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GABAergic mechanisms in the pathogenesis and treatment of epilepsy

B. S. MELDRUM
Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF

1 Evidence relating to the role of GABA in the pathogenesis of epilepsy is reviewed.
2 Impaired GABAergic function appears to contribute to seizure susceptibility in a variety of genetically-determined syndromes in animals, e.g. genetically epilepsy prone rats showing sound-induced seizures, gerbils with genetically determined epilepsy, and baboons, *Papio papio*, with photosensitive epilepsy.
3 In epilepsy secondary to a cerebral insult there is some morphological and biochemical evidence for impaired GABAergic function in experimental situations, but little definitive evidence in man.
4 Pharmacological approaches to enhancing GABAergic inhibition include the use of GABA agonists (or prodrugs), GABA-transaminase inhibition, GABA uptake inhibition, and action at the GABA/benzodiazepine allosteric site.
5 Experimental data suggest that the best prospect for potent anticonvulsant action with fewest side effects (myoclonus, sedation, ataxia) is at present offered by GABA-transaminase inhibitors or novel agents acting on the benzodiazepine receptor site.

Keywords GABA epilepsy

Introduction

GABA, 4-aminobutyric acid, is the principal inhibitory transmitter in the mammalian brain. It acts at the GABA/benzodiazepine ($GABA_A$) receptor to increase membrane chloride conductance and thereby stabilise or hyperpolarise the resting membrane potential (if extracellular chloride concentration exceeds intracellular [Cl^-]). The GABA/benzodiazepine receptor molecule has recently been purified and sequenced (Schofield *et al.*, 1987). It is composed of a and b subunits each with 4 hydrophobic (membrane spanning) sequences that provide the ion-channel and a large *N*-terminal extracellular domain that provides sites for GABA and for benzodiazepines and barbiturates to act. GABA/benzodiazepine receptors are found post-synaptically on dendrites, the somatic membrane and on the axon initial segment. Another type of receptor responding to GABA, the $GABA_B$ receptor, is found on presynaptic terminals and on postsynaptic membranes. Whereas at the $GABA_A$ receptor the effects of GABA are mimicked by muscimol and the bicyclic GABA analogue, THIP, at the $GABA_B$ receptor baclofen is a potent agonist. Bicuculline is an antagonist at the $GABA_A$ receptor but not at the $GABA_B$ receptor. The $GABA_B$ receptor increases potassium conductance and decreases calcium entry. Presynaptically, activation of the $GABA_B$ receptor decreases neurotransmitter release, as has been shown for monoamines and excitatory amino acids (Bowery *et al.*, 1980). Postsynaptically, the increase in K^+ conductance is associated with a slow inhibitory potential (Newberry & Nicoll, 1984). There are marked regional differences comparing the density of $GABA_A$ and $GABA_B$ receptors in autoradiographs (Bowery *et al.*, 1987).

Correspondence: Dr B. S. Meldrum, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF

Neurons releasing GABA fall into many structural types that have been characterised in Golgi studies or immunocytochemical studies employing antisera either to glutamic acid decarboxylase or to a GABA-glutaraldehyde-protein complex (Houser *et al.*, 1983; Somogyi & Soltesz, 1986). Intrinsic inhibitory interneurons show great structural diversity, including, for example, the chandelier cells found in cortex and hippocampus, that have very large numbers of terminals located exclusively on axon initial segments (Somogyi *et al.*, 1985). There are also GABAergic neurons that relay to more distant structures such as, for example, the striatal neurons that project to the substantia nigra (SN), and those in the SN pars reticulata that relay to the thalamus and superior colliculus. GABAergic intrinsic interneurons and extrinsic neurons both play crucial roles in the origin and spread, or in the suppression, of epileptic activity. This can be shown by the focal injection of agents impairing GABAergic neurotransmission. Such agents fall into two broad categories, those inhibiting the synthesis of GABA (such as 4-deoxypyridoxine, isoniazid, thiosemicarbazide, L-allylglycine) and those blocking its post-synaptic action (such as bicuculline, picrotoxin). Systemic injection of such agents induces generalised convulsions (Meldrum, 1975, 1985). Their injection into the neocortex produces focal cortical seizures. Their injection into the hippocampus, amygdala or prepyriform cortex produces limbic seizures. Interestingly the focal injection of bicuculline into the striatum can, by stimulating the GABA-ergic striato-nigral system, block limbic seizures induced by pilocarpine or by bicuculline itself (Turski *et al.*, 1987).

GABA in the pathogenesis of epilepsy

The proconvulsant effect of any impairment of GABA-mediated inhibition is very evident in animal models of epilepsy and in *in vitro* preparations such as the hippocampal slice. Treatment with an inhibitor of glutamic acid decarboxylase sufficient to partially block the synthesis of GABA can reproduce in normal baboons the genetically determined syndrome of photosensitive epilepsy (Meldrum *et al.*, 1975). Thus it is natural to ask if any genetic or acquired abnormality of GABAergic inhibition could be responsible for any of the diverse epileptic syndromes occurring in man.

Genetically-determined abnormalities

Impaired GABA synthesis is probably responsible for the seizures occurring in the rare genetic syndrome of pyridoxine dependency. In two genetically-determined epileptic syndromes in animals abnormalities have been found in the GABA/benzodiazepine receptor system. Thus in seizure-susceptible gerbils a reduction in the binding of [^{3}H]-flunitrazepam is found in the substantia nigra, pars reticulata(−20%) and in the midbrain periaqueductal grey (−12%) (Olsen *et al.*, 1985). This abnormality precedes developmentally the appearance of the seizures and could contribute to their occurrence. In an inbred strain of mice (DBA/2) showing sound-induced seizures at a critical age there is a reduction in the number of high-affinity GABA receptors in the brain (Horton *et al.*, 1982) and benzodiazepine binding is reduced in the substantia nigra, midbrain periaqueductal grey, caudal pons central grey, laterodorsal tegmental nucleus and inferior colliculus central nucleus (Olsen *et al.*, 1986). Using immunocytochemical methods to identify neurons containing glutamic acid decarboxylase it appears that the number of GABAergic neurons is increased in inferior colliculus central nucleus in genetically epilepsy-prone rats showing sound-induced seizures (Roberts *et al.*, 1986). A somewhat similar increase in the number of GABAergic neurons has been described in the hippocampus of seizure-sensitive gerbils (Peterson *et al.*, 1985). These increases have been interpreted by Ribak as contributing to epileptogenesis by a process of 'disinhibition' but it is perhaps more probable that they represent an attempt to compensate for a deficiency in GABAergic transmission. GABAmimetic drugs are exceptionally potent as anticonvulsants in the seizure-susceptible gerbils (Löscher *et al.*, 1983).

The genetically determined syndrome of photosensitive epilepsy in the Senegalese baboon may be caused by a deficiency in cortical GABAergic inhibition. In support of this hypothesis are the theoretical issues discussed by Meldrum & Wilkins (1984) and several direct observations. The latter include the correlation observed between the reduction in the cerebrospinal fluid GABA concentration and the degree of photosensitivity (Lloyd *et al.*, 1986) and the potent anticonvulsive action of focal cortical infusion of GABA (Brailowsky *et al.*, 1987).

Acquired abnormalities of GABAergic systems

It is possible that cerebral insults that predispose to epilepsy (such as blunt or penetrating head injuries, anoxic or ischaemic insults, and prolonged febrile convulsions) do so by selectively damaging GABAergic systems. Evidence to support this thesis comes from animal experi-

ments and from neurosurgical specimens but the results are still somewhat controversial. Focal motor epilepsy can be induced in the monkey by application of alumina gel to the sensorimotor cortex. The number of nerve terminals staining positively for glutamic acid decarboxylase is markedly reduced in the cortical focus (Ribak *et al.*, 1979). By electron microscopy there is a preferential loss of symmetric (inhibitory) synaptic junctions compared with asymmetric (excitatory) junctions (Ribak *et al.*, 1982). If such changes occurred around a traumatic or space-occupying lesion in the brain they could account for the focal initiation of seizures. It has also been proposed that GABAergic neurons are selectively vulnerable to hypoxic brain damage or to damage resulting from status epilepticus (see Meldrum & Corsellis, 1984). Evidence for this includes the report that in infant monkeys subjected to a 30 min episode of hypoxia there is a selective loss of symmetric synapses (presumed to be GABAergic) in the cortex (Sloper *et al.*, 1980). A link to increased seizure susceptibility has not, however, been demonstrated.

Some very recent studies employing immunocytochemical staining in the hippocampus suggest that in ischaemia and in status epilepticus the GABAergic interneurons tend to be preserved but certain peptide-containing interneurons (those staining for somatostatin) are selectively lost (Sloviter, 1987). It may be that one function of these interneurons is to activate the GABAergic system so that loss of somatostatin neurons could impair feedback inhibitory mechanisms. A further possibility is that insults early in life can cause long-term modifications in GABA/benzodiazepine receptor systems. For example, a change in hippocampal benzodiazepine binding in adult rats has been reported as a consequence of febrile seizures induced at 15 days of age (Chisholm *et al.*, 1985).

In man the principal studies have been of neurosurgically resected specimens of either anterior temporal lobe or neocortex. Comparison of tissue from the epileptogenic zone (determined electrographically) with non-epileptogenic tissue showed a decrease in glutamic acid decarboxylase activity in a proportion, but not all, of a group of 27 patients undergoing focal resection (Lloyd *et al.*, 1985). However, Babb (1986), performing glutamic acid decarboxylase staining on resected hippocampi (with detailed cell counts in all the hippocampal subfields), did not find preferential loss of GABAergic interneurons (in most regions principal neurons were preferentially lost). Furthermore, the staining of GABAergic terminals on surviving pyramidal neurons indicated no loss of GABAergic innervation. At present the contribution of a selective loss of GABAergic neurons to acquired epilepsy in man remains unknown.

Studies of the GABA content in lumbar CSF have shown a significant reduction in patients with a wide range of epileptic syndromes compared with non-neurological controls, both for adults (Manyam *et al.*, 1980; Wood *et al.*, 1979) and for children (Loscher *et al.*, 1981; Loscher & Siemes, 1985). These data support the concept that diminished GABAergic inhibition contributes to seizure susceptibility.

Enhancing GABA-mediated inhibition

Drugs that enhance GABA-mediated inhibition have an anticonvulsant effect in a wide range of animal models of epilepsy. The effect depends critically on the mechanism by which GABA-mediated inhibition is enhanced. Direct agonists can be proconvulsant in some models. There is not a strong preferential effect in models dependent on impaired GABA transmission (e.g. seizures due to isoniazid, bicuculline or picrotoxinin) compared with other seizure models. We shall consider sequentially the different mechanisms of enhancing GABA-mediated inhibition listed in Table 1.

Table 1 Mechanisms for enhancing GABA mediated inhibition

1	GABA, GABA agonists, GABA prodrugs e.g. liposome-entrapped GABA muscimol, THIP, cetylGABA progabide, SL 75102
2	Enhanced GABA synthesis and/or synaptic release e.g. ? benzodiazepines, ? valproate vigabatrin
3	GABA-transaminase inhibition e.g. L-cycloserine ethanolamine-*o*-sulphate γ-acetylenic GABA vigabatrin
4	GABA uptake inhibition e.g. nipecotic acid, THPO, SKF 89976A, SKF 100330A
5	Action at GABA/benzodiazepine allosteric site e.g. benzodiazepines, β-carbolines triazolopyridazines
6	Action at chloride ionophore/picrotoxinin/barbiturate site e.g. barbiturates

GABA penetrates the blood-brain barrier poorly

Cardiovascular and other side-effects prevent the therapeutic use of high systemic doses of GABA. Considerable ingenuity has been devoted to achieve brain delivery for systemically administered GABA. An anticonvulsant effect against isoniazid-induced seizures in rats has been reported following intraperitoneal injection of a liposome-entrapped solution of GABA, i.e. GABA sonicated with phosphatidyl-serine (Loeb *et al.*, 1986). Various esters of GABA have been used as prodrugs. These include benzoyl GABA, pivaloyl GABA and cetyl GABA. Anticonvulsant effects of these esters have been observed in rodent models of epilepsy (Galzigna *et al.*, 1978; Frey & Löscher, 1980). A more elaborate chemical delivery system has been devised by Bodor and colleagues. This utilises a carrier containing a pyridine ring that participates in dihydropyridine pyridinium redox reactions. GABAbenzyl ester is linked via an amide bond to the redox carrier which is lipophilic in its dihydropyridine form. Following entry to the brain it is oxidised to a charged quaternary complex that is trapped in the brain and hydrolysed to yield GABA. Administration of this complex to rats undergoing a conflict procedure produces an anxiolytic effect (Anderson *et al.*, 1987) but anticonvulsant studies have not yet been reported.

Muscimol and the synthetic bicyclic analogue of GABA, THIP, are potent specific $GABA_A$ agonists. They are anticonvulsant in several rodent models of epilepsy employing convulsant drugs. However they are proconvulsant in Wistar rats with spontaneous petit-mal-like epilepsy (Vergnes *et al.*, 1984). They also enhance spike and wave discharges and induce diffuse myoclonus in baboons with photosensitive epilepsy (Pedley *et al.*, 1979; Meldrum & Horton, 1980).

L-baclofen, the $GABA_B$ agonist, likewise appears anticonvulsant in some rodent tests but facilitates spike and wave discharges both in Wistar rats and in photosensitive baboons (Meldrum & Horton, 1974).

Progabide is a GABA receptor agonist acting on both $GABA_A$ and $GABA_B$ receptors, and is metabolised in the brain to yield SL 75102 (a more potent GABA agonist than progabide) and to GABA itself (Lloyd *et al.*, 1982). It is by no means certain that progabide acts by enhancing GABAergic transmission: in some test systems it mimics the action of phenytoin rather than that of muscimol (Fromm *et al.*, 1985). Progabide is anticonvulsant in a wide range of rodent models of epilepsy (Worms *et al.*, 1982) and has some efficacy in man.

Enhancing synaptic release of GABA

It is likely that the synaptic release of GABA can be facilitated by drugs acting either on presynaptic receptors or on the synthesis of GABA. However, definitive evidence is lacking. Benzodiazepines may facilitate GABA release (Curtis *et al.*, 1976). It has been claimed that valproate, ethanolamine-*o*-sulphate and vigabatrin all enhance synaptosomal GAD activity (Löscher, 1981). In the case of vigabatrin enhanced release of GABA into cortical superfusates has been demonstrated both at rest and during peripheral nerve stimulation (Abdul-Ghani *et al.*, 1980). This probably represents enhanced release but an indirect effect on reuptake cannot be excluded.

GABA-transaminase inhibitors

Various compounds that interact with pyridoxal phosphate inhibit GABA 2-oxoglutatarate aminotransferase (GABA-T) activity, and the activity of many other transaminases and decarboxylases. These inhibitors include aminooxyacetic acid and L-cycloserine, which have long been known to show anticonvulsant activity (Kuriyama *et al.*, 1966; Scotto *et al.*, 1963). However, because of their multiple biochemical actions a simple correlation of anticonvulsant action with elevation in brain GABA content was not found. At one time it was proposed that the anticonvulsant activity of valproate could be attributed to inhibition of GABA-T, but this enzymic action is not now thought to be a significant effect of valproate *in vivo* (Chapman *et al.*, 1982). It was the introduction of the irreversible or catalytic inhibitors of GABA-T that finally established the relationship between GABA-T inhibition and anticonvulsant action (Fowler & John, 1972; Anlezark *et al.*, 1976; Palfreyman *et al.*, 1981). The structures of some of these compounds are shown in Figure 1. Inhibition of GABA-T activity by 55–80% leads to a marked increase in brain GABA content (5–10 fold in the mouse brain) and a sustained anticonvulsant action in a wide range of rodent models, and also in photosensitive baboons (Meldrum & Horton, 1978). Among the catalytic GABA-T inhibitors vigabatrin (γ-vinyl GABA) appears to have the fewest acute toxic side-effects in the rodent, possibly because of its high specificity for GABA-T. Of the two enantiomers of vigabatrin it is the R(−) form that is the inhibitor of GABA-T and that shows the anticonvulsant effect (Danzin *et al.*, 1984; Meldrum & Murugaiah, 1983).

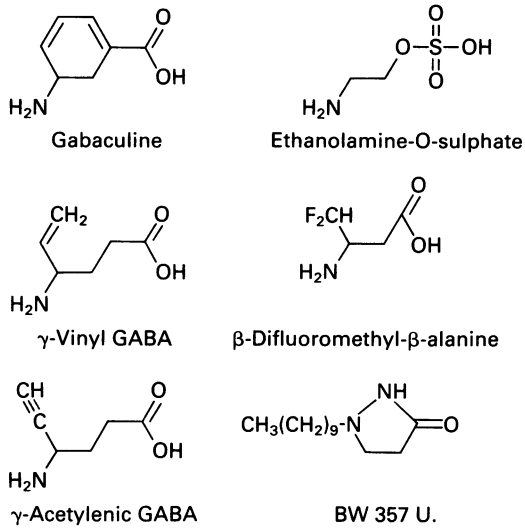

Figure 1 Molecular formulae of some irreversible inhibitors of GABA-transaminase (note that *in vivo* the diazo ring in BW 357U opens to give hydrozinopropionic acid). (Reproduced from Meldrum (1984) with permission.)

GABA uptake inhibition

The main method whereby synaptically released GABA is inactivated is by uptake into nerve terminals or into glia. Specific carriers are involved and these appear not to be identical in glia and neurons. Various GABA analogues can compete with GABA at this carrier, and the structural requirements for such competition differ from those for agonist action at the GABA receptor or for inhibitory action at the active site of GABA transaminase (Krogsgaard-Larsen, 1980). GABA uptake inhibitors administered into the ventricles block sound-induced seizures in mice, with compounds acting preferentially on glial uptake (e.g. nipecotic acid, guvacine and THPO) being the most effective (Meldrum *et al.*, 1982). These compounds do not cross the blood-brain barrier significantly, so that various prodrug or carrier molecules have been studied. Some efficacy was obtained systemically with ethyl and pivaloyloxymethyl esters (Meldrum *et al.*, 1982; Falch *et al.*, 1987), but there were significant side-effects including cholinergic effects and myoclonus (Zorn *et al.*, 1987). Diphenyl butenyl derivatives of nipecotic acid and of guvacine (SKF 89976A and SKF 100330A) are potent, orally active GABA uptake inhibitors with high anticonvulsant activity in rodent seizure models (Yunger *et al.*, 1984; Löscher, 1985). However in primates myoclonus appears as a significant toxic side-effect (Meldrum, unpublished).

Allosteric enhancement of GABAergic activity

The close functional relationship between the $GABA_A$ receptor and a benzodiazepine receptor originally defined in terms of high affinity binding to brain membrane receptors (Möhler & Okada, 1977) has been fully substantiated in recent biochemical and physiological studies (see Meldrum & Braestrup, 1986; Meldrum & Chapman, 1986; Schofield *et al.*, 1987; Haefely & Polc, 1986). Benzodiazepines and a variety of other structures such as triazolopyridazines and β-carboline derivatives act at an allosteric site on the $GABA_A$/benzodiazepine/chloride ionophore molecule to enhance the binding and efficacy of GABA. The principal electrophysiological effect is an increase in the number of channel openings induced by a given concentration of GABA (Macdonald, 1983; Barker & Owen, 1986). There is a good correlation between the potency of various benzodiazepines as anticonvulsants in the threshold pentylenetetazol test in rodents with their affinity for the GABA/benzodiazepine receptor (Meldrum & Braestrup, 1984). Some β-carboline derivatives have a partial agonist effect at the benzodiazepine receptor and produce a powerful anticonvulsant effect with very little sedative or muscle relaxant action (Meldrum *et al.*, 1983; Meldrum & Chapman, 1986). Other β-carboline derivatives act at the benzodiazepine receptor to produce an opposite effect to that of the benzodiazepines, decreasing the hyperpolarising action of GABA *in vitro*, and being

anxiogenic and proconvulsant *in vivo* (De Deyn & Macdonald, 1987; Meldrum & Chapman, 1986). Endogenous peptides have been purified from rat, bovine and human brain that appear to act on the benzodiazepine receptor in this 'inverse agonist' fashion (Guidotti *et al.*, 1983; Ferrero *et al.*, 1986; Marquardt *et al.*, 1986).

Benzodiazepines are very potent anticonvulsants in animal test systems. In clinical use they suffer from two disadvantages, impairment of motivation and complex skills and tolerance to their anticonvulsant effect that develops in one third or more of patients.

Action at the chloride ionophore

Some convulsant drugs (such as picrotoxinin) and some anticonvulsants such as barbiturates appear to act at a site separate from the GABA recognition site or the benzodiazepine site, but closely related to the chloride ionophore (Olsen, 1982). At this site anaesthetic barbiturates prolong the open time of the chloride channel (Study & Barker, 1981). Barbiturates show both a direct action on chloride conductance and a potentiation of the effect of GABA. The relationship between these effects on chloride conductance and the anticonvulsant and anaesthetic actions of barbiturates is not yet defined.

Summary

For the last 10–15 years the design of novel compounds that enhance GABA-mediated inhibition has provided a rational approach to anticonvulsant drug therapy (Meldrum, 1978). Of the various possible pharmacological approaches, the use of direct agonists has proved somewhat disappointing. Why potent agonists cause myoclonus and other proconvulsant effects is uncertain, but may involve a process of desensitisation to the inhibitory action of GABA or depolarisation at dendritic sites. The use of GABA-uptake inhibitors seems to give rise to similar problems but may merit further exploration. These problems are less significant with catalytic inhibitors of GABA-T, suggesting that the effect is probably not a sustained flooding of the synaptic and perisynaptic spaces with GABA. Enhanced synaptic release would ensure that the enhanced GABAergic activity had the correct spatial and temporal characteristics to suppress seizure activity. This is also true of benzodiazepine-like compounds that act post-synaptically to enhance the efficacy of GABA. At the present moment these two approaches offer the best prospect of providing significant new therapeutic agents in epilepsy.

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