Chapter 4. Agents Affecting GABA in the CNS

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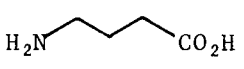
A considerable body of evidence points to the fact that γ-aminobutyric acid (GABA, 1) is a major inhibitory transmitter in the central nervous system of animals and man. A comprehensive review of GABA function and the importance of the GABA system in certain neurologic and psychiatric disorders has recently been published.[1]

It is well established that GABA itself, GABA-mimetics and certain other agents such as baclofen (2) and γ-butyrolactone (3) will slow the



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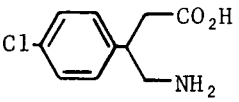

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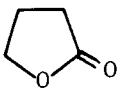

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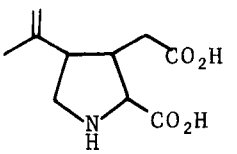
firing rate of dopaminergic neurons in two areas of the brain: those in the substantia nigra which project to the striatum[2] and those in the ventral tegmentum which project to the mesolimbic areas and cortex.[3] Since antipsychotic agents are known to block dopamine receptors in these brain areas, the use of GABA-mimetics which also attenuate dopaminergic functions alone or as an adjunct to neuroleptic therapy has received increased attention.[4]

There are several other disease states in which a disruption of a GABA neuronal system has been implicated. In Huntington's chorea a clear loss of GABA function occurs, presumably due to the destruction of the striatal-nigral GABAergic pathway.[5-10] The fact that there is no loss of GABA receptors in the striatum[11] further illustrates that the GABA pathway is not intrinsic to this extra-pyramidal area. Recently, the similarity between the cellular destruction caused by intrastriatal administration of kainic acid (4) and the observed cellular losses in Huntington's chorea has been noted.[12-14] Kainic acid is a structurally rigid



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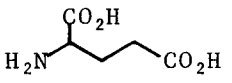

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analogue of glutamic acid (5). It is a potent neuroexcitatory agent which destroys cells containing glutamic acid receptors.

ISBN 0-12-040513-X

Due to its function as a major inhibitory transmitter in the central nervous system, GABA, or more specifically, the lack thereof, has long been suspected to play a role in epilepsy.[15] Studies have been carried out showing that agents which elevate or mimic GABA prevent seizure activity in animal models[16-18] and that agents which deplete GABA or block its actions cause seizures.[16,19-22] Surprisingly, only one anti-epileptic agent, sodium valproate (6), has been found to have a direct influence on GABA metabolism, and that effect is not the same as those mentioned above.[23,24]

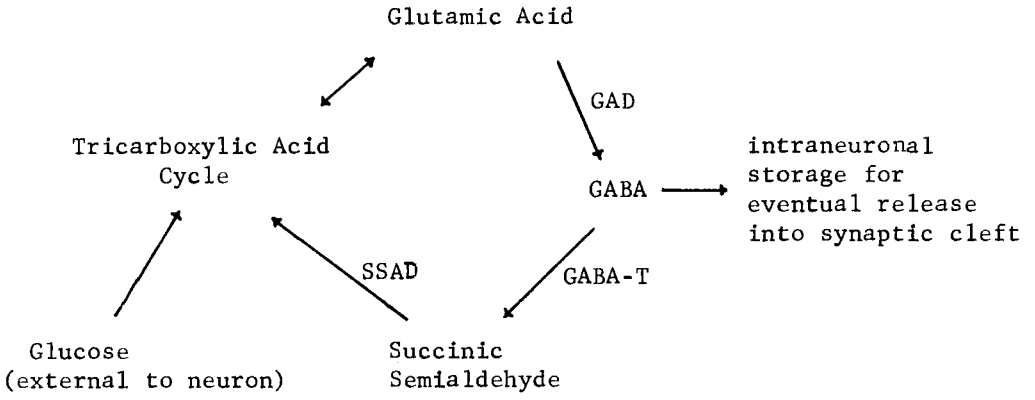
$(C_3H_7)_2CHCO_2H$

6

Sodium valproate evidently inhibits succinic semialdehyde dehydrogenase (SSAD), the enzyme necessary to metabolize the product of GABA-transaminase (GABA-T) activity (see Fig. 1 for GABA synthesis/metabolism and section on GABA-T inhibition). Without SSAD activity, GABA-T activity is presumably hampered by end-product inhibition.

The synthesis and metabolism of GABA within a presynaptic neuron is outlined in Fig. 1.

Fig. 1

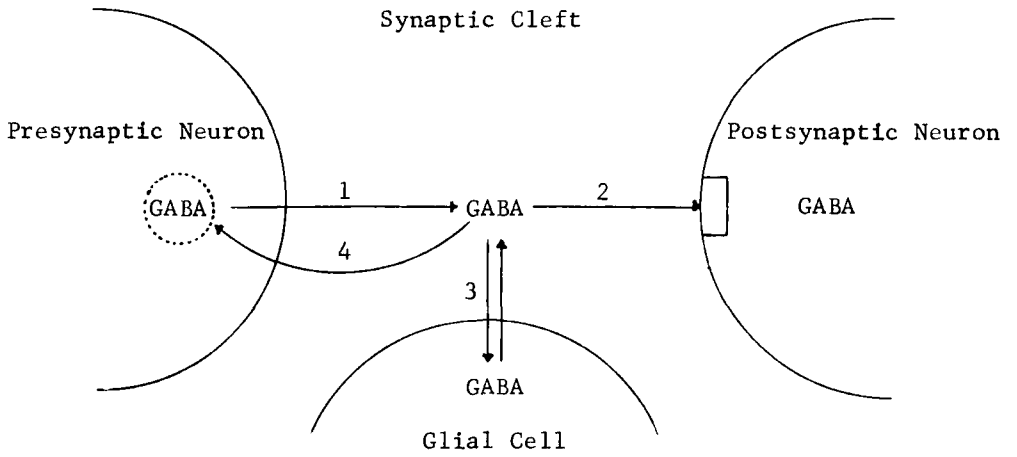


GAD ≡ glutamic acid decarboxylase
GABA-T ≡ α-aminobutyric acid-α-ketoglutarate transaminase
SSAH ≡ succinic semialdehyde dehydrogenase

As can be seen from Fig. 1, glucose from outside the neuron is the fundamental precursor of GABA. GABA itself cannot cross the blood-brain barrier and hence must be synthesized within the neuron.

A depiction of GABA-mediated neurotransmission is presented in Fig. 2.

Fig. 2



1. Release of stored GABA into synaptic cleft.
2. Receptor interaction.
3. Uptake into perisynaptic glia, or release from glia.
4. Uptake into presynaptic neurons.

In principle, GABA-mediated function could be modified by a drug which would interact with the GABA system at one or more of the locations shown in Fig. 2 and/or with GABA synthesis/metabolism (Fig. 1).

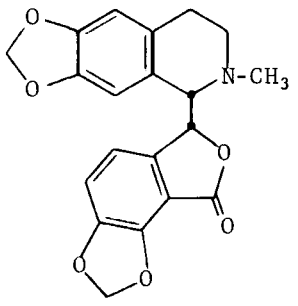
As previously mentioned, GABA is considered to be a major inhibitory transmitter. In most cases, GABA inhibits neurotransmission by increasing cell membrane permeability to chloride ions. Two types of inhibition have been identified.[25] In the first type the release of GABA from a neuron terminal partially depolarizes the terminal of an excitatory neuron. The partial depolarization causes a reduction in the release of the excitatory transmitter. This mode of inhibition between GABA interneurons and sensory neurons in the spinal cord is called presynaptic inhibition. The second, more classical mechanism of inhibition occurs at synapses between GABA neuron terminals and cell bodies. GABA hyperpolarizes the soma and/or dendrites of the next cell in line, giving rise to a higher than normal potential gradient. Since this excessive charge differential cannot be compensated for, the neuron is unable to fire.

GABA is also found in glial cells in the central nervous system.[1] Glial cells, like GABA neurons, have the capacity for high affinity uptake of GABA, and can release GABA when exposed to a sufficiently high external concentration of potassium ions (see Fig. 2). Glial GABA accounts for approximately 50% of the GABA in the brain. The exact role of glial GABA is not known, but is thought to represent a "second line of defense"

against localized excessive depolarization. If such a depolarization were to occur, the resultant rise in extracellular potassium ion concentration would induce the release of glial GABA which would, in turn, reduce the spread of depolarization.

The total number of GABAergic pathways in the brain is not known. They appear, however, to be far more ubiquitous than the pathways containing neurons which employ other known putative transmitters (e.g., norepinephrine, dopamine, serotonin and acetylcholine).[1] Theoretically, this fact could markedly reduce the selectivity of effect of agents which alter GABAergic function. Although this field of study is relatively new, numerous direct- and indirecting-acting GABA agonists and antagonists have been developed. However, only one of these agents, sodium valproate (6) has thus far become part of the physician's armamentarium to counteract any of the disease states previously mentioned.

GABA Agonists Evidence for direct postsynaptic GABA agonist activity is obtained via two main experimental methods: displacement of a GABA receptor ligand, such as H^3-GABA or H^3-bicuculline (7), from membrane fragments believed to contain GABA receptors;[27-30] microiontophoretic studies in which the suspected agonist mimics the action of GABA and where the suspected agonist's actions are blocked by a GABA antagonist such as bicuculline[31-32] (microiontophoresis is an electrophoretic technique in which the agent in question is applied very near to the neuronal receptor site). Specific binding to the GABA receptor can be distinguished from binding by the uptake carrier mechanism by omitting sodium ions from the medium. Receptor binding is sodium independent, whereas the neuronal uptake carrier mechanism requires the presence of a physiological concentration of sodium ions.[1] Some of the more potent agents capable of displacing GABA receptor ligands are muscimol (8),[29] 3-aminopropane sulphonic acid (9),[29,30] imidazole-4-acetic acid (10),[29,30] and GABA itself (1).[28-30]



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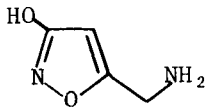

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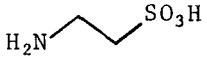

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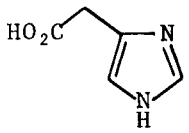

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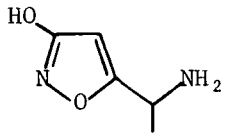
Of these four substances, only muscimol passes the blood-brain barrier in sufficient quantity to demonstrate activity after parenteral administration.[33]

A number of agents structurally related to muscimol have been synthesized but not exhaustively tested.[32] The most effective of these compounds, in terms of microiontophoretic potency relative to GABA, were 3-hydroxy-5-(1-aminoethyl)isoxazole (11) and 3-hydroxy-5-(2-aminoethyl)isoxazole (12); however, neither agent was as potent as muscimol itself.



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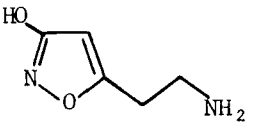

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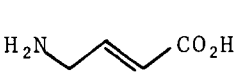
Trans-4-aminocrotonic acid (13) has also been claimed to have GABA agonist activity,[34] whereas the cis isomer (14) does not, based on microiontophoretic studies. This observation leads to the hypothesis that in order to be effective as a GABA agonist, a substance must exist in an extended (cf. 13) rather than a folded (cf. 14) conformation. The interpretation of the experimental data is confounded by the fact that trans-4-aminocrotonic acid is also a potent inhibitor of GABA uptake.[35]



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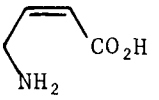

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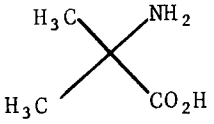
GABA Uptake Inhibitors In contrast to GABA receptor binding which is sodium independent, GABA uptake into both neuronal and glial elements is sodium dependent. The uptake mechanisms are not identical, however, since various agents differentially inhibit uptake of GABA in neurons and glial cells. Selective GABA uptake inhibitors in glial cells are α-aminoisobutyric acid (15)[36] and β-alanine (16).[27,37-39]



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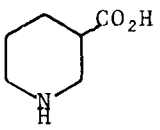
Selective GABA uptake inhibitors in neuronal elements are nipecotic acid (17),[27,40-43] guvacine (18),[43] 2,4-diaminobutyric acid (19),[30,35,44-46] and (±)-cis-1,3-aminocyclohexane carboxylic acid (20).[47]



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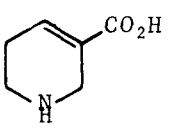

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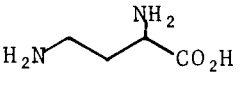

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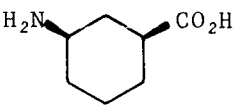

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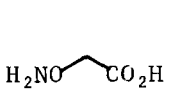
(-)-Nipecotic acid is approximately six times as potent as (+)-nipecotic acid,[41] and R(+)-2,4-diaminobutyric acid is the active component of 19, the S(-) isomer being virtually inactive.[44] The energy dependent neuronal

uptake process can also be poisoned with p-chloromercuribenzenesulphonic acid[48] and p-aminomercuribenzoic acid.[30]

γ-Aminobutyric Acid-α-Ketoglutarate Transaminase (GABA-T) Inhibitors

GABA-T is the enzyme which metabolizes and thereby inactivates GABA (see Fig. 1). GABA-T inhibitors raise brain tissue levels of GABA and presumably prolong the duration of action of presynaptically released GABA by reducing the extracellular to intracellular concentration gradient. GABA-T is a pyridoxal phosphate-dependent enzyme.[1] The most common and probably the least interesting agents which are claimed to inhibit GABA-T actually do not interfere with the function of GABA-T itself, but rather complex with its coenzyme, pyridoxal phosphate. Examples are aminooxyacetic acid (21),[49] hydrazine,[50] hydrazinopropionic acid (22),[51] isoniazid (23),[52] hydroxylamine,[53] and cycloserine (24).[54]



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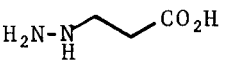

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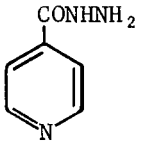

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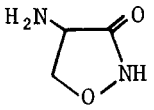

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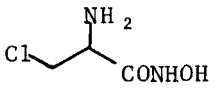
A number of irreversible inhibitors have exhibited activity in vitro and in vivo. L-α-Amino-β-chloropropionic hydroxamic acid (25), although related to the carbonyl trapping agents, is specific for GABA-T and causes an increase in mouse brain GABA levels and an increase in the onset time of isoniazid-induced seizures.[16] 4-Amino-hex-5-ynoic acid (26) increased mouse brain GABA levels,[17] decreased GABA-T activity in vitro and in vivo,[55,56] and blocked audiogenic seizures in susceptible mice, as well as electroshock in normal mice.[17] More recently, 4-amino-hex-5-enoic acid (27) was studied in mice and found to enter the brain rapidly and irreversibly inhibit GABA-T.[57,58] A sustained increase in brain GABA concentration was observed. Furthermore, this agent is more selective (GAD is only slightly affected) and longer acting than 26.



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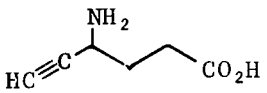

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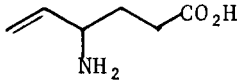

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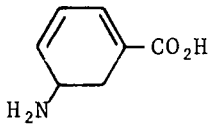
Gabaculine (28) is probably the most potent of the GABA-T inhibitors,[59] active at concentrations at least two orders of magnitude smaller than 26.

Specifically, in the equation $E + I \overset{K_I}{\rightleftarrows} EI$ (where E is the enzyme, GABA-T, and I is the inhibitor, gabaculine), K_I is 5.8×10^{-7}M at 15°C. However, the in vivo activity of 28 is not as well established. Ethanolamine-O-sulphate (29) has been extensively studied,[23,60-62] but must be given intracerebroventricularly to observe protection against audiogenic seizures and behavioral changes (piloerection, ptosis, hunched posture,

sedation and lack of spontaneous locomotor activity).



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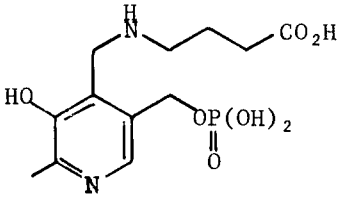

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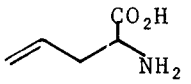
N-(5'-Phosphopyridoxyl)-4-aminobutyric acid (30), synthesized from pyridoxal-5'-phosphate and GABA, is a powerful inhibitor of rat brain GABA-T, competitive with GABA itself.[63] It is not known whether this agent can cross the blood-brain barrier.

L-Glutamic Acid Decarboxylase (GAD) Inhibitors

GAD is the neuronal and glial cytoplasmic enzyme which converts glutamic acid to GABA (cf. Fig. 1). Inhibition of GAD *in vivo* leads to seizures and convulsions which have no known or potential medical use. Many of the inhibitors of GABA-T also inhibit GAD, particularly the carbonyl trapping agents (e.g. 21-24).[19] In addition, somewhat more selective inhibitors such as allylglycine (31)[64] have been described.



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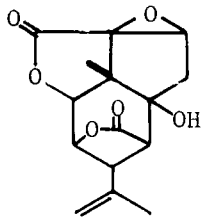
GABA Antagonists Like GAD inhibitors, GABA antagonists induce seizures and convulsions in animals and have no known or potential therapeutic value. Picrotoxin (32) was long thought to be a GABA receptor antagonist. However, more recent definitive studies have shown that picrotoxin does not bind to the GABA receptor but most likely "jams" the chloride ion channel.[28]



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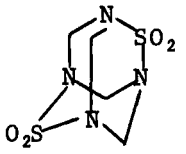
Bicuculline (7) and bicuculline methochloride are the best known, true GABA receptor antagonists[28,29,65] and have been extensively used to identify GABA receptors[28,29] and characterize GABA agonists in microiontophoretic studies.[31,32,65,66] Interestingly, (+)-bicuculline methochloride is 400 times more potent a convulsant than the (-)-isomer. In addition, the (+)-isomer is approximately 100 times more potent than the (-)-isomer in inhibiting GABA receptor binding: the IC_{50} (μM) for (+)-bicuculline methochloride is 7.0, whereas the IC_{50} for (-)-bicuculline methochloride is 500.[28] Other GABA antagonists are tetramethylenedisulphotetramine (33)[20,21] and t-butylbicyclophosphate (34).[67-68]



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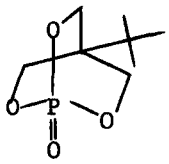

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