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COMMENTARY

THE PHARMACOLOGY OF GABA-TRANSAMINASE INHIBITORS

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γ-Aminobutyric acid (GABA) is firmly established as a major inhibitory neurotransmitter in the central nervous system [48, 80]. GABA-transaminase (GABA-T) (4-aminobutyrate:2-oxoglutarate amino transferase—EC 2.6.1.19) is the primary catabolic enzyme for GABA and its inhibition significantly elevates GABA concentrations in the brain [62]. Understanding of the physiological role of GABA in the CNS has been greatly enhanced by the use of GABA-T inhibitors and the pharmacological consequences of inhibition of this enzyme suggest several therapeutic uses for these agents.

Twenty years ago Roberts and co-workers [7] described the GABA-T inhibitory properties of the non-specific, pyridoxal phosphate "scavenger", hydroxylamine, and five years later those of the somewhat more specific aminooxyacetic acid [49]. Despite their inherent lack of selectivity amongst pyridoxal phosphate-dependent enzymes, administration of these compounds produces an increase in brain GABA concentrations with associated anticonvulsant effects [104]. They have, however, proven too toxic for general therapeutic use.

During the last decade a new concept of enzyme inhibition has been developed [94]. This concept depends on the inhibitor containing a latent reactive functionality that can be activated as a result of the normal mechanism of action of the target enzyme. These new inhibitiors, which have been called "K_{cat}" [77], suicide-enzyme inhibitors [1] or enzyme-activated inhibitors [94], may be expected to be highly specific since they should inhibit only those enzymes which can accept them as substrates *and* activate the latent functional group. Several inhibitors of GABA-T which operate by this novel mechanism have recently been designed and synthesized. Additionally, and perhaps not surprisingly in view of nature's diversity, a natural product, gabaculine, has been isolated and shown to function as a potent GABA-T inhibitor by a similar mechanism. These new compounds and their mechanisms of action have recently been reviewed by Metcalf [62].

PHARMACOLOGICAL EFFECT OF GABA-T INHIBITORS

The structures of the GABA-T inhibitors to be discussed in this commentary are shown in Fig. 1. GABA and its synthetic and catabolic enzymes occur in high concentrations in the CNS but are also found in several peripheral tissues [38, 65]. GABA's role in the periphery has been little explored and GABA-T inhibitors have seldom been employed to define it. This section deals solely with centrally-mediated pharmacologic effects of GABA-T inhibition.

Sedation, hypothermia and antinociceptive action. GABA-T inhibitors cause dose-related decreases in locomotor activity, general sedation, potentiation of barbiturate sleep, hypothermia and moderate antinociception in rats and mice. Animals treated with GABA-T inhibitors usually display a characteristic hunched posture with piloerection, ptosis and, at higher doses, ataxia, lacrimation and catatonia from which they can be easily aroused [87]. Paradoxically, following high doses of some of these inhibitors, e.g., aminooxyacetic acid, γ-acetylenic GABA, gabaculine and isogabaculine, the sedation may have interspersed brief periods of excitation consisting of rapid running, jumping, head-rearing, myoclonus and, in some cases, clonic convulsions [87]. These excitatory effects are rarely seen with ethanolamine-*O*-sulphate or γ-vinyl GABA. The antinociceptive action, while modest, is significant in several animal models [14, 16], is non-opiate like and does not induce dependence. GABA-T inhibitors do not substitute for morphine in dependent rats, nor is supersensitivity of the GABAergic system observed on morphine withdrawal when assessed using the hypothermic effect of GABA-T inhibitors [13]. Nevertheless, a role for GABA in analgesia is under investigation at this time (see [18] for review).

Anticonvulsant effects. The comparative anticonvulsant potencies of GABA-T inhibitors against various experimental seizures in mice are shown in Table 1. Anticonvulsant activity of some of these GABA-T inhibitors has also been demonstrated in rats [50, 64] and in photosensitive baboons [61]. The absence of concordance among different investigators testing the same inhibitor in the same convulsive test can probably be explained by methodological differences. Thus, seizures caused by small intravenous doses of bicuculline could be blocked effectively by relatively low doses of GABA-T inhibitors as well as by some putative direct-acting GABA agonists [15]. On the other hand, seizures induced by a range of subcutaneous doses of bicuculline were not influenced by much higher doses of the same GABA-T inhibitors [85]. Similarly, both no change (after intraperitoneal administration [104]) and a highly significant increase (after subcutaneous administration [55]) in electroshock convulsive thresholds have been found 6 hours following similar

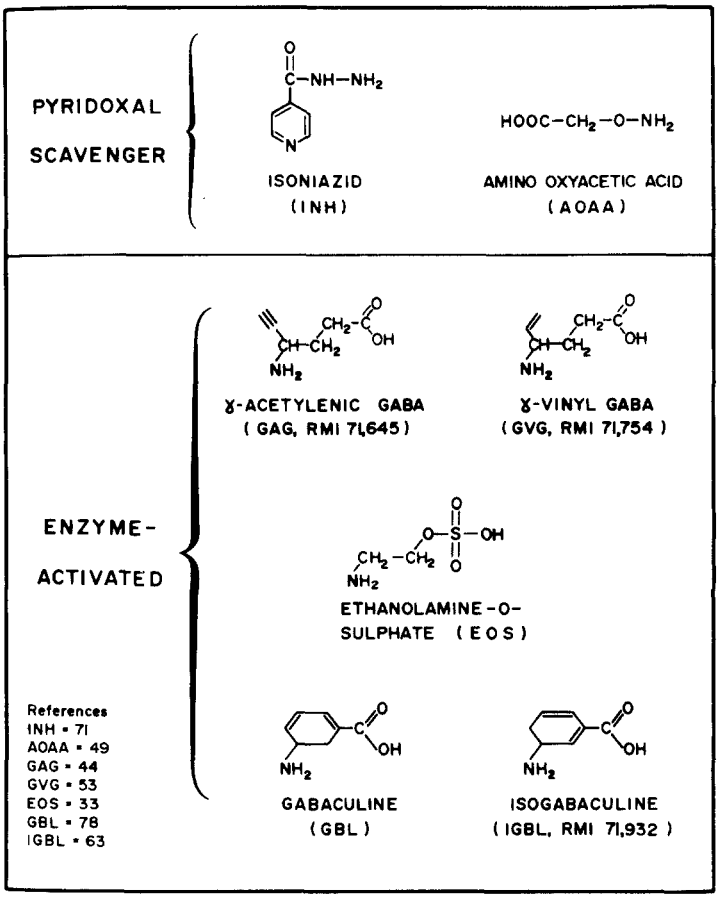

Fig. 1. Molecular structures of the inhibitors of GABA-T discussed in this commentary.

doses of aminooxyacetic acid. It has been suggested [50, 64] that GABA-T inhibitors are unable to alter the afterdischarge threshold at the epileptic focus. Rather, elevated brain GABA concentrations may act on the afterdischarge propagation. The relevance of such a hypothesis to explain the variability of anticonvulsant activity noted among different seizure models remains speculative.

In general, the potencies and activity profiles of gabaculine and isogabaculine are very similar; not surprisingly, since these two inhibitors are isomers. Aside from this similarity, there are few other common features among the GABA-T inhibitors in anticonvulsant profiles. It is likely that the differences observed are due to dissimilarities in potency and specificity among the inhibitors as well as to differences in the precise cellular and subcellular localization of the elevated GABA concentrations induced by these agents [84, 91].

Dyskinetic effects of intracerebral injections

The paradoxical excitation and myoclonic movements seen after large parenteral doses of several GABA-T inhibitors are even more evident when the inhibitors are administered into the cerebral ventricles [68]. In a preliminary study [81] we showed that all GABA-T inhibitors tested, with the sole exception of ethanolamine-*O*-sulphate, could produce dyskinetic movements when high concentrations were injected into the striatum. Subsequent studies [70, 82, 83] demonstrated that the primary locus to produce dyskinesia is on the overlying motor cortex, rather than in the striatum. The dyskinetic effect of high concentrations of GABA-T inhibitors, which is not related to their relative potencies as inhibitors of the enzyme, has been interpreted circumstantially as due to weak and reversible GABA-antagonistic actions [70, 83]. In support of this, high concentrations of several GABA-T inhibitors are able to displace [^{3}H]-GABA from its binding sites [58, 70]. Additionally, iontophoretic application of γ-acetylenic GABA can block the inhibitory effect of GABA in certain brain cells [37]. It is tempting to speculate that some of the paradoxical excitant effects of GABA-T inhibitors may be due to this antagonist action.

Alternative explanations have been proposed to account for the excitant effects of these compounds. These include inhibition of glutamic acid decarboxylase (seen with aminooxyacetic acid and γ-acetylenic GABA *in vitro* and with ethanolamine-*O*-sulphate, γ-vinyl GABA, gabaculine and isogabaculine *in vivo*, but not *in vitro* [32, 45], differential effects on neuronal and non-neuronal pools of GABA [84], or effects other than the GABAergic [69, 74, 91].

Table 1. The anticonvulsant activity of selected inhibitors of GABA-transaminase in a range of experimentally-induced seizure models in mice

Convulsant	Dose (mg/kg)	Route	Aminooxyacetic acid	γ-Acetylenic GABA	γ-Vinyl GABA	Gabaculine	Isogabaculine
Bicuculline	0.55	i.v.	19 (15–23) i.p. 5 hr [17, 19]	26 (17–36) i.p. 5 hr [17, 19]	54 (47–63) i.p. 5 hr [17, 19]	NA to 20 i.p. 5 hr [17, 19]	8 (7–12) i.p. 5 hr [17, 19]
	3	s.c.		NA to 200 i.p. 4 hr [85]	NA to 1500 i.p. 4 hr [85]	NA 40 i.p. 24 hr [89]	NA 40 i.p. 24 hr [89]
Picrotoxin	3.25	i.v.		NA to 200 i.p. 4 hr [87]	800 i.p. 5 hr [20]		
	5	i.m.	> 25 i.m. 6 hr [105]				
	6	i.v.		A 200 i.p. 4 hr [85]	A 1500 i.p. 4 hr [85]	A 40 i.p. 24 hr [89]	A 40 i.p. 24 hr [89]
Metrazol	30	i.v.		NA to 200 i.p. 4 hr [20]	40 (32–50) i.p. 5 hr [20]		
	40	i.v.		NA to 200 i.p. 4 hr [20]	51 (45–58) i.p. 5 hr [20]		
	60	i.v.		NA to 200 i.p. 4 hr [86]	NA to 1500 i.p. 4 hr [87]		
	65	i.m.	< 25 i.m. 3 hr [105]				
	70	i.v.		NA to 200 i.p. 4 hr [20]		> 40 i.p. 24 hr [89] 205 (142–300) i.p. 4 hr [56]	~ 40 i.p. 24 hr [89]
	100	s.c.	20 (11–34) s.c. 6 hr [56]				
Strychnine	0.45	i.v.	NA to 40 i.p. 6 hr [20]		1200 (1000–1400) i.p. 5 hr [20]		
	0·5	i.v.		< 200 i.p. 4 hr [86]	< 2000 i.p. 4 hr [87]		
	0.6	i.v.				NA to 40 i.p. 24 hr [89]	NA to 40 i.p. 24 hr [89]
Isoniazid	250	i.v.		< 100 i.p. 4 hr [86]	< 1500 i.p. 3 hr [87]	~ 40 i.p. 24 hr [89]	~ 40 i.p. 24 hr [89]
	300	i.m.	A 25 i.m. 6 hr [105]				
3MPA	60	s.c.	20 (14–20) s.c. 6 hr [56]			107 (63–182) i.p. 4 hr [56]	
	100	i.p.		NA to 50 i.p. 2.5 hr [79]	A 1500 i.p. 4 hr [79]		

Table 1. *(continued)*

Convulsant	Dose (mg/kg)	Route	Aminooxyacetic acid	γ-Acetylenic GABA	γ-Vinyl GABA	Gabaculine	Isogabaculine
Allylglycine	DL 700	i.m.	A 25 i.m. 3 hr [105]				
	L 173	i.p.		NA to 50 i.p. 2.5 hr [79]	~ 1500 i.p. 4 hr [79]		
	D 575	i.p.		A 50 i.p. 2.75 hr [79]	< 1500 i.p. 4 hr [79]		
Electroshock	M.E.S.					158 (87–284) i.p. 4 hr [56]	
	E.T.		25 i.p. 1.5 hr [49]	84 (60–118) i.p. 4 hr [86]	1600 (1300–1900) i.p. 5 hr [20]		
			NA at 25 i.p. 4 or 6 hr [49]	100 i.p. 4 hr [87]	1500 i.p. 4 hr [87]	NA to 50 i.p. 24 hr [89]	
Audiogenic	(100 dB)		20 i.p. 5 hr [92]	41 (33–51) i.p. 4 hr [87]	990 (750–1300) i.p. 4 hr [87]	17 (16–18) i.p. 24 hr [89]	16 (14–19) i.p. 24 hr [89]
Hyperbaric Oxygen	75 p.s.i.		A 25 i.m. 6 hr [105]				
	80 p.s.i.			> 50 i.m. 4 hr [106]	< 800 i.m. 4 hr [103]		

The figures represent ED_{50} values (with 95% limits of errors) for the protection against seizures of 50% of a group of animals. Dose expressed as base times in hours (hr) indicate the premedication intervals before testing. Compounds were administered by intravenous (i.v.), intramuscular (i.m.), intraperitoneal (i.p.) or subcutaneous (s.c.) routes. NA indicates absence of protective anticonvulsant activity to the dose shown, whereas A indicates a significant increase ($P < 0.05$) in the time of onset to seizures. Electroshock tests were either the maximal test (MES) or the threshold test (ET). dB = decibels; p.s.i. = pressure in pounds per square inch. 3 MPA = 3 mecapoproprionic acid. References are indicated by numerals in square brackets.

Effect on food intake. Several authors [24, 35, 42, 66] have shown that inhibitors of GABA-T can produce a dose-related and long-lasting decrease in food intake. This effect and the resultant reduction in weight gain can be seen with relatively small i.p. doses of inhibitors but is difficult to dissociate from the generalized depressant effects of the compounds. Recent results suggest a complex role for GABA in both the satiety and feeding centres of the hypothalamus [46, 47]. Depending on the hypothalamic area studied, both facilitatory and inhibitory effects of GABA on feeding can be demonstrated. Nevertheless, when whole brain GABA concentrations are modestly elevated with GABA-T inhibitors the overall effect on food intake is a decrease [24, 42].

Antianxiety effects. There is now an impressive body of evidence implicating GABA in numerous pharmacological effects of the benzodiazepine anxiolytics [39, 41]. Most GABA-T inhibitors are effective against metrazol-induced seizures (see previous section) a test which is indicative of potential clinical anxiolytic efficacy. However, several workers [23, 31, 43, 102] have consistently been unable to demonstrate an anxiolytic effect of GABA-T inhibitors, either alone or in combination with benzodiazepines, in various conflict models of behaviour. These conflict models, based on the models devised by Geller and Seifter [36], have been shown to be remarkably selective for anxiolytics [23].

Two general comments are perhaps apt at this point. All variants of the Geller model use alimentary reinforcement and require the experimental animal to perform a coordinated movement to obtain this reward. Secondly, the learning process may play a very significant part in this behaviour. Since GABA-T inhibitors decrease consummatory behaviour and can impair coordinated movements at higher doses, the possibility of false negative results in these rather complex model systems must not be overlooked. However, File and Hyde [31] have also been unable to show an antianxiety effect of GABA-T inhibitors in their behavioural tests and these do not involve any food-reward or learned behaviour.

Effect on dopaminergic pathways. Considerable biochemical, pharmacological and electrophysiological evidence suggests interactions between GABA and dopamine in both the extrapyramidal and limbic dopamine pathways [6, 68, 76]. Although it was originally postulated that increases in GABA would exert an inhibitory influence on the activity of various dopamine pathways, reality has proved to be more complex. After parenteral administration of GABA-T inhibitors the overall effect on the nigro-striatal and ventral tegmental-limbic pathways is inhibitory [67, 68, 107]. The complexity of this interrelationship becomes evident after local injections of GABA-T inhibitors into various component parts of these dopamine pathways [67, 68]. For example, intranigral injections of γ-acetylenic GABA or γ-vinyl GABA rapidly produce contralateral rotation which is not blocked by haloperidol. However, later on, the biochemical indices of dopaminergic activity in the striatum and the ipsilateral turning evoked by dopamine agonists are suggestive of an inhibitory action of GABA on ascending nigro-striatal dopamine fibres [67, 68].

Part of this complexity can be accounted for by the fact that GABA in the substantia nigra subserves several important functions. Its probable importance in the major output tracts from the caudate nucleus and globus pallidus [26] and its different interactions in different subdivisions of the substantia nigra [2, 4] have undoubtedly contributed to controversy in this area. In the limbic system, local elevations of GABA by injections of GABA-T inhibitors into the nucleus accumbens also suggest a major role for GABA as an inhibitory transmitter [67, 76]. However, a detailed analysis of the effect of GABA in the cell bodies of the limbic dopamine pathway, that is, the A10 or ventral tegmental area, may reveal a similar degree of complexity to that seen in the substantia nigra. In support of this prediction are the elegant studies of Arnt and Scheel-Krüger [3] with local injections of muscimol which suggest that complex interactions between GABA and dopamine occur at the cell bodies of the limbic dopamine pathway. To further add to the complexity of the subject, recent studies by Worms and Lloyd [107] have demonstrated that marked differences exist between GABA-T inhibitors and GABA-receptor agonists in their ability to modify haloperidol-induced catalepsy. The possibility that extrasynaptic receptors for GABA produce some of the pharmacological effects of GABA agonists has been discussed [107] and may be of importance in explaining the occasional lack of concordance between the pharmacological effects of GABA-T inhibitors and GABA agonists.

Animal models of Huntington's Disease. Kainic acid injected into the striatum of rats decreases the activity of glutamic acid decarboxylase and the concentration of GABA in this area and damages GABAergic projections to the substantia nigra [25]. A deficit in GAD activity is also seen in post-mortem striatum and substantia nigra from patients with Huntington's disease [8]. Systemic administration of GABA-T inhibitors to rats lesioned with intrastriatal kainic acid is able to reverse the reduction in striatal GABA content but does not modify the acute or long-term behavioural sequelae of such lesions ([93], Palyfreyman, unpublished). On the other hand, it has recently been shown that aminooxyacetic acid and γ-vinyl GABA are able to antagonize some of the excitant effects of parenterally administered kainic acid [51, 99]. Since no biochemical studies were undertaken following these parenteral injections of kainic acid, it is difficult to assess the relevance of these findings. Most likely, GABA-T inhibitors can block the neuroexcitant effects of kainic acid without modifying the neurotoxic effects of the compound.

Despite the complexities of interactions between GABA and dopamine it seems reasonable to suggest, as many authors have done, that GABA-T inhibitors could prove beneficial in the treatment of tardive dyskinesia, schizophrenia, Huntington's disease and perhaps mania. However, a cautionary note should be added, since recent data have suggested that chronic treatment with GABA-T inhibitors or GABA agonists may induce supersensitivity of the

striatal dopaminergic system [29, 30], a situation which may be conducive to the production of tardive dyskinesia.

CLINICAL STUDIES WITH GABA-T INHIBITORS

Therapeutic benefit of augmented CNS GABAergic function in a variety of clincial conditions has been the subject of speculation for many years [5, 80]. Attempts to test this hypothesis using agents which *in vitro* are direct GABA agonists, i.e. imidazole-4-acetic acid [95], muscimol [97, 100], or di-n-propyl acetate in combination with GABA [96] have been unsuccessful since these agents penetrate the blood-brain barrier poorly, may undergo pre-receptor metabolism [59], or have unacceptable toxicity.

Valproic (di-n-propyl acetic) acid and several other branched-chain fatty acids are competitive inhibitors of GABA-T and cause small increases in brain GABA concentrations in laboratory animals [98]. Valproate is an effective anticonvulsant in a variety of animal seizure models [34, 54] and has been used successfully in the treatment of petit mal epilepsy [11] as well as in other neurological syndromes [11, 12, 52]. It is questionable, however, whether GABA is involved in the clinical response of valproic acid as doses used in human therapy are unlikely to influence brain GABA concentrations [72, 88, 90].

Huntington's disease is an obvious therapeutic target for GABA-T inhibitors [93]. In an open study in Huntington's disease, Perry *et al.* [73] reported that isoniazid, a non-specific inhibitor of GABA-T capable of increasing brain GABA concentrations in experimental animals, improved mental function and the movement disorder in some patients. These observations could not be confirmed, however [27]. Furthermore, in a placebo-controlled single-blind, crossover study, Perry and collaborators found aminooxyacetic acid to be ineffective in Huntington's disease [75].

The first use of an irreversible inhibitor of GABA-T in clinical therapy involved γ-acetylenic GABA treatment of patients with Huntington's disease [101]. In an open study, 14 patients were treated with increasing oral doses ranging from 10 to 900 mg/day. No clinical improvement in the motor disability or the dementia characteristic of Huntington's disease was apparent. Tolerance to γ-acetylenic GABA therapy was generally good except for single episodes of seizures in 5 subjects preceded by a period of agitation and confusion while being treated with doses ranging from 150 to 700 mg/day. This single adverse effect limited the dosage which could be used.

During therapy with irreversible enzyme inhibitors it is clearly desirable to monitor the intensity of drug action. This is influenced not only by the dosage schedule and by the pharmacokinetic half-life of the drug but also by the half-life of the target enzyme. In the case of the GABA-T inhibitors, the degree of inhibiton of the CNS target enzyme can be assessed in laboratory animals by following the elevation of brain GABA concentrations or by measuring residual enzyme activity. While brain tissue is obviously not available in humans, cerebrospinal fluid (CSF) is. In order to use this fluid to monitor therapy with GABA-T inhibitors it was necessary to demonstrate that biochemical changes occur in the CSF during therapy and that these alterations reflect changes in GABA biochemistry in the brain itself. We demonstrated that after systemic administration of various irreversible GABA-T inhibitors to rats there were dose-related increases in the CSF concentrations of GABA [9] and homocarnosine (γ-aminobutyryl-histidine), a GABA conjugate [10, 69]. Further, we showed that there is a good linear correlation between the concentrations of GABA in CSF and brain [9] and between CSF homocarnosine and brain GABA concentrations [10]. Hence, we concluded that monitoring biochemical changes in CSF of patients undergoing therapy with GABA-T inhibitors would reflect neurochemical events occurring in the brain. A similar conclusion has been reached by other groups [28, 57].

In ten patients with Huntington's disease, concentrations of GABA and homocarnosine in the CSF were compared before and after treatment with γ-acetylenic GABA [101] (Fig. 2). Pretreatment CSF concentrations of homocarnosine were approximately 5 times those of GABA. Following γ-acetylenic GABA treatment, values of CSF GABA increased in all but one subject with the average change being 94 per cent. CSF homocarnosine concentrations increased with treatment in all patients between 2- and 12-fold over pretreatment values. Despite this evidence of the desired neurochemical modification, no amelioration of the syndrome occurred.

Recently, an increase in CSF GABA concentration in 4 Huntingtonian patients following isoniazid therapy has been reported [60], but no details of clinical response were given.

In another study with γ-acetylenic GABA, 10 patients with neuroleptic-induced tardive dyskinesia

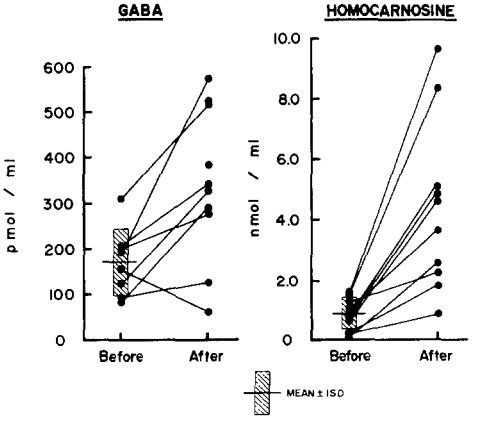

Fig. 2. Concentrations of free GABA and homocarnosine in CSF of 10 patients with Huntington's disease before and after treatment with γ-acetylenic GABA. Doses and duration of treatment were variable, ranging from 100 mg/day for 6 days to 600 mg/day for 5 days. No correlation between dose or duration of treatment and degree of change was evident.

were treated with doses of 75 to 225 mg/day [21, 22]. γ-Acetylenic GABA treatment was associated with a significant decrease in tardive dyskinesia scores compared to pre- and post-treatment placebo periods. In those patients with pre-existing parkinsonism, parkinsonian scores became worse under therapy. γ-Acetylenic GABA therapy was generally well tolerated except for confusion in 2 patients and myoclonus in one of these. The reciprocal changes between dyskinesia and parkinsonism signs in the patients under treatment are consistent with effects of augmented GABA function on dopaminergic neurons in laboratory studies [29, 30, 67, 68].

Since γ-vinyl GABA has less propensity to produce excitation and seizures in animals and a greater biochemical selectivity than γ-acetylenic GABA, it may be more useful therapeutically. In preliminary clinical studies, oral administration of γ-vinyl GABA was well tolerated and increased CSF concentrations of GABA and homocarnosine [40]. A well-tolerated GABA-T inhibitor which produces the desired neurochemical change will be useful to explore the role of GABA in normal CNS function and in the pathogenesis of some disease states.

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