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## Perspective

# γ-Aminobutyric Acid Agonists, Antagonists, and Uptake Inhibitors. Design and Therapeutic Aspects

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In recent years there has been a rapidly increasing interest in the pharmacology of the central amino acid neurotransmitters, and the complicity of γ-aminobutyric acid (GABA) in certain neurological and psychiatric diseases has brought this amino acid into focus.

The development of the pharmacology of a central amino acid neurotransmitter system involves many problems and represents a great challenge to medicinal chemists. The present article will discuss strategies for pharmacological interventions in the GABA system and for the design of agents with specific actions on GABA synaptic mechanisms.

## GABA: A Central Inhibitory Neurotransmitter

Since the demonstration of the presence of GABA in the mammalian central nervous system (CNS) some 30 years ago, the physiology and biochemistry of the GABA system has been extensively studied.[1-6] GABA has a ubiquitous distribution in the CNS, and GABA fulfills[7] the main criteria[8] established for the identification of an inhibitory neurotransmitter: (1) GABA is synthesized and stored within a limited population of nerve terminals, (2) the release of GABA from CNS tissue preparations can be induced by electric stimulation in vitro under approximate physiological conditions and in vivo by impulses in particular neuronal pathways, (3) the depressant action of GABA applied on single neurons mimics the effects of the inhibitory transmitter synaptically released after neuronal stimulation, (4) pre- and postsynaptic GABA receptor–ionophore complex(es) have been identified, and (5) carrier-mediated membrane transport systems and intracellular enzymatic processes for termination of the neurotransmission process and for the inactivation of GABA, respectively, have been characterized (see Figure 2).

At least four types of GABA-mediated inhibitory processes seem to operate in the CNS, namely, postsynaptic (Figure 1), presynaptic, recurrent, and collateral inhibition.[7,9,10] In addition, GABA disinhibition may be of importance in brain function.[11] GABA disinhibition is assumed to involve postsynaptic contact between two GABA neurons (Figure 1). Activation of G2 inhibits the firing of G1, and the reduced inhibitory input from G1 increases the firing rate of cell A. The possible involvement of disinhibitory mechanisms in the CNS makes the prediction of the consequences of pharmacological manipulations of the GABA system very complicated.

## GABA in Neurological and Psychiatric Disorders

**Pharmacological Strategies.** Huntington's chorea is characterized by progressive neuronal degeneration in some brain areas.[12] Low levels of GABA and of the GABA-synthesizing enzyme glutamate decarboxylase (GAD) have been measured in postmortem brain tissue from choreic patients.[13,14] In Parkinson's disease there is an imbalance between the GABA and the dopamine systems.[15] In some brain areas of Parkinsonian patients, especially the substantia nigra, GAD activity and GABA receptor density are below normal levels. Analyses of brain samples from sites near seizure foci in epileptics and in animals made epileptic reveal low levels of GAD and reduced GABA

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(12) T. N. Chase, N. S. Wexler, and A. Barbeau, Eds., "Huntington's Disease", Raven Press, New York, 1979.
(13) P. L. McGeer and E. G. McGeer, in ref 1, p 487.
(14) S. J. Enna, E. D. Bird, J. P. Bennett, D. B. Bylund, H. I. Yamamura, L. L. Iversen, and S. H. Snyder, *N. Engl. J. Med.*, **294**, 1305 (1976).
(15) K. G. Lloyd, L. Shemen, and O. Hornykiewicz, *Brain Res.*, **127**, 269 (1977).

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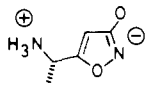
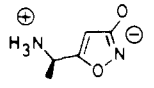
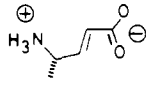
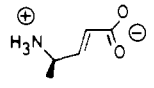
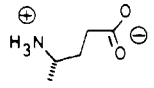
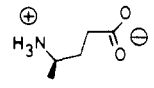
Table I. Structure and Activity of Some GABA Analogues with Known Configuration

| COMPOUND | INHIBITION OF THE RECEPTOR BINDING OF $^3$H-GABA | $^3$H-MUSCIMOL | $^3$H-ISOGUVACINE | $^3$H-THIP | $^3$H-PIS | ACTIVATION OF THE BINDING OF $^3$H-DIAZEPAM | INHIBITION OF GABA UPTAKE NEURONAL | GLIAL |
|---|---|---|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | | | | | $EC_{50}$ (μM) | $IC_{50}$ (μM) | |
|  (S)-(−)-5-Methylmuscimol | 0.64 | 0.51 | 1.6 | 0.74 | 5.8 | 230 | >1000 | >1000 |
|  (R)-(+)-5-Methylmuscimol | 19 | 8.9 | 8.2 | 9.5 | 83 | 2300 | >1000 | >1000 |
|  (S)-(−)-4-Methyl-trans-ACA | 4.1 | 3.5 | 2.5 | 2.6 | 21 | 390 | >5000 | >5000 |
|  (R)-(+)-4-Methyl-trans-ACA | 148 | 145 | 84 | 88 | 670 | >2000 | 160 | 500 |
|  (S)-(−)-4-Methyl-GABA | 4.7 | 2.5 | 5.2 | 2.9 | 24 | 550 | 750 | 1000 |
|  (R)-(+)-4-Methyl-GABA | 5.0 | 4.3 | 6.1 | 2.5 | 21 | 410 | 200 | 120 |

uptake capacity, probably reflecting degeneration of GABA neurons.[16-19] Decreased GABA activities in certain regions of brains from patients dying with schizophrenia suggest that GABA is involved in the pathophysiology of schizophrenia,[20,21] but the role of GABA in this disease is unclear.[22]

In diseases where GABA neurons are still functioning but at an abnormally low level, presynaptic mechanisms as well as postsynaptic receptors in GABA synapses are potential pharmacological sites of attack (Figure 1, IIb). In the case of extensive neuronal degeneration, GABA receptor agonist therapies appear to be of primary interest (Figure 1, IIc). Under the latter circumstances, the postsynaptic receptors probably exhibit hypersensitivity to GABA agonists.[23]

## GABA Synaptic Mechanisms as Pharmacological Sites of Attack

GABA is biosynthesized in nerve terminals (Figure 2).[24] The metabolism of GABA to succinic semialdehyde (SSA) and subsequently to succinic acid (SA), the respective enzymes being GABA:2-oxoglutarate aminotransferase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH), occurs both in nerve terminals and in glia cells.[24] The release of GABA seems to be regulated by presynaptic autoreceptors,[25,26] and the neurotransmission process, mediated by the GABA receptors, is terminated by high-affinity uptake systems in nerve terminals and in glia cells.[27,28] All of these synaptic mechanisms are potentially susceptible to pharmacological manipulation. In the present article, only GABA uptake inhibitors and GABA agonists and antagonists will be discussed.

Structure–activity (SAR) studies of conformationally restrained GABA analogues represent a major step in the pharmacological characterization of the GABA system.[29-32]

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(18) C. E. Ribak, A. B. Harris, J. E. Vaughn, and E. Roberts, *Science*, **205**, 211 (1979).
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(23) B. L. Waszczak, C. Hume, and J. R. Walters, *Life Sci.*, **28**, 2411 (1981).
(24) C. F. Baxter, in ref 1, p 61.
(25) P. R. Mitchell and I. L. Martin, *Nature (London)*, **274**, 904 (1978).
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(28) L. L. Iversen and J. S. Kelly, *Biochem. Pharmacol.*, **24**, 933 (1975).
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(30) P. Krogsgaard-Larsen, T. Honoré, and K. Thyssen, in ref 3, p 201.

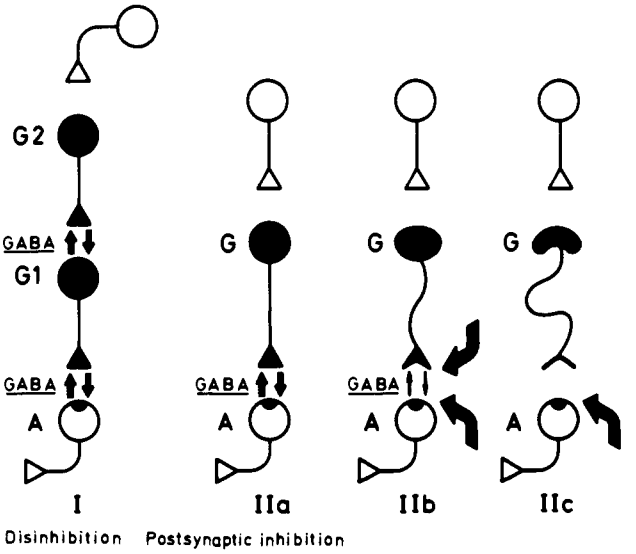


**Figure 1.** Two GABA-mediated inhibitory mechanisms (black neurons) and the potential pharmacological sites of attack after partial or total degeneration of GABA neurons.

As exemplified in Figure 2, the GABA receptors (postsynaptic receptors and autoreceptors), the uptake systems, and GABA-T have similar but distinctly different "structural specificities", and the GABA receptors and uptake systems exhibit different stereoselectivity (Table I).[33,34] These examples demonstrate that the pharmacology of different GABA synaptic mechanisms can be studied selectively, and they emphasize the importance of pharmacological studies of the enantiomers of chiral GABA-ergic compounds.

**The GABA–Receptor Complex.** As a result of extensive electrophysiological and receptor affinity binding studies, a picture of the physiological and molecular mechanisms of the GABA receptors begins to emerge.[7,35-37] The chloride ionophore is regulated by the GABA receptor consisting of multiple binding sites (Figure 2). Earlier binding studies were consistent with the presence of two binding sites with different ligand affinity but with very similar pharmacological specificity.[34,38] Recent binding studies using different GABA agonists as radioactive ligands have revealed the presence of an additional site with an affinity for GABA too low for precise characterization.[39] The physiological relevance of these multiple receptor sites is unknown, and extrasynaptic GABA recognition sites[40] may be measured together with synaptic receptors in binding studies.

The GABA receptor sites actually are structural units of a receptor complex containing constituents which bind the benzodiazepines (BZ) and the noncompetitive GABA antagonist picrotoxinin (Figure 2). The latter site appears to be the pharmacological site of action of the barbiturates.[36] Furthermore, the anthelmintic avermectin $B_{1a}$ binds with high affinity to a distinct site of the GABA–receptor complex.[41] Allosteric interactions between these units of the GABA–receptor complex detected in vitro may reflect certain aspects of the physiological receptor mechanisms.[34,36,41] Different subclasses of BZ binding sites have been identified,[42] and ther is some evidence that not all GABA receptors are coupled to BZ sites and vice versa.[43]

Since selective activation of GABA receptors in discrete brain areas is of therapeutic interest in certain diseases, mapping of the structures of these receptor complexes in different brain regions is important. In preparation for production of antibodies for immunocytochemical studies on the distribution of the GABA–receptor complexes in the brain, great efforts are being made to isolate and purify the subunits of the receptor complex.[36] Furthermore, there is an intensive search for endogenous ligands for these subunits. Various purine and pyrimidine derivatives are putative ligands for the BZ[44,45] and for the picrotoxinin site.[46] Such endogenous ligands would, if they exist,[47] be potential lead structures for the design of new drugs with GABA–receptor modulating effects.

**Design of GABA Agonists and Antagonists.** The classical definition of a GABA agonist is a depressant of neuronal firing, sensitive to the GABA antagonist bicuculline (BIC).[7] All of the GABA agonists shown in Figure 3 fulfill this criterion, except baclofen, a selective agonist for a distinct subpopulation of presynaptic GABA receptors,[48] and with this exception they are all capable of displacing radioactive GABA ([$^3$H]GABA) from GABA receptors in vitro.[49-51]

GABA and the relatively flexible GABA agonists muscimol, dihydromuscimol, and thiomuscimol stimulate the binding of BZ ([$^3$H]diazepam) in vitro.[52,53] In this test system, the conformationally more restrained agonists isoguvacine and 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) are very weak and inactive, respectively.[52,53] Piperidine-4-sulfonic acid (P4S) is an inhibitor of BZ binding[53] and reverses the muscimol-induced stimulation of BZ binding in a manner similar to that of BIC.[53] These results, which seem to conflict with generally accepted concepts, have raised a number of questions: Is P4S ac-

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(39) E. Falch and P. Krogsgaard-Larsen, *J. Neurochem.*, in press.
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(43) R. F. Squires, C. A. Klepner, and D. I. Benson, in "Receptors for Neurotransmitters and Peptide Hormones", G. Pepeu, M. J. Kuhar, and S. J. Enna, Eds., Raven Press, New York, 1980, p 285.
(44) P. J. Marangos, S. M. Paul, F. K. Goodwin, and P. Skolnick, *Life Sci.*, **25**, 1093 (1979).
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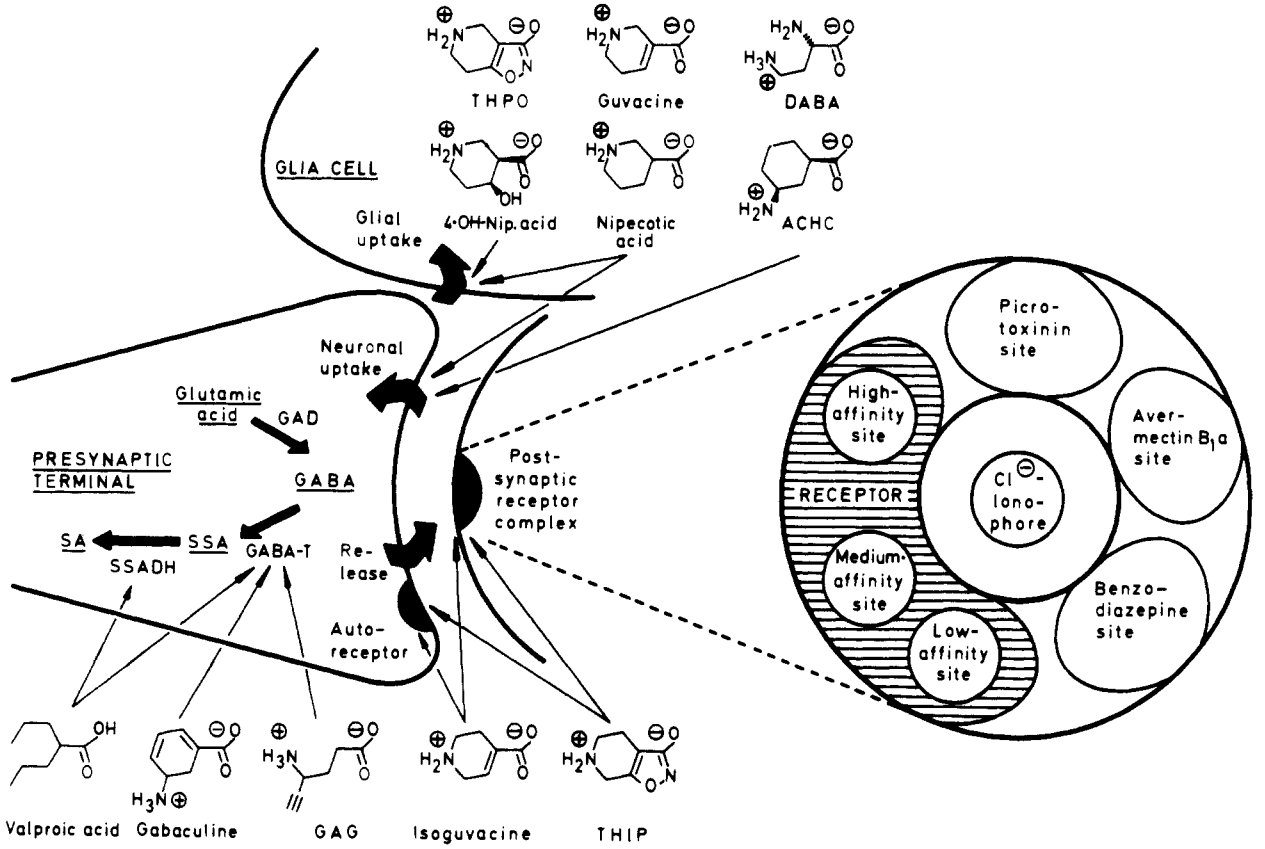


**Figure 2.** An illustration of the processes and the receptor complex in a GABA synapse and of the sites of action of various GABA analogues.

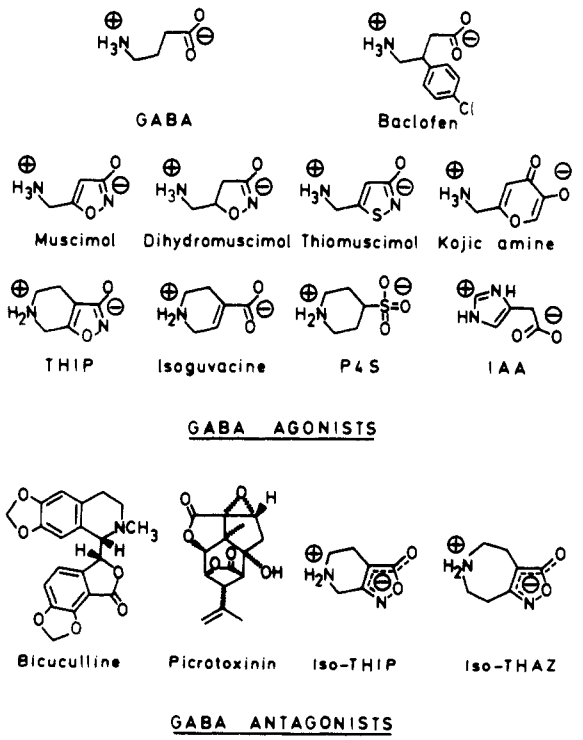


**Figure 3.** The structures of GABA and some GABA agonists and antagonists.

tually a partial GABA agonist/antagonist? Do the receptors, which are coupled to the BZ binding site, constitute a subclass of receptor different from the physiological GABA receptor?

So far, there are no indications of partial antagonistic properties of P4S.[54] The similarity of the relative potencies of the chiral GABA analogues listed in Table I as inhibitors of the binding of radioactive GABA, muscimol, isoguvacine, THIP, and P4S and as stimulators of BZ binding indicates that these GABA agonist ligands bind to the same GABA receptor[34,39,54] and that this receptor is coupled to the BZ binding site. However, the absolute potencies of these compounds suggest that P4S binds to a conformational/functional state of the receptor different from that which binds to other GABA agonists and that the BZ sites are coupled to the low-affinity site (Figure 2).

Although the physiological and pharmacological importance of these binding studies is not clear, the results strongly suggest that the GABA agonists concerned interact with the receptor complex in vivo in a different manner. Consequently, GABA agonists may be divided into groups with more or less different pharmacological profiles. Furthermore, the structural or functional state of the GABA receptors seems to vary from region to region in the brain.[55] Thus, the design of GABA agonists with desired pharmacological effects in limited parts of the brain is a realistic possibility.

Unfortunately, comparative behavioral studies of GABA, muscimol, isoguvacine, THIP, and P4S are not possible. Only THIP and muscimol are capable of penetrating the blood–brain barrier (BBB), and while THIP is stable in vivo,[56] muscimol is very rapidly decomposed after peripheral administration.[57] Such studies must await the development of more GABA agonists with desirable

(54) P. Krogsgaard-Larsen and J. Arnt, in ref 5, p 867.
(55) S. J. Enna, J. W. Ferkany, and P. Krogsgaard-Larsen, in ref 3, p 191.
(56) P. Krogsgaard-Larsen, B. Schultz, H. Mikkelsen, T. Aaes-Jørgensen, and K. P. Bøgesø, in ref 6, p 69.
(57) M. Baraldi, L. Grandison, and A. Guidotti, *Neuropharmacology*, 18, 57 (1979).

pharmacokinetic properties.

The amino and the carboxyl groups of GABA can both be replaced by other basic and acid groups without loss of affinity for, as well as intrinsic activity at, the GABA receptors (Figure 3).[49,50,58] On the other hand, even minor changes of the structures of the depicted compounds result in considerable loss of activity,[30,31,59] and the 5-isoxazolol zwitterions related to THIP, namely, 4,5,6,7-tetrahydroisoxazolo[3,4-*c*]pyridin-3-ol (iso-THIP) and 5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol (iso-THAZ), have GABA antagonistic properties.[60] The delocalization of the negative charges does not prevent these compounds from binding to the receptors, but apparently it is incompatible with receptor activation. It is evident that the design of GABA agonists and antagonists involves consideration of a variety of structural parameters, including stereochemistry, conformational mobility, and charge delocalization.

**The GABA Transport System.** GABA-mediated inhibition may be facilitated in a flexible manner via manipulation of the uptake mechanisms responsible for termination of the GABA neurotransmission process.[27,61] In principle, the strategies for such pharmacological interventions must be (1) effective blockade of both uptake systems (Figure 2) in order to enhance the effect of synaptically released GABA or (2) selective blockade of glial GABA uptake in order to increase the amount of GABA taken up by the neuronal carrier with subsequent increase of the pool of GABA in nerve terminals available for the neurotransmission process.[62]

Cultured astrocyte cells and brain tissue preparations like minislices and synaptosomes have been used as model systems for the study of glial and neuronal GABA transport, respectively.[27,61] Since these processes have dissimilar substrate specificities,[33,63] selective pharmacological studies on neuronal and glial GABA transport is possible (Figure 2). Our scanty knowledge of the mechanisms underlying GABA transport and the apparent coupling between the neuronal uptake and release mechanisms do, however, make predictions of the therapeutic effects of GABA uptake inhibitors difficult.[27,64]

**Design of GABA Uptake Inhibitors.** The GABA analogues (*S*)-(+)-2,4-diaminobutyric acid (DABA) and *cis*-3-aminocyclohexanecarboxylic acid (ACHC) are selective inhibitors of neuronal uptake (Figure 2).[27,63,65] The heterocyclic amino acids nipecotic acid and guvacine, which are not GABA analogues in the proper sense of the work, are potent inhibitors of both transport processes but with some selectivity for the glial system.[33,63,66] This selectivity is increased for the related compounds having an oxygen function in the 4 position of the piperidine ring, *cis*-4-hydroxynipecotic acid and the related isoxazole amino acid 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO) being the most potent and selective glial GABA uptake inhibitors so far known (Figure 2).[33,63,67]

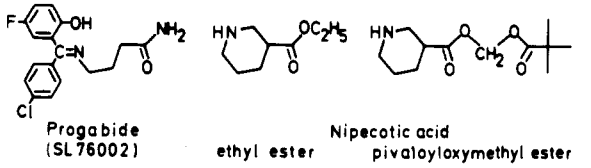


**Figure 4.** Some prodrugs of GABA and nipecotic acid.

SAR studies on conformationally restrained GABA analogues have revealed pronounced substrate specificities of the GABA transport carriers. Enlargement and contraction of the rings of nipecotic acid, guvacine, and THPO result in considerable loss of affinity for the uptake systems.[63,67] Introduction of additional amino groups in nipecotic acid gives inactive compounds.[63] X-ray and NMR spectroscopic studies on *cis*-4-hydroxynipecotic acid, which has an axial hydroxy group, and the inactive compounds *cis*-5-hydroxy- and *trans*-4-hydroxynipecotic acid indicate that the presence of a hydroxy group with a preferential equatorial orientation in the ring of nipecotic acid is incompatible with binding to the transport carriers.[32,62,63]

ACHC and nipecotic acid actually are substrates for the neuronal[65] and for the neuronal and glial uptake systems,[68,69] respectively, and it is likely that all amino acid inhibitors, so far known, are substrates for the GABA uptake carriers. The development of inhibitors of both uptake systems, which are not transported into the cells, would be an important achievement for the pharmacological development of this field. Furthermore, inhibitors/substrates capable of reacting irreversibly with the carrier macromolecules would be useful physiological and biochemical tools. So far, research along these lines has not been very fruitful. *cis*-4-Mercaptonipecotic acid and the epoxide derived from guvacine, guvacine oxide, were designed as irreversible GABA uptake inhibitors, but both compounds are only weak inhibitors.[63,70] Progress in this field is likely to provide much-needed information about the mechanisms underlying GABA transport.

## Pharmacokinetic Aspects

All compounds so far known with specific actions on GABA synaptic processes have zwitterionic structures. The ratio between the concentrations of the ionized and the un-ionized forms of amino acids in solution ($I/U$ ratio) is a function of the difference between the $pK_a^I$ and $pK_a^{II}$ values.[71] Since amino acids are likely to penetrate the BBB in the un-ionized forms, it is of interest to develop analogues of GABA-ergic amino acids with $pK_a$ values less different than those of the parent amino acids. Like GABA, P4S ($pK_a < 1$, 10.3; $I/U > 1\,000\,000$), isoguvacine ($pK_a = 3.6$, 9.8; $I/U = 200\,000$), nipecotic acid ($pK_a = 3.9$, 10.3; $I/U = 250\,000$), and *cis*-4-hydroxynipecotic acid ($pK_a = 3.4$, 10.0; $I/U = 300\,000$) do not penetrate the BBB to any significant extent, whereas THIP ($pK_a = 4.4$, 8.5; $I/U = 500$) and THPO ($pK_a = 4.3$, 9.1; $I/U = 2500$) enter the brain after systemic administration.[34,56,62] 4,5,6,7-Tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol (thio-THIP) ($pK_a = 6.1$, 8.5; $I/U = 16$) was designed as a GABA agonist with op-

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(59) J. G. Atkinson, Y. Girard, J. Rokach, C. S. McFarlane, A. Rackham, and N. N. Share, *J. Med. Chem.*, **22**, 99 (1979).
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timum pharmacokinetic properties. Thio-THIP is, however, a very weak GABA agonist, emphasizing the strict structural constraints imposed on GABA agonists.[32]

The prodrug approach, which appears to be particularly attractive in the CNS neurotransmitter amino acid field, has only been explored to a limited extent. The active amino acids, formed in the brain tissue from the prodrug after penetration of the BBB, would be "trapped" in the CNS. The duration and intensity of drug action could, in principle, be regulated by varying the pharmacokinetic properties of the prodrugs. While the ethyl ester of nipecotic acid has weak cholinergic side-effects,[72] the pivaloyloxymethyl ester (Figure 4) is devoid of pharmacological effects per se.[62] Both compounds have proved to be useful prodrugs for studies of the pharmacology of nipecotic acid.[62,72-74] Although the mechanism of action of progabide (SL 76002) is not fully elucidated, it probably acts as a prodrug of GABA.[75,76]

## Therapeutic Perspectives

Until recently, muscimol was the only GABA agonist capable of penetrating the BBB that was available for pharmacological studies, but muscimol is toxic and very susceptible to metabolism after peripheral administration.[57] THIP, on the other hand, is well tolerated by various animal species, it is active after oral administration (po), and it is excreted unchanged and to some extent in a conjugated form in the urine from humans.[31,77,78]

Muscimol has been the subject of preliminary clinical tests.[79] Progabide, THIP, valproic acid, and the irreversible inhibitors of GABA-T, γ-acetylenic-GABA (GAG) and γ-vinyl-GABA[80] (Figure 2), are at the present time under study in humans suffering from different diseases. The mechanism of interaction of valproic acid with the GABA system has not been clarified yet, but GABA-T and SSADH seem to be the primary sites of attack.[81]

**GABA Agonists and Antagonists in Neurological and Psychiatric Disorders.** Patients suffering from schizophrenia and Huntington's chorea have been treated with muscimol and progabide.[76,79] In the first category of patients, no effects or even worsening in overall psychosis ratings were observed. While muscimol did not reduce the symptoms in choreic patients, progabide seemed to ameliorate patients at an early stage of the disease, while no significant effects were observed in more advanced cases.

These preliminary results were unexpected. If the degenerated GABA neurons were exclusively involved in postsynaptic inhibition (Figure 1, IIa) and if the involved GABA receptors had developed hypersensitivity to GABA agonists, which might be expected,[23] then the results mentioned above are surprising. However, if the impaired GABA neurons served a role in disinhibitory mechanisms (Figure 1, I), degeneration of both G1 and G2 neurons would leave the GABA receptors on cell A in a hypersensitive state and without the normal stimulating input. In this situation, treatment with a GABA agonist would expose cell A to inhibition and, therefore, to an effect opposite to that obtained from the intact disinhibitory pathway. It might be worthwhile to consider treatment of the diseases concerned with low doses of GABA antagonists, for example, BIC or iso-THAZ; preliminary animal experiments seem to support this proposal.[82]

**GABA Agonists as Analgesics and Anxiolytics.** There seems to be an association between the pain process and GABA neurotransmission.[83] The neuronal pathways underlying this GABA-mediated regulation mechanism are unknown, but different types of GABA-ergic compounds, including GABA-T inhibitors, produce analgesia.[80] Surprisingly, GABA agonists and antagonists, such as baclofen, also have analgesic properties.[78,83] These effects are insensitive to naloxone, indicating a mechanism of action different from that of the opiates.[78,83] Furthermore, there is some evidence that GABA mediates the anxiolytic properties of the BZ,[84] and clinical studies have disclosed tranquilizing effects of GABA agonists.[79] This combination of effects appears to have promising clinical prospects in a variety of diseases involving pain. These aspects of THIP are at the present time the subject of clinical studies in different groups of patients.

**GABA Agonists as Potential Antiepileptics.** A number of GABA agonists, including muscimol, THIP, kojic amine, baclofen, and prodrugs of isoguvacine, have anticonvulsant properties in various animal models.[16,59,85,66] These animal studies suggest antiepileptic effects of GABA agonists, although there are arguments against future application of such compounds in epilepsy.[85] Progabide does, however, reduce or eliminate seizures in some groups of epileptic patients resistant to traditional antiepileptic medication.[76] Clinical studies in progress on progabide and THIP will provide more information about these therapeutic aspects of GABA agonists.

**Other Therapeutic Aspects of GABA Agonists.** Based on laboratory studies, the GABA system is involved in the regulation of a variety of physiological and behavioral functions, including the secretion of prolactin and other hormones,[87] blood pressure and heart rate,[88,89] feeding behavior,[90] and aggression.[91] Although more profound studies are needed before the relevance of these animal studies to the clinical situation can be estimated, all of these observations have therapeutic prospects. The first mentioned effect may have relevance to psychotic patients with neuroleptica-induced elevated prolactin

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levels. Prolactin secretion seems to be regulated by central as well as by peripheral GABA receptors, the latter receptors being located directly on the anterior pituitary.[92] Activation of the central and peripheral GABA receptors stimulates and inhibits, respectively, prolactin release,[92] suggesting that GABA agonists like P4S and isoguvacine, which do not pass the BBB, have therapeutic interest in this clinical situation.

In agreement with the involvement of GABA in feeding behavior, THIP (po) has anorexigenic actions stronger than those of cocaine (po) but ten times weaker than those of *d*-amphetamine.[93] This action of THIP may be relevant for the treatment of human obesity.

**GABA Uptake Inhibitors as Potential Antiepileptics.** The pharmacology of a number of GABA uptake inhibitors on single cells in vivo has been investigated using microelectrophoretic techniques. In experiments where inhibitor and GABA were administered simultaneously to cells in the spinal cord or in the cerebellum of cats,[94-96] all types of inhibitors, namely, DABA (neuronal) THPO (glial), and nipecotic acid, and guvacine (glial/neuronal) (Figure 2) enhanced the depressant action of GABA on neuronal firing. However, marked differences between different types of inhibitors were observed after intracerebroventricular (icv) injection into mice.[73] DABA and ACHC provoked generalized seizures, whereas THPO and nipecotic acid protected the animals against audiogenic seizures, and systemic administration of THPO or prodrugs of nipecotic acid (Figure 4) effectively protected the animals against seizures.[62]

Why are inhibitors of neuronal GABA uptake convulsants and selective glial uptake inhibitors anticonvulsants? Intramuscular injections of THPO or nipecotic acid ethyl ester into mice elevate the concentration of GABA in the nerve terminals of the brain,[74] probably because blockade of the glial uptake system results in a preferential reuptake of synaptically released GABA into the nerve terminals. This increase of the releasable pool of GABA may facilitate the GABA neurotransmission process and in this way produce anticonvulsant effects.[62]

The neuronal GABA uptake process seems to be coupled to the release system by an as yet unknown mechanism, and ACHC and related amino acids have been shown to be effective inducers of GABA release.[64] Thus, the convulsant effects of ACHC and DABA (icv) may be the consequence of interruption of GABA-mediated inhibition by depletion of GABA from the terminals. Nipecotic acid also has some effect on neuronal GABA uptake (Figure 2), although weaker than the effect on the glial system.[33] Nipecotic acid does not, however, stimulate GABA release from synaptosomes in vitro, suggesting that the mechanism of interaction of this uptake inhibitor with the neuronal transport carrier is different from that of ACHC and DABA.[64] Such a difference may contribute to the difference between the pharmacology of these amino acids and nipecotic acid. In any case, the present investigations have brought glial GABA uptake inhibitors into focus as potential antiepileptic drugs.

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# *Communications to the Editor*

## Adenosine Deaminase Inhibitors. Conversion of a Single Chiral Synthon into *erythro*- and *threo*-9-(2-Hydroxy-3-nonyl)adenines

*Sir:*

Adenosine deaminase (ADA) inhibitors are known to enhance the cytotoxic activity of a variety of adenosine analogues. Among these are adenosine arabinoside (*ara*-A), 8-azaadenosine, and formycin.[1]

Coformycin (CF) and 2′-deoxycoformycin (2′-dCF) are two naturally occurring nucleoside antibiotics which are the most potent inhibitors of the enzyme identified to date ($K_i$ = $10^{-11}$ to $10^{-12}$ M).[2,3] Among the synthetic compounds, (±)-*erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) was rationally designed and synthesized by Schaeffer and Schwender[4] and was found to be less active than the above antibiotics ($K_i = 4 \times 10^{-9}$ M).[5] However, reactivation of inhibited ADA has been observed to be much faster for (±)-EHNA than for CF and 2′-dCF.[6] It is this property that has been cited recently as being of potential importance in viral chemotherapy.[7] Prompted by these reports, the synthesis of the title compounds (1–4) was undertaken to identify the most active inhibitor and to examine the biological activity of these isomers vis-à-vis

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