0.05	(13)*
Temporal cortex	0.28 ± 0.02	(28)*
Occipital cortex	0.42 ± 0.07	(24)
Cerebellar cortex	0.62 ± 0.06	(24)
Caudate nucleus	0.22 ± 0.04	(29)
Putamen	0.42 ± 0.06	(12)
Globus pallidus I and II	0.68 ± 0.11	(11)
Nucleus accumbens	0.23 ± 0.04	(12)
Substantia nigra	0.79 ± 0.06	(28)
Inferior olive	1.05 ± 0.09	(10)
Dentate nucleus	1.34 ± 0.14	(14)
Medial dorsal thalamus	0.40 ± 0.05	(7)
Pons	0.39 ± 0.09	(6)
Cervical cord	0.57 ± 0.14	(4)

Results represent mean ± S.E.M. Figures in brackets indicate number of brains analyzed.
* Values in biopsies; all other values listed are for autopsied brain.

Table 5. Regional distribution of HCarn-Carn synthetase activity in autopsied human brain.

Brain region	Subjects*					
	M,46	M,61	F,65	M,46	M,61	F,65
	HCarn synthesis			Carn Synthesis		
Frontal cortex	3.57	3.47	2.72	16.07	16.70	12.48
Occipital cortex	4.98	3.80	6.62	21.96	16.22	22.68
Cerebellar cortex	0.10	2.23	0.54	1.68	10.43	2.64
Caudate nucleus	1.65	4.08	1.36	9.33	22.78	8.64
Putamen-globus pallidus	7.63	3.11	5.62	26.78	15.81	19.46
Substantia nigra	5.93		3.18			
Inferior olive			2.36			
Dentate nucleus		3.93			31.73	
Cervical cord	7.22	8.44	3.54	30.34		11.68

Dipeptide synthesis is expressed in nmol/h/g wet wt.

* Brains examined were from 3 patients (sex and age in years indicated) who died from hereditary cerebellar ataxias, where brain was frozen at -80°C within 60 min of death.

Table 6. Regional distribution of homocarnosinase activity in autopsied human brain.

Brain region	Subjects* F,44	M,66	F,31	M,58
	Homocarnosinase activity			
Frontal cortex	104	92	118	146
Occipital cortex		114	104	217
Cerebellar cortex	41	71	54	37
Caudate nucleus		116	90	41
Putamen-globus pallidus		122	114	149
Substantia nigra		103	150	114
Inferior olive		158	78	111
Dentate nucleus	274	355	205	184

Hydrolysis of HCarn is expressed in nmol 25 min/g wet wt.
* Brains examined were from 4 subjects (sex and age in years indicated).

enzyme activity appeared to be relatively high in the dentate nucleus and relatively low in cerebellar cortex.

HCarn and its metabolizing enzymes in the homocarnosinosis patient

Table 7 shows the concentrations of HCarn and other amino compounds found in the CSF of the homocarnosinosis patient (L.S, Case 1) and of 43 control patients undergoing lumbar puncture as part of the diagnostic study of their illnesses. None of these patients were known to suffer from any disorder involving abnormalities in the concentration of any amino acid. Although it would have been preferable to have obtained data from CSF of healthy subjects only, ethical considerations made it virtually impossible to secure such data. The concentration of HCarn was found to be markedly elevated in the patient's CSF (25 standard deviations above the mean control value). In contrast, the concentrations of other measured amino compounds in the patient's CSF were within 1-2 standard deviations of the mean. CSF collected from the subarachnoid space overlying the biopsy site immediately before removal of the frontal cortical biopsy contained HCarn in a concentration of 52.6 µmol/l, almost the same as that found in this patient's CSF obtained by lumbar puncture.

The identification of HCarn in the CSF of the homocarnosinosis patient was established by isolating the dipeptide from CSF by using the amino acid analyzer as a preparative instrument. Strong acid hydrolysis of the isolated compound yielded equimolar amounts of GABA and histidine, but no trace of β-alanine. In addition, when the unhydrolyzed HCarn-Carn fraction was subjected to high voltage paper electrophoresis, only one spot of radioactivity was observed on electrophoretograms which co-migrated with authentic HCarn. The presumed HCarn in this patient's brain biopsy (see below) was similarly shown to consist entirely of HCarn.

Table 7. Free amino compounds in CSF of control subjects and in CSF of homocarnosinosis patient.

Amino compound	43 control subjects*	Homocarnosinosis Patient (Case 1)
Taurine	6.6 ± 1.7	7.9
Phosphoethanolamine	4.9 ± 1.6	6.8
Aspartic acid	0.3 ± 0.3	0.1
Threonine	31.4 ± 8.3	34.0
Serine	26.8 + 6.0	30.4
Asparagine	8.5 ± 2.5	7.4
Glutamic acid	1.8 ± 0.7	0.8
Glutamine	602.1 ± 118.6	576.6
Glycine	6.4 ± 1.7	9.8
Alanine	30.0 ± 6.5	38.8
Citrulline	2.1 ± 0.8	1.6
α-Amino-n-butyric acid	18.1 ± 4.7	25.3
Valine	0.1 ± 0.2	trace
Methionine	3.0 ± 0.9	3.7
Isoleucine	4.8 ± 1.5	5.5
Leucine	12.9 ± 3.5	15.7
Tyrosine	8.0 ± 2.1	7.9
Phenylalanine	8.7 ± 3.5	9.4
GABA	ND	ND
Tryptophan	1.0 ± 0.7	0.5
Ethanolamine	15.4 ± 4.8	17.6
Ornithine	5.5 ± 1.9	5.8
Lysine	27.5 ± 5.1	27.4
ε-N-Methyllysine	1.5 ± 2.0	2.8
Histidine	12.7 ± 2.7	11.2
Homocarnosine	1.8 ± 1.8	48.2←
γ-Aminobutyryl-lysine	0.1 ± 0.2	ND
Arginine	19.7 ± 4.3	22.6

Results (mean ± S.D.) are expessed in μmol/l.
* From Perry et al., 1975b.
ND - not detectable

Table 8 shows the contents of HCarn and other ninhydrin positive amino compounds found in the frontal cortical biopsy of the homocarnosinosis patient (Case 1) and in frontal cortical biopsies from 13 control subjects. These control biopsies consisted of epileptic foci as well as presumably normal tissue which had to be sacrificed by the neurosurgeon to gain access to a deep-seated tumour. The mean content of HCarn found in these frontal cortical biopsies (0.34 ± 0.19 μmol/g, n = 13) was similar to that observed in autopsied frontal cortex (0.28 ± 0.18 μmol/g, n = 25) obtained from neurologically normal subjects. HCarn content was elevated approximately 4-fold (5 standard deviations above the mean control value) in the patient's frontal cortex, while the contents of GABA and histidine, the constituent amino acids of HCarn, as well as the contents of other measured amino compounds were within 1-2 standard deviations of the mean control levels. Although HCarn content was markedly elevated in the patient's brain, a related GABA-containing dipeptide, GABA-lysine, was present in only trace amounts.

Figure 1 shows the activity of HCarn-Carn synthetase found in the biopsy of frontal cortex from the homocarnosinosis patient and in single biopsies of frontal or temporal cortex from 12 control subjects. Dipeptide formed in vitro was approximately 3-5 times greater when β-alanine was used as a substrate in the assay than when GABA was used as substrate. It can be seen that HCarn-Carn synthetase activity varied widely among the control subjects. This variability did not appear to result from variability in the assay methodology, since assays of the same biopsy on different days produced almost identical results. It is apparent, however, that the activity of this enzyme in the homocarnosinosis patient's biopsy is not excessive and in fact is relatively low in comparison to the control specimens.

Table 8. Amino compounds in frontal cortical biopsies of control patients and of the homocarnosinosis patient.

Amino compound	Controls*	Homocarnosinosis Patient Case 1
Glycerophosphoethanolamine	0.93 ± 0.38	0.88
Taurine	0.51 ± 0.52	1.33
Phosphoethanolamine	1.87 ± 0.51	1.95
Aspartic acid	1.16 ± 0.29	1.74
Glutathione**	2.39 ± 0.46	2.98
Threonine	0.26 ± 0.14	0.17
Serine	0.42 ± 0.07	0.45
Aspargine	0.06 ± 0.03	0.02
Glutamic acid	7.38 ± 1.79	9.94
Glutamine	5.25 ± 1.21	4.22
Proline	0.08 ± 0.05	0.03
Glycine	0.52 ± 0.17	0.60
Alanine	0.35 ± 0.16	0.29
Citrulline	0.08 ± 0.04	0.08
α-Amino-n-butyric acid	0.04 ± 0.03	0.02
Valine	0.14 ± 0.04	0.09
Cystine	0.01 ± 0.01	tr
Methionine	0.02 ± 0.01	0.01
Cystathionine	0.69 ± 0.52	0.36
Isoleucine	0.03 ± 0.01	0.01
Leucine	0.08 ± 0.02	0.04
Tyrosine	0.05 ± 0.02	0.04
Phenylalanine	0.05 ± 0.02	0.03
β-Alanine	0.01 ± 0.01	tr
γ-Aminobutyric acid	0.69 ± 0.19	0.76
Tryptophan	0.01 ± 0.01	0.01
Ethanolamine	0.12 ± 0.07	0.07
Ornithine	0.03 ± 0.02	tr
Lysine	0.12 ± 0.04	0.08
Histidine	0.09 ± 0.04	0.07
Homocarnosine	0.34 ± 0.19	1.29 ←
γ-Aminobutyryl-lysine	0.03 ± 0.02	tr
Putrescine	0.01 ± 0.01	tr
Arginine	0.08 ± 0.03	0.05

Results (mean ± S.D.) are expressed in μmol/g wet wt.
* 16 biopsies from 13 patients mean ± S.D.
** Total glutathione, expressed as the reduced compound

Fig. 1. Synthesis of HCarn (left column) and Carn (right column) in vitro in biopsy specimens of frontal or temporal cerebellar cortex. The arrows indicate the values found in the biopsy from the homocarnosinosis patient.

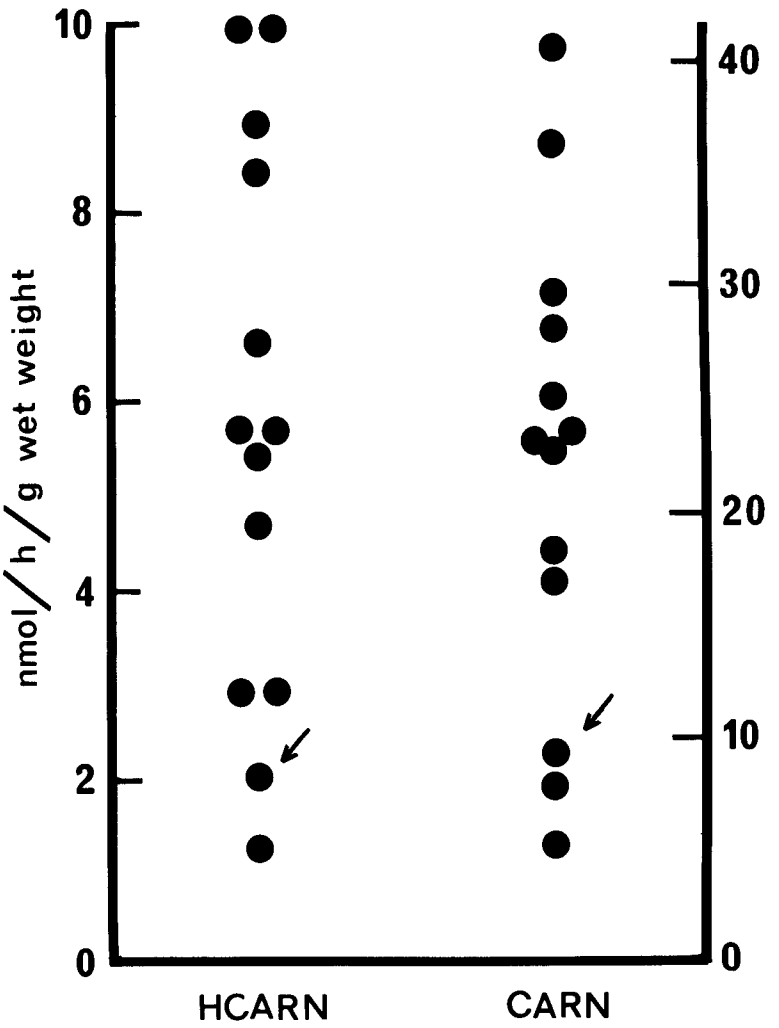

10
8
6
4
2
0
nmol / h / g wet weight
40
30
20
10
0
HCARN
CARN

Figure 2 shows the activities of the HCarn catabolizing enzyme found in the cortical biopsy from the homocarnosinosis patient, and in single frontal or temporal cortical biopsies from 15 control subjects. Homocarnosinase activity varied between 69 and 287 nmol/25 min/g wet wt among the control biopsies, whereas hydrolysis of HCarn was undetectable in the biopsy from the homocarnosinosis patient. When equal portions of biopsies from the homocarnosinosis patient and from a control subject were mixed and incubated, the homocarnosinase activity of the control specimen was not reduced. Thus, the absent homocarnosinase activity in the patient's brain was probably not due to the presence of an enzyme inhibitor present in the brain supernatant. For complete details of results see Perry et al., 1979d.

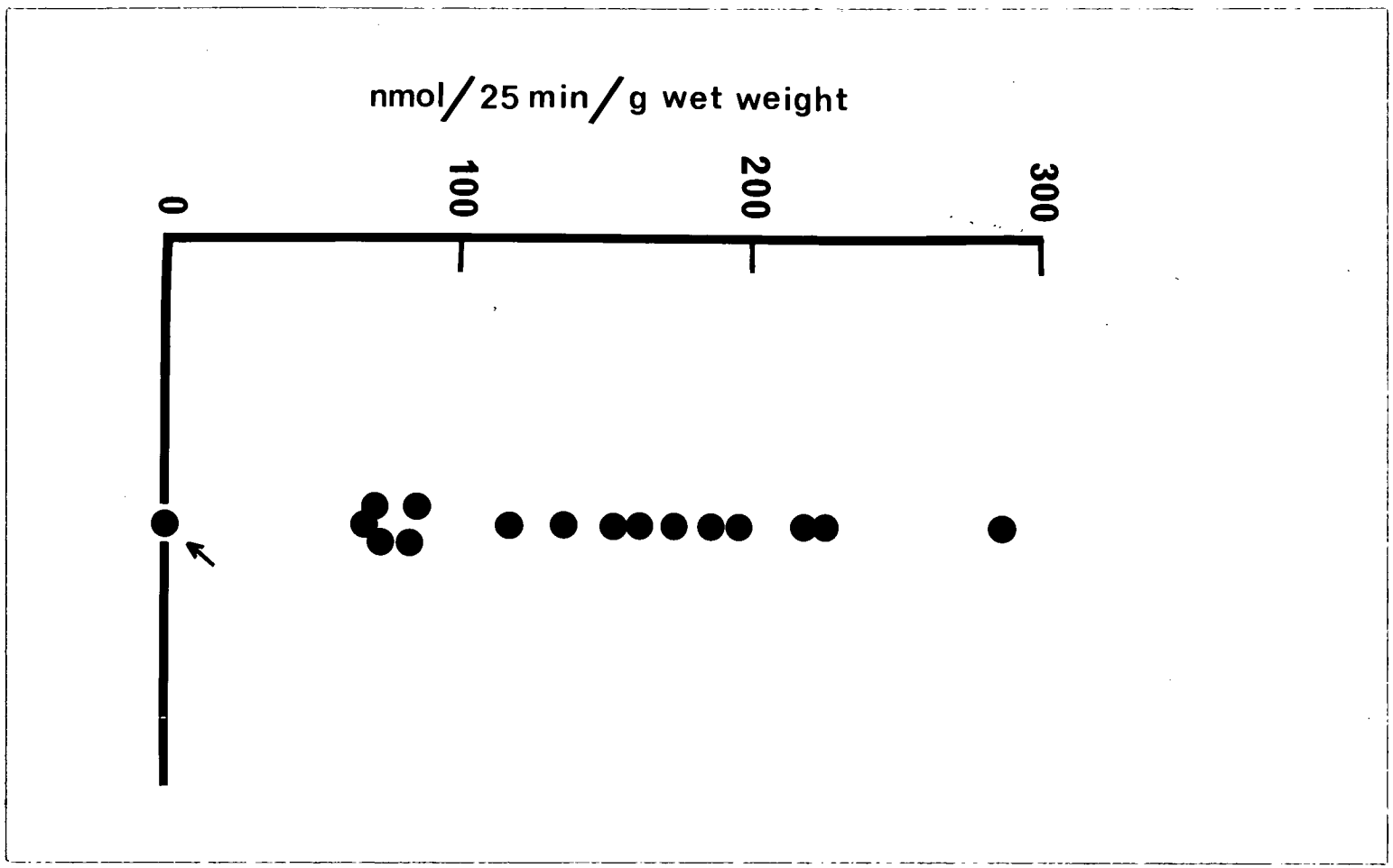
Neuropathology of homocarnosinosis brain

During the neurosurgical removal of the brain specimen from the homocarnosinosis patient, marked atrophy of the cortical gyri was observed, and there was marked enlargement of the subarachnoid space over the exposed brain area. However, histological examination of the brain biopsy from the homocarnosinosis patient was unremarkable. The meninges were normal and uninflamed. The cortex had a normal structural appearance without neuronal depletion or astrocytic proliferation. The neurons were normal, and neurofibrillary tangles and senile plaques were not found. The underlying white matter was normal and without demyelination.

Pharmacological properties of HCarn

In preliminary experiments 6 rats and 6 mice received twice daily intraperitoneal injections of 500 mg/kg HCarn sulfate for 21 days. In addition, 5 rats received a single intravenous injection of 120-150 mg/kg

Fig. 2. Homocarnosinase activities *in vitro* in biopsy specimens of frontal or temporal cortex. The arrow indicates the value for the homocarnosinosis patient.


nmol/25 min/g wet weight
0
100
200
300

HCarn sulfate. No behavioural or neurological abnormalities could be observed in any of the animals treated with HCarn. Amino acid analyses performed on the brains of several of the rats chronically treated with HCarn indicated that their brain HCarn content was within the normal range. Therefore, since the lack of behavioural response following peripheral HCarn administration may have been due to the failure of the dipeptide to penetrate the blood brain barrier, further experiments were designed to examine the behavioural effects resulting from administration of HCarn directly into the rat CNS.

Intraventricular injection of HCarn and related compounds

The behavioural effects following injection of HCarn sulfate into the right lateral cerebral ventricle of the rat are shown in Table 9. Two to three minutes following an intraventricular injection of 250 nmol HCarn sulfate, the rat would rapidly circle the cage for 15-20 seconds, lie flat on its stomach for 5-10 seconds, and then repeat this sequence for 5-10 minutes. Intraventricular injection of higher doses of HCarn sulfate (500-1000 nmol) resulted in teeth chattering, twisting of the body on its long axis and generalized tonic-clonic convulsions lasting 20-25 minutes. No attempt was made to quantify the hyperactivity or the convulsions produced by HCarn. An equimolar injection of NaCl, Na_2SO_4 (to control for the sulfate present in the Sigma Chemical preparation of HCarn) or two structurally related dipeptides, Carn and glycyl-L-histidine, had no effect on the behaviour of the animals.

Intrastriatal injection of HCarn and related compounds

The intrastriatal injection of 300 nmol NaCl or Na_2SO_4 had no behavioural effect on any of the animals tested (Table 10). The injection of

Table 9. Effects of intraventricular injection of HCarn and related compounds on behaviour in the rat.

Drug	Dose of Drug (nmol)	Number of Rats	Behavioural Effect	Duration
HCarn	100	4	No effect	
HCarn	250	4	↑ Locomotor activity	5-10 min
HCarn	500	4	↑↑↑ Locomotor activity, clonic convulsions	10-15 min
HCarn	1000	4	Jumping, teeth chattering, Tonic-clonic convulsions	20-25 min
NaCl	1000	4	No effect	
Na_2SO_4	1000	4	No effect	
β-Alanyl-L histidine	1000	4	No effect	
Glycyl-L histidine	1000	4	No effect	

All drugs were injected in a volume of 4 µl into the right lateral cerebral ventricle over a three minute period.

Table 10. Repetitive myoclonic jerking induced by intrastriatal injection of HCarn and related dipeptides.

Drug	Dose (nmol)	Number of rats responding	Onset (min)	Duration (min)	Intensity
NaCl	300	0/12			
Na_2SO_4	300	0/10			
HCarn-sulfate	75	1/10	2	9	1
HCarn-sulfate	150	9/11	2.6 ± 1.7	26 ± 15	2.2 ± 1.6
HCarn-sulfate	300	10/10	4.8 ± 3.8	52 ± 29	4.0 ± 1.3
HCarn-sulfate-free*	300	6/6	4.0 ± 1.0	59 ± 26	3.5 ± 1.2
L-histidine*	300	0/6			
GABA-DL-alanine	300	6/6	12 ± 4	21 ± 8	2.3 ± 1.4
α-aminobutyryl -DL-alanine	300	0/6			
GABA-DL-leucine	300	2/6	5,8	16,23	1,1
α-aminobutyryl DL-leucine	300	0/6			
GABA-choline	300	0/6			
GABA-L-lysine	300	0/6			
glycyl-GABA	300	0/6			
Carnosine	300	2/10	0,4	23,47	1,2
L-alanyl-L-histidine	300	0/10			
glycyl-L-histidine	300	0/10			
L-histidine	300	0/10			
L-glutamyl-L- histidine	300	0/10			

Values represent mean ± S.E.M.

* Subjected to washing on Dowex cation exchange resin.

All drugs where injected in a volume of 1 µl/3 min into the right anterior striatum. Intensity of myoclonus was scored using a 5-point scale (5 max, see methodology).

150-300 nmol HCarn sulfate into the right anterior striatum of the rat produced rhythmic jerking movements of the contralateral forepaw and the head and neck. The myoclonus (terminology of Marsden et al., 1975) always began in the left forepaw which would abruptly lift off the floor and return somewhat more slowly. After approximately 7-10 minutes following the onset, the myoclonus would spread to the head and the neck, which turned in a direction opposite to the jerking forelimb. The myoclonus occurred at variable frequencies ranging from 10-60 movements/minute. In addition, most of the rats receiving the highest dose of HCarn experienced periods of tonic convulsions primarily involving the left forepaw. Several animals receiving this dose of HCarn showed markedly reduced motor activity following the end of the dyskinesias as well as salivation and teeth chattering. The behavioural syndrome observed following intrastriatal injection of 150-300 µmol HCarn was found to be qualitatively identical to that produced by intrastriatal injection of 3 nmol picrotoxin in two rats studied.

A further experiment was performed to test the possibility that the myoclonus produced by HCarn sulfate may have resulted from an interaction between the sulfate ion and the HCarn rather than from the dipeptide itself. In this experiment, HCarn-free of sulfate was prepared by applying the dipeptide to a Dowex 50 cation exchange resin (H^+ form) and extensively washing with H_2O to remove the sulfate. The sulfate-free HCarn was subsequently eluted from the column with NH_4OH and taken to dryness in a rotary evaporator. The intensity and duration of the myoclonus produced by intrastriatal injection of the sulfate-free HCarn was found to be similar to that resulting from administration of HCarn sulfate, (see Table 10) thus indicating that the contralateral myoclonus was a consequence of injection of the dipeptide itself. Intrastriatal injection of L-histidine

(free base) carried through the same washing procedure as HCarn sulfate had no effect on the behaviour of the animals (Table 10).

In additional experiments, the effects of intrastriatal injection of compounds structurally related to HCarn was explored. Replacement of the histidine in HCarn by DL-alanine (GABA-DL-alanine) or by DL-leucine (GABA-DL-leucine) reduced but did not abolish all myoclonic activity. However, no myoclonic activity was detected when L-histidine was replaced by the basic amino acid L-lysine (GABA-L-lysine) or by choline in an ester linkage (GABA-choline). Alterations in the N-terminal residue of HCarn resulted in a drastic loss of activity. Reduction in the separation of the amino group and the peptide bond from 4 carbons (HCarn) to three carbons (Carn) almost completely abolished the myoclonus while a further reduction in the separation to two carbons (L-alanyl-L-histidine, glycyl-L-histidine) or complete removal of the GABA (L-histidine) resulted in a total loss of activity. Myoclonus was also absent following injection of γ-glutamyl-L-histidine or the α-aminobutyryl dipeptides of DL-alanine and DL-leucine.

Modification of HCarn-induced myoclonus by GABA and the potent GABA-mimetic muscimol

GABA (300 or 3000 nmol in 1 µl H_2O) was injected into the right striatum of 6 rats 5-10 minutes after the myoclonus had been established by intrastriatal injection of 300 nmol HCarn. Neither dose of GABA was found to have any effect on the intensity or the frequency of the myoclonic convulsions. Likewise, intrastriatal injection of 3000 nmol GABA three minutes before (n = 3) or after (n = 3) injection of 300 nmol HCarn failed to prevent the development of normal myoclonus within 7 minutes of the HCarn injection. On the other hand, intrastriatal injection of 150 nmol muscimol administered 5-10 minutes after the myoclonus had been established

completely blocked the myoclonus in 3 of 3 animals studied within two minutes of injection, while a lower dose of 30 nmol muscimol blocked the involuntary movements in 2 of 3 animals, also within two minutes of injection. Injections of 300 or 3000 nmol NaCl had no effect on the intensity or frequency of the jerking activity.

Extent of diffusion of injected HCarn in rat brain

In a separate experiment the extent of diffusion of intrastriatally injected HCarn was determined. Four rats received an intrastriatal injection of 300 nmol ^{14}C-labelled HCarn in a volume of 1 µl and were subsequently sacrificed 10 or 30 minutes following the injection. Ten minutes following injection 60% of the injected radioactivity was found in the ipsilateral striatum while a total of 11% was detected in other adjacent ipsilateral areas, primarily the cerebral cortex overlying the striatum. No radioactivity could be found in the contralateral 1/2 forebrain. Thirty minutes after intrastriatal infusion 41% of the radiolabel was detected in the ipsilateral striatum while 9% was found in adjacent regions, again primarily in the overlying cortex.

Crayfish claw preparation

Continuous perfusion of HCarn at concentrations of 1-25 mM for periods of up to 15 minutes had no effect on the amplitude of the contraction of the claw during electrical stimulation of the excitatory nerve. Figure 3 shows the effect of HCarn on the GABA-induced inhibition of the contraction of the claw from one of five preparations studied. Unfortunately, in all the crayfish claw preparations studied, the inhibitory response to GABA gradually and slightly increased with time. This upward shift of the dose-

response curve for GABA was similar in the presence or absence of continuous perfusion of HCarn. Thus, although the dose-response curves for GABA taken before and after perfusion with HCarn are not identical, the data from Fig. 3 indicate that perfusion with a high (25 mM) concentration of HCarn did not block the inhibitory action of GABA. HCarn in concentrations of 1-25 mM was also found to be ineffective in antagonizing the action of GABA in four other claw preparations examined. In contrast, as shown in Fig. 3 the inhibitory response to GABA was completely blocked by the known GABA antagonist for the crayfish, picrotoxin, at a concentration of 1 mM.

Crayfish stretch receptor preparation

Administration of HCarn (.001-20 mM) had no effect itself on the firing rate of the stretch receptor in the 7 preparations studied. The effect of HCarn and of Carn on the GABA-induced inhibition of the firing rate from one of 5 preparations studied is shown in Fig. 4. HCarn in a concentration of 1 mM had no effect on the inhibitory response to GABA, whereas higher concentrations of 3 and 10 mM HCarn slightly potentiated the response to GABA. However, this potentiation was also observed with the same high concentration of the related dipeptide, Carn. Both HCarn and Carn, in concentrations of 3-10 mM were also found to slightly potentiate the action of GABA in the 4 other stretch receptor preparations examined.

Electropharmacology of HCarn in rat brain

The iontophoretic application of HCarn or Carn alone (80-200 nA, 0.5 to 5 minute application) had no striking effect on the discharge rate of the 8 cortical neurons investigated. The mean firing rate was usually unchanged although in 2 of the 8 cortical neurons HCarn and Carn produced a slight

Fig. 3. Effect of HCarn and of picrotoxin on the GABA-induced inhibition of the amplitude of contraction of the crayfish claw. GABA was administered in van Harreveld's solution by a 0.5 ml injection made into a rubber tube attached to the tip of the propodite. HCarn and picrotoxin were administered in van Harreveld's solution by a constant 10 minute perfusion. The excitatory nerve to the claw was electrically stimulated once every 50 sec. Isometric contractions of the dactytopodite were recorded using a Grass transducer and polygraph. Control dose response curves for GABA were made before and 10 minutes after perfusion with HCarn. The degree of inhibition was quantitated by measuring the area (mm^2) on the polygraph recording of the GABA-induced depression of the control contraction amplitudes.

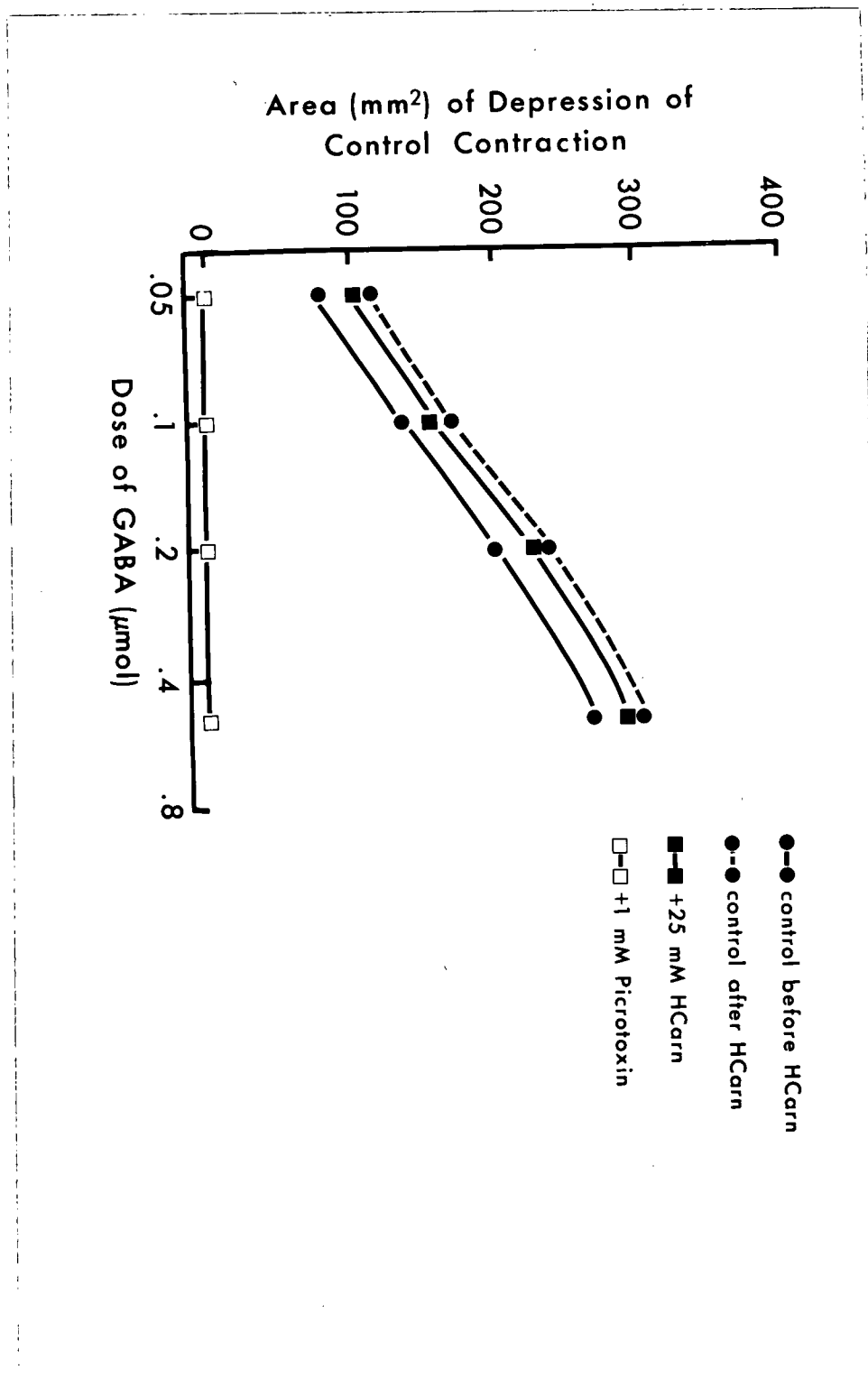

Area (mm²) of Depression of Control Contraction
400
300
200
100
0
.05
.1
.2
.4
.8
Dose of GABA (μmol)
●—● control before HCarn
●--● control after HCarn
■—■ +25 mM HCarn
□—□ +1 mM Picrotoxin

Fig. 4. The effect of HCarn and Carn on the GABA-induced inhibition of the crayfish stretch receptor firing rate. All drugs were administered in van Harreveld's solution and were applied as described in the methodology. Control dose-response curves for GABA made before and after dipeptide applications were identical.

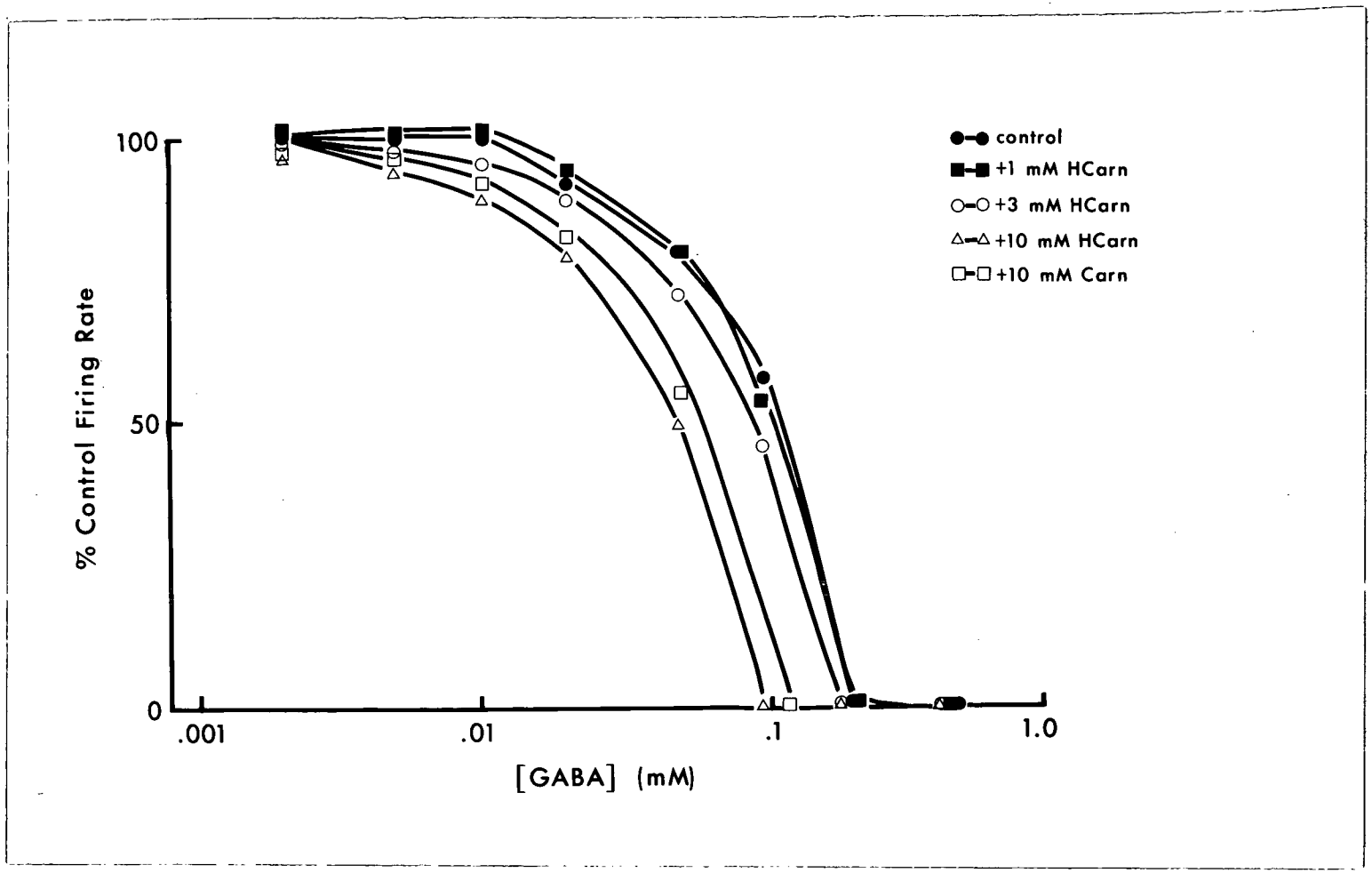
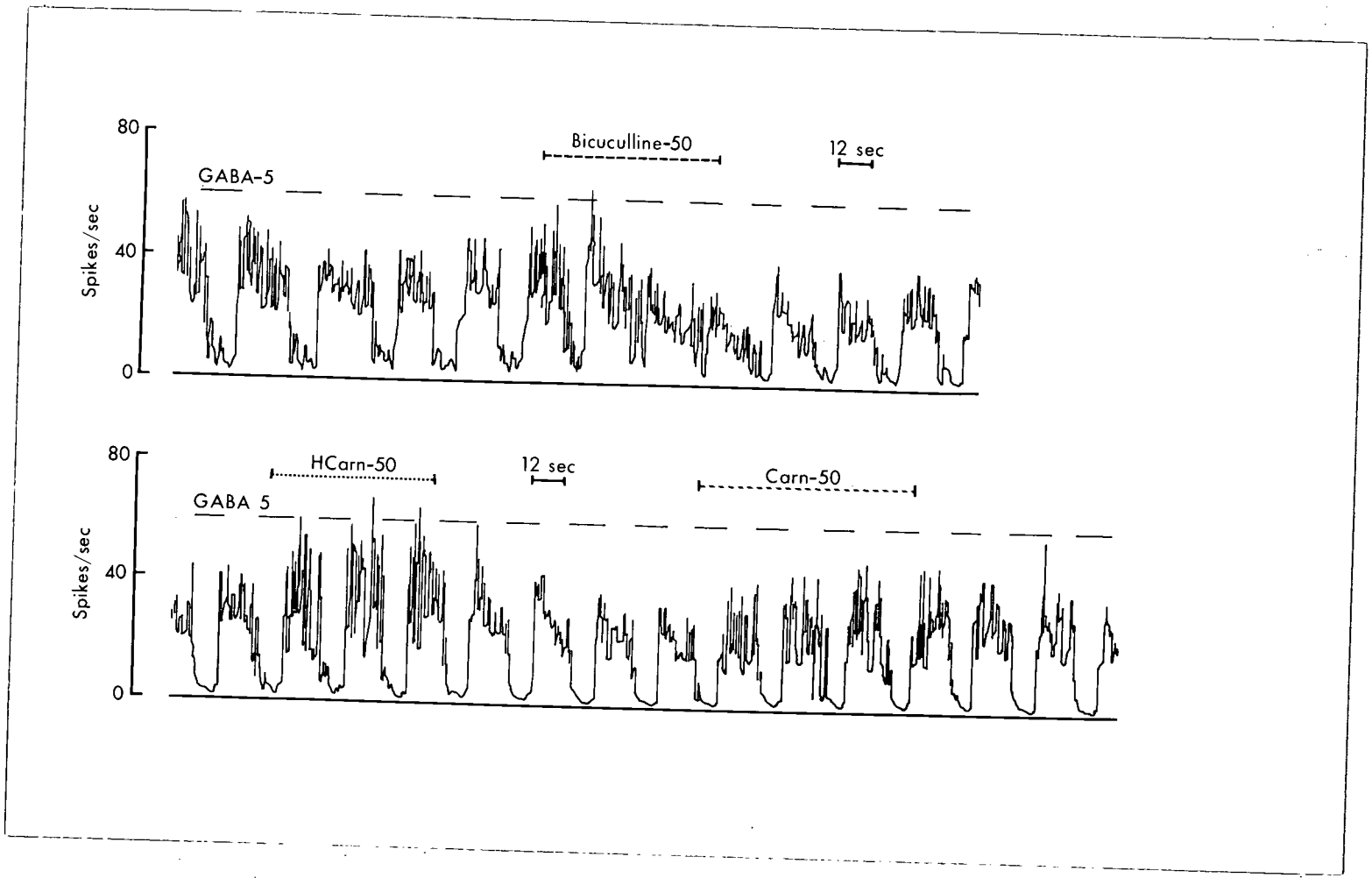

100
50
0
% Control Firing Rate
.001
.01
.1
1.0
[GABA] (mM)
control
+1 mM HCarn
+3 mM HCarn
+10 mM HCarn
+10 mM Carn

Fig. 5. Extracellular recording from a rat cerebral cortical neuron. The effects of HCarn (50 nA), Carn (50 nA) and of bicuculline (50 nA) on the GABA-induced (5 nA) inhibition of firing rate. Lower trace is a continuation of upper trace. The cortical neuron was driven with a continuous application of 3 nA DL-homocysteate.


80
40
0
Spikes/sec
GABA-5
Bicuculline-50
12 sec
80
40
0
Spikes/sec
GABA 5
HCarn-50
12 sec
Carn-50

increase in the discharge. A more consistent effect (as shown in the example in Fig. 5) was an increase in the irregularity of the firing rate produced by HCarn, and Carn to a lesser extent. In 4 of 4 thalamic neurons studied application of HCarn (80-100 nA) and Carn, to a somewhat lesser extent resulted in a slight increase in the firing frequency.

As illustrated in Fig. 5, HCarn and Carn (50-200 nA) did not block the depressant response to GABA (1-20 nA) in any of the 8 cortical or 4 thalamic neurons studied. In contrast, application of bicuculline (50 nA) partially antagonized the GABA-induced depression of spike rate in the three cortical neurons tested. In addition, as shown in the example in Fig. 5, application of bicuculline markedly depressed the firing frequency of the cortical neurons.

VI. DISCUSSION

HCarn and its metabolizing enzymes in control human brain

The content of HCarn in human brain varies considerably among different regions, being 6 times higher in the dentate nucleus than in the caudate nucleus. Since experimentally induced alterations in brain GABA content (Perry and Hansen, 1973a; Perry et al., 1979b), and in histidine content (Quinn and Fisher, 1977) have been shown to alter HCarn content in brain in several mammalian species, it was expected that the distribution of HCarn in brain might parallel that of GABA and/or histidine. However, in 10 regions of 19 autopsied brains studied, no statistically significant correlation ($p > 0.05$) could be found between brain GABA or histidine content and HCarn levels. For example, in some brain regions, such as the globus pallidus, substantia nigra and dentate nucleus, the content of both GABA and HCarn is high. However, in the nucleus accumbens, and olfactory tubercle where GABA content is high, HCarn content is low. The inferior olive,

which has the second highest HCarn content of the brain regions studied, has a relatively low GABA content. A similar lack of correlation between GABA and histidine levels and HCarn content has also been reported by Young and Snyder (1973) in guinea pig brain, suggesting that HCarn levels are not determined solely by the availability of its constituent amino acids. This lack of correlation might be the result of formation of HCarn from a pool of GABA and/or histidine which bears no consistent relationship to the total content of GABA measured in each brain region. For example, HCarn may possibly be formed *in vivo* from a pool of GABA derived from putrescine, as suggested by Nakajima *et al.* (1974), rather than from the pool of GABA synthesized by glutamic acid decarboxylase. A second possibility which has yet to be tested is that HCarn is not formed *in vivo* from GABA and histidine but is synthesized by the decarboxylation of γ-glutamyl-L-histidine, a compound which has been detected in bovine brain (Kakimoto and Konishi, 1976). If this were the case, then one might expect that the regional distribution of γ-glutamyl histidine should parallel that of HCarn. It was also expected that the distribution of activities of the presumed HCarn synthesizing enzyme might parallel that of HCarn content. However, a statistical analysis showed no significant correlation ($p > 0.05$) between the activity of HCarn-Carn synthetase measured *in vitro* and HCarn content in the three brains studied. This lack of correlation may have been due to the fact that the *in vitro* assay conditions employed did not accurately reflect the chemical environment of the enzyme *in vivo*. Alternatively, it is conceivable that HCarn may be synthesized *in vivo* by a completely different enzyme than by the one measured *in vitro*.

The reason for the overwhelming preponderance of HCarn in human brain in the face of greater *in vitro* synthesis of Carn, is not clear. GABA content in biopsied human brain is about 100 times greater than that of

β-alanine, so that the relative concentrations of the two substrates available for HCarn-Carn synthetase may determine the amounts of the two dipeptides formed. However, in the olfactory bulb, where Carn is present in moderate amounts, the relative proportions of GABA and β-alanine are the same as in other regions of human brain. It may also be that subcellular location of the synthetic enzyme favours HCarn synthesis, or that differences in activity between carnosinase and homocarnosinase account for HCarn being the chief or only imidazole dipeptide found in most brain regions in man.

The finding that HCarn-Carn synthetase in human brain could only be detected in supernatants and not in crude extracts is in agreement with the observations of Skaper et al. (1973) in rat brain. HCarn-Carn synthetase in human brain supernatants forms Carn about 3-5 times as rapidly as it forms HCarn under the assay conditions used. This ratio is similar to that found by Ng and Marshall (1978) in various regions of rat brain, where about two times as much Carn as HCarn was synthesized in vitro. The apparent K_m values of the synthetic enzyme for β-alanine (1.8 mM) and for GABA (8.8 mM) found in human brain are similar to those reported for this enzyme in rat brain (Ng and Marshall, 1978). Although activities of HCarn-Carn synthetase varied widely among the 12 biopsied human cortical specimens assayed (Fig. 1), the mean syntheses of HCarn (6.0 ± 2.9 nmol/h/g wet wt) and of Carn (23.1 ± 10.3 nmol/h/g wet wt) were, respectively, 7 and 14 times greater than those reported for cerebrum in the rat (Ng and Marshall, 1978).

Lenney and co-workers have purified both carnosinase (Lenney, 1976) and homocarnosinase (Lenney et al., 1977) from hog kidney. Carnosinase is activated by several metal ions, especially Co^{2+} and Mn^{2+}, is relatively heat-stable, and hydrolyzes Carn but not HCarn. Homocarnosinase is

activated best by Co^{2+}, and to a lesser extent by Mn^{2+}, is unstable at 50°C, and hydrolyzes both Carn and HCarn. Lenney and co-workers (Lenney, 1976; Lenney et al., 1977) detected carnosinase activity but no homocarnosinase activity in rat brain. Ng et al. (1977) and Ng and Marshall (1978) reported the degradation of both radioactive Carn and HCarn in the brains of rabbits and rats. However, they did not use any activating metal ions in their degradative enzyme assays, and their incubation mixtures contained dithioerythritol, which would probably have chelated any Co^{2+} and Mn^{2+} ions naturally present in the brain extracts they used. There is therefore considerable doubt as to whether they measured activity of the same dipeptidase purified by Lenney. On average, cleavage of HCarn in the present in vitro conditions was about 1000 times greater than has been reported by Ng and Marshall (1978) for the cerebrum of the rat.

The present results confirm the claim of Lenney et al. (1977) that homocarnosinase is activated by Co^{2+}, and that it is markedly inhibited by addition of dithiothreitol. The finding of readily measurable homocarnosinase activity in both biopsy and autopsy samples of human brain, where Lenney et al. (1977) were unable to detect activity in rat (Lenney et al., 1977) or human brain (Lenney, personal communication) can probably be explained by the increased sensitivity of the homocarnosinase assay when ^{14}C-HCarn is used as substrate. Lenney et al. (1977) used non-radioactive HCarn as a substrate in their homocarnosinase assay, and measured the histidine formed by a fluorometric technique, after reaction with o-phthaladehyde. However, when homocarnosinase activity is determined in hog kidney, the richest known source of homocarnosinase, the values obtained using either the present assay system or that of Lenney et al., (1977) are similar.

In general, homocarnosinase activity was found to be evenly distributed among different regions of human brain. The two exceptions were the dentate nucleus, which had the highest activity of all regions tested, and the cerebellar cortex in which homocarnosinase values were uniformly low. Although no statistically significant correlation was obtained between homocarnosinase activity and HCarn content in the 4 brains studied, homocarnosinase is most likely an important enzyme in the catabolism of HCarn since a lack of this enzyme is associated with an excessive amount of HCarn in brain and CSF (see below).

HCarn and its metabolizing enzymes in the homocarnosinosis patient

Before these biochemical studies were undertaken, several possibilities existed to account for the 20-40-fold increases in HCarn concentration found in the CSF of the 4 individuals with homocarnosinosis. HCarn might have leaked from the CNS into the CSF as a result of failure of some high affinity reuptake mechanism for HCarn that is normally present. Excessive amounts of HCarn might have been formed in brain as a result of increased activity of the enzyme HCarn-Carn synthetase, or as a result of an abnormally high content in brain of one of its substrates, GABA or histidine. Finally, the elevated CSF concentrations of HCarn could have been the consequence of an elevated brain HCarn content secondary to failure of the enzyme that degrades this dipeptide.

Since the content of HCarn itself was found to be markedly elevated in frontal cortex of the biopsied patient, it is unlikely that an abnormality of transport of HCarn between CSF and brain is involved in the disorder. As well, the normal content of GABA and histidine in the patient's brain makes it unlikely that the excessive HCarn was the result of an abnormally high content of either of these two precursors.

Finally, the results of the *in vitro* assays of HCarn-Carn synthetase and of homocarnosinase in the patient's brain homogenates strongly suggest that it is a marked deficiency of the latter enzyme that is responsible for the increased HCarn content in brain. The fact that the related dipeptide, GABA-Lys, was not increased in the patient's brain (Table 8) is in agreement with a report by Kumon *et al.* (1970) that GABA-Lys is normally hydrolyzed by a dipeptidase different from homocarnosinase.

Initially it was unclear whether the progressive neurological disorder present in the three siblings with homocarnosinosis was causally related to the elevated levels of HCarn found in their CSF specimens. It is likely that a deficiency of homocarnosinase is also present in the brain of their mother, since on two occasions, 4 years apart, she has had HCarn concentrations in her CSF as high as those of her three neurologically affected children. The observations that she continues to present no evidence of neurological or mental deterioration, and that her retinae remain normal, suggest that homocarnosinase deficiency with resultant elevation of HCarn content in brain is unlikely to be the *sole* cause of the progressive neurological disorder in her children.

On the other hand, it is conceivable, although unlikely, that the elevated brain HCarn is causally related to the neurological syndrome, but that there is a marked variability in the expressivity of the neurological abnormalities, such as seen with the genetically-inherited disorder phenylketonuria (Perry *et al.*, 1973c). Such a variability in expressivity of the neurological symptoms in the Norwegian family might result from the presence or absence of other genetic traits influencing the expression of the neurological syndrome.

It is likely that two different hereditary disorders are present in the homocarnosinosis family. The first, a neurological disease of unknown

etiology, is likely to have been inherited as an autosomal recessive, and has affected 3 of 4 children in the sibship. Both parents are presumably clinically unaffected heterozygotes. The second disorder, homocarnosinosis, probably does not by itself produce any clinical symptoms. Since it is present in family members of both sexes and has occurred in two successive generations, homocarnosinosis might conceivably be inherited in autosomal dominant fashion, with the mother and three of her children being heterozygotes for brain homocarnosinase deficiency.

However, as Harris (1975) has pointed out, in most inborn errors of metabolism where enzyme activity is completely or almost completely absent in affected homozygotes, one expects to find in heterozygotes about 50% of the enzyme activity that is present in normal homozygotes. Gene mutations causing enzyme deficiencies are unlikely to produce virtual absence of enzyme activity in heterozygous individuals, and thus are unlikely to be inherited in autosomal dominant fashion. The mother of the three neurologically affected homocarnosinosis patients presumably lacks homocarnosinase activity in her brain, and therefore must have received a double dose of the mutant gene. Although there is no known consanguinity between the parents in the affected pedigree (Sjasstad et al., 1976), it is possible that the father (whose HCarn concentration in CSF is normal) is actually a heterozygote for homocarnosinase deficiency . Thus, three of their children may have inherited two different disorders as autosomal recessives, one of them homocarnosinosis, and the other a degenerative brain disorder of unknown etiology. The second of the three neurologically affected patients is the father of two clinically normal young children. When these children become old enough for diagnostic lumbar punctures to be ethically suitable, measurement of HCarn in their CSF may help clarify the mode of inheritance of homocarnosinase deficiency.

Intraventricular and intrastriatal injection of HCarn

Intraventricular injection of HCarn in the rat produced hyperexcitability and in high doses convulsions. These findings are similar to the results of Turnbull et al. (1972) for the rat and are also consistent with observations that intracisternal administration of HCarn produces convulsions in the dog (Hayashi, 1965) and shortens the pentobarbital sleeping time in the mouse (Bissette et al., 1978). Since a feature common to many convulsant agents is the ability to reduce brain GABAergic activity (Woodbury, 1980) it seemed reasonable to test whether HCarn has the properties of a GABA antagonist. This hypothesis is strengthened by the observations that HCarn is able to displace ^{3}H-GABA from binding sites in rat and human membrane fragments.

Intrastriatal injection of HCarn into the unanaesthesized rat was found to produce repetitive myoclonic jerking activity identical to that produced by picrotoxin, a convulsant which blocks many of the electrophysiological effects of GABA in the mammalian CNS (Hill et al., 1973; Okamoto and Quastel, 1976; Frederickson et al., 1978). This observation, as well as the demonstration that the myoclonus could be suppressed by the potent GABA-mimetic muscimol is consistent with the hypothesis that HCarn possesses an antagonist effect on the action of GABA. The fact that GABA itself had no effect on the HCarn-induced myoclonus is likely due in part to the rapid uptake of this amino acid. Although the mechanism by which intrastriatally administered drugs produce myoclonus is not known, most, if not all, compounds which have been found to produce contralateral myoclonus in the rat following intrastriatal injection are either antagonists of the action of GABA or inhibitors of GABA synthesis. For example, compounds such as picrotoxin, bicuculline, d-tubocurare and benzyl-penicillin, which produce myoclonic jerking activity upon intrastriatal injection in the rat

(McKenzie and Viik, 1975; Tarsey et al., 1978) have been shown to block the GABA-induced depression of firing rate of cat cerebral cortical neurons (Hill et al., 1973). Likewise, allyl-glycine and thiosemicarbazide, which produce forelimb myoclonus following intrastriatal injection (Tarsey et al., 1978) are known to be inhibitors of the enzyme responsible for the synthesis of GABA, glutamic acid decarboxylase (Woodbury, 1980). Intrastriatal administration of another compound, 1-(3,4-dihydroxyphenyl) piperazine also induces similar dyskinetic movements in the rat (Costall et al., 1976). However, this compound has not yet been tested for any possible antagonism of the depressant action of GABA.

The data presented in Table 10 show the effects of changing various chemical substituents of the HCarn molecule on myoclonic activity. Unfortunately, this phase of the study was limited to a large degree by the lack of availability of compounds structurally related to HCarn. Based on the small number of compounds studied, these data demonstrate that a free amino group separated by three methylenic spacers from a substituted amide group is essential, although not sufficient for maximal myoclonic activity. Thus, one might hypothesize that HCarn and GABA-DL-alanine, by virtue of their free amino group and structural similarity to GABA might be capable of binding to GABA receptors, but have no or little intrinsic inhibitory activity as a result of replacement of the ionizable COOH group of GABA (which is essential for neuronal inhibitory activity [Buu et al., 1975]) by the substituted amide group.

About 40-60% of the injected dose appeared in the striatum 10-30 minutes following injection of ^{14}-C-labelled HCarn, while about 10% was found in adjacent structures, primarily in the overlying cortex. Although Tarsey et al. (1978) have been unable to demonstrate a myoclonic response to picrotoxin following injection into the overlying cortex and other brain

regions of the rat, Robin and co-workers (1980) have recently found that topical application of picrotoxin to cortex overlying the striatum of the rat produces the dyskinetic syndrome. Thus, the possibility exists that the contralateral myoclonus observed in the present study may have been partially a consequence of an action of HCarn at cortical and/or other extrastriatal brain areas.

Pharmacological properties of HCarn in crayfish and in cortex and thalamus of the rat

The results of the two types of invertebrate experiments and the preliminary microiontophoretic experiment in the rat do not support the hypothesis that HCarn is an antagonist at receptors for GABA. Furthermore, the observations that HCarn does not exhibit antagonism of the action of GABA or has GABA-like properties in the three preparations studied despite the fact that the dipeptide is moderately potent in mammalian ^{3}H-GABA binding experiments, renders questionable the use of data obtained in *in vitro* GABA-binding studies as predictors of biological potency *in vivo*. However, much more work is needed before reaching any final conclusion. For example, the possibility exists that HCarn may in fact act as an antagonist of GABA-induced depression in other regions of mammalian CNS which were not studied in the present investigation. For example, several laboratories have been unable to convincingly demonstrate GABA antagonism in the mammalian cortex using picrotoxin and bicuculline (Curtis and Felix, 1971; Krnjevic *et al.*, 1966; Godfraind, *et al.*, 1970) although these compounds effectively block GABA-mediated inhibition in other regions of the central and peripheral nervous system (Krnjevic, 1974). This finding suggests that CNS receptors for GABA vary in their susceptibility to blockade by antagonists. Thus, it is conceivable that while HCarn may not block the effects

of GABA in the crayfish, or at the rat cortical or thalamic neurons studied in the present investigation, or at the cat brain stem neurons studied by Turnbull et al. (1972), it might block GABA-mediated inhibition at rat striatal GABA receptors. In order to study the effects of HCarn on GABA-mediated inhibition in the striatum one might wish to employ intracellular techniques in an in vitro striatal slice preparation in which known concentrations of HCarn could be applied. Using such a procedure to record membrane conductance and potential changes one would be able to describe more quantitatively how HCarn might alter the interaction of GABA with its receptor.

Another possibility is that the convulsive and myoclonic properties of HCarn are due in part to an antagonism of GABA-mediated inhibition in certain key areas of the brain through a mechanism other than direct pharmacological antagonism of the action of GABA such as depletion of the store of GABA at the nerve endings or blockade of release of this neurotransmitter. This has been proposed as the mechanism of convulsive action of tetanus toxin (Woodbury, 1980; Collingridge et al., 1980).

Alternatively, HCarn may produce its CNS stimulant action through a mechanism not directly related to the GABAergic system of the brain. Further experiments will be required to test these and other hypotheses.

SECTION B -BRAIN GABA AND PSYCHIATRIC DISEASE

I. ABSTRACT

GABA content was measured in the nucleus accumbens, caudate nucleus, frontal cortex and thalamus of autopsied brains from patients who had died with schizophrenia or Huntington's chorea (HC) and in brains from control subjects. The mean content of GABA was reduced by 20-25% in all 4 brain regions of the schizophrenic patients as compared to the control group. However, the differences were found to be statistically significant for only the caudate nucleus and thalamus. A similar reduction in GABA content was observed in the nucleus accumbens and thalamus of the HC patients. Extraneous factors such as age of patient at death, interval from death-to-freezing of brain, immediate cause of death, and prolonged neuroleptic drug treatment did not readily explain the observed reduction in GABA content in schizophrenic brain, although the possible influence of other complicating factors has yet to be resolved. The results of the investigation are suggestive of an association between a deficiency of GABAergic function in brain and some forms of schizophrenia.

II. HISTORICAL REVIEW

Dopamine hypothesis of schizophrenia

Schizophrenia[1] is defined as a group of chronic psychotic disorders involving certain characteristic delusions, hallucinations or types of thought disorders (Draft version of the Third Diagnostic and Statistical Manual of the American Psychiatric Asssociation, Spitzer et al., 1978). Recent investigations into the causes of schizophrenia have been predicated on the belief that a biochemical lesion is present in these disorders. Such a notion is supported but not proven by observations that the symptoms of schizophrenia can be ameliorated or exacerbated by drugs which act on neurotransmitter systems in the CNS (Hornykiewicz, 1978).

Currently the most popular biochemical theory of schizophrenia is the dopamine hypothesis. This hypothesis essentially states that the behavioural alterations observed in schizophrenia are the result of either overactivity of dopaminergic neurons or of dopamine receptor "supersensitivity". There is evidence from histochemical and biochemical studies for the existence of at least four distinct dopaminergic neuronal pathways (Hornykiewicz, 1978): the nigrostriatal system, originating in the pars compacta of the substantia nigra and terminating in the caudate nucleus, putamen, and possibly the nucleus accumbens; the mesolimbic system, which originates in the ventral tegmental area (nucleus paranigralis in human brain) and projects to the nucleus accumbens, olfactory tubercle, amygdala, and frontal cortex; the mesocortical system, also arising in midbrain and projecting to prefrontal and temporal areas of the cerebral cortex; and the

---

[1] Throughout my thesis whenever the term "schizophrenia" is used, it is used to refer to the group of disorders classified as schizophrenia.

tubero-infundibular system, originating in the arcuate nucleus of the hypothalamus and terminating in the median eminence.

Evidence in support of the dopamine hypothesis of schizophrenia is entirely circumstantial in nature and is primarily derived from pharmacological observations employing agents which have an action on brain dopaminergic mechanisms. For example, all drugs which are clinically effective in the treatment of schizophrenia have been shown to block many of the biochemical (Iversen et al., 1976; Scatton et al., 1977), behavioural (Pijnenburg et al., 1976; Cools et al., 1976; Gianutsos and Lal, 1976; Lal, 1976) and electrophysiological (Ben-Ari and Kelly, 1976; Spehlmann and Norcross, 1978; but see Feltz, 1978 and Skirball and Bunney, 1979) actions of dopamine. In addition, schizophrenic symptoms can often be exacerbated in psychiatric patients or even produced in normal subjects as a result of administration of drugs such as L-DOPA and amphetamine which increase brain dopaminergic activity (Meltzer and Stahl, 1976).

The pharmacological evidence implicating brain dopamine in schizophrenia has led to numerous studies seeking more direct biochemical evidence in support of this hypothesis. Measurement of cerebrospinal fluid (CSF) levels of dopamine metabolites offers one method for measuring central dopaminergic activity in patients with schizophrenia. To date, however, the concentrations of two such dopamine metabolites, homovanillic acid and 3,4 dihydroxyphenylacetic acid, have not been shown to be significantly different in the CSF of schizophrenic patients from control values (Persson and Ross, 1969; Berger et al., 1980).

A second means of examining the possible neuronal mechanisms underlying neurological and psychiatric disorders is through biochemical analyses performed on autopsied human brain. Unfortunately, much of the data arising from such studies is contradictory and inconclusive, probably due in part

to the lack of an accurate and reproducible dissection procedure for the human brain. For example, Bird et al. (1979) have recently reported a significant increase in the concentration of dopamine in the nucleus accumbens and anterior perforated substance in autopsied brains of schizophrenic patients as compared to controls, while no significant differences were found in other areas of the limbic striatum or in the caudate nucleus. On the other hand, Crow and co-workers (Crow et al., 1979) failed to detect any significant increase in dopamine content in the nucleus accumbens, but did observe a significant increase in the caudate nucleus. In contrast to both of these studies, Farley et al. (1977) were unable to detect any significant difference in dopamine content in either of these two regions of schizophrenic as compared to control brain.

A more consistent finding is the observation of increased density of "dopamine receptors" in several regions of schizophrenic brain as measured by ^{3}H-haloperidol and ^{3}H-spiperone binding to brain membrane fragments (Owen et al., 1978; Lee and Seeman, 1980). However, the results of such binding studies must be interpreted with caution since no evidence is available which conclusively demonstrates that ^{3}H-haloperidol binding sites are identical with functional dopamine receptors. Furthermore, the important question of whether the increased receptor density is secondary to prolonged neuroleptic medication remains to be resolved (Clow et al., 1980).

GABA-dopamine interaction in the CNS: A GABA hypothesis of schizophrenia

It has also been suggested that one of the biochemical disturbances in schizophrenia might involve an excess or a deficiency of other neurohumoural substances which normally oppose or modulate the effects of dopamine. For example, Roberts (1972) has speculated that schizophrenia might

arise from a deficiency of the neurotransmitter γ-aminobutyric acid (GABA) which might normally exert an inhibitory action upon dopaminergic neurons. Although the precise nature of the interaction between GABA and dopaminergic systems in the mammalian CNS is not understood, some pharmacological evidence is available suggesting an inhibitory action of GABA on neurons in areas of the brain such as the nucleus accumbens and caudate nucleus receiving a dopaminergic input as well as a direct inhibitory action on the dopaminergic neurons themselves. For example, the potent GABA-mimetic muscimol has been shown to antagonize the increase in locomotor activity resulting from injection of either dopamine or of the dopamine agonists ergometrine and apomorphine in the rat nucleus accumbens (Scheel-Kruger et al., 1977a). Moreover, the contraversive head turning evoked by intrastriatal dopamine is blocked by intrastriatal injection of GABA (Cools and Janssen, 1976). In an electrophysiological study, Bernardi et al. (1975) demonstrated that iontophoretically applied GABA consistently depressed neuronal activity of caudate neurons producing an increase in membrane conductance and hyperpolarization.

Experiments designed to test the possibility that GABAergic neurons may modulate transmitter release from dopaminergic nerve terminals have so far yielded conflicting results. Both GABA and the GABA antagonist picrotoxin have been shown to facilitate ^{3}H-dopamine release evoked by 20 mM K^{+} in rat striatal slices (Starr, 1979). On the other hand, in an in vivo study, Bartholini and Stadler (1977) demonstrated that perfusion of the head of the cat caudate nucleus reduced the spontaneous release of dopamine, this effect being blocked by the GABA antagonists bicuculline and picrotoxin.

The demonstration of neurons containing glutamic acid decarboxylase (the marker enzyme for GABAergic neurons) originating in the rat striatum

and nucleus accumbens and terminating in the substantia nigra and ventral tegmental area (VTA), (Fonnum et al., 1978; Walaas and Fonnum, 1980) suggests that GABAergic neurons may provide a tonic inhibitory influence upon the dopaminergic cell bodies or dendrites of the substantia nigra and VTA. However, recent observations have shown that the interactions between the GABA and dopamine systems in these two brain regions are likely to be exceedingly complex. Increasing the content of GABA in the pars reticulata of the substantia nigra produces an increase in the firing rate of pars compacta neurons and behavioural stimulation similar to that produced by dopaminergic agonists (Dray et al., 1977; Scheel-Kruger et al., 1977b; Grace and Bunney, 1979). The latter findings imply that the effects of GABA on dopaminergic transmission in the substantia nigra may be mediated through the involvement of an inhibitory interneuron. The possible influence of GABAergic neuronal activity upon the dopaminergic cell bodies or dendrites of the VTA is also unclear. Injection of muscimol into the caudal VTA of the rat produces an increase in locomotor behaviour and aggression while injection of this GABA agonist into the rostral VTA results in behavioural sedation (Scheel-Kruger 1979). Thus, in view of the complex and incompletely understood relationship between the GABA and dopamine neuronal systems in the mammalian CNS, it becomes apparent that one cannot assume that the net effect of decreased brain GABAergic activity will necessarily be a corresponding increase in dopaminergic activity. In fact, one might logically argue that the symptoms of schizophrenia could even arise from excessive activity of certain brain GABAergic neurons in key areas of the brain in which GABAergic neurons inhibit tonically active inhibitory interneurons.

Neuroanatomical sites underlying the biochemical disturbances of schizophrenia

The anatomical sites of the biochemical disturbances which cause schizophrenia have not yet been determined. However, if excessive dopaminergic activity is related to schizophrenia then it seems reasonable to expect to find in schizophrenic brain pathological changes in those areas receiving a dopaminergic input such as the basal ganglia and the limbic system, as well as in areas of the frontal cortex receiving dopaminergic projections. This proposal finds some support in the observations that schizophrenic or other psychiatric symptoms have been reported in patients having lesions in limbic brain regions (Malamud, 1967; Escobar and Chandel, 1977; Brun and Gustafson, 1978; Torrey and Peterson, 1974) and in frontal cerebral cortical areas (Avery et al., 1971; Carlson, 1977; Ruff and Russakoff, 1980).

With respect to basal ganglia involvement in schizophrenia, Weinberger, et al. (1979) have recently observed lateral cerebral ventricular enlargement in a group of chronic schizophrenia patients, implying cerebral atrophy in brain areas such as the striatum. There also exists neurobehavioural evidence suggesting that disturbances of the basal ganglia may be related to the symptoms of some schizophrenic patients. For example, large lesions of the caudate nucleus of cats produce a bizarre behavioural syndrome in which the animal will compulsively seek out and follow any moving stimulus (Villablanca et al., 1978). Such behaviour is similar to that found in those schizophrenic patients suffering from a disorder of attention. An additional observation is the finding of stereotyped motor behaviour in both schizophrenic patients, and in experimental animals upon chemical stimulation of the basal ganglia (Ungerstedt et al., 1969). Furthermore, patients with Huntington's chorea (HC), a disorder characterized

by gross atrophy of the caudate as well as other less marked neuropathological changes in other brain areas, often manifest a broad range of psychiatric syndromes and may present with schizophrenic-like symptoms before the onset of any choreic movements (Folstein, 1979).

Thus, although schizophrenia is unlikely to be associated with a dysfunction in any single brain region, circumstantial evidence from the neuropathological and the neurobehavioural literature suggests an involvement of limbic and basal ganglia structures in some forms of schizophrenia.

III. HYPOTHESES TO BE TESTED AND EXPERIMENTAL OUTLINE

In the following pages I will describe a series of experiments designed to test the hypothesis that the content of GABA is significantly altered in the autopsied brains of patients dying with schizophrenia. The objective of this study was to further our knowledge regarding the neurochemical disturbances underlying schizophrenia and ultimately to lead to the development of more rational treatments for these disorders.

Four brain regions were examined: the nucleus accumbens, a structure of the mesolimbic system; the caudate nucleus, a basal ganglia region, the frontal cortex, and the thalamus. The first three areas had been circumstantially implicated in the pathogenesis of schizophrenia on the basis of neuropathological and pharmacological evidence. The choice of the thalamus was fortuitous, since it was the only other region of schizophrenic brain available to us for study.

The brain content of GABA in schizophrenic brain has been compared to the levels found in a control group of subjects as well as in patients dying with Huntington's chorea (HC). The HC group serves as an additional group having many features common to schizophrenia such as psychiatric and cognitive disturbances and prolonged neuroleptic medication.

In an additional series of experiments, we explored the effects of chronic administration of two antipsychotic drugs, chlorpromazine and haloperidol, on the content of GABA in the mesolimbic region of rat brain as well as on the activity of the GABA-synthesizing enzyme, GAD, in the rat striatum. The objective of this part of the study to determine whether long-term administration of two commonly used antipsychotic drugs could itself reduce brain GABA content in the mesolimbic area.

IV. METHODOLOGY

Chemicals

For sources of chemicals see Perry et al., 1979c.

Human brain specimens

The following brain specimens were obtained from W. W. Tourtellotte, Human Neurospecimen Bank, Los Angeles, California from dissections carried out there: nucleus accumbens from 7 control subjects, 8 schizophrenic patients and 5 HC patients; caudate nucleus and thalamus from one control subject and 7 schizophrenic subjects; thalamus from three schizophrenic patients; and frontal cortex from 9 schizophrenic patients. Thalamus specimens from 13 control subjects, 15 schizophrenic patients and 14 HC patients were supplied by Dr. E. D. Bird, Addenbrookes Hospital, Cambridge, England from dissections carried out there. In addition, caudate nucleus specimens from 3 schizophrenic patients were received from Dr. P. Seeman, University of Toronto, Ontario. The remaining brain specimens from the control and patient groups were dissected in our laboratory in Vancouver. All frozen brains obtained in Los Angeles and in Vancouver were dissected with the aid of the Atlas of the Basal Ganglia by Riley.

Nucleus accumbens was examined from a total of 19 control subjects, 12 patients with schizophrenia, and 12 patients with HC whose mean ages in

years (52 ± 4 S.E.M., 48 ± 4 and 54 ± 3 respectively) did not differ significantly ($p > 0.05$, F-test). Caudate nucleus was examined from 30 control subjects, 14 schizophrenic patients and 22 HC patients whose mean ages (55 ± 3, 49 ± 4 and 52 ± 2) did not differ significantly. Frontal cortex was examined from 25 control subjects, 13 schizophrenic patients and 19 HC patients, whose mean ages (52 ± 3, 48 ± 3 and 54 ± 2) did not differ significantly. Thalamus was examined from 31 control subjects, 22 schizophrenic patients, and 29 HC patients whose mean ages (58 ± 3, 62 ± 3 and 54 ± 2) did not differ significantly. The intervals between death of the patient and freezing of the brain ranged from 2 to 80 hours for the nucleus accumbens, and 2 to 56 hours for the caudate, thalamus, and frontal cortex. The male/female ratios in the control and patient groups for each brain region were not found to be significantly different ($p > 0.05$, chi-squared test) for any of the 4 brain regions.

Animals

Female Wistar rats (starting weight 100 g) were injected subcutaneously with either chlorpromazine, haloperidol, or 0.15 M NaCl once daily for 100 days. Injection volumes ranged between 0.1 and 0.25 ml. Chlorpromazine was given in a daily dosage of 20 mg/kg. Haloperidol for injection was given in a daily dosage of 3 mg/kg. Haloperidol-treated rats showed no local effects from the injections, and they gained weight over 100 days of treatment as well as did the saline-injected control animals. Chlorpromazine-treated rats developed subcutaneous inflammatory and fibrotic changes after repeated injections, and it was necessary to rotate injection sites to avoid sloughing of skin. The mean gain in body weight of the chlorpromazine-treated rats over 100 days was 10% less than that of rats injected with haloperidol or saline solution. Preliminary experiments had

shown that a daily chlorpromazine dosage of 25 mg/kg resulted in rats gaining weight very slowly, and that repeated intraperitoneal injections of chlorpromazine produced abdominal adhesions which led to intestinal obstruction and increased mortality.

Rat brain dissection

Most of the animals in each treatment group were sacrified 24 hours after the last drug injection. The remaining animals in each group were sacrified 35 days later, after a recovery period during which they received no treatment. Rats were killed by cervical dislocation. The entire brain was removed and immersed in liquid nitrogen within 25 to 45 seconds of the animal's death. 20 to 25 seconds following immersion, brains were removed from liquid nitrogen and allowed to thaw to a waxy consistency in a cold room at 4°C for approximately one minute. (If rat brains remained in nitrogen longer than 30 seconds, they cracked spontaneously, rendering accurate dissection impossible.)

Frozen rat brains were then dissected, with the aid of the atlas of Konig and Klippel (1963), in such a manner as to separate out both the mesolimbic area and the striatum. For complete details of brain dissection see Perry et al., 1979c.

Amino acid analyses

Amino acid analyses of human brain specimens and of mesolimbic areas of individual rat brains were performed as described in Section A.

Glutamic acid decarboxylase assay

GAD activity in rat striatum of individual rats was determined by measuring the rate of formation of $^{14}CO_2$ from L-[1-^{14}C]glutamic acid, as

described in Perry et al., 1979c.

Statistical analysis of data

Statistical analysis of the data derived from human brain specimens was carried out by using an analysis of variance followed by appropriate tests for comparisons between group means.

The biochemical data from all control and patient groups were first checked for homogeneity of within group variances and for normality of distribution using the Bartlett test and the Lilliefors-Kolmogorov-Smirnov goodness of fit test, respectively. If these two assumptions underlying the parametric statistical analysis were met then a one-way analysis of variance (F-test) was performed on the data for each brain region. If the level of significance of the F-ratio exceeded the critical value for the 0.05 significance level then control vs. schizophrenic and control vs. HC comparisons were made using Dunnett's two-way multiple comparison test at the 0.05 significance level. The numerator for the t-statistic used in the Dunnett's test is that of the two-sample t-test. However, the denominator uses the error mean square from the analysis of variance.

Since a regression analysis revealed that GABA values significantly declined with advancing age ($p < 0.05$) for the frontal cortex and thalamus, an analysis of covariance was performed on the data derived from these brain regions, using age as the covariate.

V. RESULTS

Brain GABA in schizophrenic and HC brain

Our earlier results were published in Perry et al. (1979a) and Table 11 brings the results from our human brain data up to date.

Table 11. GABA content (μmol/g wet wt) in post-mortem brain.

Brain region	Controls	Schizophrenia	Huntington's chorea
Nucleus accumbens	4.13 ± 0.33 (19)	3.00 ± 0.45 (12)	3.15 ± 0.42 (12)
Caudate nucleus	2.93 ± 0.15 (30)	2.20 ± 0.24* (14)	1.38 ± 0.17* (22)
Frontal cortex	1.69 ± 0.09 (25)	1.36 ± 0.12 (13)	1.40 ± 0.18 (19)
Thalamus	2.11 ± 0.13 (31)	1.70 ± 0.08* (22)	1.71 ± 0.10* (29)

Values are mean ± S.E.M. Number of subjects shown in parentheses.
* Values significantly different from controls, $p < 0.05$, (one-way analysis of variance followed by two-tailed Dunnett's test).

No statistically significant differences were found for the within-group variances of the control and patient groups for any of the four brain regions ($p > 0.05$, Bartlett test). As well, the distribution of GABA values for each group was not found to deviate significantly from normality ($p > 0.05$, Lillifors-Kolmogorov-Smirnov goodness of fit test). Therefore, since the assumptions of homogeneity of within-group variances and normality of distribution appeared to be met all biochemical data were subjected to the parametric statistical analysis.

The mean content of GABA was reduced by 20-25% in all 4 brain regions examined of the schizophrenic patients as compared to the control group (Table 11 and Figs. 6,7,8). However, the differences were found to be statistically significant for only the caudate nucleus and thalamus ($p < 0.05$). A similar reduction in GABA content was also observed in the nucleus accumbens, and thalamus of the HC patients while, as expected, (Perry et al., 1973b) the content of GABA in HC caudate was markedly reduced by about 50%. All of the values for GABA in the 4 regions of schizophrenic brain were found to be within two standard deviations of the control group mean, with the exception of one nucleus accumbens, one frontal cortex, and two caudate specimens with very low GABA levels.

Neuroleptic drug treatment and rat brain GABA content

Table 12 shows the mean contents of amino compounds in the mesolimbic area of brain of rats injected daily for 100 days with saline solution, chlorpromazine, or haloperidol. These rats were killed 24 hours after the last injection. Values are shown only for the 11 amino compounds present in sufficiently high concentration to guarantee accurate quantitation in the small specimens of mesolimbic tissue. GABA content was not altered by chronic treatment with chlorpromazine or haloperidol in high dosage. The

Fig. 6. Individual GABA values in nucleus accumbens of controls and patients. Horizontal lines represent mean values.

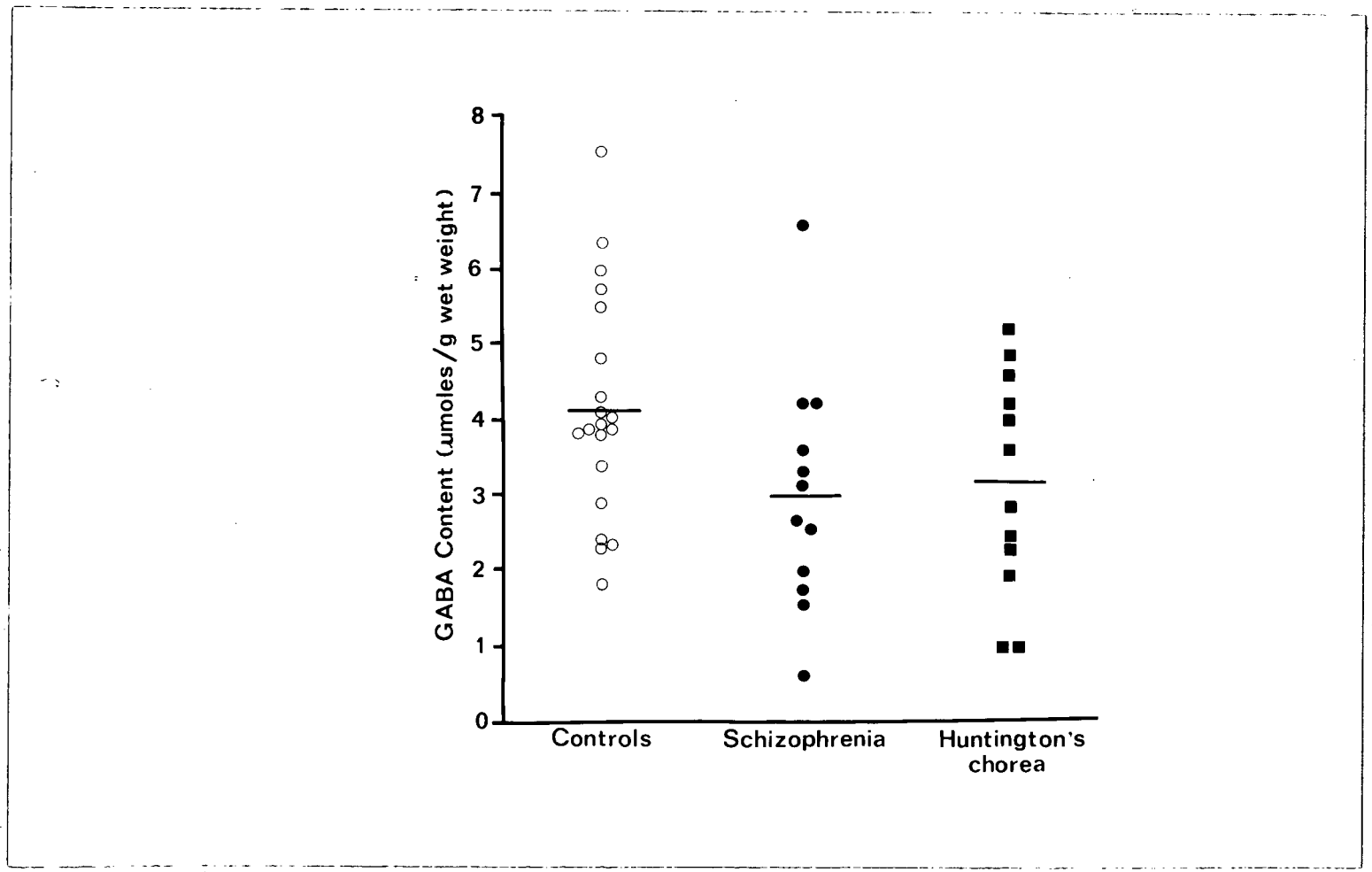

GABA Content (μmoles/g wet weight)
8
7
6
5
4
3
2
1
0
Controls
Schizophrenia
Huntington's chorea

Fig. 7. Individual GABA values in caudate nucleus of controls and patients. Horizontal lines represent mean values.

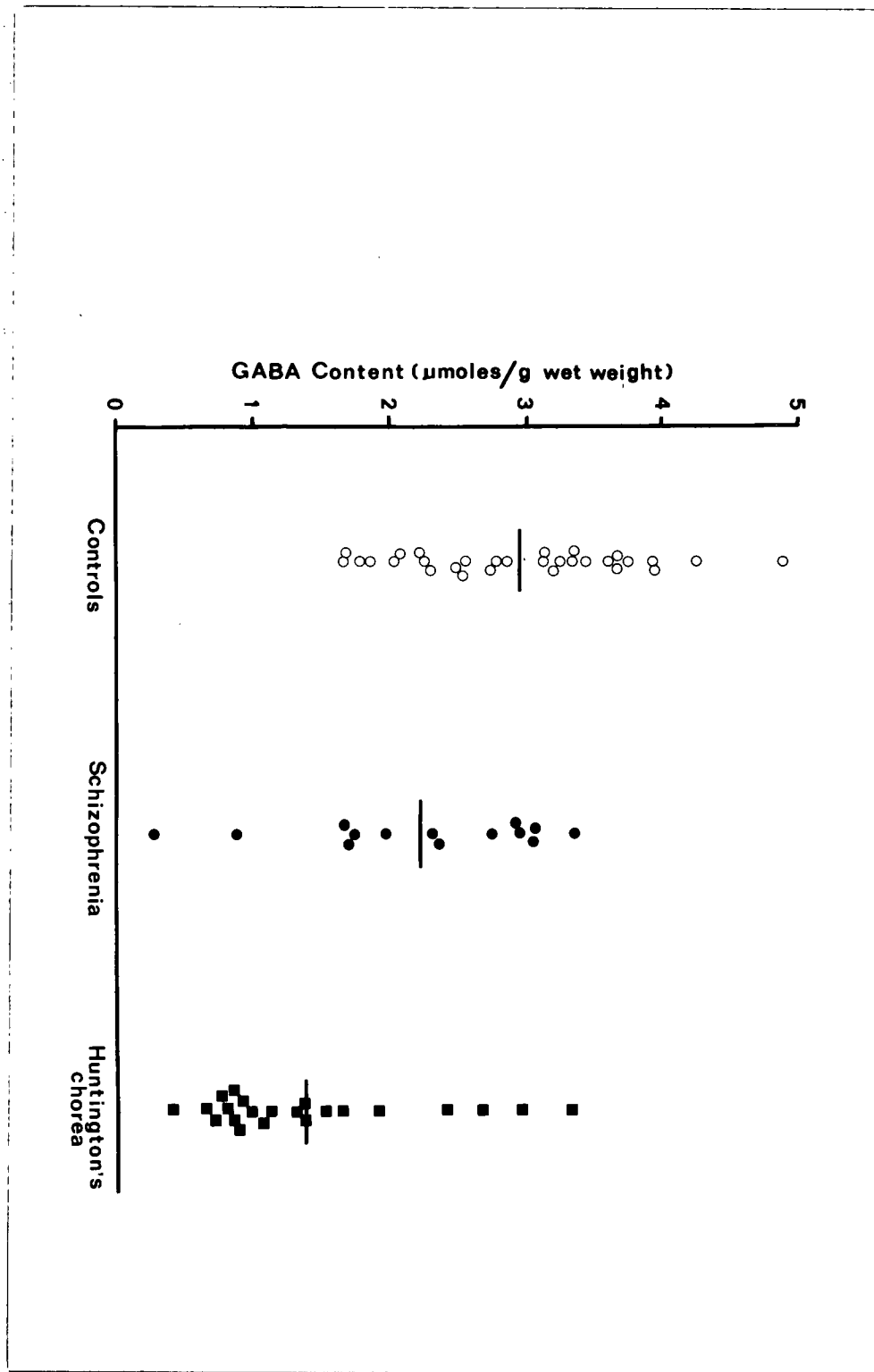

GABA Content (µmoles/g wet weight)
0
1
2
3
4
5
Controls
Schizophrenia
Huntington's chorea

Fig. 8. Individual GABA values in thalamus of controls and patients. Horizontal lines represent mean values.

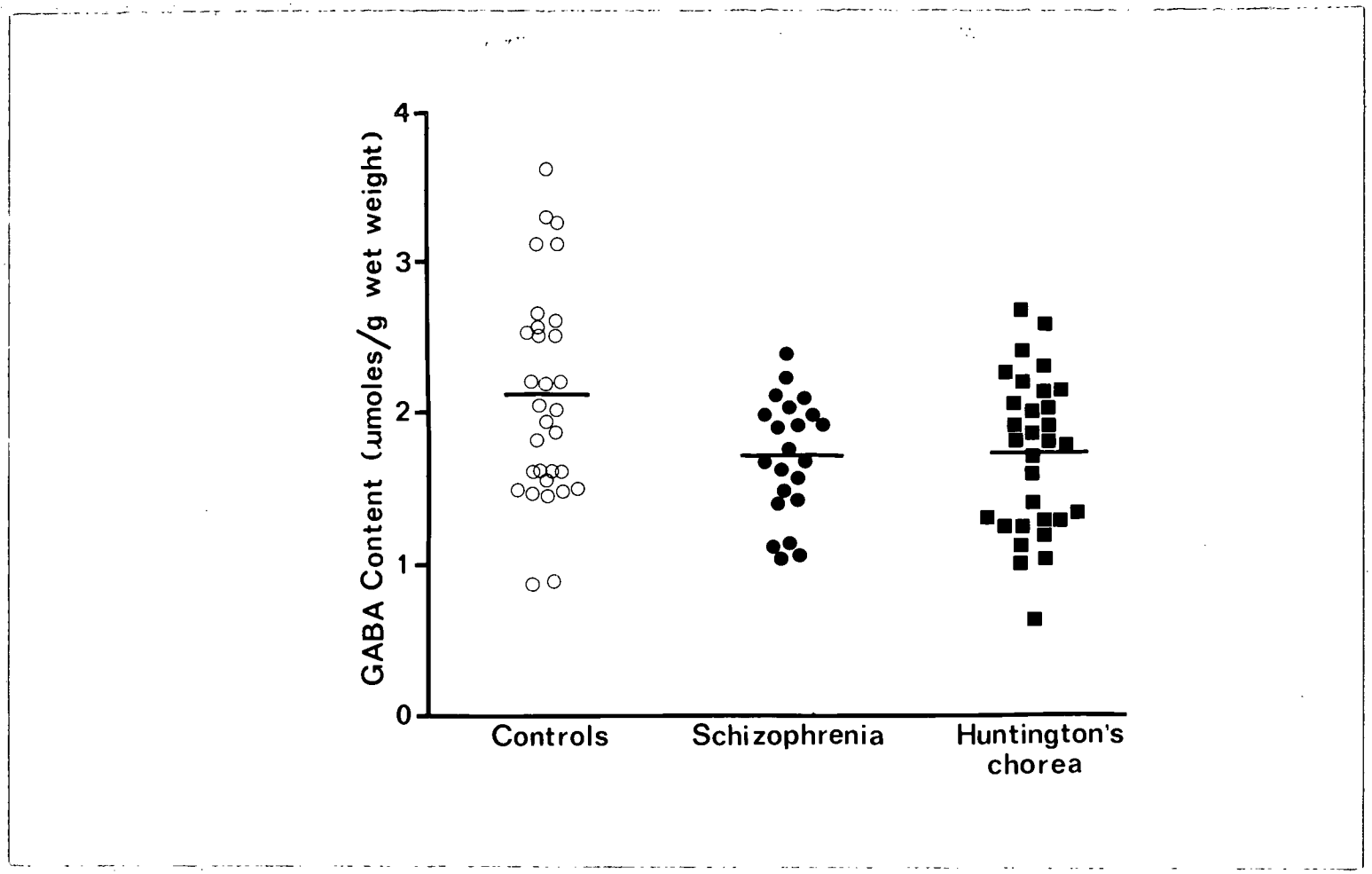

GABA Content (μmoles/g wet weight)
4
3
2
1
0
Controls
Schizophrenia
Huntington's chorea

Table 12. Amino compounds in mesolimbic area of rat brain 24 hours after 100 days chronic drug treatment*.

Compound	Controls (10)	Chlorpromazine (9)	Haloperidol (10)
Glycerophosphoethanolamine	0.37 ± 0.03	0.43 ± 0.03	0.40 ± 0.02
Taurine	5.43 ± 0.25	5.74 ± 0.28	5.26 ± 0.26
Phosphoethanolamine	1.22 ± 0.06	1.35 ± 0.09	1.28 ± 0.02
Aspartic acid	2.49 ± 0.06	2.56 ± 0.05	2.70 ± 0.05
Threonine	0.69 ± 0.04	0.62 ± 0.03	0.61 ± 0.03
Serine	0.97 ± 0.04	1.00 ± 0.03	0.95 ± 0.03
Glutathione	2.54 ± 0.10	2.76 ± 0.10	2.59 ± 0.09
Glutamic acid	10.97 ± 0.38	11.54 ± 0.26	11.56 ± 0.23
Glutamine	5.39 ± 0.15	5.36 ± 0.27	5.26 ± 0.19
Glycine	0.72 ± 0.02	0.68 ± 0.02	0.61 ± 0.02*
Alanine	0.67 ± 0.02	0.62 ± 0.03	0.61 ± 0.03
GABA	2.64 ± 0.11	2.65 ± 0.06	2.72 ± 0.11

* Values (mean ± S.E.M.) are expressed in µmol/g wet wt. Number of animals in parentheses. Total glutathione is expressed as the reduced compound. Chlorpromazine (20 mg/kg, s.c.) and haloperidol (3 mg/kg, s.c.) were administered daily for 100 days.
$p < 0.05$ (one way analysis of variance followed by Dunnett's test)

only change found was a small but significant reduction in glycine content in the haloperidol-treated rats.

Table 13 shows amino compound contents in the mesolimbic area of brain in rats which had received the same treatments, but which had been sacrificed 35 days after the last injection. Again there were no alterations of mean GABA content by the two antipsychotic drugs, and the haloperidol-induced change in glycine content was no longer present.

Mean striatal GAD activities are shown in the three groups of rats in Table 14, both for animals killed one day and 35 days after the end of drug treatment. Chronic treatment with chlorpromazine or haloperidol did not alter GAD activity in the striatum. For complete details of results see Perry et al., 1979c.

VI. DISCUSSION

GABA deficiency in schizophrenic brain

The results of our study demonstrate that mean GABA content is significantly reduced in thalamus and caudate of schizophrenic brain. The observation of low GABA in schizophrenic thalamus supports the findings of Roberts (1977) who found a significant decrease in GAD activity in the thalamus of schizophrenic brain. Although a reduction in GAD activity has also been reported for the schizophrenic caudate (Bird et al., 1979; Spokes, 1980) the authors concluded that the GAD deficiency was likely the result of ante-mortem hypoxia, which was more common in their schizophrenic patients than in their control subjects. In our preliminary report (Perry et al., 1979a) based on a smaller number of subjects, we found a significantly reduced GABA content in nucleus accumbens of schizophrenic brain. The finding that this reduction is now no longer statistically significant in the larger number of cases now examined emphasizes the importance of

Table 13. Amino compounds in mesolimbic area of rat brain 35 days after chronic drug treatment*

Compound	Controls (6)	Chlorpromazine (5)	Haloperidol (7)
Glycerophosphoethanolamine	0.38 ± 0.02	0.38 ± 0.02	0.43 ± 0.04
Taurine	6.29 ± 0.25	6.10 ± 0.42	5.92 ± 0.30
Phosphoethanolamine	1.33 ± 0.07	1.39 ± 0.08	1.38 ± 0.06
Aspartic acid	2.63 ± 0.06	2.43 ± 0.09	2.62 ± 0.10
Threonine	0.64 ± 0.05	0.51 ± 0.06	0.55 ± 0.04
Serine	0.92 ± 0.02	0.95 ± 0.04	0.96 ± 0.06
Glutathione	2.75 ± 0.08	2.81 ± 0.07	2.79 ± 0.08
Glutamic acid	11.73 ± 0.29	11.43 ± 0.27	11.33 ± 0.36
Glutamine	5.02 ± 0.24	4.68 ± 0.27	4.96 ± 0.13
Glycine	0.63 ± 0.03	0.62 ± 0.03	0.67 ± 0.03
Alanine	0.66 ± 0.01	0.70 ± 0.02	0.66 ± 0.03
GABA	2.48 ± 0.14	2.41 ± 0.08	2.59 ± 0.13

* Values (mean ± S.E.M.) are expressed in μmol/g wet weight. Number of animals in parentheses. Total glutathione is expressed as the reduced compound. Chlorpromazine (20 mg/kg, s.c.) and haloperidol (3 mg/kg, s.c.) were administered daily for 100 days, after which rats were given no treatment for 35 days until sacrificed. No values in the experimental groups differed significantly from those in the control group. ($p > 0.05$).

Table 14. Glutamic acid decarboxylase activity in striatum of rats after chronic drug treatment*.

Time of Sacrifice	Controls	Chlorpromazine	Haloperidol
24 hours after end of treatment	2.88 ± 0.10 (10)	2.89 ± 0.11 (10)	3.11 ± 0.15 (10)
35 days after end of treatment	3.23 ± 0.14 (6)	3.11 ± 0.04 (5)	3.28 ± 0.10 (7)

* Enzyme activities (mean ± S.E.M.) are expressed in μmol/min/g protein. Number of animals in parentheses. Chlorpromazine (20 mg/kg, s.c.) and haloperidol (3 mg/kg, s.c.) were administered daily for 100 days. GAD activity in the experimental groups was not significantly different from that in the control group. ($p > 0.05$).

amassing data from a large number of autopsied brains. Cross et al. (1979) have also recently reported normal concentrations of GABA in thalamus and nucleus accumbens of schizophrenic brain. However, the results of their study and our own investigation for the nucleus accumbens can not be directly compared since there were marked differences in the dissection procedure for this brain area. Whereas our laboratory excises a region dorsal to the anterior commissure (p. 386, Atlas of the Basal Ganglia by Riley) Cross and co-workers select an area which lies ventral to the anterior commissure (Waddington, personal communication). Unfortunately, a standardized dissection technique for the nucleus accumbens in human brain is not presently available.

Possible confounding variables

We explored various factors which might have accounted for the reduction in mean GABA content that was observed in the two brain areas of schizophrenic patients.

Age:

Biochemical data from the 4 brain regions were analyzed as a function of age. Although advancing age was negatively correlated with GABA content for all 4 brain areas, the correlation coefficient reached statistical significance for only the frontal cortex and the thalamus. When the data for the control and patient groups for these two brain regions were analyzed using an analysis of covariance (using age as the covariate) the statistical significance of the data was not altered.

Brain dissection:

It was also possible that differences in the way brain nuclei were dis-

sected in the three main centers that contributed autopsy specimens might have skewed the data. This seemed especially likely for the thalamus, a large anatomical region containing many brain nuclei. However, when mean GABA values for the control and patient groups were compared for only those thalamus specimens dissected in Cambridge, the significant reduction in mean GABA content persisted for the schizophrenic patients.

Immediate cause of death:

Another possible confounding factor is the immediate cause of death. For example, the activity of the GABA-synthesizing enzyme, glutamic acid decarboxylase has been shown to be reduced in the brains of patients dying with prolonged anoxia, e.g. in bronchopneumonia as compared to patients suffering a sudden death (Bird et al., 1979). However, an analysis of our data revealed no significant differences in mean GABA content in the brains of patients with slow anoxic deaths vs. those with sudden deaths. The failure of agonal status to influence GABA content in human brain has also been recently reported by Spokes et al. (1979).

Post-mortem changes:

The interval from death-to-freezing of the brain varied from 2-80 hours for the brains specimens studied. Thus, it seemed possible that this factor may have influenced the biochemical measurements. However, GABA content in human brain rises rapidly after brain death, reaches a plateau by two hours, and remains unchanged for up to 120 hours (Perry et al., 1981 in press). Therefore, the GABA content of brain from the controls and patients could be validly compared even though the intervals between death and freezing of brain ranged from 2-80 hours.

Since GABA levels rise after death, measurements of GABA content in autopsied brain may not necessarily reflect the content of GABA that was present during life. Thus, it is conceivable that the reduction in GABA that was observed in schizophrenic and HC brain could have resulted from a decrease in the post-mortem rise of brain GABA content, rather than from any ante-mortem GABA deficiency. Although this possibility seems unlikely, particularly for the HC caudate region in which a loss of GABA-containing neurons occurs during life, no conclusive evidence is available in schizophrenic brain to exclude this possibility.

Drug treatment:

Another possible explanation for the observed GABA deficiency in schizophrenic brain was that the deficiency was drug-induced - the result of chronic treatment with antipsychotic drugs. We were unable to obtain complete drug histories from some of the case records. However, it is likely that most, if not all of the schizophrenic and HC patients received these drugs. Lloyd and Hornykiewicz (1977) found that, while a single injection of haloperidol or clozapine lowered the GABA content of the substantia nigra in rats, chronic administration of haloperidol (167 days) or clozapine (100 days) produced no significant change from control values in the GABA content of this brain area. Neither did chronic administration of these two antipsychotic drugs alter GAD enzyme activity in the rat substantia nigra.

In the present study, we sacrified some rats immediately after chronic treatment with two commonly employed antipsychotic drugs, and others after a substantial period off drugs, in an effort to mimic the conditions of drug use which might prevail in human psychotic patients who come to autopsy. The demonstration of a lack of effect of chronic neuroleptic drug

treatment on brain GABA content in rodents in our study and in the investigation of Lloyd and Hornykiewicz (1977) suggests, although it does not prove, that changes in GABA content which may be found in the brains of human psychiatric patients are not artifacts produced by previous antipsychotic drug therapy.

However, if one is to exclude conclusively the possibility that GABA deficiencies found in schizophrenic patients may be drug-induced, other experiments will be required. GABA content and GAD activity could be measured in brain of primates after long-term administration of different antipsychotic drugs. Most convincing would be the demonstration of abnormalities in the GABA system in brain of psychotic patients who have never received drug treatment. Such patients, however, are very unlikely to be encountered any longer.

Disease diagnosis:

Another possible confounding variable which we could not control was variation in the diagnosis of schizophrenia. At the present time the diagnosis of schizophrenia depends to some extent on the country and even the city in which the diagnosis is made. The brain specimens used in this investigation were obtained from patients with a hospital diagnosis of schizophrenia. It is likely that the diagnostic criteria for this disorder varied somewhat among the different hospitals concerned, which in turn might have reduced the significance of the data. The American Psychiatric Association has recently developed a new definition of schizophrenia (Spitzer et al., 1978) which is a more precise definition and is likely to be more reliable than previous North American diagnostic criteria. Unfortunately, however, no information yet exists which indicates that this newly

developed definition of schizophrenia is any more valid than the older, less precise diagnostic criteria.

In conclusion, I believe that we have been able to rule out a number of potentially confounding variables which might have explained the observed reduction in mean GABA found in two brain areas of schizophrenic patients. However, the possible influence of other complicating factors has yet to be entirely resolved and thus, the possibility exists that the observed GABA deficiency may have been related to some subsidiary phenomenon of the psychiatric disorder.

Biological significance of the brain GABA deficiency

Although the deficiency of GABA observed in schizophrenic thalamus and caudate was statistically significant, the magnitude of the change was relatively small (about 25%) and thus, the biological significance of our findings is uncertain. Furthermore, most of the schizophrenic specimens studied clearly did not have a marked deficiency of GABA in any of the 4 brain regions studied. However, the absence of a GABA deficiency in some individual brain specimens of the schizophrenic patients we studied need not invalidate the possibility that brain GABA deficiency plays an important role in some forms of schizophrenia. Since schizophrenia represents a group of disorders among which there may be many different biochemical causes, then such etiological heterogeneity should markedly reduce the likelihood of finding an association between this or any other biochemical alteration and the schizophrenic syndromes. In this regard, Buchsbaum and Rieder (1979) have demonstrated, through computer simulation, the low probability of finding a statistically significant biochemical difference even when a relatively large proportion (20%) of the pathological group has the biochemical abnormality.

The results of the present investigation provide some support to the hypothesis of Roberts (1972) that a brain GABA deficiency may be a biochemical characteristic of certain forms of schizophrenia. Additionally, it is possible that the GABA deficiency previously observed in HC caudate (as well as other regions of HC brain, Perry et al., 1973b) and now in thalamus, might be linked not only to the choreic symptoms of this disorder, but also to the psychiatric symptoms which are often observed in HC. Although the thalamus has not traditionally been thought of as an anatomical site which might contain the biochemical abnormality responsible for schizophrenic psychosis, such a possibility certainly seems plausible in view of the many anatomical connections between the thalamus and limbic and basal ganglia regions. Thus, the GABA system in certain key areas of brain might be the vulnerable component in a complex balance of dopaminergic, cholinergic and other neurons, as well as environmental factors, in which it plays a critical role in behavioural regulation. Such a notion is supported by the interesting observations of Stevens (1974) in which an injection of the GABA antagonist bicuculline into the ventral tegmental area of the cat, which contains the cell bodies of the mesolimbic dopaminergic system, produced an unusual state of behaviour suggesting intense fear as well as visual and auditory hallucinations. These behavioral responses were found to be prevented by pretreatment of the animal with the antipsychotic agent, haloperidol.

In conclusion, the results of our study are suggestive of an association between a deficiency of GABAergic function in certain brain areas and some forms of schizophrenia. However, it will be necessary to obtain biochemical data from a much larger number of patients in which the diagnosis of schizophrenia has been carefully determined and preferably classified into subtypes, before a more definitive statement can be made. Ideally, in

the rare instances in which this is possible, brain specimens should be obtained at autopsy from schizophrenic patients in whom antipsychotic drugs have not been used. Careful attention should be given to the physiological conditions immediately preceding death, and ideally patient and control brains should be dissected by the same neuropathologist. Furthermore, post-mortem brain should be obtained from a more adequate control group which is comparable to the schizophrenic group in terms of institutionalization and long-term stress. If a brain GABA deficiency can be confirmed for some forms of schizophrenia, new therapeutic possibilities for this disorder may unfold.

SECTION C - AN EXAMINATION INTO THE ETIOLOGY OF THE ASPARTATE DEFICIENCY IN HEREDITARY CEREBELLAR DISORDERS

I. ABSTRACT

Perry and his associates have recently observed a deficiency in aspartate content in the cerebellar cortex of some patients with dominantly-inherited cerebellar disorders. The present study was undertaken to test whether the deficiency of brain aspartate was associated with a reduction in the activities of two enzymes involved in the synthesis of aspartate, namely aspartate aminotransferase, the enzyme generally assumed to be responsible for the synthesis of aspartate, and pyruvate carboxylase, an enzyme which synthesizes oxaloacetic acid, a precursor of aspartate. A new and sensitive radiochemical technique was developed for the measurement of brain aspartate aminotransferase activity. Normal activities of aspartate aminotransferase in autopsied and biopsied cerebellar cortex were found in all of the cerebellar disease patients as compared to a large control group. Pyruvate carboxylase activity deteriorates in brain rapidly after death and thus could usually be detected only in biopsied cerebellum. No deficiency of pyruvate carboxylase activity was observed in biopsied cerebellum of a single patient with dominantly-inherited cerebellar disease and aspartate deficiency. It is concluded that the aspartate deficiency in cerebellar cortex of the cerebellar disease patients was unlikely to be a consequence of a deficiency of either of these two brain enzymes.

II INTRODUCTION

Perry and his associates have recently described several abnormalities of amino acid content in the brains of 11 patients with dominantly-inherited cerebellar disorders (Perry, et al., 1977b; Perry et al., 1980 in press). Despite the clinical similarities among the patients, three biochemically different disorders were found. One disorder was characterized by moderate reduction of aspartate and glutamate contents in cerebellar cortex alone. In a second disorder, aspartate and glutamate contents were markedly reduced in cerebellar cortex as well as in other brain areas. In the third cerebellar disorder, aspartate and glutamate contents were normal in cerebellar cortex. Additionally, GABA content in cerebellar cortex and dentate nucleus was reduced in some patients with each disorder.

The deficiency of aspartate in the cerebellum of many of these patients may be physiologically significant in view of the biochemical (Nadi et al., 1977; Rea et al., 1980) and electrophysiological (Hackett et al., 1979) evidence that aspartate may be the excitatory neurotransmitter of the cerebellar climbing fibers. Neuropathological examination of autopsied brain of several of the patients with cerebellar disorders showed a marked decrease in the number of cells in the inferior olivary nucleus, the site of origin of most or all of the climbing fibers (Courville and Faraco-Cantin, 1978), as well as loss of Purkinje cells upon which the climbing fibers terminate.

The deficiency of aspartate in the cerebellar cortex of some of the patients with dominantly-inherited cerebellar disorders might be explained by a loss of a population of neurons which utilize aspartate as a neurotransmitter. Alternatively, aspartate content might be reduced in brain as a result of a deficiency of the synthetic enzyme(s) for aspartate. In order to test the latter hypothesis, I have measured the activity of the

enzyme which is generally assumed to be responsible for the synthesis of aspartate, aspartate aminotransferase as well as the activity of pyruvate carboxylase, an enzyme which synthesizes oxaloacetic acid, a precursor of aspartate, in the autopsied brains of the cerebellar disease patients.

III. METHODOLOGY

Patients

Three patients whose autopsied brain was examined, and one living patient from whom a cerebellar cortical biopsy was obtained, were members of a large pedigree in the southern United States and suffered from olivo-pontocerebellar atrophy (OPCA) I of Konigsmark and Weiner (1970). The disorder has occurred in many members of at least 5 successive generations in this pedigree (Currier et al., 1972), and it has been shown to be HLA-linked, with the mutant gene being located on chromosome 6 (Jackson et al., 1977; 1978). The clinical picture and the neuropathological changes in 2 of the 4 patients in this pedigree (hereafter designated Pedigree C) have been previously described by Perry et al. (1977b).

Two patients (Pedigree O) belonged to an unrelated family in the northern United States, in which numerous individuals in three successive generations have been affected by a cerebellar disorder which appears to be OPCA I (Konigsmark and Weiner, 1979), based on its clinical and neuropathological features. Linkage of the mutant gene to the HLA locus has neither been established nor excluded in Pedigree O (Whittington et al., in press).

Two patients (Pedigree A) belonged to an unrelated family in the southern United States, in which numerous individuals in 4 successive generations have been affected by a cerebellar disorder which is indistinguishable from OPCA I in its clinical and neuropathological characteristics. In

Pedigree A, there is no evidence for linkage of the mutant gene to the HLA locus (Jackson et al., 1978).

One patient (Pedigree S) belonged to an unrelated family in the northern United States in which many individuals in 5 successive generations have been affected by a cerebellar disorder which has been classified as OPCA IV (Konigsmark and Weiner, 1970). The clinical details of this family's hereditary disorder were described 30 years ago by Schut (1950). There is no HLA linkage in this pedigree.

Single patients belonged to two unrelated Canadian families (Pedigree R and G), in each of which unclassified cerebellar disorders had occurred in several members of at least two successive generations. Possible HLA linkage has not been explored in Pedigrees R and G.

Collection of brain specimens

Autopsied brain specimens from the 10 patients were frozen at -80°C within 1 to 4 hours after death, were shipped to our laboratory on dry ice, and were maintained frozen at -80°C until they were dissected and processed for amino acid analysis. A single neuropathologist dissected 9 of the 10 brains from patients with cerebellar disorders, as well as most of the control brains, thus assuring reasonable anatomical consistency between the brain regions compared.

Biopsy specimens of apparently normal cerebellar cortex were obtained from 8 control subjects who underwent neurosurgical removal of posterior fossa tumors, and (with informed consent) from one patient in Pedigree C. The cerebellar biopsy specimens were immersed in liquid nitrogen within 10 seconds of their neurosurgical removal, and then were held at -80°C or lower until processed.

Aspartate and glutamate contents were found by Perry and his team (Perry et al., 1977b, 1980 in press) to be moderately reduced in the cerebellar cortex from patients of Pedigrees C and O, and markedly reduced in cerebellar cortex and other brain regions of patients S and R. Aspartate content was normal in the cerebellar cortex of the two patients in Pedigree A. A single patient (G) was found to have reduced aspartate in cerebellar cortex but normal aspartate and glutamate in other brain regions. GABA content was found to be decreased in the cerebellar cortex and dentate nucleus of 5 patients (from 5 different pedigrees).

Enzyme assays

Two enzymes likely to be important for the synthesis of aspartic acid were assayed in cerebellar cortical tissue. Pyruvate carboxylase activity was determined by measuring the rate of formation of [^{14}-C]-oxaloacetate from [^{14}-C]-sodium bicarbonate and unlabelled pyruvate as described for brain by Atkin et al. (1979).

A new and sensitive radiochemical technique was developed for measuring aspartate aminotransferase activity, both in the direction of aspartate formation, and in the reverse direction towards oxalacetate. Frozen brain was homogenized at 4°C in 400 to 500 vol of 0.1 M potassium phosphate buffer, pH 7.5, containing 0.5% Triton-X-100. All incubation mixtures contained 600 µl of brain homogenate, 100 µl of potassium phosphate buffer (0.41 M, pH 7.5), and 10 µl of pyridoxal-5'-phosphate (12.5 mM). For assays of the enzyme in the direction of aspartate formation, incubation mixtures also contained 190 µl of potassium glutamate (0.313 M) which included 0.2 µCi of U-[^{14}C]-glutamate, and either 100 µl of cis-oxaloacetic acid (30 mM, Sigma Chemicals) or (for blanks) 100 µl of water. When the enzyme was measured in the direction of aspartate degradation, incubation mixtures

instead contained 190 µl of potassium aspartate (0.215 M), which included 0.2 µCi of U-[^{14}C]-aspartate, and either 100 µl of potassium α-ketoglutarate (0.2 M) or (for blanks) 100 µl of water.

Reaction mixtures were incubated for 10 min at 37°C, after which the reaction was stopped by adding 100 µl of 2 N HCl. The [^{14}C]-labelled α-ketoglutarate formed (forward reaction), or the [^{14}C]-labelled oxaloacetate formed (reverse reaction) were then separated from their respective radioactive precursors by applying incubation mixtures to 5 x 0.75 cm columns of Dowex 50-X8, H^+ form, and eluting with 3 ml of water. Eluates were then counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer.

The apparent Michaelis constants for the substrates of aspartate aminotransferase were calculated to be K oxaloacetate = 1.0 mM, K glutamate = 18 mM, K aspartate = 3.3 mM, and K α-ketoglutarate = 0.78 mM. The V_{max} for the reaction using oxaloacetate and glutamate as substrates was found to be 8.9 µmol/10 min/mg protein, whereas a value of 3.4 µmol/10 min/mg protein was obtained when aspartate and α-ketoglutarate were used as substrates.

Protein concentrations in brain homogenates were determined by the Lowry method (Lowry, 1951).

IV. RESULTS

Enzyme Activities

Table 15 shows the activities of two enzymes in cerebellar cortex, deficiencies of which might conceivably cause reductions in aspartic acid content. The activity of pyruvate carboxylase, the enzyme that converts pyruvate to oxaloacetate, was normal in the biopsied cerebellum of patient C4. Pyruvate carboxylase activity deteriorates in brain rapidly after

Table 15. Enzyme activities in autopsied and biopsied cerebellar cortex.

Subjects	Aspartate aminotransferase		Pyruvate** carboxylase
	Aspartate formation*	Oxaloacetate formation*	
Controls	8.20 ± 1.44 (18)	3.43 ± 0.56 (18)	
Controls†	7.53 ± 1.18† (4)	2.84 ± 0.73† (4)	30.8 ± 5.5† (4)
Patients			
C1	5.64	2.42	
C2	7.01	2.69	
C3	6.77	2.65	
C4†	9.08†	3.27†	38.8†
O1	6.86	2.98	
O2	7.40	3.13	
A1	8.93	3.34	
A2	6.70	2.92	
S	8.17	3.20	
R	7.98	3.23	
G	10.40	4.08	

* Aspartate aminotransferase activity expressed in μmol/10 min/mg protein.

** Pyruvate carboxylase activity expressed in μmol/10 min/g protein

† Enzyme assays performed on biopsied tissue.

death, and enzyme activity was usually undetectable in autopsied brain frozen more than 2 1/2 hours after death. Hence, enzyme activities for autopsied cerebellum are not listed in Table 15. Appreciable pyruvate carboxylase activity was found, however, in the cerebellar cortex of Patients S and G, suggesting at least that the aspartate deficiency observed in these two patients was not related to an abnormality in this enzyme.

Aspartate aminotransferase enzyme activity appeared to be stable in brain with death-to-freezing intervals up to 12 hours, and the control values for autopsied and biopsied cerebellum were similar (Table 15). The rate of reaction was about 2 1/2 times greater in the direction of aspartate formation, than in the direction of oxaloacetate. None of the 11 cerebellar disorder patients were found to have a deficiency in the activity of aspartate aminotransferase in the cerebellar cortex.

V. DISCUSSION

The demonstration of normal activities of aspartate aminotransferase in all of the cerebellar disease patients studied by Perry et al., (1977b, 1980 in press) as well as normal pyruvate carboxylase activity in one of the patients with reduced cerebellar aspartate content, strongly suggests that the aspartate deficiency observed in some of these patients did not result from a deficiency of either of the two enzymes. Another possible explanation for the aspartate deficiency is that the reduction was a consequence of a deficiency of another enzyme which is responsible for the synthesis of oxaloacetate, such as malate dehydrogenase, the citrate cleavage enzyme, or phosphoenolpyruvate carboxylase. Alternatively, it is conceivable that aspartate may be synthesized in human brain via a completely different enzymatic pathway than the one studied in the present investigation. Although it has generally been assumed in the literature that aspar-

tate is synthesized in mammals by the enzyme aspartate aminotransferase, the metabolic pathway for aspartate formation in human brain, has not been clearly defined. Moreover, in the present study no statistically significant correlation could be found between aspartate content and either aspartate aminotransferase or pyruvate carboxylase activity in biopsied cerebellar cortex.

A more likely explanation for the aspartate deficiency in cerebellar cortex is that the abnormality reflects a loss of a specific type of cerebellar neuron which utilizes aspartate as a neurotransmitter. Such a possibility would be analogous to the marked reduction in GABA content in the caudate of the brains of patients with Huntington's chorea (Perry et al., 1973b) in which the biochemical abnormality is probably a consequence of the loss of GABAergic neurons. The possible causes of the loss of neurons in the cerebellar disease patients are many and include abnormal proteins in cell membranes, defective DNA repair mechanisms, and endogenous or exogenous neurotoxins which might be incompletely destroyed by reduced enzyme activity in patients heterozygous for the mutant genes.

SECTION D - γ-VINYL GABA AND HYDRAZINE: EFFECTS OF CHRONIC ADMINISTRATION ON THE CONTENTS OF GABA AND OTHER AMINO COMPOUNDS IN RAT BRAIN

I. ABSTRACT

Experiments were undertaken to determine the degree of biochemical selectivity of γ-vinyl GABA and hydrazine, two GABA aminotransferase (GABA-T) inhibitors which are presently under consideration for use in clinical trials on patients with Huntington's chorea. Chronic administration of either γ-vinyl GABA or of hydrazine produced many biochemical changes in rat brain other than the desired inhibition of the GABA catabolizing enzyme and consequent elevation of brain GABA content. Prolonged treatment with γ-vinyl GABA produced a marked increase in the brain contents of hypotaurine, β-alanine and homocarnosine, and a reduction in brain glutamine and threonine levels. In addition, administration of γ-vinyl GABA resulted in a 22% decrease in the activity of glutamic acid decarboxylase (GAD) in rat brain. Since GAD is localized to a large extent in nerve endings, the possibility therefore exists that γ-vinyl GABA might actually reduce the amount of GABA available for release at synapses. Chronic administration of hydrazine had no effect on GAD activity measured in whole brain. However, the contents of many brain amino compounds other than GABA were altered by hydrazine, including hypotaurine, alanine, tyrosine, ornithine, cystathionine, α-aminoadipate, glutamate and glutamine. No gross changes were observed in the behaviour of the animals treated with γ-vinyl GABA and with hydrazine, or in the livers of the hydrazine-treated rats. However, the physiological role and neurotoxicity of the amino compounds which were altered in brain by both drugs have not been well described. Thus, it remains to be determined whether the additional non-specific effects of the two GABA-T inhibitors are in fact harmful.

II. INTRODUCTION

A deficiency of GABA has been demonstrated in certain regions of autopsied brain obtained from patients dying with Huntington's chorea (HC) (Perry et al., 1973b; 1979a) and schizophrenia (Perry et al., 1979a). Since GABA is a major inhibitory neurotransmitter in the mammalian central nervous system (Krnjevic et al., 1974), these biochemical findings have stimulated much interest in designing pharmacological agents capable of increasing GABAergic activity in brain. One of several possible strategies for treating patients with a deficiency of GABA content in brain would be to increase endogenous brain GABA levels by inhibiting the first of two enzymes in the degradative pathway of GABA: 4 aminobutyrate:2-oxoglutarate aminotransferase (GABA aminotransferase, GABA-T). Two such compounds which have been shown to elevate brain GABA content in vivo in experimental animals are isonicotinic acid hydrazide (isoniazid) and the new irreversible GABA-T inhibitor, γ-vinyl GABA (Perry et al., 1973a, 1974; Jung et al., 1977). Recently, efforts to elevate brain GABA content by administering high doses of isoniazid, have produced clinical improvement in a minority of patients with HC (Perry et al., 1979e). As well, γ-vinyl GABA is presently being tested by a pharmaceutical firm in France in HC patients.

A disturbing problem with many of the drugs used as GABA-T inhibitors is that they are relatively non-specific and can inhibit one or more additional brain enzymes, including the enzyme responsible for synthesizing GABA, glutamic acid decarboxylase (GAD). GAD is localized to a large extent in presynaptic nerve endings (Kuriyama, 1976; Wood et al., 1976). Thus, inhibition of this enzyme, which can occur with high doses of GABA-T inhibitors such as amino-oxyacetic acid (Roberts and Simonsen, 1963) and γ-acetylenic GABA (Jung et al., 1977b); Schechter et al., 1977) might actually reduce the amount of GABA available for release at the synapse, even

though overall brain GABA content was elevated. It would therefore be potentially important to have available for human use a highly specific GABA-T inhibitor which has no inhibitory effect on GAD or other brain enzymes.

III. HYPOTHESES TO BE TESTED AND EXPERIMENTAL OUTLINE

The present study was divided into two parts. In the first part, we measured amino acid content and the activities of the GABA-synthesizing and catabolizing enzymes in rat brain following repeated doses of γ-vinyl GABA, a drug which has been claimed to be a selective inhibitor of brain GABA-T (Sarhan and Seiler, 1979). The effects of γ-vinyl GABA have previously been examined on only a limited number of brain enzymes (Jung et al., 1977a) following short-term (three days) administration. Thus, the major objective of this study was to measure the effects of chronic administration of γ-vinyl GABA on a large number of potentially important brain amino compounds as well as GABA and its metabolizing enzymes in order to determine the degree of biochemical selectivity of γ-vinyl GABA and thereby its potential usefulness as a chemotherapeutic agent in humans.

In the second part of the study, we explored in vitro the effects of isoniazid, and several of its metabolites on the enzymes that synthesize and degrade brain GABA. Although administration of isoniazid elevates brain GABA in experimental animals in vivo, the compound had no significant action on either the GABA-synthesizing or catabolizing enzymes in vitro. Moreover, of the isoniazid metabolites tested only hydrazine was capable of significantly inhibiting brain GABA-T at relatively low concentrations at which no inhibition of GAD activity occurred. Based on these preliminary findings and after we found that short-term administration of hydrazine elevated brain GABA in vivo, we then undertook a study to determine whether

GABA levels in brain would remain elevated following long-term treatment with hydrazine, and also to assess whether the drug produced any toxic or potentially undesirable effects. Additionally, since it is likely that administration of hydrazine to humans would result in less formation of the potent hepatotoxin acetylhydrazine than when isoniazid is given (Mitchell et al., 1975; Wright and Timbrell, 1978), we thought that hydrazine might prove to be less toxic than isoniazid, and might be useful in future clinical trials on patients with disorders involving a brain GABA deficiency.

IV. METHODOLOGY

γ-vinyl GABA experiments:

Chemicals

Sources of chemicals for the γ-vinyl GABA experiments can be found in Perry et al., 1979b.

Animals

Female Wistar rats (150-200 g) were used in the experiments described. Ten rats were injected subcutaneously once every 24 hours for 11 days with 100 mg/kg of γ-vinyl GABA dissolved in 0.2-0.5 ml water. An additional five rats were given a single subcutaneous injection of γ-vinyl GABA in the same dosage. Control animals were litter-mates who were given daily subcutaneous injections of comparable volumes of 0.15 M-NaCl. Rats were killed by cervical dislocation 24 hours after the last injection of γ-vinyl GABA or of saline solution. The entire brain was removed, divided sagitally into two equal halves, and immersed in liquid nitrogen within 24-45 sec of the animal's death. Brain specimens were then maintained at -80°C until analysed. One-half brain was utilized for amino acid analysis. The other half was ground to a homogeneous powder in liquid nitrogen with a mortar

and pestle, and the frozen powder was used for the two enzyme assays performed.

Enzyme assays

GAD and GABA-T enzyme activities were determined as described in Perry et al., 1979b. GAD and GABA-T enzyme activities were found to be similar in fresh whole rat brain and in the powdered frozen rat brain used in this study.

Experiments with isoniazid and its metabolites:

Chemicals

L-[1-^{14}C] glutamic acid (50 mCi/mmol) was obtained from New England Nuclear, Boston, MA. ^{14}C-U-GABA (224 mCi/mmol and [2,3-^{14}C] succinic acid (17.5 mCi/mmol) were obtained from Amersham Corp., Arlington Heights, IL. INH and isonicotinic acid were obtained from Eastman Organic Chemicals, Rochester, N.Y. Acetylhydrazine (95% pure) and m-anisaldehyde were obtained from Aldrich Chemical Co., Milwaukee, WI. Hydrazine hydrate (85% pure) and hydrazine sulfate were obtained from Fisher Chemical Co., Fair Lawn, N.J. N,N'-diacetylhydrazine was obtained from ICN Pharmaceuticals, Plainview, N.Y.

The following compounds were prepared by Dr. T. L. Perry:

Acetylisoniazid was prepared by treating INH dissolved in glacial acetic acid with acetic anhydride as described by Yale et al. (1953).

Acetylhydrazine, 95% pure was recrystallized twice from chloroform diethylether 91/2, v/v), with thorough drying in a vacuum desiccator between crystallizations. The purified compound consisted of colorless needle-like crystals, having a melting point of 67-68.5°C (uncorrected).

Hydrazine monohydrochloride was prepared by dissolving 1.35 mol of acetylhydrazine (95% pure) in 300 ml of water, and adding gradually with constant stirring 1.35 mol of HCl. The solution was allowed to stand at room temperature overnight, and then was lyophilized to dryness. The major product obtained was soluble in boiling methanol, and precipitated as needle-like crystals on chilling the methanolic solution, or on adding an equal portion of diethyl ether. After three recrystallizations from methanol-diethyl ether (1/1, v/v), 48 g of a colorless crystalline compound was obtained which had a melting point of 94-97°C. When examined by high performance liquid chromatography techniques, this material gave a peak for hydrazine, but showed no evidence of acetylhydrazine or other impurities. Mass spectrometry of the synthesized compound showed no fragment derived from an acetyl group. Finally, the identity of the synthetic compound as hydrazine monohydrochloride, rather than the dihydrochloride salt, was attested by finding only a single molar equivalent of chlorine when the product was titrated with a solution of silver nitrate.

Animals

Female litter-mate Wistar rats (125-140 g) were used in the experiments described. Rats were injected subcutaneously once every 24 hours for 109 days with aqueous solutions of hydrazine monohydrochloride, together with pyridoxine hydrochloride, or in the case of control animals, pyridoxine hydrochloride alone. Solutions were made so that each rat received 5 mg of pyridoxine hydrochloride per kg daily, and that injection volumes were 0.2 ml/100 g body weight. Fourteen control animals received pyridoxine only, 14 animals received hydrazine monohydrochloride 0.08 mmol/kg/day, and 14 animals received hydrazine monohydrochloride 0.16 mmol/kg/day. In

addition, a small group of litter-mate rats were given no injections. All rats were weighed daily prior to injections.

At the end of the experiment, rats were killed by cervical dislocation, some of them 6 hours and the remainder 24 hours after the last of the 109 daily injections. The entire brain was removed, divided sagitally into two equal halves, and immersed in liquid nitrogen within 25-45 sec of the animals's death. Brain specimens were then maintained at -80°C until analyzed. One-half brain was utilized for amino acid analysis. The other half was ground to a homogeneous powder in liquid nitrogen with a mortar and pestle, and the frozen powder was used for the two enzyme assays performed.

Enzyme assays

GAD enzyme activity was determined as described in Perry et al., 1979b.

GABA-T activity was determined by a modification of the radiometric procedure of White and Sato (1978), in which ^{14}C-GABA is converted to ^{14}C-succinic semialdehyde and ^{14}C-succinic acid. Frozen brain was homogenized in 150-200 vol of ice-cold 50 mM potassium phosphate buffer (pH 8.0) containing 40 µM pyridoxal phosphate, 0.1 M EDTA, 0.5 mM dithiothreitol, and 0.2% (v/v) Triton X-100. Reaction mixtures (final vol 1.0 ml) contained 50 µl (0.2 µCi) ^{14}C-U-GABA (60 mM), 50 µl α-ketoglutaric acid (40 mM), 50 µl potassium phosphate (400 mM, pH 8.0), and 600 µl of brain homogenate. After incubation at 37°C for 30 min, the reaction was stopped by adding 100 µl of 2 M-HCl. Assay blanks contained 100 µl of 2 M HCl added prior to incubation, and they gave values identical to enzyme blanks which had been boiled, or to blanks containing no α-ketoglutarate. Incubation mixtures were then applied to small columns (5 x 0.75 cm) of Dowex 50 x 8, 200-400 mesh, H^+ form, and the ^{14}C-succinate and ^{14}C-succinic

semialdehyde were separated from the unreacted ^{14}C-GABA by washing the columns with 3.0 ml of 0.2 M-HCl. The column effluents were counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. Recovery of authentic ^{14}C-succinate carried through the assay procedure was 90%. GABA-T assays were performed in duplicate on each brain specimen.

GAD and GABA-T enzyme activities were found to be similar in fresh whole rat brain and in the powdered frozen rat brain used in this study.

The effects of isoniazid and its metabolites on GAD and GABA-T enzyme activities were determined by adding .0005-5 mM concentrations of each of these compounds to homogenates of brain from freshly killed rats that had received no drug treatment. The assay procedures were as described above. Protein in brain homogenates was determined by the method of Lowry et al. (1951).

Amino acid analyses

All amino acid analyses were performed as described in Section A.

V. RESULTS

Effects of γ-vinyl GABA on amino compounds in brain

Table 16 shows the mean contents of 31 different amino acids and related compounds in whole brain of rats injected once daily for 11 days with 100 mg/kg of γ-vinyl GABA, and in whole brain of saline-injected littermate controls. Three compounds present in rat brain in amounts too small for reliable quantitation (α-amino-n-butyric acid, cystine, and putreanine) have been omitted from Table 16. Although the dosage of γ-vinyl GABA used was low by comparison with previous studies (Schechter et al., 1977; Jung et al., 1977a), marked changes were produced in a number of brain amino compounds. The content of GABA was more than doubled. β-alanine and homo-

Table 16. Free amino compounds in whole brain of rats treated with γ-vinyl GABA for 11 days.

Compound	Controls (12)	γ-vinyl GABA (10)
Glycerophosphoethanolamine	0.43 ± 0.02	0.36 ± 0.01
Taurine	4.60 ± 0.18	4.45 ± 0.21
Phosphoethanolamine	1.36 ± 0.03	1.36 ± 0.06
Hypotaurine	0.06 ± 0.00	0.23 ± 0.03*
Aspartic acid	2.78 ± 0.03	2.61 ± 0.09
GSH	2.23 ± 0.04	2.30 ± 0.06
Threonine	0.61 ± 0.02	0.54 ± 0.01*
Serine	0.86 ± 0.02	0.86 ± 0.02
Asparagine	0.09 ± 0.01	0.09 ± 0.01
Glutamic acid	12.46 ± 0.23	12.07 ± 0.23
Glutamine	5.02 ± 0.14	4.37 ± 0.11*
Proline	0.06 ± 0.01	0.08 ± 0.02
Glycine	1.02 ± 0.03	1.03 ± 0.05
Alanine	0.48 ± 0.02	0.47 ± 0.01
Citrulline	0.06 ± 0.00	0.06 ± 0.01
Valine	0.06 ± 0.00	0.05 ± 0.00
Methionine	0.02 ± 0.00	0.02 ± 0.00
Cystathionine	0.06 ± 0.00	0.07 ± 0.01
Isoleucine	0.02 ± 0.00	0.02 ± 0.00
Leucine	0.05 ± 0.00	0.05 ± 0.00
Tyrosine	0.05 ± 0.00	0.06 ± 0.00
Phenylalanine	0.03 ± 0.00	0.03 ± 0.00
β-Alanine	0.01 ± 0.00	0.04 ± 0.00*
GABA	1.90 ± 0.03	4.65 ± 0.11*
Tryptophan	0.01 ± 0.00	0.02 ± 0.00
Ethanolamine	0.11 ± 0.01	0.12 ± 0.00
Ornithine	0.01 ± 0.00	0.02 ± 0.00*
Lysine	0.22 ± 0.01	0.24 ± 0.01
Histidine	0.05 ± 0.00	0.05 ± 0.00
Homocarnosine	0.08 ± 0.01	0.21 ± 0.01*
Arginine	0.10 ± 0.00	0.11 ± 0.01

Values (mean ± S.E.M.) are those found 24 hours after the last injection, and are expressed in μmol/g wet wt.
Figures in brackets indicate number of animals in each group.
GSH indicates total glutathione, expressed as the reduced compound.
* $P < 0.01$ (Student's t-test, as compared with controls).

Table 17. GAD and GABA-T enzyme activity in whole brain of rats treated with γ-vinyl GABA for 11 days.

Enzyme activity	Controls	γ-vinyl GABA
GAD	251 ± 6 (11)	196 ± 9* (10)
GABA-T	485 ± 7 (9)	359 ± 12* (8)

Enzyme activities (24 hours after last injection) are expressed in μmol/h/g protein, and represent mean ± S.E.M.
Figures in brackets indicate number of animals in each group.
* $p < 0.01$ (Student's t-test)

carnosine contents in brain increased significantly. Unexpected changes in brain amino compounds produced by γ-vinyl GABA were a significant increase in the content of hypotaurine, and significant decreases in the contents of threonine and glutamine.

In five rats given a single injection of γ-vinyl GABA (100 mg/kg) and killed 24 hours later, significant but less marked increases in brain contents of GABA, homocarnosine, β-alanine and hypotaurine were found (not shown in Table 16). Mean GABA content was 2.58 μmol/g wet wt, as compared to 4.65 μmol/g wet wt in the brains of rats treated for 11 days. No significant decreases in brain contents of threonine or glutamine were observed in the rats given a single injection of γ-vinyl GABA.

Free γ-vinyl GABA was not detectable in the brains of any of the treated rats 24 hours after the last injection of this drug, regardless of whether they had received 1 or 11 daily injections. γ-vinyl GABA was well separated from GABA and other brain amino compounds with the chromatographic technique used (Perry et al., 1968), and the compound should have been detectable if as much as 0.05 μmol/g wet wt had been present.

Effects of γ-vinyl GABA on brain enzymes

Table 17 shows the changes in GAD and GABA-T enzyme activity produced in rat brain by γ-vinyl GABA injected for 11 days in a daily dosage of 100 mg/kg. Mean activities of both enzymes were significantly decreased compared to saline-injected litter-mate controls. GAD activity had been reduced by 22% and GABA-T activity by 26%.

Effects of γ-vinyl GABA on rat behaviour

No evidence of toxicity or changes in behaviour were observed in any of the rats treated with γ-vinyl GABA. They gained weight as rapidly as the

saline-injected control animals. The limited supply of γ-vinyl GABA available to us prevented exploration of the possible toxic effects of more prolonged administration. For complete details of results see Perry et al., 1979b.

In vitro effects of isoniazid and its metabolites on GABA-T and GAD activity

Table 18 shows the concentrations of isoniazid metabolites required to produce a 50% inhibition of enzyme activities of GABA-T and GAD in vitro. When varying concentrations of isoniazid and 5 of its metabolic degradation products were added to fresh rat brain homogenates, only hydrazine was found to inhibit GABA-T in relatively low concentrations. GAD activity in vitro was significantly inhibited only by hydrazine and at concentrations two orders of magnitude greater than that required to inhibit GABA-T.

Effects of chronic administration of hydrazine to rats

When in vitro enzyme assays showed that hydrazine was a potent inhibitor of GABA-T, but not of GAD, and that isoniazid and other metabolites did not inhibit GABA-T in concentrations up to 5 mM, this suggested that hydrazine was the active component in rats treated with isoniazid which was responsible for the elevation of brain GABA. After preliminary experiments showed that brief administration of hydrazine to rats caused marked increases in brain GABA content, we then undertook studies of the biochemical effects of chronic administration of this isoniazid metabolite. The two dosages of hydrazine monohydrochloride chosen (0.08 and 0.16 mmol/kg/day) were comparable to the daily doses of isoniazid used in efforts to increase brain GABA content in Huntington's chorea patients (Perry et al., 1979e), had all of the isoniazid been converted to hydrazine. Pyridoxine was injected together with hydrazine, since pyridoxine is routinely given to

Table 18. Concentrations of isoniazid and metabolites (mM) required for 50% inhibition of GABA-T and GAD activity in rat whole brain homogenates.

Drug	GABA-T	GAD
Isoniazid	>5	>5
Isonicotinic acid	>5	>5
Acetylisoniazid	>5	>5
Acetylhydrazine	>5	2.5
Diacetylhydrazine	>5	>5
Hydrazine hydrate	0.008	0.3
Hydrazine sulfate	0.005	0.2
Hydrazine monohydrochloride	0.005	0.2

Table 19. Free amino compounds in whole brain of rats treated with hydrazine for 109 days.

Compound	Controls (12)	Hydrazine-treated rats			
		0.08 mmol/kg/day		0.16 mmol/kg/day	
		6h (7)	24h (7)	6h (7)	24h (7)
Taurine	5.61 ± 0.13	5.09 ± 0.31	5.45 ± 0.23	4.95 ± 0.22	5.17 ± 0.32
Hypotaurine	0.03 ± 0	0.10 ± 0.01*	0.13 ± 0.01*	0.10 ± 0.01*	0.08 ± 0.01*
Glutamic acid	12.92 ± 0.15	12.26 ± 0.21*	12.99 ± 0.35	12.16 ± 0.22*	12.26 ± 0.14*
Glutamine	5.75 ± 0.13	4.75 ± 0.10*	5.11 ± 0.14*	3.92 ± 0.11	4.76 ± 0.10*
α-aminoadipic acid	tr	0.04 ± 0.01*	0.04 ± 0*	0.06 ± 0.01*	0.03 ± 0.01*
Alanine	0.45 ± 0.01	0.72 ± 0.03*	0.65 ± 0.02*	1.43 ± 0.06*	0.99 ± 0.04*
Cystathionine	0.04 ± 0	0.06 ± 0*	0.06 ± 0*	0.09 ± 0.01*	0.06 ± 0*
Tyrosine	0.03 ± 0	0.06 ± 0.01*	0.04 ± 0.01*	0.16 ± 0.01*	0.03 ± 0
β-Alanine	0.01 ± 0	0.03 ± 0.01*	0.01 ± 0	0.07 ± 0*	0.03 ± 0.01*
GABA	2.03 ± 0.03	3.34 ± 0.12*	2.77 ± 0.09*	4.81 ± 0.18*	3.07 ± 0.11*
Ornithine	0.01 ± 0	0.02 ± 0*	0.01 ± 0	0.03 ± 0.01*	0.01 ± 0
Homocarnosine	0.08 ± 0.01	0.14 ± 0.02*	0.17 ± 0.01*	0.08 ± 0.01	0.09 ± 0.01

Values (mean ± S.E.M.) are expressed in μmol/g wet wt. Figures in parentheses indicate number of animals in each group, and hours indicate times animals were killed after last hydrazine injection.

* $p < 0.05$ as compared to controls (one way analysis of variance followed by Dunnett's 2-tailed multiple t-test).

Table 20. GABA-T and GAD enzyme activity in whole brain of rats treated with hydrazine for 109 days.

Enzyme activity	Controls (17)	Hydrazine-treated rats			
		0.08 mmol/kg/day		0.16 mmol/kg/day	
		6h (7)	24h (7)	6h (7)	24h (7)
GABA-T	400 ± 10	307 ± 7*	306 ± 20*	263 ± 4*	280 ± 12*
GAD	298 ± 5	301 ± 14	296 ± 9	294 ± 7	297 ± 9

Enzyme activities are expressed in μmol/h/g protein, and represent mean ± S.E.M.
Figures in parentheses indicate number of animals in each group, and hours indicate times animals were killed after last hydrazine injection.
* $p < 0.05$ as compared with controls (one way analysis of variance followed by Dunnett's two-tailed multiple t-test).

human patients treated with isoniazid in order to prevent peripheral neuropathy.

All rats tolerated daily subcutaneous injections of hydrazine and pyridoxine well, and there was no evidence of local tissue damage. Hydrazine-treated rats gained weight normally, the mean individual weight gains over 109 days for the hydrazine high and low dose rats, pyridoxine-injected controls, and uninjected controls being 149 g, 161 g, 156 g, and 151 g respectively. No differences in behavior were noted in the hydrazine-treated rats as compared to rats in either control group.

Histological examination by light microscopy and electron microscopy were performed by Dr. L. Dunn on the liver specimens from rats treated with the high dose of hydrazine monohydrochloride (0.16 mmol/kg/day) for 109 days. No evidence of any irreversible pathological changes were observed in the livers of any of the hydrazine-treated rats. Although some of the rats showed evidence of fatty infiltration there was no significant difference in this effect between the hydrazine-treated and control rats.

Effects of hydrazine on brain amino compounds and relevant enzymes

Table 19 shows the effects of chronic hydrazine treatment on the contents of 12 amino compounds in whole rat brain. No changes were observed in 23 other free amino acids and related compounds that were quantitated on the amino acid analyzer, and these are therefore omitted from Table 19. Values are shown for brain amino acid contents 6 hours and 24 hours after the last hydrazine injection on both the lower and higher dosages. The contents of all brain amino acids were similar in pyridoxine-injected controls killed either 6 hours or 24 hours after the last injection, and in control rats which had not been injected ($p > 0.05$, one-way analysis of

variance). To simplify Table 19, values for all the control groups have been pooled.

Brain GABA content was significantly increased at both 6 hours and 24 hours after the last injection by the low and the high doses of hydrazine, and GABA content was greater with the higher dose. β-alanine content also followed a similar pattern. Homocarnosine content in brain was elevated by the low dose of hydrazine, but surprisingly not by the higher dose.

Increases also occurred in the whole brain contents of alanine, hypotaurine, α-aminoadipic acid, cystathionine, tyrosine and ornithine, which were usually most marked in rats sacrificed 6 hours after the last injection on the high hydrazine dosage schedule.

Finally, brain glutamine content was lowered by hydrazine administration, more by the higher dose, and this was accompanied by a slight decrease in glutamic acid content.

Table 20 shows the mean activities of GABA-T and GAD in the whole brain of control rats and of rats sacrificed 6 or 24 hours after the last of 109 daily injections of hydrazine monohydrochloride. Activities of both enzymes were not significantly different in pyridoxine-injected control rats killed either 6 hours or 24 hours after the last injection, or in control rats which had received no injection ($p > 0.05$, one way analysis of variance). Therefore, values were pooled for the controls. GABA-T activity was significantly reduced in brain of hydrazine-treated rats, and enzyme activity was similar 6 hours and 24 hours after the last hydrazine injection. Chronic hydrazine administration did not alter brain GAD activity (Table 20).

VI. DISCUSSION

Effects of γ-vinyl GABA and hydrazine on GABA and its metabolizing enzymes

The observations that chronic administration of γ-vinyl GABA reduced the activity of the GABA catabolizing enzyme, GABA-T, and markedly elevated the content of GABA in whole rat brain are in accord with the findings of Jung et al. (1977a) for mouse brain. Likewise, the effects of chronic hydrazine treatment on GABA content and GABA-T activity in rat brain are in agreement with those detailed by Maynert and Kaji (1962), Medina (1963), and Wood and Peesker (1975) in which the biochemical effects of hydrazine on GABA metabolism were examined following a single injection. The observations that isoniazid itself had no significant inhibitory effect on GABA-T in vitro, and that hydrazine was the only isoniazid metabolite tested which significantly inhibited GABA-T in vitro, suggest that the isoniazid-induced elevation of brain GABA in rat and monkey brain observed in vivo (Perry et al., 1973a; 1974) may be a consequence of GABA-T inhibition by hydrazine. Ellard and Gammon (1976) present evidence suggesting that hydrazine may be produced from the hydrolysis of isoniazid in vivo. Furthermore, Wright and Timbrell (1978) have suggested that hydrazine may be a product of the hydrolysis of acetylhydrazine as well.

GAD activity in brain was almost as greatly reduced as was GABA-T activity after 11 days treatment with γ-vinyl GABA. However, no reduction in brain GAD activity was observed in the animals receiving chronic hydrazine treatment. The reduction of brain GAD activity following administration of GABA-T inhibitors has been postulated to be due to the unavoidable consequence of feedback inhibition of the synthesis of new enzyme protein caused by prolonged high levels of GABA in brain (Jung, 1978). The observation that long-term administration of hydrazine elevated brain GABA levels without significant inhibition of the GABA-synthesizing enzyme makes

such a postulate unlikely. Although several investigators (Medina, 1963; Wood and Peesker, 1975; Abe and Matsuda, 1977) found that hydrazine produced marked inhibition of GAD activity in mouse brain *in vivo* after acute administration, the doses of hydrazine employed by these investigators were much higher (0.5-4 mmol/kg) than those used in the present study. Thus, the results of our study demonstrate that it is possible to produce a prolonged elevation of whole brain GABA *in vivo* without a concomitant and potentially deleterious decrease in GAD activity.

Effects of γ-vinyl GABA and hydrazine on other brain amino compounds

The contents of both β-alanine and homocarnosine were increased in brain following administration of either γ-vinyl GABA or hydrazine. This could be expected because β-alanine is degraded to malonic acid semialdehyde by GABA-T (Wu *et al.*, 1976) and homocarnosine is probably synthesized from GABA and histidine by the enzyme homocarnosine-carnosine synthetase (Skaper *et al.*, 1973) and thus would be expected to increase when the brain content of its substrate GABA increased. The fact that brain homocarnosine content was only elevated in rats receiving the lower dose of hydrazine suggests that higher concentrations of this agent may directly inhibit the homocarnosine synthesizing enzyme. The hydrazine-induced reduction in brain glutamate content may have been the result of inhibition of GABA-T, in which GABA degradation is coupled to glutamate formation.

γ-Vinyl GABA and hydrazine obviously have more widespread effects in brain than on the enzymes that synthesize and degrade GABA. The increases in the hypotaurine content of brain produced by both of these agents was not anticipated. Hypotaurine is the intermediate between cysteine sulfinic acid and taurine in the metabolic pathway from cysteine to taurine in mammalian brain (Jacobsen and Smith, 1968). Oxidation of hypotaurine to

taurine is carried out by hypotaurine dehydrogenase (Sumizu, 1962; Oja et al., 1973; Di Giorgio et al., 1977). Oja et al. (1973) found activity of hypotaurine dehydrogenase present in infant rat brain, but could not detect it in adult rat brain. However, Perry and Hansen (1973d) have found hypotaurine routinely present in adult rat brain. It seems likely that γ-vinyl GABA and hydrazine inhibit hypotaurine dehydrogenase and in this way produce the marked increase in hypotaurine in rat brain. It is of interest that a smaller increase in hypotaurine content in rat brain was observed in an earlier study with another GABA-T inhibitor, aminooxyacetic acid (Perry and Hansen, 1973a).

The decrease in the mean content of glutamine produced by repeated injections of both γ-vinyl GABA and hydrazine was also unexpected. The lowered glutamine content suggests the possibility that these agents may inhibit the enzyme glutamine synthetase. Alternatively, the low glutamine content may have been a consequence of the reduced amount of its substrate, glutamate in brain.

Hydrazine is a pyridoxal phosphate scavenger (Roberts et al., 1964) and thus would be expected to have an inhibitory action on enzymes utilizing pyridoxal phosphate as a cofactor. In this regard, the elevation of alanine, tyrosine, ornithine and cystathionine might be explained by the inhibition of their respective degrading enzymes which employ pyridoxal phosphate as a cofactor: alanine aminotransferase, tyrosine aminotransferase, ornithine aminotransferase and cystathionine γ-lyase. Likewise, the elevation of α-aminoadipic acid might be due to the inhibition of α-aminoadipic transaminase, an enzyme which converts α-aminoadipic acid to α-ketoadipic acid, and which is likely to use pyridoxal phosphate as a cofactor.

Biological significance of the lack of selectivity of γ-vinyl GABA and hydrazine

The results of our investigation demonstrate that chronic administration of either γ-vinyl GABA or hydrazine can produce many effects in brain other than the desired inhibition of the GABA catabolizing enzyme. The ability of γ-vinyl GABA to inhibit brain GAD activity is clearly an undesired side effect, since a decrease of GABA concentration in nerve endings as a consequence of GAD inhibition might result in a reduction in the amount of GABA available for release at synapses, even though the GABA content was elevated overall in the brain. In this respect, hydrazine, which had no effect on GAD activity in whole brain, appears to be the preferred drug. However, in the present investigation we were limited to a small supply of γ-vinyl GABA available to us for study and thus were unable to explore the biochemical effects in brain following administration of lower doses of γ-vinyl GABA. It may be that chronic administration of γ-vinyl GABA in doses considerably lower than we used would cause a similar elevation of brain GABA, without reducing GAD activity.

A new approach to the study of the relationship between brain GABAergic activity and neurological alterations has been to correlate drug-induced changes in behaviour with GABA content in certain subcellular fractions of brain, rather than in whole brain homogenates. In this regard, several groups of investigators have found a greater correlation between the excitability of brain, as measured by the time to onset and intensity of drug-induced seizures, and GABA levels determined in brain synaptosomal fractions, than with GABA levels in whole brain homogenates (Abe and Matsuda, 1977; Matsuda et al., 1979; Sarhan and Seiler, 1979; Wood et al., 1979). Thus, it would be interesting to examine the chronic effects of γ-vinyl GABA and hydrazine, and other GABA-T inhibitors, on GABA content

and GAD activity in the brain synaptosomal fraction, a subcellular fraction which may better reflect the critical biochemical events at the nerve ending.

Both γ-vinyl GABA and hydrazine were found to alter the contents of many brain amino compounds other than GABA. Some of the amino compounds which were elevated in brain such as β-alanine, hypotaurine, and cystathionine have a depressant action when applied iontophoretically onto mammalian neurons (Curtis and Johnston, 1974), while other compounds such as α-aminoadipic acid (L-isomer) have an excitatory action (Hall et al., 1979). Thus, it is conceivable that drug-induced changes in these brain amino compounds might have important electrophysiological as well as metabolic consequences in brain. Although we observed no gross changes in either the behaviour of the animals treated with γ-vinyl GABA and hydrazine, the potential toxicity of the changes in the brain amino compounds is unknown. Thus, it remains to be determined whether the additional non-specific actions of the two GABA-T inhibitors are in fact harmful.

It should also be emphasized that, in future clinical trials, should administration of one or the other of these two compounds be found to have a beneficial effect in patients with certain neurological disorders, such an effect could not be automatically attributed to an action of the drug in elevating brain GABA content.

References

Abe, M. and Matsuda, M. (1977) γ-aminobutyric acid metabolism in subcellular particles of mouse brain and its relationship to convulsions. J. Biochem. 82, 195-200.

Abraham, D., Pisano, J. J. and Udenfriend, S. (1961) Synthesis of homocarnosine in muscle in vivo. Biochim. Biophys. Acta 50, 570-572.

Abraham, D., Pisano, J. J. and Udenfriend S. (1962) The distribution of homocarnosine in mammals. Arch. Bioch. Biophys. 99, 210-213.

Abraham, D., Pisano, J. J. and Udenfriend, S. (1964) Uptake of carnosine and homocarnosine by rat brain slices. Arch. Bioch. Biophy. 104, 160-165.

Amend, J. F., Strumeyer, D. H. and Fisher, H. (1979) Effect of dietary histidine on tissue concentrations of histidine-containing dipeptides in adult cockerels. J. Nutr. 109, 1779-1786.

Anastasi, A. and Erspamer, V. (1964) Occurrence of homocarnosine (γ-aminobutyrylhistidine) in the brain of Rana esculenta. J. Neurochem. 11, 619.

Arnt, J., Scheel-Kruger, J., Magelund, G. and Krogsgaard-Larsen, P. (1979) Muscimol and related GABA receptor agonists: the potency of GABA-ergic drugs in vivo determined after intranigral injection. J. Pharm. Pharmacol. 31, 306-313.

Atkin, B. M., Buist, N. R. M., Utter, M. F., et al. (1979) Pyruvate carboxylase deficiency and lactic acidosis in a retarded child without Leigh's disease. Pediat. Res. 13, 109-116.

Avery, T. L. (1971) Seven cases of frontal tumour with psychiatric presentation. Br. J. Psychiat. 119, 19-23.

Bartholini, G. and Stadler, H. (1977) Evidence for an intrastriatal GABA-ergic influence on dopamine neurones of the cat. Neuropharmacol. 16, 343-347.

Ben-Ari, Y. and Kelly, J. S. (1976) Dopamine evoked inhibition of single cells of the feline putamen and basolateral amygdala. J. Physiol. 256, 1-21.

Berger, P. A., Faull, K. F., Kilkowski, J., Anderson, P. J., Kraemer, H., Davis, K. L. and Barchas, J. D. (1980) CSF monoamine metabolites in depression and schizophrenia. Am. J. Psychiat. 137, 174-180.

Bernardi, G., Marciani, M. G., Norocutti, C. and Giacomini, P. (1975) The action of GABA on rat caudate neurones recorded intracellularly. Brain Res. 92, 511-515.

Bird, E. D., Spokes, E. G. S. and Iversen, L. L. (1979) Increased dopamine concentration in limbic areas of brain from patients dying with schizophrenia. Brain 102, 347-360.

Bissette, G., Nemeroff, C. B., Loosen, P. T., Breese, G. R., Burnett, G. B., Lipton, M. A. and Prange, Jr. A. J. (1978) Modification of pentobarbital-induced sedation by natural and synthetic peptides. Neuropharmacol. 17, 229-237.

Brun, A. and Gustafson, L. (1978) Limbic lobe involvement in presenile dementia. Arch. Psychiat. Nervenkr. 226, 79-93.

Buchsbaum, M. S. and Reider, R. O. (1979) Biologic heterogeneity and psychiatric research. Arch. Gen. Psychiat. 36, 1163-1169.

Buu, N. T., Puil, E. and van Gelder, N. M. (1975) Receptors for amino acids in excitable tissues. Gen. Pharmac. 7, 5-14.

Carlson, R. J. (1977) Frontal lobe lesions masquerading as psychiatric disturbances. Can. Psychiat. Assoc. J. 22, 315-318.

Chung-Hwang, E., Khurana, H. and Fisher, H. (1976) The effect of dietary histidine level on the carnosine concentration of rat olfactory bulbs. J. Neurochem. 26, 1087-1091.

Clow, A., Jenner, P., Marsden, C. D. and Theodorou, A. (1980) Striatal dopamine receptor activity during 6 months withdrawal following one years neuroleptic administration to rats. Brit. J. Pharmacol. 69: 277P-278P.

Collingridge, G. L., Collins, C. G. S., Davies, J., James, T. A., Neal, M. J. and Tongroach, P. (1980) Effect of tetanus toxin on transmitter release from the substantia nigra and striatum in vitro. J. Neurochem. 34, 540-547.

Cools, A. R. and Janssen, H. J. (1976) γ-aminobutyric acid: the essential mediator of behaviour triggered by neostriatally applied apomorphine and haloperidol. J. Pharm. Pharmacol. 28, 70-74.

Cools, A. R., Struyker Boudier, H. A. J. and van Rossum, J. M. (1976) Dopamine receptors: selective agonists and antagonists of functionally distinct types within the feline brain. Eur. J. Pharm. 37, 283-293.

Costall, B., Naylor, R. J. and Pinder, R. M. (1976) Mechanisms of dyskinesia induction by 1-(3,4-dihydroxyphenyl) piperazine in the rat. Neuropharm. 15, 203-210.

Courville, J. and Faraco-Cantin, F. (1978) On the origin of the climbing fibers of the cerebellum: an experimental study in the cat with an autoradiographic tracing method. Neurosci. 3, 797-809.

Cross, A. J., Crow, T. J. and Owen, F. (1979) Gamma-aminobutyric acid in the brain in schizophrenia. Lancet 560-561.

Crow, T. J., Baker, H. F., Cross, A. J., Joseph, M. H., Lofthouse, R., Longden, A., Owen, F., Riley, G. J., Glover, V. and Killpack, W. S. (1979) Monoamine mechanisms in chronic schizophrenia: post mortem neurochemical findings. Brit. J. Psychiat. 134, 249-256.

Currier, R. D., Glover, G., Jackson, J. F., et al. (1972) Spinocerebellar ataxia: study of a large kindred. I. General information and genetics. Neurology (Minneap.) 22, 1040-1043.

Curtis, D. R. and Felix, D. (1971) The effects of bicuculline upon synaptic inhibition in the cerebral and cerebellar cortices of the cat. Brain Res. 34, 301-321.

Curtis, D. R. and Johnston, G. A. R. (1974) Amino acid transmitters in the mammalian central nervous system. Ergeb. der Physiol. 69, 97-188.

Di Giorgio, R. M., Macaione, S. and De Luca, G. (1977) Subcellular distribution of hypotaurine oxidase activity in ox retina. Life. Sci. 20, 1657-1662.

Dray, A., Fowler, L. J., Oakley, N. R., Simmonds, M. A. and Tanner, T. (1977) Regulation of nigro-striatal dopaminergic neurotransmission in the rat. Neuropharmacology 16, 511-518.

Dudel, J. (1965) Presynaptic and postsynaptic effects of inhibitory drugs on the crayfish neuromuscular junction. Pflugers Archiv. 283, 104-118.

Dudel, J. (1975) Potentiation and desensitization after glutamate induced postsynaptic currents at the crayfish neuromuscular junction. Pflugers Arch. 356, 317-327.

Ellard, G. A. and Gammon, P. T. (1976) Pharmacokinetics of isoniazid metabolism in man. J. Pharm and Biopharm. 4, 83-113.

Elliott, K. A. C. and Florey, E. (1956) Factor I - inhibitory factor from brain. J. Neurochem. I, 181-191.

Enna, S. J. and Snyder, S. H. (1976) A simple, sensitive and specific radioreceptor assay for endogenous GABA in brain tissue. J. Neurochem. 26, 221-224.

Enwonwu, C. O. and Worthington, B. S. (1973) Accumulation of histidine, 3-methylhistidine and homocarnosine in the brains of protein-calorie deficient monkeys. J. Neurochem. 21, 799-807.

Escobar, J. I. and Chandel, V. (1977) Nuclear symptoms of schizophrenia after cingulotomy: a case report. Am. J. Psychiat. 134, 11.

Farley, I. J., Price, K. S. and Hornykiewicz, O. (1977) Dopamine in the limbic regions of the human brain: normal and abnormal. In: Advances in Biochemical Psychopharmacology, Vol. 16. Eds. E. Costa and G. L. Gessa, Raven Press, New York.

Feltz, P. (1978) Pharmacological considerations about establishing true actions of dopamine on striatal neurones with nigral inputs. In: Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System, ed. Ryall and Kelly. Elsevier/North-Holland Biomedical Press, pp. 25-37.

Folstein, S. E., Folstein, M. F., and McHugh, P. R. (1979) Psychiatric syndromes in Huntington's disease. In: Advances in Neurology, Vol. 23, ed. T. N. Chase et al. Raven Press, New York, pp. 281-290.

Fonnum, F., Gottesfeld, Z. and Grofova, I. (1978) Distribution of glutamate decarboxylase, choline acetyltransferase and aromatic amino acid decarboxylase in the basal ganglia of normal and operated rats. Evidence for striatopallidal, striatoentopeduncular and striatonigral GABAergic fibres. Brain Res. 143, 125-138.

Frederickson, R. C. A., Neuss, M., Morzorati, S. L. and McBride, W. J. (1978) A comparison of the inhibitory effects of taurine and GABA on identified Purkinje cells and other neurons in the cerebellar cortex of the rat. Brain Res. 145, 117-126.

Gianutsos, G. and Lal, H. (1976) Blockade of apomorphine-induced aggression by morphine or neuroleptics: differential alteration by antimuscarinics and naloxone. Pharm. Bioch. and Behav. 4, 639-642.

Gjessing, L. R., Gjesdahl, P. and Sjaastad, O. (1972) The free amino acids in human cerebropsinal fluid. J. Neurochem. 19, 1807-1808.

Gjessing, L. R. and Sjaastad, O. (1974) Homocarnosinosis: a new metabolic disorder associated with spasticity and mental retardation. Lancet 2, 1028.

Godfraind, J. M., Krnjevic, K. and Pumain, R. (1970) Doubtful value of bicuculline as a specific antagonist of GABA. Nature 228, 675-676.

Grace, A. A. and Bunney, B. S. (1979) Paradoxical GABA excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons. Eur. J. Pharm. 59, 211-218.

Greenlee, D. V., van Ness, P. C. and Olsen, R. W. (1978) Gamma-aminobutyric acid binding in mammalian brain: receptor-like specificity of sodium-independent sites. J. Neurochem. 31, 933-938.

Hackett, J. T., Cochran, S. L. and Brown, D. L. Amino acids that excite cerebellar Purkinje cells and their potential role as climbing fiber and parallel fiber neurotransmitters. Abstract. Soc. for Neurosci. November 2-6, 1979. pp. 742.

Hall, J. G., Hicks, T. P., McLennan, H., Richardson, T. L. and Wheal, H. V. (1979) The excitation of mammalian central neurones by amino acids. J. Physiol. 286, 29-39.

Hansen, S., Perry, T.L., Wada, J. A. and Sokol, M. (1973) Brain amino acids in baboons in light-induced epilepsy. Brain Res. 50, 480-483.

Harding, J. and Margolis, F. L. (1976) Denervation in the primary olfactory pathway of mice. III. Effect on enzymes of carnosine metabolism. Brain Res. 110, 351-360.

Harris, H. (1975) The Principles of Human Biochemical Genetics, 2nd ed., pp. 232-241. North-Holland Publishing Co., Amsterdam.

Hayashi, T., (1965) Physiological action of carnitine on nerve cells, nervous pathway, and muscle. In: Recent Research on Carnitine: its Relation to Lipid Metabolism, ed. G. Wolf. The M.I.T. Press, Cambridge, Massachusetts, pp. 183-191.

Hayashi, T. and Namba, H. (1963) Treatment of epileptic patients by precursor substances of the inhibitory transmitter in the central nervous system. Biochem. Pharmacol. Supp 12, 258-259.

Hill, R. G., Simmonds, M. A. and Straughan, D. W. (1973) A comparative study of some convulsant substances as γ-aminobutyric acid antagonists in the feline cerebral cortex. Br. J. Pharmac. 49, 37-51.

Horinishi, H., Grillo, M. and Margolis, F. L. (1978) Purification and characterization of carnosine synthetase from mouse olfactory bulbs. J. Neurochem. 31, 909-919.

Hornykiewicz, O. (1978) Psychopharmacological implications of dopamine and dopamine antagonists: a critical evaluation of current evidence. Neurosci. 3, 773-783.

Iversen, L. L., Rogawski, M. A. and Miller, R. J. (1976) Comparison of the effects of neuroleptic drugs on pre- and postsynaptic dopaminergic mechanisms in the rat striatum. Mol. Pharm. 12, 251-262.

Jackson, J. F., Currier, R. D., Terasaki, P. I., et al. (1977) Spinocerebellar ataxia and HLA linkage. Risk prediction by HLA typing. New Engl. J. Med. 296, 1138-1141.

Jackson, J. F., Whittington, J. E., Currier, R. D. et al: (1978) Genetic linkage and spinocerebellar ataxia. In: Advances in Neurology, Vol. 21, The Inherited Ataxias: Biochemical, Viral, and Pathological Studies. Eds. Kark R.A.P., Rosenberg, R. N., Schut, L. J. Raven Press, New York, pp. 315-318.

Jacobsen, J. G. and Smith, L. H. (1968) Biochemistry and physiology of taurine and taurine derivatives. Physiol. Rev. 48, 424-511.

Jung, M. J. (1978) In vivo biochemistry of GABA transaminase inhibition. In: Enzyme-Activated Irreversible Inhibitors, ed. N. Seiler, M. J. Jung and J. Koch-Weser. Elsevier/North-Holland Biomedical Press, pp. 135-162.

Jung, M. J., Lippert, B., Metcalf, B. W. and Schechter, P. J. (1977a) γ-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: Effects on brain GABA metabolism in mice. J. Neurochem. 29, 797-802.

Jung, M. J., Lippert, B., Metcalf, B. W., Schechter, P. J., Bohlen, P. and Sjoerdsma, A. (1977b) The effect of 4-amino hex-5-ynoic acid (γ-acetylenic GABA, γ-ethynyl GABA) a catalytic inhibitor of GABA transaminase, on brain GABA metabolism in vivo. J. Neurochem. 28, 717-723.

Kakimoto, Y. and Konishi, H. (1976) Occurrence of γ-glutamylhistidine in bovine brain. J. Neurochem. 26, 1263-1265.

Kalyankar, G. D. and Meister, A. (1959) Enzymatic synthesis of carnosine and related β-alanyl and γ-aminobutyryl peptides. J. Biol. Chem. 234, 3210-3218

Kanazawa, A. and Sano, I. (1967) A method of determination of homocarnosine and its distribution in mammalian tissues. J. Neurochem. 14, 211-214.

Kish, S. J., Perry, T. L. and Hansen, S. (1979) Regional distribution of homocarnosine, homocarnosine-carnosine synthetase and homocarnosinase in human brain. J. Neurochem. 32, 1629-1636.

Konig, J. R. F. and Klippel, R. A. The Rat Brain, A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore. (1963).

Konigsmark, B. W. and Weiner, L. P. (1970) The olivopontocerebellar atrophies: a review. Medicine 49, 227-241.

Krnjevic, K. (1974) Chemical nature of synaptic transmission in vertebrates. Phys. Review 54, 419-540.

Krnjevic, K., Randic, M. and Straughan, D. W. (1966) Pharmacology of cortical inhibition. J. Physiol. (London) 184, 78-105.

Kumon, A., Matsuoka, Y., Kakimoto, Y., Nakajima, T., and Sano, I. (1970) A peptidase that hydrolyzes Nα-(γ-aminobutyryl)lysine. Biochim. Biophys. Acta 200, 466-474.

Kuriyama, K. (1976) Subcellular localization of the GABA system in brain. In: GABA in Nervous System Function, ed. E. Roberts, T. N. Chase and D. B. Tower. Raven Press, New York, pp. 187-196.

Lal, H., Marky, M. and Fielding, S. (1976) Effect of neuroleptic drugs on mouse jumping induced by L-DOPA in amphetamine treated mice. Neuropharm. 15, 669-671.

Lee, T. and Seeman, P. (1980) Elevation of brain neuroleptic/dopamine receptors in schizophrenia. Am. J. Psychiat. 137, 191-197.

Lenney, J. F. (1976) Specificity and distribution of mammalian carnosinase. Biochem. Biophys. Acta 429, 214-219.

Lenney, J. F., Kan, S.-C., Siu, K. and Sugiyama, G. H. (1977) Homocarnosinase: a hog kidney dipeptidase with a broader specificity than carnosinase. Archs. Biochem. Biophys. 184, 257-266.

Lidsky, T.I., Weinhold, P. M. and Levine, F. M. (1979) Implications of basal ganglionic dysfunction for schizophrenia. Biol. Psych. 14, 3-12.

Lloyd, K. G. and Dreksler, S. (1978) An analysis of [^{3}H]Gamma-aminobutyric acid (GABA) binding in the human brain. Brain Res. 163, 77-87.

Lloyd, K. G. and Hornykiewicz, O. (1977) Effect of chronic neuroleptic or L-DOPA administration on GABA levels in the rat substantia nigra. Life Sci. 21, 1489-1496.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

MacLeod, N. K. and Straughan, D. W. (1979) Responses of olfactory bulb neurones to the dipeptide carnosine. Exp. Brain Res. 34, 183-188.

MacLeod, N. (1978) Is carnosine (β-Ala-L-His) a neurotransmitter in the primary olfactory pathway ? In: Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System. Ed. Ryall and Kelly. Elsevier/-North-Holland Biomedical Press, pp. 117-119.

Malamud, N. C. (1967) Psychiatric disorder with intracranial tumors of the limbic system. Arch. Neurol. 17, 113-124.

Margolis, F. L. (1974) Carnosine in the primary olfactory pathway. Science 184, 909-911.

Marsden, C., Meldrum, B. S., Pycock, C. and Tarsy, D. (1975) Focal myoclonus produced by injection of picrotoxin into the caudate nucleus of the rat. J. Physiol. Lond. 249, 96P.

Marshall, Jr., F. D. (1973) The effect of reserpine, chlorpromazine and nembutal on levels of dipeptides in rat brain and muscle. Life Sci. 13, 135-140.

Marstein, S., Jellum, E., Halpern, B., Eldjarn, L., and Perry, T. L. (1976) Biochemical studies of erythrocytes in a patient with pyroglutamic acidemia (5-oxoprolinemia). New Eng. J. Med. 295, 406-412.

Mathison, R. D., and Dreifuss, J. J. (1980) Structure-activity relationships of a neurohypophysial GABA receptor. Brain Res. 187, 476-480.

Matsuda, M., Abe, M., Hoshino, M. and Sakurai, T. (1979) γ-aminobutyric acid in subcellular fractions of mouse brain and its relation to convulsions. Biochem. Pharm. 28, 2785-2789.

Maynert, E. W. and Kaji, H. K. (1962) On the relationship of brain γ-aminobutyric acid to convulsions. J. Pharm. Exp. Therap. 137, 114-121.

McGeer, E. G., McGeer, P. L. and McLennan, H. (1961) The inhibitory action of 3-hydroxytyramine, gamma-aminobutyric acid (GABA) and some other compounds towards the crayfish stretch receptor neuron. J. Neurochem. 8, 36-49.

McKenzie, G. M., Gordon, R. J. and Viik, K. (1972) Some biochemical and behavioural correlates of a possible animal model of human hyperkinetic syndromes. Brain Res. 47, 439-456.

McKenzie, G. M. and Viik, K. (1975) Chemically induced choreiform activity: Antagonism by GABA and EEG patterns. Exp. Neurol. 46, 229-243.

Medina, M. A. (1963) The in vivo effects of hydrazines and vitamin B_6 on the metabolism of gamma-aminobutyric acid. J. Pharm. Exp. Therap. 140, 133-137.

Meltzer, H. Y. and Stahl, S. M. (1976) The dopamine hypothesis of schizophrenia: a review. Schizophrenia Bulletin 2, 19-76.

Mitchell, J. R., Thorgeirsson, U. P., Black, M., Timbrell, J. A., Snodgrass, W. R., Potter, W. Z., Jollow, D. J. and Keiser, H. R. (1975) Increased incidence of isoniazid hepatitis in rapid acetylators: Possible relation to hydrazine metabolites. Clin. Pharmac. and Ther. 18, 70-79.

Nadi, N. S., Kanter, D., McBride, W. J. et al. (1977) Effects of 3-acetylpyridine on several putative neurotransmitter amino acids in the cerebellum and medulla of the rat. J. Neurochem. 28, 661-662.

Nakajima, T., Kakimoto, Y., Tsuji, M., Konishi, H. and Sano, Y. (1974) Metabolism of polyamines in mammals: metabolic pathway of putrescine. Bull. Jap. Neurochem. Soc. 13, 174-177.

Neidle, A. and Kandera, J. (1974) Carnosine - an olfactory bulb peptide. Brain Res. 80, 359-364.

Ng, R. H. and Marshall, F. D. (1976a) Distribution of homocarnosine-carnosine synthetase in tissues of rat, mouse, chick and frog. Comp. Biochem. Physiol. 54B, 519-521.

Ng, R. H. and Marshall, F. D. (1976b) The effects of various agents in vitro on homocarnosine-carnosine synthetase from rat brain. Experientia 32, 839-841.

Ng, R. H. and Marshall, F. D. (1978) Regional and subcellular distribution of homocarnosine-carnosine synthetase in the central nervous system of rats. J. Neurochem. 30, 187-190.

Ng, R. H., Marshall, F. D., Henn, F. A. and Sellstrom, A. (1977) Metabolism of carnosine and homocarnosine in subcellular fractions and neuronal and glial cell-enriched fractions of rabbit brain. J. Neurochem. 28, 449-452.

Oja, S. S., Karvonen, M. -L. and Lahdesmaki, P. (1973) Biosynthesis of taurine and enhancement of decarboxylation of cysteine sulphinate and glutamate by the electrical stimulation of rat brain slices. Brain Res. 55, 173-178.

Okamoto, K. and Quastel, J. H. (1976) Effects of amino acids and convulsants on spontaneous action potentials in cerebellar cortex slices. Br. J. Pharmac. 57, 3-15.

Owen, F., Crow, T. J., Poulter, M., Cross, A. J., Longden, A., and Riley, G. J. (1978) Increased dopamine-receptor sensitivity in schizophrenia. Lancet 2, 223-226.

Pellegrino, L. J. and Cushman, A. J. (1967) A Stereotaxic Atlas of the Rat Brain. Appleton-Century Crofts, New York.

Perrson, T., Ross, B. E. (1969) Acid metabolites from monoamines in CSF of chronic schizophrenics. Br. J. Psychiatry 115, 95-98.

Perry, T. L., Stedman, D., and Hansen, S. (1968) A versatile lithium buffer elution system for single column automatic amino acid chromatography. J. Chromat. 38, 460-466.

Perry, T. L., Berry, K., Hansen, S., Diamond, S. and Mok, C. (1971) Regional distribution of amino acids in human brain obtained at autopsy. J. Neurochem. 18, 513-519.

Perry, T. L., Sanders, H. D., Hansen, S., Lesk, D., Kloster, M. and Gravlin, L. (1972) Free amino acids and related compounds in five regions of biopsied cat brain. J. Neurochem. 19, 1651-1656.

Perry, T. L. and Hansen, S. (1973a) Sustained drug-induced elevation of brain GABA in the rat. J. Neurochem. 21, 1167-1175.

Perry, T. L., Hansen, S. and Kloster, M. (1973b) Huntington's chorea: deficiency of γ-aminobutyric acid in brain. N. Eng. J. Med. 288, 337-342.

Perry, T. L., Hansen, S., Tischler, B., Richards, F. M. and Sokol, M. (1973c) Unrecognized adult phenylketonuria: implications for obstetrics and psychiatry. New Eng. J. Med. 289, 395-398.

Perry, T. L. and Hansen, S. (1973d) Quantification of free amino compounds of rat brain: identification of hypotaurine. J. Neurochem. 21, 1009-1011.

Perry, T. L., Urquhart, N., Hansen, S. and Kennedy, J. (1974) γ-Aminobutyric acid: drug-induced elevation in monkey brain. J. Neurochem. 23, 443-445.

Perry, T. L., Urquhart, N., MacLean, J., Evans, M. E., Hansen, S., Davidson, A. G. F., Applegarth, D. A., MacLeod, P. J. and Lock, J. E. (1975a) Nonketotic hyperglycinemia: glycine accumulation due to absence of glycine cleavage in brain. New Eng. J. Med. 292, 1269-1273.

Perry, T. L., Hansen, S. and Kennedy, J. (1975b) CSF amino acids and plasma-CSF amino acid ratios in adults. J. Neurochem. 24, 587-589.

Perry, T. L., Hansen, S., Schier, G. M. and Halpern, B. (1977a) Isolation and identification of γ-aminobutyryl-cystathionine from human brain and CSF. J. Neurochem. 29, 791-795.

Perry, T. L., Currier, R. D., Hansen, S. et al. (1977b) Aspartate-taurine imbalance in dominantly inherited olivopontocerebellar atrophy. Neurology (Minneap.) 27, 257-261.

Perry, T. L., Kish, S. J., Buchanan, J. and Hansen, S. (1979a) Gamma-aminobutyric acid in the brain in schizophrenia. Lancet I, 560-561.

Perry, T. L., Kish, S. J. and Hansen, S. (1979b) γ-vinyl GABA: effects of chronic administration on the metabolism of GABA and other amino compounds in rat brain. J. Neurochem. 32, 1641-1645.

Perry, T. L., Hansen, S. and Kish, S. J. (1979c) Effects of chronic administration of antipsychotic drugs on GABA and other amino acids in the mesolimbic area of rat brain. Life Sci. 24, 283.

Perry, T. L., Kish, S. J., Sjaastad, O., Gjessing, L. R., Nesbakken, R., Schrader, H. and Loken, A. C. (1979d) Homocarnosinosis: increased content of homocarnosine and deficiency of homocarnosinase in brain. J. Neurochem. 32, 1637-1640.

Perry, T. L., Wright, J. M., Hansen, S. and MacLeod, P. M. (1979e) Isoniazid therapy of Huntington disease. Neurology 29, 370-375.

Perry, T. L., Kish, S. J., Hansen, S. and Currier, R. D. (1980) Neurotransmitter amino acids in dominantly inherited cerebellar disorders. Neurology (in press).

Perry, T. L., Hansen, S. and Gandham, S. (1981) Post-mortem changes of amino compounds in human and rat brain. J. Neurochem. (in press).

Pijnenburg, A. J. J., Honig, W. M. M., van Der Heyden, J. A. M. and van Rossum, J. M. (1976) Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharm. 35, 45-58.

Pisano, J. J., Wilson, J. D., Cohen, L., Abraham, D. and Udenfriend, S. (1961) Isolation of γ-aminobutyrylhistidine (homocarnosine) from brain. J. Biol. Chem. 236, 499-502.

Quinn, M. R. and Fisher, H. (1977) Effect of dietary histidine on olfaction, and rat brain and muscle concentration of histidine-containing dipeptides. J. Neurochem. 29, 717-728.

Rea, M. A., McBride, W. J. and Rohde, B. H. (1980) Regional and synaptosomal levels of amino acid neurotransmitters in the 3-acetylpyridine deafferentated rat cerebellum. J. Neurochem. 35, 1106-1108.

Riley, L. (1960) An Atlas of the Basal Ganglia, Brain Stem and Spinal Cord. Hafner Publishing Co., New York.

Robbins, J. (1959) The excitation and inhibition of crustacean muscle by amino acids. J. Physiol. 148, 39-50.

Robbins, J. and van der Kloot, W. G. (1958) The effect of picrotoxin on peripheral inhibiton in the crayfish. J. Physiol. 143, 541-552.

Roberts, E. (1972) An hypothesis suggesting that there is a defect in the GABA system in schizophrenia. Neurosci. Res. Program. Bull. 10, 468-483.

Roberts, E. The γ-aminobutyric acid system in schizophrenia. In: Neuroregulators and Psychiatric Disorders, ed. Usden, E. et al. Oxford University Press, N.Y. (1977), pp. 347-357.

Roberts, E. and Simonsen, D. G. (1963) Some properties of L-glutamate decarboxylase in mouse brain. Biochem. Pharmac. 12, 113-134.

Roberts, E., Wein, J. and Simonsen, D. G. (1964) γ-aminobutyric acid (γ-ABA), vitamin B_6, and neuronal function - a speculative synthesis. Vitam. Norm. 22, 503-559.

Roberts, E., Wong, E., Krause, D.N., Ikeda, K. and Degener, P. New directions in GABA research II: some observations relevant to GABA action and inactivation. In: GABA-Neurotransmitters. Eds. Krogsgaard-Larsen, P. et al. Academic Press, New York, (1978) pp. 46-59.

Robin, M. M., Palfreyman, M. G. and Schechter, P. J. (1980) Dyskinetic effects of intrastriatally injected GABA-transaminase inhibitors. Life Sci. 25, 1103-1110.

Ruff, R. L. and Russakoff, L. M. (1980) Catatonia with frontal lobe atrophy. J. Neurol. Neurosurg. and Psychiat. 43, 185-187.

Scatton, B., Bischoff, S., Dedek, J. and Korf, J. (1977) Regional effects of neuroleptics on dopamine metabolism and dopamine-sensitive adenylate cyclase activity. Eur. J. Pharm. 44, 287-292.

Sarhan, S. and Seiler, N. (1979) Metabolic inhibitors and subcellular distribution of GABA. J. Neurosci. Res. 4, 399-421.

Schechter, P. J., Tranier, Y., Jung, M. J. and Bohlen, P. (1977) Audiogenic seizure protection by elevated brain GABA concentration in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. Eur. J. Pharmac. 45, 319-328.

Scheel-Kruger, J., Cools, A. R. and Honig, W. (1977a) Muscimol antagonizes the ergometrine-induced locomotor activity in nucleus accumbens: evidence for a GABA-dopaminergic interaction. Eur. J. Pharm. 42, 311-313.

Scheel-Kruger, J., Arnt, J. and Magelund, G. (1977b) Behavioural stimulation induced by muscimol and other GABA agonists injected into the substantia nigra. Neurosci. Lett. 4, 351-356.

Scheel-Kruger, J., Arnt, J., Magelund, G., Olianas, M., Przewlocka, B. and Christensen, A. V. (1979) Behavioural functions of GABA in basal ganglia and limbic system. Abstract. Brain Res. Bull. 4, 709.

Schut, J. W. (1950) Hereditary ataxia: Clinical study through six generations. Arch. Neurol. Psychiat. 63, 535-568.

Sjaastad, O., Berstad, J., Gjesdahl, P. and Gjessing, L. (1976) Homocarnosinosis. 2. A familial metabolic disorder associated with spastic paraplegia, progressive mental deficiency, and retinal pigmentation. Acta Neurol. Scand. 53, 275-290.

Sjaastad, O., Gjessing, L., Berstad, J. R. and Gjesdahl, P. (1977) Homocarnosinosis. 3. Spinal fluid amino acids in familial spastic paraplegia. Acta Neurol. Scand. 55, 158-162.

Skaper, S. D., Das, S. and Marshall, F. D. (1973) Some properties of a homocarnosine-carnosine synthetase isolated from rat brain. J. Neurochem. 21, 1429-1445.

Skirboll, L. R. and Bunney, B. S. (1979) The effects of acute and chronic haloperidol treatment on spontaneously firing neurons in the caudate nucleus of the rat. Life Sci. 25, 1419-1434.

Slater, P. and Turnbull, M. J. (1969) Hypothermia following intraventricular injection of homocarnosine in the rat. Life Sci. 6, 441-447.

Spehlmann, R. and Norcross, K. Dopamine antagonists selectively block excitatory nigral input to caudate neurons. In: Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System, ed. Ryall and Kelly. Elsevier/North-Holland Biomedical Press, (1978) pp. 62-64.

Spitzer, R. L., Andreasen, N. C. and Endicott, J. (1978) Schizophrenia and other psychotic disorders in DSM-III. Schizophrenia Bull. 4, 489-509.

Spokes, E. G. S. (1980) Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain 103, 179-210.

Spokes, E. G. S., Garrett, N. J. and Iversen, L. L. (1979) Differential effects of agonal status on measurements of GABA and glutamate decarboxylase in human post-mortem brain tissue from control and Huntington's chorea subjects. J. Neurochem. 33, 773-778.

Starr, M. S. (1979) GABA-mediated potentiation of amine release from nigrostriatal dopamine neurones in vitro. Eur. J. Pharmacol. 53, 215-226.

Stevens, J., Wilson, K. and Foote, W. (1974) GABA blockade, dopamine and schizophrenia: experimental studies in the cat. Psychopharmacologia 39, 105-119.

Sumizu, K. (1962) Oxidation of hypotaurine in rat liver. Biochim. Biophys. Acta 63, 210-212.

Tardy, M., Rolland, B., Bardakdjian, J. and Gonnard, P. (1978) Action of homocarnosine, carnosine and anserine on uptake and metabolism of GABA in different subcellular fractions of rat brain. Experientia 34, 823-962.

Tarsy, D., Pycock, C. J., Meldrum, B. S. and Marsden, C. D. (1978) Focal contralateral myoclonus produced by inhibition of GABA action in the caudate nucleus of rats. Brain 101, 143-162.

Torrey, E. F. and Peterson, M. R. (1974) Schizophrenia and the limbic system. Lancet 2, 942-946.

Turnbull, M. J. and Slater, P. (1970) Further studies on homocarnosine induced hypothermia. Life Sci. 9, 83-89.

Turnbull, M. J., Slater, P. and Briggs, I. (1972) An investigation of the pharmacological properties of homocarnosine. Arch. Int. Pharmacodyn. 196, 127-132.

Ungerstedt, U., Butcher, L. L., Butcher, S. G., Anden, N. E. and Fuxe, K. (1969) Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. Brain Res. 14, 461.

Urquhart, N., Perry, T. L., Hansen, S. and Kennedy, J. (1975) GABA content and glutamic acid decarboxylase activity in brain of Huntington's chorea patients and control subjects. J. Neurochem. 24, 1071-1075.

van Balgooy, J. N. A. and Roberts, E. (1975) Studies of metabolism of imidazole compounds in mouse brain. Biochem. Pharmacol. 24, 1421-1425.

van der Kloot, W. G. (1960) Factor S - a substance which excites crustacean muscle. J. Neurochem. 5, 245-252.

Villablanca, J. R., Elmstead, C. E., Levine, M. A. and Marcus, R. J. (1978) Effects of caudate nuclei or frontal cortical ablations in kittens: neurology and gross behaviour. Exp. Neurol. 61, 615-634.

Wachtel, H. and Anden, N.-E. (1978) Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. Arch. Pharmacol. 302, 133-139.

Walaas, I. and Fonnum, F. (1980) Biochemical evidence for γ-aminobutyrate containing fibres from the nucleus accumbens to the substantia nigra and ventral tegmental area in the rat. Neurosci. 5, 63-72.

Waszczak, B. L. and Walters, J. R. (1979) Single unit recording studies of effects of GABA agonists and antagonists on dopaminergic neurons. Abstract. Brain Res. Bull. 4, 690.

Weinberger, D. R., Torrey, E. F., Neophytides, A. N. and Wyatt, R. J. (1979) Lateral cerebral ventricular enlargement in chronic schizophrenia. Arch. Gen. Psychiatry 36, 735-739.

White, H. L. and Sato, T. L. (1978) GABA-transaminases of human brain and peripheral tissues - kinetic and molecular properties. J. Neurochem. 31, 41-47.

Whittington, J. L., Keats, B. J. B., and Jackson, J. F. Linkage studies on glyoxalase-1, pepsinogen-5, spinocerebellar ataxia, and HLA. Cytogenet. Cell. Genet. (in press).

Wood, J. D. and Peesker, S. J. (1975) The anticonvulsant action of GABA-elevating agents: a re-evaluation. J. Neurochem. 25, 277-282.

Wood, J. D., Russell, M. P., Kurylo, E. and Newstead, J. D. (1979) Stability of synaptosomal GABA levels and their use in determining the *in vivo* effects of drugs: Convulsant agents. J. Neurochem. 33, 61-68.

Wood, J. G., McLaughlin, B. J. and Vaughn, J. E. Immunocytochemical localization of GAD in electron microscopic preparations of rodent CNS. In: GABA in Nervous System Function, ed. E. Roberts, T. N. Chase and D. B. Tower. Raven Press, New York, (1976) pp. 133-148.

Woodbury, D. M. Convulsant drugs: mechanisms of action. In: Antiepileptic Drugs: Mechanisms of Action, ed. G. H. Glaser, J. K. Perry and D. M. Woodbury. Raven Press, New York, (1980) pp. 249-309.

Wright, J. M. and Timbrell, J. A. (1978) Factors affecting the metabolism of [^{14}C]acetylhydrazine in rats. Drug Metab. Dispos. 6, 561-566.

Wu, J.-Y. Purification, characterization, and kinetic studies of GAD and GABA-T from mouse brain. In: GABA in Nervous System Function, ed. E. Roberts, T. N. Chase, and D. B. Tower. Raven Press, New York, (1976) pp. 7-55.

Yale, H. C., Losee, K., Martins, J., Holsing, M., Perry, F. M. and Bernstein, J. (1953) Chemotherapy of experimental tuberculosis: the synthesis of acid hydrazides, their derivatives and related compounds. Am. Chem. Soc. J. 75, 1933-1942.

Yockey, W. C. and Marshall, F. D. (1969) Incorporation of [^{14}C]histidine into homocarnosine and carnosine of frog brain in vivo and in vitro. Biochem. J. 114, 585-588.

Young, A. B. and Snyder, S. H. (1973) A sensitive specific enzymatic-fluorometric assay for homocarnosine. J. Neurochem. 21, 387-396.