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NEUROPHARMACOLOGY OF AMINO ACID INHIBITORY TRANSMITTERS

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In the three years since the review on amino acids as central neurotransmitters by DeFeudis (1) there has been a profusion of neurophysiological, neuropharmacological, and neurochemical studies concerned with simple amino acids and the CNS. While γ-aminobutyric acid (GABA) and glycine are generally accepted as inhibitory transmitters of major significance, there are some problems regarding possible functions of the excitatory amino acids, glutamate and aspartate (2). Glutamate may well be the major excitatory transmitter in the brain but it does appear to be a somewhat gregarious substance being involved in much, e.g. γ-glutamyl compounds (3), that is not directly related to excitatory transmission. Some other amino acids, including taurine (4, 5) and proline (6), may function as inhibitory transmitters.

Major activities in the field of amino acid transmitters during the past three years might be grouped into several general areas. There has been continued and widespread use of postsynaptic antagonists such as bicuculline and strychnine to study synaptic inhibitions, and a thus far unsuccessful search for suitably selective antagonists of synaptic excitations that might be mediated by amino acid excitants (7). Neurochemical studies on the localization of amino acid transmitters have been greatly aided by the development of immunocytochemical techniques for specific enzymes and the increasing use of degeneration experiments (8). Studies of the binding of amino acid agonists and antagonists to synaptic membrane fractions have opened up new vistas and greatly accelerated investigations of isolated receptors that exhibit many of the properties anticipated from in vivo studies (9). Much work has been devoted to studies of amino acid compartmentation (10), of the processes whereby amino acids are taken up into, and released from, the various compartments that include glial as well as neuronal elements (11–14) and of the active transport processes involved in synaptic transmission mediated by amino acids. There has been some progress in our understanding of the effects of some widely used centrally active drugs, and in the development of new agents that may lead to

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advances in the therapy of neurological disorders that involve abnormal functioning of amino acid transmitter systems (15). The present review concentrates on the more purely pharmacological aspects of these investigations and deals mainly with recent developments concerning agents that have relatively specific actions on inhibitory synaptic transmission mediated by GABA, and to a lesser extent glycine, in the mammalian CNS.

GABA

There is evidence that GABA functions as an inhibitory synaptic transmitter in most areas of the mammalian CNS. Abnormalities in the GABA system may be associated with such disorders as Parkinson's disease and Huntington's chorea (16, 17). Agents active at GABA synapses may be useful for alleviating the clinical manifestations of these, and of other disorders such as schizophrenia (15, 18) and epilepsy (19). The interactions of GABA with other transmitter systems, e.g. the dopamine nigrostriatal system (20), are being investigated intensively. The proceedings of a conference on GABA in nervous system function illustrate the enormous expansion in the field since the first GABA conference was held 15 years earlier (21).

GABA Agonists

Many substances inhibit the firing of central neurons when administered extracellularly from micropipettes. It is, however, difficult to determine whether or not such inhibitory actions result from the activation of specific GABA receptors as distinct from those, for example, for glycine or noradrenaline. Heavy reliance has been placed on the use of selective antagonists and on studies on the displacement of GABA bound to synaptic membranes in vitro. The inhibitory action of GABA is generally antagonized by bicuculline, but not by strychnine, when these alkaloids are administered from adjacent barrels of multibarreled micropipettes. Thus a working description of a GABA agonist is one having bicuculline-sensitive, strychnine-insensitive inhibitory action on neuronal firing. Bicuculline-insensitive GABA receptors may well exist (22), and both species and regional differences appear likely in GABA receptors. The use of more selective antagonists could lead to further classifications of GABA receptors and thus of GABA agonists. A further complicating problem is that a substance may act indirectly by releasing GABA from presynaptic terminals or from glial cells (23).

CONFORMATIONALLY RESTRICTED GABA ANALOGUES GABA is a flexible molecule, and both theoretical calculations and spectroscopic studies indicate that it can exist in a variety of energetically favorable conformations. Different conformations of GABA might interact with different receptors, and the active conformations of GABA can be studied indirectly by structure-activity correlations of GABA analogues of restricted conformation. A number of these GABA analogues have bicuculline-sensitive, strychnine-insensitive inhibitory actions (7, 22). As the actual flexibility of the GABA molecule could be important for the inhibitory action of this amino acid, the development of relatively rigid GABA agonists is particularly

interesting (24). By analogy with similar studies on acetylcholine analogues, however, more GABA analogues will have to be synthesized and tested before an acceptable understanding of this aspect of GABA-receptor interactions can be attained.

In general, these conformationally restricted analogues of GABA, like GABA itself, do not pass the blood-brain barriers to influence central GABA receptors after systemic administration. The exceptions appear to be those analogues, such as muscimol, which contain 3-isoxazolol moieties as masked carboxy groups.

MUSCIMOL Mushrooms of the genus *Amanita* contain numerous biologically active substances, perhaps the most well known being the acetylcholine analogue muscarine. The hallucinogenic effects of these mushrooms are not due to muscarine, however, but at least in part are associated with the GABA analogue muscimol. In man, 10 mg of muscimol taken orally results in considerable psychic disturbance. In experimental animals, muscimol appears to act as a potent GABA agonist with respect to bicuculline-sensitive, strychnine-insensitive receptors (22). Muscimol is about 10 times more potent than GABA in displacing either radioactive GABA or radioactive bicuculline methiodide bound to membranes from rat brain (25, 26). A number of structurally related analogues of muscimol have interesting properties, and their study has led to the development of new classes of relatively rigid GABA agonists (24) and inhibitors of GABA uptake (27).

Muscimol injected intravenously into rats potentiates morphine analgesia (28), decreases acetylcholine turnover in some but not all brain areas (29), antagonizes isoniazid-induced seizures and lowers the cyclic GMP content of the cerebellum (30). These effects may be interpreted on the basis of activation of GABA receptors. Muscimol injected into the nucleus accumbens or the substantia nigra has been used to study GABA-dopaminergic interactions in rats (31, 32).

GABA Antagonists

Dating from 1970 a variety of substances have been shown to antagonize the postsynaptic action of GABA and to reduce certain synaptic inhibitions in all areas of the CNS (7). Membrane binding studies suggest that some of these substances, e.g. bicuculline (26), compete with GABA for postsynaptic receptors while others, e.g. picrotoxin, may act at the level of the GABA ionophore (33).

BICUCULLINE AND RELATED COMPOUNDS Bicuculline hydrochloride, methiodide, and methochloride are widely used as selective GABA antagonists (22). Some early doubts about the usefulness of bicuculline may have been related to the instability of bicuculline under certain conditions (34) and bicuculline has been recently described as "an important weapon for the neurophysiologist's arsenal" (35). Bicuculline-sensitive synaptic inhibitions are found in all areas of the CNS.

Bicuculline-sensitive GABA binding has been observed with various preparations of nervous tissue. The first observations were made by Peck and his colleagues (36), who described the binding of GABA (apparent dissociation constant, K_D 21 μM) to a synaptosomal fraction of rat cerebellar cortex in the presence of chlorpromazine

which could be competitively inhibited by bicuculline (apparent inhibition constant, K_i 80 μM); furthermore the binding component could be solubilized with Triton X-100® with retention of sensitivity to bicuculline (37). Snyder and his colleagues (9, 38, 39) investigated the binding of GABA (K_D 100 nM) to a synaptic membrane fraction of rat brain in the absence of sodium ions which could be displaced by bicuculline (concentration producing 50% inhibition, IC_{50} 5 μM) and related alkaloids in a stereoselective manner (25). DeFeudis and his colleagues (40) have found that in the presence of physiological concentrations of ions, 100 nM bicuculline methiodide significantly inhibits the binding of GABA to a synaptosomal fraction of rat cerebral cortex. The binding of GABA in a bicuculline-sensitive manner has also been investigated using junctional complexes of rat cerebellum (41), a hydrophobic protein purified from shrimp muscle (42), and a particulate fraction from crayfish muscle (43). Recently it has been shown that treatment with Triton X-100 of synaptic membranes from rat brain increases both the apparent affinity and the apparent density of sodium-independent binding sites for GABA, and reveals biphasic binding kinetics (44, 45).

The concentrations of bicuculline required to displace bound GABA in the above studies seem high when compared to the reported antagonism of GABA-induced inhibition in explants of rat cerebellum by 10 nM bicuculline (46) and of possible GABA-mediated "presynaptic" inhibition in slices of rat olfactory cortex by 20 nM bicuculline (47). Recently, GABA-sensitive binding of bicuculline methiodide to synaptic membranes of rat cerebellum has been investigated (26): half maximal binding occurred at 380 nM while K_i values of 420 and 68 nM were found for GABA and bicuculline hydrochloride respectively as inhibitors of binding. It thus appears that studies of GABA-sensitive bicuculline binding, rather than of bicuculline-sensitive GABA binding, might more accurately reflect pharmacological experiments using bicuculline to investigate synaptic processes. As the rank order of regional GABA-binding and bicuculline-binding are not identical (26), it seems likely that all agonist and antagonist sites are not identical. This is supported by the recent finding that certain anions (thiocyanate, iodide, and nitrate) increase the potency of bicuculline tenfold in displacing bound GABA without affecting the potency of GABA agonists (45). The existence of bicuculline-insensitive GABA receptors, proposed on other grounds (22), might explain some of the observed differences between GABA and bicuculline binding. Also bicuculline has other actions besides being a GABA antagonist (7, 22).

PICROTOXIN This convulsant (a 1:1 mixture of the more potent picrotoxinin and the less potent picrotin) is known to antagonize a number of synaptic inhibitions on systemic administration that can also be antagonized by bicuculline. Despite difficulties in the microelectrophoretic administration of picrotoxin due to its low aqueous solubility and the lack of readily ionizable groups on the molecules, picrotoxin applied to single neurons appears to have similar effects to those of similarly applied bicuculline in antagonizing the inhibitory action of GABA in a relatively selective manner (22). There are differences since picrotoxin also antagonizes the inhibitory action of 5-hydroxytryptamine in rat hippocampus (48).

Picrotoxin and bicuculline would appear to antagonize GABA-induced neuronal inhibition by different mechanisms since in general picrotoxin does not influence the binding of GABA to synaptic membranes. Picrotoxinin has been shown by Olsen and his colleagues to inhibit (IC_{50} 4 μM) the GABA-induced increase in chloride permeability in isolated strips of crayfish abdominal muscle (33), while 500 μM picrotoxin does not inhibit the binding of GABA to membranes isolated from the same muscle (43). In the same system, bicuculline inhibits both GABA binding (IC_{50} 350 μM) and GABA-induced increase in chloride permeability (IC_{50} 500 μM); the crayfish abdominal stretch receptor is known to be relatively insensitive to bicuculline (49). With respect to crayfish muscle, it would thus appear that picrotoxin acts not on the interaction between GABA and GABA receptors but on the membrane molecule(s) responsible for controlling the GABA-induced chloride flux, the GABA ionophores, or on the link between receptors and ionophores (33).

PENICILLINS Antagonism of GABA-mediated inhibition may be an important factor in the epileptogenic action of benzylpenicillin. Recent studies on crab neuromuscular junction indicate that in doses lower than 2 mM benzylpenicillin antagonizes GABA-induced chloride conductance increases by a weaker competitive inhibition, which may be receptor antagonism, and a more powerful noncompetitive inhibition, which may be ionophore blockade (50). Much higher concentrations of benzylpenicillin are needed for effects on excitatory synapses. Some recent structure-activity studies on the epileptogenic properties of various penicillins have been reported (51, 52). GABA synthesis and transport are impaired in pencillin-induced epileptogenic foci (53).

BICYCLIC PHOSPHATES A number of 4-alkyl-substituted derivatives of 1-phospha-2,6,7-trioxabicyclo[2,2,2]octane-1-oxide (PTBO) are potent convulsants and antagonize the depolarizing responses to GABA in the isolated frog spinal cord and rat superior cervical ganglion (54). The most potent are the isopropyl (IPTBO) and t-butyl (t-BPTBO) derivatives, these being more potent than bicuculline both as convulsants when injected intravenously into mice and as GABA antagonists in the isolated frog spinal cord (55).

OTHER GABA ANTAGONISTS A structural similarity between GABA and acetylcholine (56) may be reflected in the reported actions of tubocurarine and nicotine as weak GABA antagonists (57). Certain caprolactam derivatives which are potent convulsants appear to act as GABA antagonists (58). High concentrations of naloxone have been reported to antagonize GABA-evoked inhibitions in the rat olfactory tuberole and GABA binding (IC_{50} 300 μM) to membranes from human cerebellum (59). The potent convulsant tetramethylenedisulfotetramine may antagonize an ionophore common to GABA and glycine (60).

Inhibitors of GABA Synthesis

The main metabolic pathway of GABA synthesis involves the decarboxylation of L-glutamate catalyzed by glutamate decarboxylase (GAD), a pyridoxal phosphate-

dependent enzyme which appears to be localized in nerve terminals. GAD is considered to be the rate-limiting enzyme that normally determines the steady state levels of brain GABA. It can be used as a marker for the localization of GABA terminals (8). GAD accumulates in the proximal part of presumed GABA neurons after axotomy (61), consistent with the enzyme being synthesized in the cell body and being transported via the axon to the terminals. The enzyme from mouse brain has been extensively purified enabling detailed inhibition studies to be carried out (62) and providing immunogenic material for immunocytochemical localization studies (63).

There is much evidence that GAD activity can act as a regulator of cerebral excitability. An equation has been developed which relates excitability to both GAD activity and GABA levels, with the former being the major factor (64). There appears to be a threshold level of GAD activity below which convulsions occur (65). This implies that there is a relatively rapid turnover of transmitter GABA and that newly synthesized GABA is usually readily available for release from GABA terminals. 3-Mercaptopropionic acid, a potent competitive inhibitor (62) of GAD (K_i 2 μM), produces convulsions, decreased GAD activity, and decreased GABA levels in rats seven minutes after intraperitoneal injection (66).

Many endogenous factors can inhibit GAD. Zinc ions are potent inhibitors (IC_{50} 10 μM); since zinc is one of the richest divalent metals in brain it may be involved in the regulation of cerebral excitability (62). Sulfhydryl agents such as glutathione and cysteine could also be important in modulating GAD activity. Folic acid is a weak competitive inhibitor of GAD (K_i 2 mM) and this may be associated with some forms of epilepsy where folate levels increase (67). Inhibition of GAD by glutarate, glutaconate, and β-hydroxyglutarate may explain some of the manifestations of glutaric aciduria (68). Huntington's chorea is associated with a decrease in GAD activity in the basal ganglia (16). Similar decreases, initially thought to be associated with senile dementia, now appear to be the result of hypoxic brain damage (69).

There is an extensive literature on carbonyl-trapping agents as GAD inhibitors which reflects
coenzyme (62, 70). Many structural analogues of glutamate inhibit GAD (71). Investigation of the stereoisomers of the convulsant allylglycine has provided evidence that a common intermediate derived from either isomer may be responsible for the in vivo inhibition of GAD (72).

GAD activity may be measured by the rate of CO_2 production or by the rate of GABA production. While both methods usually give the same result, the latter is likely to be more reliable particularly when low levels of activity are measured, such as those in peripheral tissues (73, 74).

Inhibitors of GABA Degradation

GABA is metabolized to succinate by transamination to succinic semialdehyde catalyzed by GABA transaminase (GABA-T) and subsequent oxidation catalyzed by succinic semialdehyde dehydrogenase (SSAD). Selective inhibition of either

enzyme generally leads to increased levels of GABA accompanied by an anticonvulsant action. GABA-T is a mitochondrial enzyme of seemingly ubiquitous distribution in CNS tissue (75), which has been purified from mouse brain in order to study its subunit structure and kinetic properties (76). It is possible that GABA-T is also involved in the synthesis of GABA by a NAD^+-dependent route from glutamate (77).

AMINOOXYACETIC ACID This potent competitive inhibitor (K_i 60 nM) of GABA-T (76) is one of the most widely used agents for increasing the levels of GABA in the brain. Unfortunately aminooxyacetic acid (AOAA) seems to have other effects that must be taken into account. The anticonvulsant action of AOAA appears to involve at least two mechanisms, only one of which is concerned with the GABA system (78, 79). The depressant action of AOAA on spinal reflexes does not appear to involve GABA (80). AOAA is a carbonyl-trapping agent which is a quite potent competitive inhibitor (K_i 2 μM) of GAD (62) and which inhibits most, if not all, transaminases (76) and the transport of many amino acids and amines (81).

ETHANOLAMINE-O-SULFATE This active-site directed irreversible inhibitor of GABA-T, introduced by Fowler & John (82), is being used extensively to study the effects of increased levels of GABA. Ethanolamine-O-sulfate (EOS) forms an initial reversible complex with the enzyme (K_i 440 μM) followed by irreversible inactivation by a pseudo first
(82). Like AOAA, EOS (under the name aminoethyl hydrogen sulfate) is a weak inhibitor of GABA uptake in rat brain slices (83). EOS does not inhibit SSAD (84). EOS does not elevate brain GABA after systemic administration and must be injected directly into the brain for in vivo studies. Examples of its use include unilateral injection into the substantia nigra to study the role of GABA in rotational behavior (85) and bilateral injection into the globus pallidus to assess the possible control by GABA of an accumbens-pallidal pathway (86).

SODIUM DI-n-PROPYLACETATE Sodium Di-*n*-propylacetate (DPA, sodium valproate) is used clinically in the treatment of epilepsy (87). Systemic administration of DPA protects against seizures and increases brain levels of GABA (88). Although DPA has been reported to be a competitive inhibitor of GABA-T (88), it has a more potent action on SSAD (K_i 1.5 mM) than on GABA-T (K_i 18 mM) (84, 89). DPA does not influence GABA uptake at 1 mM (90). A pharmacokinetic study on the anticonvulsant action of DPA in monkeys indicates that DPA has multiple effects (91). A recent study using mice has shown a clear anticonvulsant action of DPA in the absence of significant changes in the levels of brain GABA (84). From the above work it seems probable that DPA has other actions, besides effects on GABA degradation, that contribute to its anticonvulsant action. DPA has some effects on monoamine metabolism, but these can be also dissociated from its anticonvulsant action (92).

γ-ACETYLENIC GABA Consideration of the mechanism of action of GABA-T led to the synthesis of γ-acetylenic GABA (GAG) (4-aminohex-5-ynoic acid) as a

catalytic inhibitor of GABA-T (93). It appears to act in a manner somewhat similar to EOS, binding to the active site of GABA-T (K_i 340 μM) and causing a subsequent time-dependent irreversible inhibition. Unlike EOS, GAG is active after systemic administration, increasing brain levels of GABA and protecting against seizures induced by a variety of agents (94).

GABACULINE This active site catalytic inhibitor of GABA-T was discovered in a culture filtrate of *Streptomyces toyocaemis* subsp. 1039, and was characterized as (–)-5-aminocyclohexa-1,3-diene-1-carboxylic acid (95). Gabaculine is much more potent than EOS or GAG having a K_i of the order of 1 μM (96, 97). It is a relatively weak inhibitor of GAD (IC_{50} 1 mM) and a moderately potent inhibitor of GABA uptake (IC_{50} 69 μM), and it does not act as a GABA agonist with respect either to the sodium-independent binding of GABA to membranes from rat brain or to GABA receptors on cat spinal interneurons (98). Systemically administered gabaculine increases brain levels of GABA and protects against picrotoxin-induced convulsions (99).

OTHER GABA-T INHIBITORS Inhibitors of GABA-T continue to be discovered; these include the substrate-coenzyme analogue, N-(5'-phosphopyridoxyl)-4-aminobutanoic acid, which is a potent competitive inhibitor (K_i 1.4 μM) in vitro (100), and L-α-amino-
T but not GAD in vivo and which protects against isoniazid-induced seizures (101).

Inhibitors of GABA Uptake

GABA is transported into various CNS tissue preparations by structurally specific, sodium-dependent uptake systems (102). Autoradiographic studies show that GABA is taken up mainly by nerve terminals and glial cells with L-2,4-diaminobutyric acid and β-alanine, being relatively selective substrates for the neuronal and glial uptake systems respectively in rat cerebral cortex and cerebellum (11). Degeneration studies have shown that the neuronal uptake of GABA may be associated with the axon terminals of some, but not necessarily all, GABA neurons (103). There has been some debate as to whether GABA uptake, as measured with synaptosomal preparations, represents net uptake only or whether it includes exchange with endogenous GABA; high intracellular sodium ion levels appear to promote a high rate of GABA efflux and thus exchange in these preparations (104), and net uptake of GABA does appear to be mediated by physiologically operational high affinity transport (105).

The cellular uptake of GABA might have several important functions. It might be important for preventing accumulation of GABA in the extracellular space where GABA might otherwise exert a tonic effect on GABA receptors. Uptake by synaptic terminals could provide a supply of GABA for reuse; there is some evidence for this in that GABA, newly taken up into synaptosomes, is released by potassium depolarization in preference to endogenous GABA (106), but the relatively rapid effects

following inhibition of GABA synthesis (66) indicates that any reuse of GABA by nerve terminals is not adequate to maintain presynaptic transmitter stores and that loss of GABA to glial cells is appreciable. Much recent work has emphasized the importance of glial transport systems (11–14, 107). There has been considerable speculation that uptake into perisynaptic structures contributes to the inactivation of synaptically released GABA. A number of inhibitors of GABA uptake have been shown to both prolong and potentiate the inhibitory action of electrophoretic GABA on the firing of CNS neurons (108, 109), but these uptake inhibitors have not yet been shown to have any direct influence on GABA-mediated synaptic inhibition. The failure to influence the action of synaptically released GABA may be due to the failure of the uptake inhibitors to reach the physiological site(s) of inactivation since these inhibitors themselves appear to be substrates for the uptake systems. At the present time there is thus no direct evidence that GABA uptake terminates the action of synaptically released GABA. Studies on crayfish muscle indicate that the time course of inhibition induced by GABA may be determined mainly by the time course of the interaction of GABA with its receptors (110).

L-2,4-DIAMINOBUTYRIC ACID This neurotoxic amino acid found in various species of *Lathyrus* and *Vicia* appears to be a substrate-competitive inhibitor (K_M, K_i ca 30 μM) of the neuronal uptake of GABA (11, 102). DABA has a weak depressant action on the firing of cat spinal, cerebellar, and cerebral neurons and potentiates the action of electrophoretically administered GABA on these neurons (108). The D-stereoisomer of DABA is much less potent than L-DABA as an inhibitor of sodium-dependent GABA uptake, but is equipotent as a weak inhibitor (IC_{50} 200 μM) of sodium-independent binding of GABA to synaptic membranes; as both stereoisomers are equally neurotoxic on intracisternal injection, it appears that activation of GABA receptors rather than interaction with GABA transport is responsible for some of the toxic effects (111).

NIPECOTIC ACID Like DABA, nipecotic acid appears to be a substrate-competitive inhibitor (K_M, K_i ca 10 μM) of the neuronal uptake of GABA (112, 113). On the basis of the absolute structures of the more active stereoisomers of DABA, nipecotic acid and the related piperazic acid (114), these substances are proposed to interact in a similar way with the neuronal transport system. It is interesting that such a relatively bulky and inflexible molecule as nipecotic acid may replace GABA in an uptake system, and indeed nipecotic acid appears to be transported with higher efficiency than does GABA (112). Both isomers of nipecotic acid enhance the action of electrophoretically administered, but not synaptically released, GABA on cat spinal, cerebellar, and cerebral neurons (108).

ARECAIDINE AND GUVACINE These constituents of *Areca catechu* nuts are structurally related to nipecotic acid and are also inhibitors (K_i 141 and 14 μM respectively) of the neuronal uptake of GABA (115). It is possible that some of the psychopharmacological effects of ingestion of *Areca* nuts are related to interaction of arecaidine and guvacine with GABA transport systems. Arecaidine, administered

electrophoretically, does enhance the action of similarly administered GABA on neurons in the cat spinal cord and cerebellum, but appears not to influence synaptic inhibition mediated by GABA in these tissues after either microelectrophoretic or intravenous administration (109).

CIS-3-AMINOCYCLOHEXANECARBOXYLIC ACID This conformationally restricted analogue of GABA, which is a competitive inhibitor (K_i 100 μM) of GABA uptake (116), appears to be more selective for neuronal compared to glial uptake of GABA than either DABA or nipecotic acid (117).

β-ALANINE The uptake of β-alanine into macroglia in the rat cerebellum and cerebral cortex may represent uptake mediated by a transport system for GABA equivalent to that found in sensory ganglia (11, 118). The inhibitory action of microelectrophoretically administered β-alanine on the firing of cat spinal, cerebellar, and cerebral neurons can be potentiated by similarly administered DABA and (–)-nipecotic acid (108). The latter results differed from those predicted on the basis of uptake studies using rat brain slices, and this led to the finding of regional differences in the susceptibility of GABA and β-alanine uptake to inhibitors (119). In the spinal cord, β-alanine may be associated with the neuronal uptake of GABA being taken up into a compartment from which it can be released by potassium stimulation in a calcium-dependent manner (120).

DRUGS AND GABA UPTAKE A number of centrally active drugs including butyrophenones, phenothiazines (121), and benzodiazepines (122) inhibit GABA uptake in vitro. The variations in relative potencies of 14 butyrophenones as inhibitors of GABA uptake correlate well with the relative clinical potencies of these drugs, but the same was not found for the 9 phenothiazines tested (121). At the present time it is not clear as to whether or not inhibition of GABA uptake contributes to the therapeutic actions of any of these drugs.

Inhibitors of GABA Release

Recent studies using in vitro models have indicated that many drugs might act on the synaptic release of GABA. Imipramine, haloperidol, chlorpromazine, and diazepam, at 1 μM, inhibit the calcium-stimulated release of radioactive GABA from mouse brain synaptosomes (122), while pentobarbitone (200 μM) inhibits this release, acting possibly on "late" calcium ionophores (123). The convulsions that follow intraventricular injection of ruthenium red may result from inhibition of GABA release, possibly due to antagonism of calcium influx (124).

GLYCINE

The concept of glycine acting as a major inhibitory transmitter in the spinal cord and brain stem owes much to the work of Aprison and his colleagues (125). Glycine appears to be an exclusively vertebrate transmitter which would make it unique among the known synaptic transmitters. Abnormalities in the glycine system may be associated with spinal spasticity (126).

There has been little recent progress concerning drugs that may act on the glycine system. Glycine metabolism is still poorly understood, and only nonspecific effects of centrally active drugs have been described on glycine uptake systems (127). Strychnine-sensitive synaptic inhibitions which may be mediated by glycine continue to be discovered, e.g. inhibition of intralaminar thalamic neurons following stimulation of cortical neurones (128), and there is increasing evidence that glycine may be a transmitter in the retina (129). Not all neuronal effects of glycine are antagonized by strychnine. Glycine has a bimodal action on frog spinal motoneurons; the hyperpolarizing response is blocked by strychnine but the depolarizing action which is usually the major effect is not influenced by strychnine (130).

Studies on the binding of strychnine to spinal membranes have indicated that glycine and strychnine bind to distinct sites that interact in a cooperative fashion, the strychnine binding site being possibly associated with the glycine ionophore (9). The displacement of bound strychnine by a series of strychnine analogues has been correlated with the convulsant and lethal effects of these alkaloids (131). A wide variety of substances are now known to act as glycine antagonists (7). Tetanus toxin, which blocks glycine release, has been shown to bind like glycine to synaptic terminals containing flattened and pleomorphic vesicles (132).

DRUGS THAT MAY ACT ON AMINO ACID–MEDIATED INHIBITION

In addition to those drugs already mentioned, there are a number of widely used drugs that may act, at least in part, on synaptic inhibition mediated by amino acid transmitters. The prolongation of GABA-mediated inhibition may contribute to general anesthesia (133), while the convulsant properties of dissociative anesthetics may be the result of inhibition of GABA synthesis (134). Some anticonvulsants may act by potentiating GABA uptake (135) release (136) or by prolonging GABA action (137). Ethanol intoxication has been related to GABA metabolism (138), and GABA may be involved in morphine analgesia (28, 139). The possible actions of benzodiazepines, barbiturates, butyrophenones and β-p-chlorophenyl-GABA on amino acid inhibition are discussed in more detail below.

Benzodiazepines

Based on their ability to displace strychnine bound to spinal membranes, it has been proposed that the antianxiety, anticonvulsant, and muscle-relaxant effects of benzodiazepines results from glycine agonist activity (140). No evidence has been obtained, however, for an action of benzodiazepines at glycine receptors in vivo (141–143). The results of the in vitro binding studies can be interpreted as showing that benzodiazepines bind to the strychnine rather than the glycine binding site. The affinity of benzodiazepines for the strychnine binding site would be some four orders of magnitude lower than that of strychnine (K_D 4 nM), suggesting that benzodiazepines could be regarded as showing rather weak strychnine agonist or antagonist activity which is not relevant to their therapeutic effects (7).

There is considerable evidence that benzodiazepines facilitate GABA-mediated synaptic inhibition (144, 145). Diazepam enhances synaptic inhibitions in many CNS regions likely to be mediated by GABA (146–148) and provides more protection against the convulsant effects of the GABA antagonists picrotoxin and bicuculline than against those of strychnine (142, 144). The reduction of cerebellar cGMP by diazepam is consistent with stimulation of GABA-mediated inhibition (149). Diazepam and the potent GABA agonist muscimol have similar effects on acetylcholine turnover in some but not all regions of rat brain (29). Not all of the effects of diazepam involve GABA, however, since for example, picrotoxin does not modify the decreased spontaneous activity elicited in mice by diazepam (150). Flurazepam has been shown to have a bicuculline-sensitive inhibitory action on rat medullary neurons when administered microelectrophoretically (143). Such GABA agonist activity has not been found for benzodiazepines by other workers (143, 148, 151), and indeed GABA antagonist actions have been reported (143, 151). Benzodiazepines do not influence the binding of GABA to membranes from rat cerebral cortex (38) or the binding of bicuculline methiodide to membranes from rat cerebellum (26). Diazepam has been shown to bind to rat brain membranes with high affinity (K_D 2.6 nM) and can be displaced by other benzodiazepines whose displacement potencies correlate very well with their muscle relaxant properties (152). The benzodiazepines were some four orders of magnitude more potent as displacers of diazepam binding than of strychnine binding. Bound diazepam could not be displaced by GABA, muscimol, bicuculline, picrotoxin, glycine, or strychnine. Studies on frog sympathetic ganglia suggest that diazepam has an indirect action perhaps stimulating GABA release, since diazepam did not depolarize preganglionic terminals in ganglia depleted in GABA content although this treatment did not influence the ability of exogenous GABA to depolarize these terminals (153). Effects on GABA release may underlie the facilitation of GABA-mediated inhibition by benzodiazepines which have been shown to inhibit calcium-dependent efflux and stimulate calcium-independent efflux of GABA from mouse brain synaptosomes (122).

Barbiturates

Pentobarbitone appears to have both post- and presynaptic effects on GABA-mediated inhibitions. Evidence that pentobarbitone prolongs and potentiates postsynaptic conductance changes induced by GABA comes from in vivo studies on synaptic inhibition of cat hippocampal neurons (154) and in vitro studies on the action of GABA on hemisected frog spinal cord (155), mouse spinal neurons in tissue culture (156), and slices of guinea pig olfactory cortex (157). Changes in the reversal potential of the conductance changes induced by GABA and a direct hyperpolarizing effect of pentobarbitone may be involved in these postsynaptic actions. Evidence for presynaptic actions comes from in vitro release studies, although varying effects of barbituates on GABA uptake and release processes have been described. Pentobarbitone inhibits the uptake of GABA by rat brain slices (158) and stimulates the uptake of GABA by rat brain synaptosomes (135). Pentobarbitone potentiates electrically stimulated release of GABA from rat brain slices

but inhibits potassium-stimulated release (158). Phenobarbitone inhibits GABA uptake and potentiates protoveratrine-stimulated release of GABA from rat brain slices (136). Pentobarbitone inhibits calcium-dependent release of GABA from mouse brain synaptosomes at the level of the "late" calcium ionophores (123).

Pentobarbitone and related barbiturates have been shown to reverse the antagonism of GABA responses produced by bicuculline, picrotoxin, and isopropylbicyclophosphate on isolated rat superior cervical ganglia, and similar results have been obtained with pentobarbitone, GABA, and bicuculline methochloride administered microelectrophoretically to rat medullary neurons (159). It is unlikely that barbiturates act directly on GABA or bicuculline receptors since pentobarbitone does not influence the binding of either GABA or bicuculline methiodide to rat brain membranes (26, 38, 160). The reported reversal of GABA antagonism by barbiturates could involve GABA ionophores in view of the known effects of barbiturates on GABA-induced conductance changes.

Butyrophenones

While haloperidol and related antipsychotic butyrophenones were originally investigated because of their structural similarity to GABA (161), the effects of these drugs are usually interpreted on the basis of dopamine antagonism. There are, however, a number of recent findings which suggest that some of the observed effects on dopaminergic systems could be secondary to effects on GABA neurons. It has been reported that droperidol has a bicuculline-sensitive inhibitory action on the firing of Purkinje cells in cat cerebellum (162), although haloperidol has a different action from that of GABA on cat spinal motoneurons in that, like dopamine, haloperidol causes an increase in membrane resistance (163). Haloperidol displaces radioactive bicuculline methiodide (18% at 10 μM) bound to membranes from rat cerebellum (26), inhibits the electrically stimulated release of preloaded radioactive GABA (66% at 10 μM) from slices of rat corpus striatum (164), and inhibits GABA uptake (48% at 200 μM) into slices of rat cerebral cortex (165). Haloperidol causes a decrease in GABA levels in rat striatum and substantia nigra, and a stimulation of dopamine turnover which can be blocked by pretreatment with aminooxyacetic acid (166). When injected into the neostriatum of cats via implanted cannulae, haloperidol and GABA produce identical effects which can be antagonized by picrotoxin (167).

β-p-Chlorophenyl-GABA (Lioresal, Baclofen)

The inhibitory action of this muscle relaxant, which is effective orally in the treatment of human spasticity, on the firing of CNS neurons does *not* appear to involve bicuculline-sensitive receptors (168, 169). The pharmacological profile of β-*p*-chlorophenyl-GABA administered intravenously to rats differs from that expected of a GABA agonist (30), and β-*p*-chlorophenyl-GABA does not influence the binding of radioactive bicuculline methiodide to membranes from rat cerebellum (26). The reported selective antagonism by β-*p*-chlorophenyl-GABA of the action of substance P on spinal neurons (170) has not been supported by more recent work (171–173), and β-*p*-chlorophenyl-GABA appears to have a general depressant

action on the firing of neurons in many areas of the CNS. This depressant action may be more a result of its structural resemblance to phenylethylamine derivatives than to GABA (168, 169), although interaction with bicuculline-insensitive GABA receptors cannot be ruled out.

CONCLUSION

This review has dealt mainly with the neuropharmacology of GABA which appears to be in a phase of exponential growth. The neuropharmacology of glycine on the other hand appears to be in something of a lag phase perhaps awaiting a breakthrough in our understanding of glycine metabolism. There is a huge amount of effort going into the probable excitatory amino acid transmitters, but breakthroughs are needed in this area too, particularly with respect to the development of specific antagonists. Ligand binding studies are likely to become an ever increasing part of amino acid neuropharmacology, and are already providing data that are difficult to obtain in any other way. As our appreciation of the essential role of amino acid transmitters grows, chemical manipulation of amino acid transmitters for therapeutic purposes will be put on a sounder experimental basis and we may learn a little more about brain function.

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