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COMMENTARY

γ-AMINOBUTYRIC ACID AND NERVOUS SYSTEM FUNCTION—A PERSPECTIVE*

EUGENE ROBERTS
Division of Neurosciences, City of Hope National Medical Center, Duarte, Calif. 91090, U.S.A.

HISTORICAL

IN THE COURSE of the study of free amino acids of various normal and neoplastic tissues in several species of animals by paper chromatography,[1,2] relatively large amounts of an unidentified ninhydrin-reactive material were found in extracts of fresh mouse, rat, rabbit, guinea-pig, human, frog, salamander, turtle, alligator and chick brains. At most, only traces of this material were found in a large number of extracts of many other normal and neoplastic tissues and in urine and blood. The unknown material was isolated from suitably prepared paper chromatograms and a study of the properties of the substance revealed it to be γ-aminobutyric acid (GABA).[3–5] An independent report of the occurrence of GABA in brain tissue was made by Awapara *et al.*[6] For several years this finding remained a biochemical curiosity and a physiological enigma. My continued efforts to convince neurophysiologists to test GABA on various preparations at the end of their planned experiments met with complete failure. In the first review on the subject, in desperation, I concluded,[7] "Perhaps the most difficult question to answer would be whether the presence in the gray matter of the central nervous system of uniquely high concentrations of γ-aminobutyric acid and the enzyme which forms it from glutamic acid has a direct or indirect connection to conduction of the nerve impulse in this tissue." However, later in that year, the first suggestion that GABA might have an inhibitory function in the vertebrate nervous system came from studies in which it was found that topically applied solutions of GABA exerted inhibitory effects on electrical activity in the brain.[8,9] Then in 1957, the suggestion was made that GABA might have an inhibitory function in the central nervous system from studies with convulsant hydrazides.[10,11] Also in 1957, definitive evidence for an inhibitory function for GABA at synapses came from studies that established GABA as the major factor in brain extracts responsible for the inhibitory action of these extracts on the crayfish stretch receptor system.[12] Within a brief period the activity in this field increased greatly, so that the research being carried out ranged all the way from the study of the effects of GABA on ionic movements in single neurons to clinical

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evaluation of the role of the GABA system in epilepsy, schizophrenia, mental retardation, etc. This warranted the convocation of a memorable interdisciplinary conference in 1959 in which we were fortunate in having present most of the individuals who had had a role in opening up this exciting field.[13] Today there are many lines of evidence that make it seem probable that GABA is a major inhibitory neurotransmitter in the vertebrate central nervous system and in invertebrate central and peripheral nervous systems. (See references 14–22 for representative reviews.)

METABOLISM

An outline of the metabolism of GABA is shown in Fig. 1. Following only the main lines in Fig. 1, it is seen that GABA is formed in the CNS of vertebrate organisms to a large extent, if not entirely, from L-glutamic acid. The reaction is catalyzed by an L-glutamic acid decarboxylase (GAD I), an enzyme found in mammalian organisms only in the CNS, largely in gray matter. For a number of years it was assumed that there was only one GAD in the vertebrate organism and that it was located entirely in neurons in the CNS. With more sensitive methods, it was found that GAD activity can be detected in glial cells, kidney, heart, adrenal and pituitary glands, and in blood vessels. The GAD in the latter tissues shows different properties from the neuronal enzyme[23] and does not cross-react with antibodies to it.

We now have succeeded in purifying to homogeneity neuronal GAD from mouse brain.[24] It has a molecular weight of 85,000, a sharp pH optimum at 7·0, and catalyzes the rapid α-decarboxylation only of L-glutamic acid of the naturally occurring amino

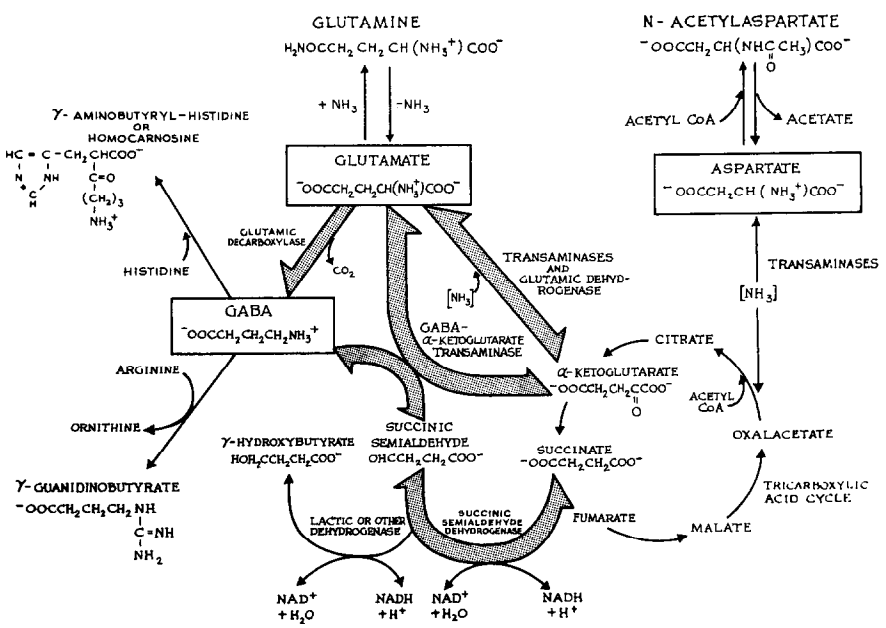

FIG. 1. Outline of chief known reactions of GABA, glutamate and aspartate in the nervous system. The reactions pertinent to GABA metabolism are emphasized by the large arrows.

acids and, to a very slight extent, that of L-aspartic acid. Preliminary evidence suggests that GAD may be a hexamer consisting of 15,000-dalton subunits.[25] Immunochemical studies with antibodies to purified mouse brain GAD indicated that the rat brain enzyme is most closely related to the mouse enzyme, followed in order by human, rabbit, calf, guinea-pig, quail, frog and trout.[26] Henceforth, when mention is made of GAD, it will be assumed that reference is being made to neuronal GAD. Further work also is in progress in our laboratories on the purification and properties of the non-neuronal enzyme. The reversible transamination of GABA with α-ketoglutarate is catalyzed by an aminotransferase, GABA-T, which in the CNS is found chiefly in the gray matter, but also is found in other tissues. The products of the transaminase reaction are succinic semialdehyde and glutamic acid. A dehydrogenase is present in excess which catalyzes the oxidation of succinic semialdehyde to succinic acid, which in turn can be oxidized via the reactions of the tricarboxylic acid cycle. Recently we have succeeded in purifying GABA-T to homogeneity, and its properties are being studied in detail.[27] The enzyme has a molecular weight of 109,000, a pH optimum of 8·05, and can be split into two unequal subunits. Of the keto acids tested, only α-ketoglutarate was an amino group acceptor. However, of a series of amino acids tested, β-alanine showed activity equal to that of GABA as substrate; δ-aminovaleric acid and β-aminoisobutyric acid also were effective amino donors. Therefore, GABA-T may be important in several aspects of metabolism of ω-amino acids and not just related to the GABA system. Antibodies to mouse brain GABA-T were found to cross-react with the enzyme from human, calf, mouse, rat, guinea-pig, rabbit and frog.[28] Microcomplement fixation tests showed that only the enzyme from rat brain may be very similar to the mouse brain enzyme in terms of protein structure. Accordingly, this enzyme seems to be highly species specific.

Steady state concentrations of GABA in various brain areas normally are governed by the GAD activity and not by the GABA-T. In many inhibitory nerves, both GAD and GABA are present and are distributed throughout the neuron, the GAD being somewhat more highly concentrated in the presynaptic endings than elsewhere. The GABA-T is contained in mitochondria of all neuronal regions, but it seems to be richer in the mitochondria of those neuronal sites onto which GABA might be liberated. Such regions would be expected to exist in perikarya and dendrites that receive GABA inputs and possibly in the glial and endothelial cells that are in the vicinity of GABA synapses.[18,21]

Recently it has become possible to visualize GAD[29] on sections of the central nervous system of rat and mouse at the light and electron microscopic levels, employing rabbit antisera to the purified mouse brain enzyme and peroxidase-labeling techniques. This has led to much more definitive data than were hitherto available through cell fractionation studies and, when coupled with similar procedures for enzymes that are rate-limiting in the biosynthesis of other transmitters, could give detailed information of interrelationships of various neuronal systems at the ultrastructural level.

PHYSIOLOGY

There is evidence for presynaptic release of GABA. Stimulation of axons of several nerves inhibiting different lobster muscles was shown to result in the release of GABA in amounts related to the extent of stimulation, while stimulation of the excitatory

nerve did not produce GABA release.[30] Data showing the liberation of GABA on stimulation of specific inhibitory neurons in the vertebrate nervous system are extremely difficult to obtain, but there are many experiments that suggest that this does take place.[31–34]

An ionic basis of the inhibitory effect of GABA on the postsynaptic regions of vertebrate and invertebrate neurons is known, at least in some instances. (See references 14–22 for reviews.) Applied GABA alters the membrane conductance to chloride ions with the membrane potential staying near the resting level. GABA also has a presynaptic inhibitory action at the crayfish neuromuscular junction, imitating the action of the natural inhibitory transmitter by increasing permeability to chloride, thus decreasing the probability of release of quanta of excitatory transmitter.[35] There is as yet no hint about the manner in which chemical or physical interaction of GABA with membranes produces increases in the chloride ion conductance of the membranes and, to date, all of our attempts to isolate the GABA receptor from mouse brain have failed, nor has convincing evidence for purification of the receptor come from any other laboratories. What is lacking is a high-affinity label for the active site. Recent evidence suggests that GABA may be a transmitter mediating presynaptic inhibition in a number of regions in the vertebrate CNS[36,37] and that it acts by producing depolarization of primary afferent terminals. Although it was suggested that sodium may be the chief ion involved in the latter mechanism,[36] recent unpublished data in several laboratories suggest the participation of the chloride ion. However, all of the data with regard to the role of GABA in presynaptic inhibition are indirect and must be viewed with caution. At this juncture, the reader should be reminded that the key to the action of transmitter substances always lies in the nature of the changes they cause in the conformations of receptive membrane regions and in the consequences of these changes. It is, therefore, conceivable that instances may be found in which GABA causes changes in permeability to ions other than chloride and that at some membrane sites the effects may be excitatory rather than inhibitory. Indeed, there is convincing evidence that GABA is the excitatory transmitter liberated from hair cells onto the first afferent neurons in the inner ear and lateral line sense organs.*

Detailed quantitative studies of GABA receptors from the physiological point of view have not been undertaken in vertebrate systems because of currently insurmountable technical difficulties. Employing more manageable invertebrate preparations, it has been proposed that the combination of two molecules of GABA is required to activate GABA receptors at the crayfish neuromuscular junction.[38] It appears that three molecules of GABA are required to activate the receptors on the membrane of locust muscle fibers,[39] and the latter data appear to be consistent with the interpretation that receptor activation occurs only after more than one molecule of GABA is bound to the active site. The latter data would make it seem unlikely that simple analogues of GABA would turn out to be the most effective blockers of GABA action at postsynaptic sites.

The cessation of action of a synaptically active substance could be brought about by the removal of the substance from the sensitive sites by destruction, by transport

* A. FLOCK and D. LAM, personal communication and since published (A. FLOCK and D. M. K. LAM, *Nature, Lond* **249**, 142 (1974).

or by diffusion. In the case of GABA, it is likely that the active transport out of the synaptic gap is the major inactivating mechanism.[18]

Although by now the GABA system has been studied with varying degrees of thoroughness in almost every region of the vertebrate CNS, the cerebellum has been by far the most favorable site for investigation of possible substances which may mediate the activity of neurons with inhibitory functions. More extensive correlative neuroanatomical and neurophysiological analyses have been made of the cerebellum than of any other structure in the vertebrate brain (see reference 21). The overall function of the cerebellum probably is entirely inhibitory. The only output cells of the cerebellar cortex, the Purkinje cells, inhibit monosynaptically in Deiters' and intracerebellar nuclei. Cells that lie entirely in the cerebellar cortex—the basket, stellate and Golgi type-II cells—are believed to play inhibitory roles within the cerebellum. The basket cells make numerous powerful inhibitory synapses on the lower region of the somata of the Purkinje cells and on their basal processes, or "preaxons." The superficial stellate cells form inhibitory synapses on the dendrites of Purkinje cells. The Golgi cells make inhibitory synapses on the dendrites of the granule cells. Afferent excitatory inputs reach the cerebellum via the climbing and mossy fibers, which excite the dendrites of the Purkinje and granule cells respectively. The latter are believed to be the only cells that lie entirely within the cerebellum that have an excitatory function. An afferent inhibitory noradrenergic input is believed to reach the Purkinje cells from cells in the locus coeruleus.[40] Even the first comprehensive biochemical laminar analyses of the GABA system suggested the possibility that all of the inhibitory cells of the cerebellum (Purkinje, basket, stellate and Golgi) might use GABA as transmitter,[41] and considerable subsequent experimental work has lent indirect support to this view.[21] Now more direct evidence for the above supposition has come from the immunocytochemical localization of GAD in rat cerebellum, using antibody against the purified mouse brain enzyme. At the light level, the enzyme was visualized in bouton-like punctate structures,[29] while at the electron microscopic level, the enzyme was found to be highly localized in certain synaptic terminals in close association with the membranes of synaptic vesicles and mitochondria, but not within these organelles.* The location of the visualized endings is in complete agreement with the above earlier deductions. Similar work is being carried out on spinal cord, hippocampus, retina, basal ganglia and other regions of the central nervous system. However, in no instance have the functional relationships been worked out to the same extent as they have been in cerebellum; and, therefore, the immunocytochemical data would tend to have less precise value in terms of elucidating modes of information processing in other neural regions.

DISINHIBITION AS AN ORGANIZING PRINCIPLE IN THE NERVOUS SYSTEM—THE ROLE OF GABA

All normal or adaptive activity in nervous systems is a result of the coordinated dynamic interplay of excitation and inhibition, within and between neuronal subsystems. A basic inadequacy in this interplay could lead to an abnormal (disease) process such as is found in Parkinson's disease, Huntington's chorea, schizophrenia, epilepsy, depression, manic-depressive disorder, hyperkinetic behavior in children,

* B. J. McLaughlin, J. G. Wood, K. Saito, R. Barber, J. E. Vaughan, E. Roberts and J.-Y. Wu, *Brain Res.* **67** (1974), in Press.

psychosomatic disorders, etc. It seems that a nervous system and its subunits consist largely of poised genetically preprogrammed circuits which are released for action by pacemaker or command neurons that are strategically located at junctions in neuronal hierarchies dealing with both sensory input and effector output. In some instances, it has been possible to identify a hierarchy of controls within a neuronal population. A pacemaker neuron seems to be in control of the whole unit (a ganglion, for example), and its activity is the key to the activity of the ganglion.

In a hierarchical segmental system, such as has been found in crustacea and analyzed in the case of the system involved in control of swimmeret movement in the crayfish,[42,43] the activity of a pacemaker neuron of a particular segment is controlled to a considerable extent by the neuronal activities of the segments above it in the hierarchy, with the head ganglion or brain exerting the highest level of control. From considerable experimental evidence, it appears that segmental pacemaker neurons, like the circuits they control, largely are inhibited and that the degree of inhibition is controlled from above. A decrease in inhibition allows them to fire, thereby releasing the preprogrammed circuits over whose activity they preside. A striking instance of this is the release of the highly coordinated stereotypical sexual behavior of the male praying mantis when his head is bitten off by the female.[44] At successive stages of development of the guppy embryo, strychnine enhances motility of progressively more complex and coordinated nature, suggesting that active inhibitory processes are responsible for the early relative immotility rather than lack of development of excitatory synaptic functions.[45] A headless chicken running is another example. Although similar patterns of relationships are discernible in the nervous systems of higher vertebrate forms, the order of complexity is greatly increased. An example of this in humans may be the stereotypical postural effects and paranoia produced by overdoses of amphetamines.[46] Paranoid thinking may be a complicated, but stereotypical, genetically preprogrammed process that can be evoked by skillful demagogues as well as by drugs.

In behavioral sequences, innate or learned, many genetically preprogrammed circuits may be released to function at varying rates and in various combinations by inhibition of neurons which are tonically holding in check pacemaker cells with capacity for spontaneous activity. If the pacemaker neurons are triggering a circuit related to the regulation of a vital function such as heart action or respiration, the inhibitory neurons might act in such a way as to vary the rate of the discharge of the pacemaker neuron. On the other hand, if a behavioral sequence involves the voluntary movement of a limb muscle, the pacemaker neuron might be held in complete check by the tonic action of inhibitory neurons and might be allowed to discharge in a graded manner only related to demand. In this view, excitatory input to pacemaker neurons would have largely a modulatory role. Thus, disinhibition, acting in conjunction with intrinsic pacemaker activity and often with modulatory excitatory input, appears to be one of the major organizing principles in nervous system function. Disinhibition may act as a switch, turning on a specific coherent neural pattern which is otherwise actively and continuously inhibited, as well as play a role in the organization of sequential and alternating discharges among separate groups of elements. Recent findings have uncovered neural circuitry that operates to a considerable extent with inhibitory neurons that liberate inhibitory transmitter. Almost all of the activity within the stomatogastric ganglion in the lobster is integrated by mechanisms in-

volving inhibition and disinhibition rather than by direct excitation, while the final motor messages to the stomach muscles are excitatory.[47] As seen above, the cerebellar cortex also operates to a considerable extent with inhibitory neurons.

Inhibitory neurons interact with other inhibitory neurons regardless of the transmitter which they employ. Thus, inhibitory neurons utilizing GABA as a transmitter in the cerebellum can be inhibited by other GABA neurons and by noradrenergic neurons. Serotonin-releasing neurons from the Raphé nuclei inhibit noradrenergic neurons in the brain stem.[48] It is likely that in the complex arrangements of various regions of the CNS a variety of combinations of inhibitory neurons can act upon each other. In most instances in which they have been studied by iontophoretic application, the biogenic amines have been found to exert an inhibitory action on neurons.[49,50]

GABA neurons are present ubiquitously in the CNS of vertebrate species, and on a quantitative basis GABA is much more extensively and relatively more evenly distributed throughout the various brain regions than the neural systems that employ other known neurotransmitters such as acetylcholine, the catecholamines or serotonin. A consideration of the biochemical, pharmacological and physiological data subsequently developed about the function of GABA and currently available about nervous system function, in general, suggested to me that the major neural system exerting tonic inhibition on pacemaker neurons might be the system of inhibitory neurons utilizing GABA as transmitter.[51] GABA neurons are envisioned to play a key role at all levels, from setting the gain on the sensitivity of sensory receptors to coordinating the function of the systems involved in perceptual integration and in reaching the decisions with regard to which neural circuits should be released for use at a particular time. For example, if in an individual there should be a paucity or defective function of horizontal GABA neurons in layer IV of the motor cortex, this individual might be expected to be more susceptible than normal to occurrence of grand mal seizures. If such a problem should exist in the region of the globus pallidus, postural control would be expected to be defective. If the GABA system were inadequate in those regions of the hypothalamus dealing with food intake, hyperphagia or anorexia nervosa might result. If GABA neurons in the dorsal horn of the spinal cord were inadequately functional, there might be an inordinately great sensitivity to tactile and thermal stimulation and inadequate spatial and temporal discrimination of the stimuli. If there were a defect in GABA function in the retina, visual perception and integration might be faulty.

Recently, relatively specific and remarkable decreases in GABA levels[52] and GAD activities[53] have been found in the substantia nigra, putamen, globus pallidus and caudate nucleus of patients with Huntington's chorea, an autosomal-dominant, hereditary disease in which, among other symptoms, slight inconstant irregular choreiform movements eventually progress into constant twitching, stretching, gesturing, facial grimaces, etc. Although the nature of all the connections among the basal ganglia and between them and other brain structures is not known and all of their functions have not been delineated, it appears that they largely are concerned with processing information related to proprioceptive, vestibular and visual stimuli in the service of coordinating postural mechanisms. As far as it is possible for me to understand current neuroanatomical opinion, it seems that the caudate nucleus, putamen and substantia nigra all exchange fibers with each other and that efferent

outputs from the caudate and putamen go to the globus pallidus, which also may receive some fibers from the substantia nigra. The globus pallidus has two-way communication with the subthalamic nucleus. There are thalamic and cortical inputs to the caudate and putamen. The final results of the computations in the basal ganglia are sent out via a fiber system from the globus pallidus to the ventral lateral nuclei of the thalamus. The globus pallidus and substantia nigra have the highest contents of GABA and highest activities of GAD in the brain.[54–57] Normal relations in the basal ganglia must involve minimally a coordinated functioning of different groups of inter- and intra-system neurons whose transmitters are GABA, dopamine, acetylcholine[58] and an excitatory transmitter, the action of which can be mimicked by externally applied glutamic acid.[59]

It seems to me reasonable to assume from the present data, and employing the concept of disinhibition, that the basal ganglia contain preprogrammed neural circuits for patterned postural control that are held in tonic inhibition by indigenous, closely lying GABA neurons. The chief switching mechanisms for turning on the patterned activities within the non-nigral regions may be the dopamine fibers emanating from neurons in the substantia nigra that receive a variety of afferent inputs. The nigro-fugal fibers release dopamine in the caudate and putamen, inhibiting indigenous tonically inhibitory GABA neurons and, acting together with excitatory and/or disinhibitory inputs from the thalamus and cortex, release specific coded neural patterns in a sequential manner, for which the pacesetters may be acetylcholine neurons. The results of this activity are communicated to the pallidum and thence to regions in the thalamus where integration with other incoming information takes place. The final postural instructions are then sent to the appropriate regions of the cortex where, after further refinement, the activities of appropriate pyramidal neurons in the motor cortex are released to signal the effectors. The circuits that are fired in the basal ganglia inform the other units about their activity via intersystem inhibitory fibers employing GABA or other inhibitory transmitters, particularly to the appropriate nigral neurons, thus preventing their own further activation until the need arises again.

It is easy to see from the above model how specific loss of GABA neurons might lead to some of the symptoms of Huntington's chorea. The occasionally helpful effects of haloperidol, a blocker of dopamine receptors, in patients with Huntington's chorea might be attributable to a decreased extent of inhibition of GABA neurons, allowing the less than normally effective GABA neurons to maintain their tenuous hold on the neurons requiring their restraints. Since orally or parenterally administered GABA usually does not pass the blood–brain barrier and often causes undesirable peripheral effects, the search is on for a suitable GABA-mimetic substance as a possible treatment in Huntington's chorea. Currently, clinical tests are in progress with imidazoleacetic acid, a GABA-mimetic substance that passes the blood–brain barrier. (See reference 62 for pertinent references.)

PHARMACOLOGY

There is a vast amount of, as yet, poorly correlated data which purport to deal with the roles of GABA in the control of neuronal excitability and behavior in intact animals. Some of the reasons for the difficulties encountered at this level of analysis should be readily apparent to the reader from the preceding discussion. Often,

measurements of GABA levels of whole brain or of grossly dissected regions are correlated with global phenomena such as occurrence of convulsive seizures, susceptibility to electroconvulsive shock, EEG changes, etc. In line with experience with studies of other putative neurotransmitters such as the catecholamines, serotonin and acetylcholine, measurements of levels of GABA generally are not functionally meaningful, since decreases or increases in total content may be attributable to many factors not relevant to use of GABA in informational transactions at synapses. Measurements of turnover rates of GABA in specific brain regions would be potentially much more meaningful and functionally significant. However, adequate techniques for performing this kind of measurement have not yet been developed. It is difficult to envision at this time how one could measure specifically the turnover rates of GABA in transmitter pools. A number of the technical tools that are available in comparable studies with the catecholamines and serotonin are not applicable to GABA, which is made from ubiquitously occurring glutamic acid and which is metabolized extremely rapidly, yielding the common and nondescript metabolic products, CO_2 and H_2O.

The use of drugs in the manipulation of the GABA system to date has yielded no substances with the requisite degree of specificity to be able to reach more than very tentative conclusions. The first pharmacologically active compounds to have been studied in relation to the GABA system were the convulsant hydrazides, substances that inhibit GAD, GABA-T and a host of other B_6-enzymes. These were first related to the GABA system because the hydrazides were shown to inhibit GAD. In some instances it was shown that there were correlations of the extent of lowering of GABA content and decrease of GAD activity in the brains of animals with the occurrence of convulsive states observed either electrographically or by observing overt convulsions. However, other experiments were performed that showed that decreases in GABA levels could be prevented, but that the convulsions attributable to hydrazides would occur nonetheless. It would indeed be surprising if there were a perfect correlation of the decrease in GABA content with the occurrence of seizures produced by the hydrazides, since these highly reactive substances can react with carbonyl and other groups present in many simple and complex molecules in tissues, inhibiting enzymes, altering connective tissue structure and permeability of blood vessels, and forming free radicals which can become alkylating agents. They can participate in the formation of a variety of derivatives, which themselves could have enzyme-inhibitory and pharmacological effects. (The degree of complexity of pharmacological action of these substances is discussed in some detail in reference 15.) The study of the properties of purified GAD has not yet led to a completely specific chemical inhibitor of the enzyme that could be used for depletion of GABA, *in vivo*, but work is continuing in this direction. Antibodies to GAD could be the most specific inactivators of the enzyme, but to date the animals producing antibodies to the injected enzyme have not shown any symptoms at all, suggesting that the antibodies do not pass the blood–brain barrier. Employing various techniques now available for increasing permeability of the barrier to large molecules and/or direct administration of the antibodies into specific brain regions may prove to be of value in the subsequent pharmacological analysis of the GABA system.

Since the convulsants, bicuculline and picrotoxin, have been shown to have antagonistic effects to GABA on firing rates of neurons in various preparations,[1–3]

it has become commonplace to infer the demonstration of GABA-mediated synapses in both vertebrate and invertebrate nervous systems on the basis of such effects. However, when conductance measurements were made, it became obvious that neither bicuculline nor picrotoxin is a competitive antagonist of GABA action on postsynaptic membranes at the crayfish neuromuscular junction.[64,65] Bicuculline seems not to be antagonistic at all to GABA-induced conductance increases of the postsynaptic membrane of the slowly adapting stretch receptor neuron of the crayfish;[66] nor does it block neurally evoked inhibition at the neuromuscular junction in the hermit crab,[67] where applied GABA is active. Also, bicuculline has been found both to antagonize and potentiate effects of GABA on cat cerebral cortical neurons.[68] To illustrate further the complexity of the situation, bicuculline and picrotoxin have been shown to act on the lobster giant axon in such a manner as to cause depolarization and a broadening of the action potential contour, an increase in excitability, and sometimes even repetitive firing.[69] Obviously, when effects on firing rates are observed, nonsynaptic actions, as well as synaptic ones, must be considered. In addition, bicuculline has been shown to be a moderately potent competitive inhibitor of brain acetylcholinesterase.[70] It has been suggested from the latter and structural considerations that the physiological effect of bicuculline might be found to be more directly related to some function of the cholinergic system than to the GABA system *in vivo*.[70] To my knowledge there have been no quantitative intracellular studies of GABA–bicuculline and picrotoxin antagonism on conductance changes in vertebrate neurons. Although there often is a close relationship between the generator potential and frequency of firing, it is well known that a variety of chemical and physical changes can produce a dissociation or uncoupling between the two. Thus, the above results with bicuculline and picrotoxin bring us face to face with the possibility that, at least in some instances, the latter two substances may be acting at different neuronal membrane sites than those at which GABA exerts its effects. Thus, at this time it appears that a proven specific competitive antagonist to GABA at postsynaptic sites has yet to be discovered.

What will be necessary for the development of a rational pharmacology of the GABA system? First of all, it is apparent from current work that probably there will be more than one type of GABA receptor. Although GABA plays largely an inhibitory role in the vertebrate central nervous system, it also can play a depolarizing or excitatory one. From experience with other transmitters, it is likely that the nature of the receptor complexes at the sites on which GABA exerts different effects also will differ. Thus, included in whatever test systems are used, should be examples of sites on which GABA has both inhibitory and excitatory functions. There may even be more than one type of inhibitory or excitatory site. It is highly desirable to define by electrophysiological methods the nature of the changes in ionic conductance that GABA produces in a particular instance. From a technical point of view, the latter type of information is much more readily obtained in invertebrate preparations than from studies in a vertebrate nervous system.

With a proper choice of the system, it is possible to begin to look for antagonists and mimetics of the postsynaptic action of GABA. Sufficient work already has been done with compounds closely related in structure to GABA to indicate that it is unlikely that a simple structural alteration of the linear GABA molecule will yield highly active compounds. As alluded to previously, one should begin to consider

molecular structures whose three-dimensional configuration will resemble two or three GABA molecules in different arrays, because probably more than one GABA molecule is required to activate one receptor site.

From the point of view of therapy, it would be worthwhile to establish conditions by which it would be possible to enhance the inhibitory function of GABA in the nervous system in a variety of clinical conditions such as Huntington's disease,[52,53] epilepsy and schizophrenia,[54] in which there are some possibilities that there are defects in key inhibitory elements either in the whole brain or in specific regions. However, the administration of GABA-mimetic substances that pass the blood–brain barrier might not prove to be a generally useful strategy, since GABA has been found to be active on membranes of neurons that may not have an input from GABA-releasing nerve endings. The nonspecific flooding of the CNS with such substances might produce chaotic results. An alternative strategy should be sought by which the effects of GABA liberated from nerve endings that normally use GABA as a transmitter could be amplified in pertinent synapses. Since GABA largely is inactivated at synapses by a mechanism that involves the binding of GABA to membranes and the subsequent transport of the GABA out of the synaptic junction, it would seem that substances that can retard the rate of uptake of GABA while themselves having no GABA-mimetic or GABA-antagonist properties should be sought. This kind of action would be analogous to that proposed for the action of tricyclic antidepressants on catecholamines. Although hundreds of compounds have been tested as inhibitors of GABA uptake in test systems employing brain slices or subcellular particles, none has yet been found with an affinity approaching that required for such an agent. It is possible that the use of a combination of test systems *in vitro* for the uptake mechanism, employing brain subcellular particles and invertebrate preparations that allow quantitative measurements of responses of nerve membranes to known concentrations of GABA, could be used to approach the development of suitable drugs for use in this field.

CONCLUSION

In the 25 years since the discovery of GABA as a constituent of rodent brain, the status of the compound has passed from that of a biochemical curiosity and physiological enigma to that of a major inhibitory transmitter in the vertebrate central nervous system and in some invertebrate central and peripheral nervous systems. The chief enzymes in its metabolism have been purified and immunocytochemical techniques have been developed for identifying them at the light and electron microscopic levels. By localizing GAD it has been possible to identify those synaptic endings that use GABA as transmitter. The first disease in which there is a disturbance in function of the GABA system, Huntington's chorea, has been reported, and it has been suggested that also in other neurological and mental disorders there may be disturbances in GABA function.

Much remains to be learned about all aspects of the function of the GABA system. The most glaring deficit in our armamentarium is the availability of specific pharmacological tools and approaches. Most of the substances used to date for manipulation of the GABA system have proven to be nonspecific and have given equivocal results. It is anticipated that a rich theoretical and practical harvest will be reaped by those who accept the challenge to enter the field at the present time, when more highly

purified enzyme preparations and more precise physiological systems are available for study than before.

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