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Perspective

$GABA_A$ Receptor Agonists, Partial Agonists, and Antagonists. Design and Therapeutic Prospects

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GABA Inhibition and Disinhibition

The neutral amino acid, 4-aminobutanoic acid (GABA, **1**), is an inhibitory transmitter in the central nervous system (CNS). GABA is also involved as a neurotransmitter and/or a paracrine effector in the regulation of a variety of physiological mechanisms in the periphery. Some of these latter functions may be under central GABA control, whereas others are managed by local GABA neurons (see later section). A large percentage, perhaps the majority, of central neurons are under GABA control. The complex mechanisms underlying the GABA-mediated neurotransmission have been extensively studied using a broad spectrum of electrophysiological, neurochemical, pharmacological, and, in recent years, molecular biological techniques.[1-8]

The overall activity of the brain is basically determined by two superior functions: (1) excitation by the major excitatory amino acid transmitter, glutamic acid (Glu) (Figure 1), which depolarizes neurons through a large number of receptor subtypes,[9-11] and (2) inhibition by GABA, which hyperpolarizes neurons, likewise through multiple receptors (see later sections). It may be mentioned, however, that depolarizing actions of GABA may occur, particularly during early postnatal development of the brain.[12]

It has been proposed that a third mechanism may play a fundamental role in the function of the brain,

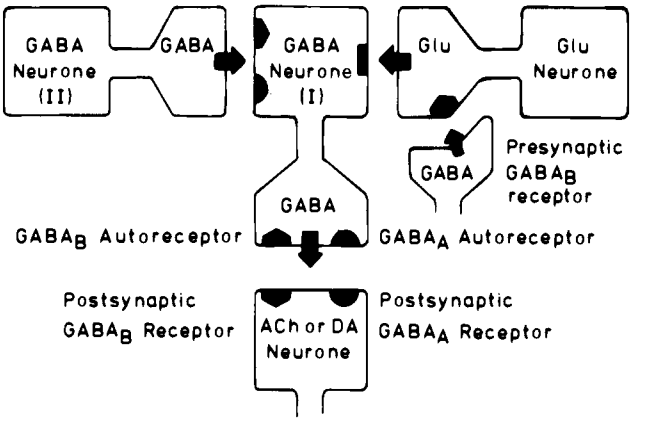

Figure 1. Schematic illustration of neuronal interactions in the CNS and the location of different types of GABA receptors. The synaptic contact between two inhibitory GABA neurons results in the type of functional excitation named disinhibition, which may play an important role in the function of the CNS. Synaptic contact between a GABA neuron and an acetylcholine (ACh) or a dopamine (DA) neuron is illustrated.

namely disinhibition.[13,14] This indirect neuronal excitation implies synaptic contact between two inhibitory neurons, as exemplified in Figure 1. Thus, activation of GABA neuron II inhibits neuron I, resulting in relief of the inhibition by this neuron of the subsequent neuron, in Figure 1 an acetylcholine (ACh) or a dopamine (DA) neuron. The operation of this indirect excitatory mechanism in the CNS has never been unequivocally proved or disproved, but many apparently paradoxical observations have been explained on the basis of disinhibition.

A detailed discussion of the function of the mammalian CNS is outside the scope of this paper. However,

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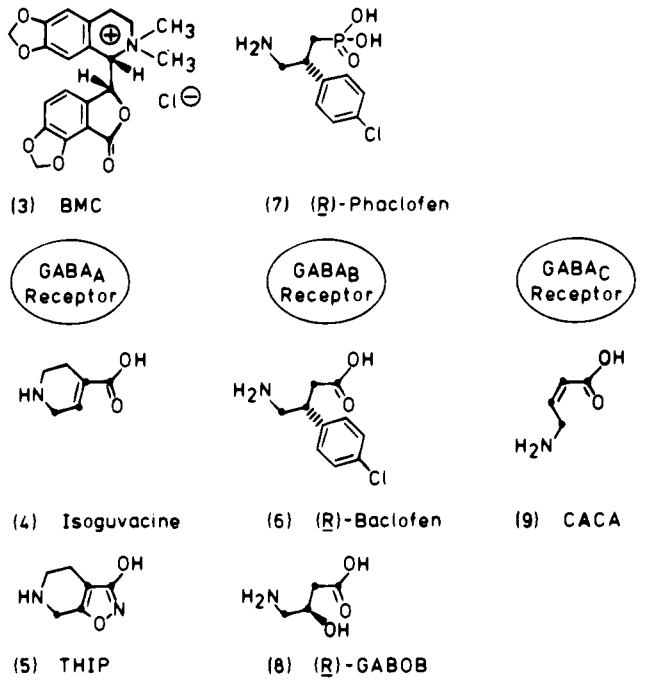


Figure 2. Schematic illustration of different classes of GABA receptors and the structures of some key agonists (bottom) and antagonists (top).

the recently observed[15] apparent disinhibitory interaction between different types of GABA receptors, located on the same neuron, will be discussed briefly in a later section.

Multiplicity of GABA Receptors

The discovery of GABA in the early 1950s[16] and the identification of the alkaloid bicuculline (**2**)[17] and its quaternized analogue bicuculline methochloride (BMC, **3**)[18] as competitive GABA antagonists in CNS tissues initiated the pharmacological characterization of GABA receptors. The subsequent design of isoguvacine (**4**), 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP, **5**)[19] (Figure 2), and piperidine-4-sulfonic acid (P4S)[20] as a novel class of specific GABA agonists further stimulated studies of the pharmacology of the GABA receptors.

The GABA analog baclofen did, however, disturb the picture of a uniform class of GABA receptors. This compound, which was designed as a lipophilic analogue of GABA capable of penetrating the blood–brain barrier (BBB), is an antispastic agent,[21] but its GABA agonistic effect could not be antagonized by BMC.[22] In the early 1980s, it was demonstrated that baclofen, or rather (*R*)-(−)-baclofen (**6**), was selectively recognized as an agonist by a distinct subpopulation of GABA receptors, which were termed GABA$_B$ receptors.[23,24] The "classical" GABA receptors were thus designated as GABA$_A$ receptors. This receptor classification represents an important step in the development of the pharmacology of GABA.

During this period the exploration of the GABA$_A$ receptors was dramatically intensified by the observation that the binding site for the benzodiazepines (BZDs)[25–27] was associated with the latter class of GABA receptors.[7,28,29] This area of the pharmacology of GABA, which is not reviewed in this article, continues to be in a state of almost explosive development, which was further stimulated by the cloning of a large number of GABA$_A$ receptor subunits (see subsequent section).

Substitution of a phosphono group for the carboxyl group of **6** gives the GABA$_B$ antagonist, phaclofen,[30] and in agreement with the competitive nature of this antagonism the GABA$_B$ receptor affinity of phaclofen resides in the (*R*)-enantiomer (**7**)[31] (Figure 2). On the other hand, replacement of the aromatic group of **6** by a hydroxy group to give 3-hydroxy-4-aminobutanoic acid (GABOB) results in a GABA$_B$ agonist. It is the (*R*)-form of GABOB (**8**) that interacts with the GABA$_B$ receptors, and since the aromatic substituent of **6** and the hydroxy group of **8** have opposite orientations, these groups probably bind to different substructures of the GABA$_B$ receptor site.[32] This observation has been exploited in the GABA$_B$ antagonist field and has led to the development of new effective antagonists.[33,34]

In connection with the design of conformationally restricted analogues of GABA another "disturber of the peace" appeared on the GABA scene, namely *cis*-4-aminopent-2-enoic acid (CACA, **9**). This compound is a GABA-like neuronal depressant that is not sensitive to BMC,[35] and it binds to a class of GABA receptor sites, which do not recognize isoguvacine (**4**) or (*R*)-baclofen (**6**).[36] These receptors have accordingly been termed GABA$_C$ receptors[36] or "non-GABA$_A$, non-GABA$_B$" (NANB) receptors for GABA.[37] It is possible that this not very well understood class of GABA receptors is heterogeneous.[36] It has been proposed that a recently cloned GABA receptor subunit (ϱ_1) showing some homology with the α and β subunits of GABA$_A$ receptors[38] (see subsequent section) may confer BMC-resistant properties of ionotropic GABA receptors structurally related to GABA$_A$ receptors.[37] GABA$_A$-like receptors containing this subunit may thus be identical with or similar to the proposed GABA$_C$ receptors. Interestingly, a NANB receptor sensitive to **9** (Figure 2) has been identified in the retina, and this ionotropic receptor probably comprises the ϱ_1 subunit.[39,40]

The physiology and pharmacology of NANB GABA receptors are still very incompletely elucidated, but these receptors, which seem to exist in the peripheral nervous system (PNS) as well as the CNS,[37] may be interesting novel targets for drug development.

Recombinant GABA$_A$ Receptors—Structure and Function

The introduction of molecular biological techniques has revolutionized receptor research, and during the past few years the number of papers describing the structure and function of G protein-coupled receptors as well as ligand-gated ion channels has virtually exploded.[7,41–46] A detailed review of this research area is beyond the scope of this article, and only a few aspects of particular relevance to medicinal chemists interested in the GABA$_A$ ligand-gated ion channel(s) will be mentioned.

GABA$_A$ receptors are known from cDNA cloning and expression studies to contain a combination of homologous subunits primarily from three classes, α, β, and γ, whereas additional types, notably δ and ϱ (see previous section), have been identified in certain types of neurons. Each subunit is present in the brain in several independently expressed isoforms, and so far six α-subunits (α_1–α_6), three β-subunits (β_1–β_3), three γ-subunits (γ_1–γ_3), and one δ-subunit have been identified. The GABA$_A$ receptor is probably assembled as a pentameric structure[47] (Figure 3) from different subunit families, making it possible that a very large number of such heteromeric GABA$_A$ receptors exist in the mammalian CNS and PNS. The number of physiologically relevant

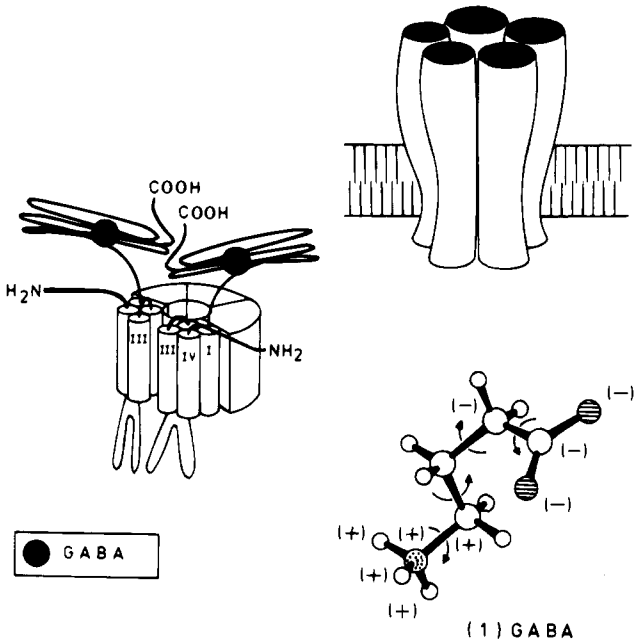


Figure 3. The structure of GABA and a schematic illustration of the pentameric $GABA_A$ receptor complex.

$GABA_A$ receptors, their subunit stoichiometry, and their regional distributions are, however, far from being fully elucidated.[42]

The gating properties of recombinant $GABA_A$ receptors vary markedly with subunit subtype combinations.[48] Subunits assemble with different efficiencies, and, when expressed in fibroblasts, $\alpha_1\beta_1$ for example, but not $\alpha_1\gamma_2$ or $\beta_1\gamma_2$, subtypes assemble to produce BZD-insensitive $GABA_A$ receptor channels. In contrast to $\alpha_1\beta_1$ channels, which have only two open states, $\alpha_1\beta_1\gamma_2$ $GABA_A$ channels have gating properties similar to those of neuronal $GABA_A$ receptors. The importance of the α subunit is emphasized by the observation that $\alpha_6\beta_1\gamma_2$ channels show different properties.

The $GABA_A$ receptor complex comprises a large number of binding sites for drugs, notably BZDs, barbiturates, and steroids,[3,27-29] and virtually all steps in the recognition and gating processes of the $GABA_A$ receptor have been shown to be subject to modulation by such agents.[48] The binding step(s) appear to be modified by BZDs, β-carbolines acting at the BZD site, and possibly by steroids including endogenous as well as synthetic steroids. The gating process is apparently regulated by steroids and barbiturates, and the open state of the channel can be occluded by penicillin. The sensitivity of the desensitization mechanism(s) and state(s) to such drugs has, so far, not been studied in detail. This degree of complexity of the $GABA_A$ receptor function is comparable with that of the *N*-methyl-D-aspartic acid (NMDA) subtype of Glu receptor channels.[9-11]

Recombinant techniques have made it possible to determine the primary structure of receptor glycoproteins and to disclose a degree of heterogeneity of all classes of receptors, which was beyond imagination about a decade ago. Although the present models of ligand-gated ion channels, including $GABA_A$ and NMDA receptors (see Figure 3), as well as G protein-coupled receptors probably represent oversimplifications of the structure and structural diversity of these membrane bound receptors, they are useful working models. A broad spectrum of problems regarding structure and function of receptors remain to be elucidated,[48] and molecular biologists and pharmacologists are faced with a number of unanswered questions: Which type of cells express the individual receptor protein mRNAs? Do all subunit subtypes assemble into $GABA_A$ pentameric receptors? How many different heteromeric $GABA_A$ receptors do actually become inserted into the cell membrane? Do all $GABA_A$ receptors assembled form functional and physiologically relevant $GABA_A$ and, perhaps, $GABA_C$ receptors?

These and many other problems regarding structure and function of receptors will be intensively studied during the next decade. Specific $GABA_A$ agonist and antagonists (see later sections) as well as BZDs, barbiturates, and steroids interacting specifically with distinct binding sites at the $GABA_A$ receptor complex are indispensable tools for such studies. *In this regard it must be emphasized that there is no acceptable alternative to tools showing specific effects on the mechanisms under study. Medicinal chemists and molecular pharmacologists share the responsibility in this regard.*

A major goal of such studies is to uncover the mechanisms underlying the extremely complex operational and regulatory mechanisms of the $GABA_A$ receptor complex. Such future studies undoubtedly will uncover the molecular mechanisms of key importance for receptor activation and desensitization. Elucidation of these aspects of $GABA_A$ receptors may make rational design of non-desensitizing partial agonists and novel types of $GABA_A$ receptor modulating drugs possible.

The relationship between $GABA_A$ receptor subunit composition and molecular pharmacology of the $GABA_A$ receptor modulating BZDs has been extensively studied,[49] and on the basis of mutation studies it has been possible to identify a single amino acid residue of key importance for the binding of BZD ligands.[50] It may be possible to identify and localize distinct subtypes of $GABA_A$ receptors associated with different physiological and pathophysiological functions. The aim of such studies in the BZD field is to design compounds with appropriately balanced agonist/antagonist or inverse agonist/antagonist properties capable of interacting selectively with $GABA_A$ receptors of particular relevance to anxiety, epilepsy, and sleep disorders.[49]

$GABA_A$ and $GABA_B$ Receptors: Regulation and Apparent Disinhibitory Interaction at the Cellular Level

The $GABA_A$ as well as the $GABA_B$ receptor families include postsynaptic receptors, presynaptic receptors on non-GABA terminals (heteroreceptors), and autoreceptors (homoreceptors) as exemplified in Figure 1.[5,6] There is growing evidence of the existence of pharmacologically distinct subclasses of receptors within each of these types of receptors.[36,37,51,52] The mechanisms of action and interaction of these receptors are far from being fully elucidated, but studies in recent years on neurons from the cerebellum, the anatomically best characterized part of the brain,[53] have shed new light on these aspects.

Neurons intrinsic to cerebellum utilize either Glu or GABA as the neurotransmitter, forming complicated excitatory–inhibitory loops by which incoming excitatory signals are modulated, generating inhibitory outputs of varying intensity.[53] The glutamatergic excitatory innervation of the Purkinje neurons by granule cell parallel fibers is thus fine tuned by GABAergic

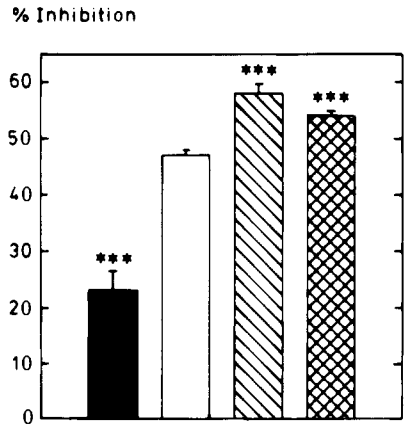


Figure 4. Effect of GABA receptor agonists on KCl (40 mM) stimulated [^{3}H]D-aspartic acid release from cerebellar granule cells cultured for 7 days in plain culture media. Filled column, 50 μM (*R*)-baclofen (**6**); open column, 100 μM isoguvacine (**4**); hatched column, 50 μM **6** plus 100 μM **4**; and cross-hatched column, 100 μM GABA. Results are averages of 26 experiments with SEM values shown as vertical bars. Inhibition is expressed as percentage of control stimulations. Asterisks indicate a statistically significant difference from inhibition mediated by **4** ($p < 0.001$; Student's *t*-test). From ref 15.

interneurons. In other words, GABA exerts a modulatory action inhibiting glutamatergic activity, a process involving activation of both $GABA_A$ and $GABA_B$ receptors. The molecular mechanisms for this fine tuning of the excitatory glutamatergic activity are as yet not fully clarified but are likely to involve an interaction between $GABA_A$ and $GABA_B$ receptors leading to disinhibitory phenomena at the level of single neurons, i.e., the granule cells.[15]

Cerebellar granular neurons have been shown to have specific binding sites for baclofen,[15,54-56] and using cultured cells it has recently been demonstrated that the number of binding sites can be increased by exposure of the neurons to THIP (**5**) during the culture period.[15] This appears to be analogous to the ability of THIP (**5**) to induce low-affinity $GABA_A$ receptors on these neurons (see below). The $GABA_B$ receptors on the granule cells are functionally involved in regulation of transmitter release since (*R*)-baclofen (**6**) has been shown to inhibit K^+-stimulated Glu release from these neurons.[15,54,55,57,58] The inhibitory actions of $GABA_A$ and $GABA_B$ receptors on evoked Glu release in cerebellar granule neurons were recently characterized. It was shown (Figure 4) that the inhibitory actions of baclofen and isoguvacine (**4**) were not additive, which strongly indicates that the two receptors are functionally coupled to each other.[15] An inhibitory action of $GABA_B$ receptors on $GABA_A$ receptors, as also previously suggested,[59] will result in a disinhibitory action of the $GABA_B$ receptors at the cellular level, which could possibly explain numerous reports on excitatory actions of GABA or baclofen in multicellular systems or in the intact brain.[60-63] Such a disinhibitory interaction between $GABA_A$ and $GABA_B$ receptors at the cellular level may be functionally indistinguishable from the originally described disinhibitory organization of neuronal networks (Figure 1).[13,14]

Cerebellar granule neurons are rich in $GABA_A$ receptors which based on kinetic analysis of GABA binding can be divided into high- and low-affinity receptors with affinity constants of 5–10 and 500 nM, respectively.[64] Using a monotypic cerebellar culture system,[65] it has been shown that expression of the low-affinity receptors

Table 1. Ability of GABA to Inhibit K^+-Induced Transmitter Release in Cerebellar Granule Cells Expressing High-Affinity or High- and Low-Affinity GABA Receptors[a]

	inhibitory action of GABA (%)	
depolarizing condition	high-affinity receptors	high- and low-affinity receptors
30 mM KCl	37	70
55 mM KCl	0	60

[a] Modified from ref 68.

is dependent upon whether or not the neurons are exposed to GABA or THIP during early development.[64] Since cerebellar granule neurons in culture therefore can be grown under conditions where either high-affinity GABA receptors are expressed alone, or the two kinetically distinct receptors are expressed together, it is possible to obtain information about the functional properties of these receptors.

As mentioned above, the granule neurons in cerebellum are excitatory in nature using Glu as the neurotransmitter.[66] This property can be conveniently studied in culture monitoring depolarization-evoked, Ca^{2+}-dependent release of preloaded [^{3}H]D-aspartic acid which labels the neurotransmitter pool of Glu.[67] As shown in Table 1, this transmitter release can be inhibited by GABA, dependent upon the expression of the two types of $GABA_A$ receptors as well as the depolarizing signal. In cells expressing only high-affinity GABA receptors, GABA is able to inhibit transmitter release evoked by a moderately depolarizing signal (30 mM KCl), whereas that evoked by a strong depolarizing pulse (55 mM KCl) cannot be inhibited by GABA. On the contrary, in neurons expressing both high- and low-affinity GABA receptors, GABA is able to inhibit transmitter release regardless of the depolarizing condition. This action of GABA can be mimicked by THIP (**5**) and muscimol (**14**) and blocked by bicuculline (**2**) in keeping with the notion that the low-affinity GABA receptors are $GABA_A$ receptors. Interestingly, the action of GABA mediated by the low-affinity GABA receptor has been shown to be insensitive to the chloride channel blocker, picrotoxinin, indicating that these receptors may be mechanistically different from the high-affinity receptors which are clearly coupled to a chloride channel and blocked by picrotoxinin.[68] A more detailed discussion of this aspect will be given below.

As mentioned above, previous studies using vesicles from mouse cerebellum have also shown that activation of $GABA_B$ receptors can inhibit the function of $GABA_A$ receptors.[59] This action was suggested to involve either a non-desensitizing subtype of $GABA_A$ receptor or the rate of recycling of desensitized to non-desensitized receptors. Such mechanisms may play a key role in the observed inhibitory effects using cerebellar granule neurons.[15]

The inhibition of evoked Glu release mediated by high-affinity $GABA_A$ receptors clearly is dependent upon the $GABA_A$ receptor gated chloride channels, since this action of GABA can be blocked by picrotoxinin.[68] It is not clear how the inhibitory action of GABA mediated by the inducible low-affinity receptors may be mediated. Since this action of GABA cannot be inhibited by picrotoxinin, it is unlikely that the classical mechanism involving the chloride channel is playing a major role.[68] It was, however, shown that the induction of the low-

affinity $GABA_A$ receptors is closely associated with a similar increase in the number of voltage-gated calcium channels.[69] More importantly, it was observed that in nerve processes, but not in cell bodies, there was a tight spatial coupling between $GABA_A$ receptors and calcium channels in neurons expressing the low-affinity GABA receptors but not in cells expressing high-affinity receptors alone.[69] This led to the suggestion that the inhibitory action of GABA mediated by the low-affinity receptors could involve a regulation of the activity of voltage-gated calcium channels.[68,70] Attempts to show this directly by demonstrating a facilitation by GABA of the ability of the calcium channel blockers ω-conotoxin and verapamil[71] to inhibit evoked Glu release have, however, failed.[72] It should be emphasized that ω-conotoxin only blocks a fraction of the many types of calcium channels,[73] leaving the possibility open that GABA may regulate other types of calcium channels. In keeping with this, it has been shown that GABA exerts a regulatory action on intracellular calcium levels in hippocampal neurons.[74] In this context is should also be emphasized that the $GABA_B$ receptors in cerebellar granule neurons via a G protein dependent mechanism regulate the intracellular calcium level.[15,75] If a coupling between $GABA_A$ and $GABA_B$ receptors in these neurons is of functional importance, this could explain how $GABA_A$ receptors may modulate transmitter release in a manner involving calcium channels.

Transformation of Biological Information into Specific Drugs

The structure and function of the nervous system, not least the CNS, are characterized by an extreme degree of complexity, globally and also at the cellular and molecular level, as exemplified. It is virtually impossible to predict behavioral effects of new drugs, even compounds with a specific effect at a particular targe-treceptor. Nevertheless, simple models or cartoons are indispensable elements of drug design and development projects. Such oversimplified illustrations of complex neurobiological interactions and phenomena are valuable components of the communication between neurobiologists and medicinal chemists. They often catalyze the complex process of translating biological information into a "chemical language" with subsequent transformation into specific drugs. Models, which have to be revised repeatedly during such projects, may be visualized as convertible drug design chess boards and the specific pharmacological tools as the requisite chessmen. A prerequisite for an inspired drug design game of chess is the design of specific tools, which frequently represent key steps in the development of novel specific therapeutic agents.

Specific $GABA_A$ Receptor Agonists

The basically inhibitory nature of the central GABA neurotransmission prompted the design and development of different structural types of GABA agonists. Conformational restriction of various parts of the molecule of GABA and bioisosteric replacements of the functional groups of this amino acid have led to a broad spectrum of specific $GABA_A$ agonists. Some of these molecules have played a key role in the development of the pharmacology of the $GABA_A$ receptor, or rather, receptor family.

The histamine metabolite imidazole-4-ethanoic acid (IAA, **10**) (Scheme 1) is a relatively potent $GABA_A$ agonist, which may play a role as a central and/or peripheral endogenous $GABA_A$ receptor ligand. Like the imidazole group of **10**, the structurally related isothiouronium elements of (*RS*)-2-amino-2-thiazoline-4-ethanoic acid (**11**)[76] and (*Z*)-3-[(aminoiminomethyl)-thio]prop-2-enoic acid (ZAPA, **12**)[77] are effectively recognized by the $GABA_A$ receptor sites, compound **12** showing preferential affinity for low-affinity $GABA_A$ sites. (1*S*,3*S*)-3-Aminocyclopentane-1-carboxylic acid [(1*S*,3*S*)-TACP, **13**], which is a specific $GABA_A$ agonist,[78] is a GABA analogue containing a conformationally restricted carbon backbone.

Muscimol (**14**), a constituent of the mushroom *Amanita muscaria*, has been extensively used as a lead for the design of different classes of GABA analogues (Scheme 1). The 3-hydroxyisoxazole carboxyl group bioisostere of **14** can be replaced by a 3-hydroxyisothiazole or 3-hydroxyisoxazoline group to give thiomuscimol (**15**) and dihydromuscimol (DHM), respectively, without significant loss of $GABA_A$ receptor agonism.[79] (*S*)-DHM (**16**) is the most potent $GABA_A$ agonist so far described.[80]

Conversion of **14** into THIP (**5**)[19] and the isomeric compound 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THPO, **17**) effectively separated $GABA_A$ receptor and GABA uptake affinity, THIP being a specific $GABA_A$ agonist and THPO a GABA uptake inhibitor.[81] Using THIP as a lead, a series of specific monoheterocyclic $GABA_A$ agonists, including isoguvacine (**4**) and isonipecotic acid (**18**), were developed.[82,83] Thio-THIP (**19**) is weaker than THIP (**5**) as a $GABA_A$ agonist (Figure 5),[84] but recent studies have disclosed a unique pharmacological profile of **19** (see later section).

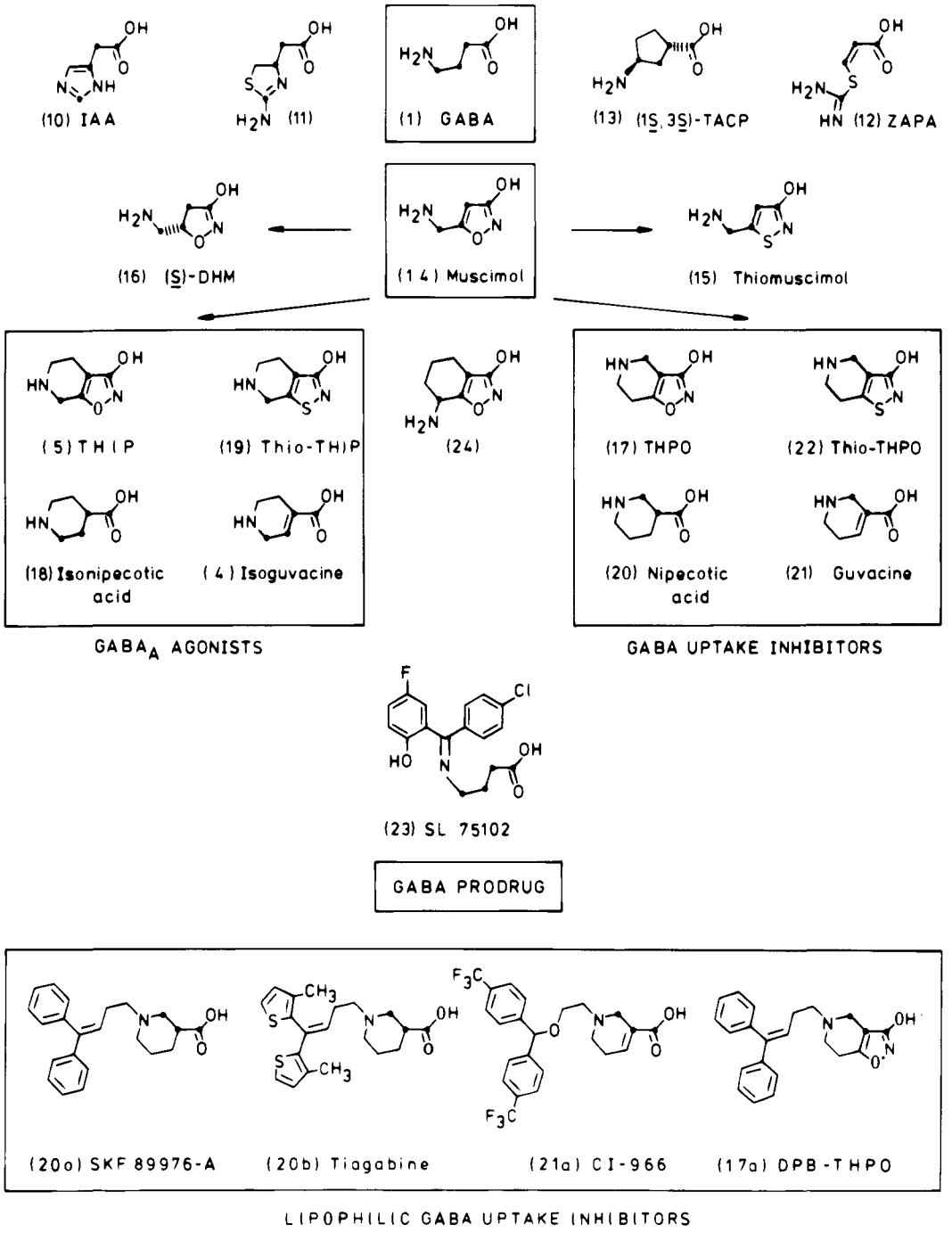
Analogously, a series of cyclic amino acids derived from THPO (**17**), including nipecotic acid (**20**)[81] and guvacine (**21**),[85] was developed as GABA uptake inhibitors. Whereas **20** and **21** potently inhibit neuronal as well as glial GABA uptake,[86] THPO (**17**) interacts selectively with the latter uptake system.[87,88] Thio-THPO (**22**) is slightly weaker than **17** as an inhibitor of GABA uptake.[84] The benzophenone imine derivative of GABA, SL 75102 (**23**), is a prodrug of GABA, which, like THIP (**5**),[89] has been the object of quite extensive clinical studies.[90] Since **23** is a prodrug of GABA, its effects in animals and humans are mediated by all types of GABA receptors present in the tissues accessible to the compound.

A structure–activity analysis on the $GABA_A$ agonists shown in Scheme 1 leads to a rather paradoxical result. The $GABA_A$ receptors evidently can tolerate quite extensive bioisosteric modification of the functional groups of GABA, whereas even minor structural alteration of the individual agonist molecules normally results in a marked or complete loss of activity.[82,83] Thus, all analogues of IAA (**10**) so far described are very weak or inactive as $GABA_A$ agonists, and the aromatic analogue of **11** shows very little effect on $GABA_A$ receptors.[76] The stereochemistry of **13** clearly is optimal for $GABA_A$ receptor affinity,[78] and analogues of **12** are much weaker than the parent compound.[77]

Although thiomuscimol (**15**) is almost equipotent with muscimol (**14**) as a $GABA_A$ agonist, thio-THIP (**19**) is much weaker than THIP (**5**) (Figure 5).[84] Furthermore, substituted analogues or ring homologs of these compounds, of isoguvacine (**4**), and of isonipecotic acid (**18**) only interact weakly with $GABA_A$ receptor sites.[82]

It should be emphasized that each receptor, or biological recognition site in general, normally exhibits a

Scheme 1



unique pattern of "bioisosteric tolerance". A successful bioisosteric replacement strategy in a particular receptor system normally cannot be applied for the design of effective ligands for other types of receptors, or for other recognition sites within the same biological system. Thus, whereas P4S (**25**) and also 1,2,3,6-tetrahydropyridine-4-sulfonic acid (DH-P4S, **26**) are very potent partial $GABA_A$ agonists[91] (see later section), the sulfonic acid analogue of nipecotic acid (**20**) does not interact significantly with neuronal or glial GABA uptake.[92]

Compound **24**,[93] which essentially reflects the conformation(s) of muscimol (**14**) in the crystalline state,[94] does not affect $GABA_A$ receptor binding detectably. On the other hand, the proposed receptor-active conformation of **14**, as reflected by THIP (**5**), has somewhat higher energy than low-energy conformations of **14** (Figure 6). It seems likely that this energy difference is of importance for the very effective activation of the $GABA_A$ receptor by muscimol (**14**).

Although a broad spectrum of $GABA_A$ agonists is now available for molecular pharmacological studies, this field still is open for design of novel types of agonist molecules. Medicinal chemists with solid insight in heterocyclic chemistry and computer modeling techniques[95] almost certainly will be able to construct novel active molecules in this still rather young research field.

GABA Uptake Inhibitors as Indirect GABAergic Modulators

Inhibitors of GABA uptake are indispensable tools for studies of GABA synaptic mechanisms, and such compounds have growing therapeutic interest as indirect GABAergic modulators.[5,83,86-88] Pharmacological inhibition of the neuronal and/or glial GABA transport

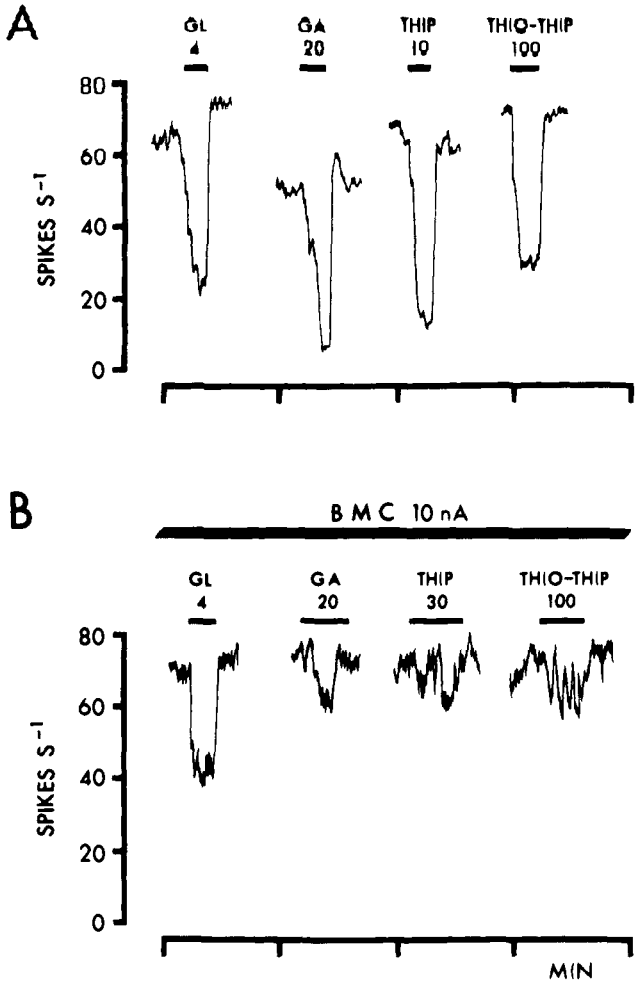


Figure 5. Comparison of the inhibitory effects of glycine (GL), GABA (GA), THIP (**5**), and thio-THIP (**19**) on the firing of a spinal dorsal horn interneuron of a cat anaesthetized with pentobarbitone sodium, maintained with continuously ejected DL-homocysteic acid (DLH) before (A, DLH 15 nA) and during (B, DLH 3 nA) the ejection of BMC (**3**) (10 nA). The amino acids were ejected electrophoretically with the indicated currents (nA) from aqueous solutions of glycine (0.5 M, pH 3), GABA (0.2 M, pH 3), THIP (0.2 M, pH 3.5), and thio-THIP (0.2 M, pH 3.2). The ratios for equieffective currents for GABA, THIP, and thio-THIP (20, 10, 100) for this particular cell were unusual, indicating that THIP was twice as potent as GABA and 10 times as potent as thio-THIP. With 7 other cells (3 cats), THIP was approximately equipotent to GABA, whereas thio-THIP was approximately half as potent as THIP. The reduction by BMC of the actions of GABA, THIP, and thio-THIP recovered within 10 min of terminating the administration of BMC (not illustrated). Ordinates: firing rate, spikes s^{-1}. Abscissae: Time, min. (Reproduced with permission from ref 84.)

mechanisms provides a mechanism for sustaining levels of synaptically released GABA in the synapse and thereby increasing GABA-mediated transmission.[88]

The classical GABA uptake inhibitors nipecotic acid (**20**) and guvacine (**21**) (Scheme 1) are fully zwitterionic at physiological pH, which explains their very poor penetration of the blood–brain barrier (BBB) (see later section).[88] Furthermore, these amino acids are substrates for the GABA transport carriers, making interpretation of pharmacological data difficult.[81,84–88] In an effort to overcome the pharmacokinetic obstacle for in vivo studies of these and related compounds, Ali and co-workers[96] examined the effect of adding bulky lipophilic side chains to the nitrogen atoms of nipecotic acid (**20**), guvacine (**21**), and structurally related GABA uptake inhibitors. Whereas introduction of small substituents on the amino groups of these compounds normally results in a decrease in or complete loss of GABA transport affinity,[83,88] the addition of the 4,4-diphenyl-3-butenyl (**DPB**) side chain to nipecotic acid (**20**) to give SKF 89976-A (**20a**) (Scheme 1) resulted in a 20-fold increase in potency when tested in brain synaptosomes.[96] Compound **20a** was subsequently shown not to be a substrate for GABA transport carriers.[97] Since the original report, a number of substituents structurally related to the DPB group have been introduced to produce similar derivatives such as tiagabine (**20b**),[98] CI-966 (**21a**),[99] and the DPB analogue of THPO (**17a**).[100]

All of these compounds display anticonvulsant effects in laboratory animals.[98–102] The guvacine derivative **21a** has been shown to cause severe adverse effects after administration to human volunteers,[103] but such effects have not been observed for the nipecotic acid derivative tiagabine (**20b**). This GABA uptake inhibitor has demonstrated antiseizure activity in epileptic patients (for references, see ref 98), thus demonstrating the therapeutic potential of this class of GABAergic compounds.

Like most, if not all, other biomechanisms, GABA transport carriers exist in multiple forms.[104] This observation is likely to further stimulate the exploration of GABA uptake mechanisms as novel targets for GABA-modulating drugs. It may be possible to identify and localize distinct subtypes of GABA transport carriers in brain areas of primary importance in for example epilepsy, making the development of effectively targeted antiepileptic drugs possible. It is possible that different subtypes of GABA carriers are associated with synapses operated by $GABA_A$ or $GABA_B$ receptors, and, if so, GABA uptake inhibitors showing receptor-selective modulatory effects may be developed.

GABA in the Periphery—Transmitter and Paracrine Effector

The role of GABA as a central transmitter is fully established, and there is rapidly growing evidence that GABA also has a broad spectrum of physiological functions in the periphery.[5,6,105–107] In a wide range of peripheral tissues, notably parts of the PNS, endocrine glands, smooth muscles, and the female reproductive systems, GABA receptors have been detected. In all tissues so far analyzed, $GABA_A$ as well as $GABA_B$ receptors have been identified.

There are many unsolved questions regarding the peripheral actions of GABA and its interactions with other physiological mechanisms using ACh, norepinephrine, serotonin, and various peptides as transmitter or paracrine mediators.[107] It is possible that disinhibitory mechanisms between GABA neurons or between $GABA_A$ and $GABA_B$ receptors at the cellular level, as described above, also contribute to the apparently very complex functions of GABA in the periphery.

The peripheral GABA receptors, or other GABAergic synaptic mechanisms, obviously have interest as drug targets. So far, GABA drug design projects have been focused on sites at central GABA-operated synapses. It should, however, be emphasized that even for GABAergic drugs, which easily penetrate the BBB, most of the drug administered is found in the periphery, where it may cause adverse effects. On the other hand, most GABA analogues of pharmacological interest do not

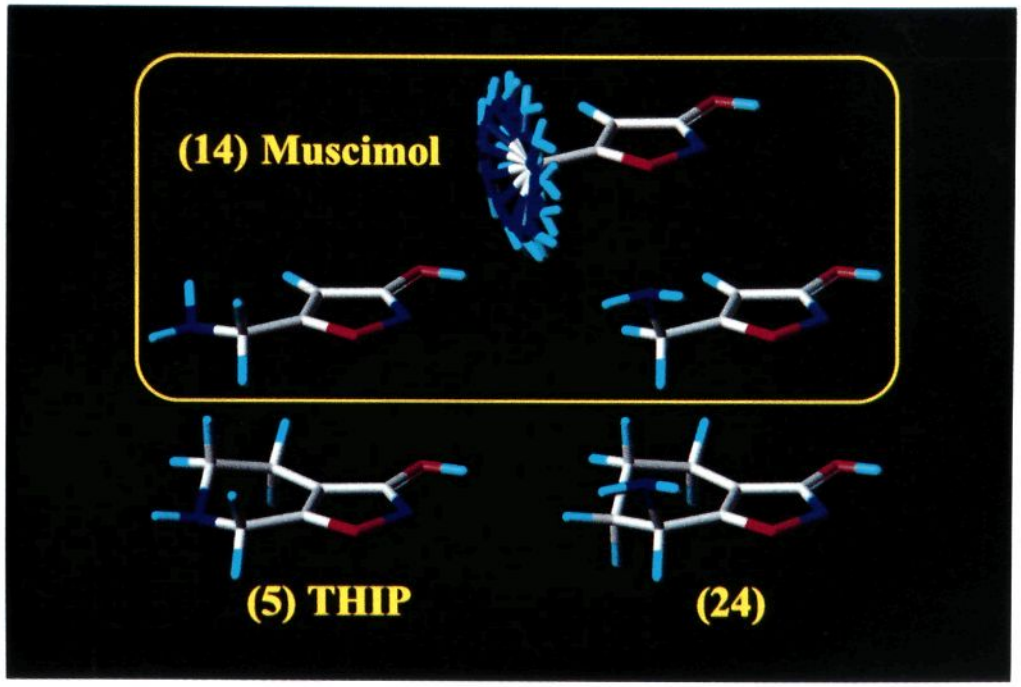


Figure 6. The flexibility of the $GABA_A$ agonist muscimol (**14**) illustrated by a superposition of conformations with different orientation of the side chain (top). Two of these conformations (middle) correspond to the conformation of the muscimol moiety in the conformationally restricted compounds THIP (**5**) and **24**, respectively (bottom).

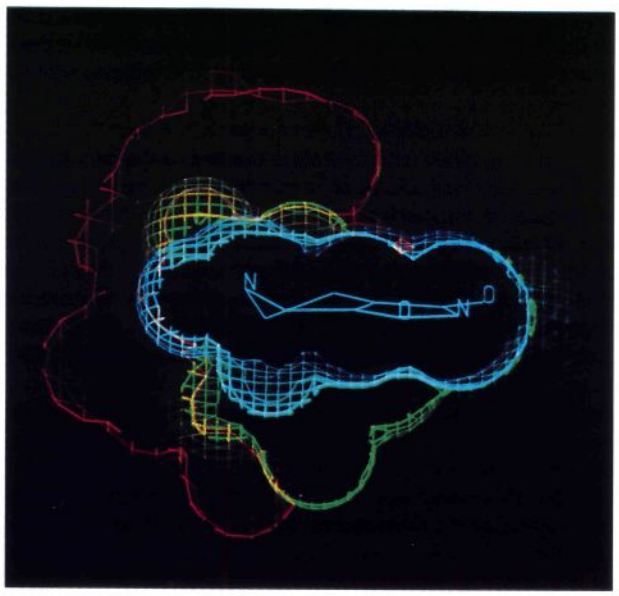


Figure 7. The volume of the $GABA_A$ agonist THIP (**5**) (blue) together with the extra volume occupied by the partial $GABA_A$ agonist 4-PIOL (**33**) (green). The extra volume occupied by the receptor relevant conformations of the inactive compound, 2-PIOL (**32**), relative to THIP (**5**) and 4-PIOL (**33**) is shown in red. The volumes have been z-clipped and only the non-hydrogen atoms of THIP are shown. (Reproduced with permission from ref 159.)

easily penetrate the BBB, making it possible to develop GABAergic drugs specifically targeted at peripheral GABA receptors.

In light of the identification of NANB GABA receptors, probably of the proposed $GABA_C$ type, in the retina (see earlier section), it seems likely that other types of NANB GABA receptors may be identified in peripheral tissues. There is, for example, circumstantial evidence of an involvement of nonclassical $GABA_A$ receptors in the regulation of the release of luteinizing hormone from rat pituitary cells.[108] Such atypical GABA receptors in tissues showing specific physiological responsiveness to GABA may be particularly interesting targets for drug design.

The function of GABA, mediated by $GABA_A$ as well as $GABA_B$ receptors, in the enteric nervous system has been quite extensively studied.[109] While it seems unlikely that GABA is playing a major role in intestinal secretory activity, $GABA_A$ and, perhaps in particular, $GABA_B$ receptors may play important roles in the control of gut motility. Thus, peristalsis can be markedly reduced and ultimately stopped after administration of $GABA_A$ and $GABA_B$ antagonists, separately or in combination.[107]

GABA receptors in the gut as well as GABA receptors in the gallbladder, the lung, and the urinary bladder[106] may give rise to adverse effects in GABA drug therapies or may be important new therapeutic targets in drug-induced or pathological dysfunctions of these organs.

The involvement of GABA in the regulation of blood pressure has been the object of numerous studies.[110] The relative importance of $GABA_A$ and $GABA_B$ receptors in these very complex mechanisms is still unclear, and the effects of GABA mimetics are, to some extent, species-dependent. In this area, the $GABA_A$ receptor-mediated dilatation by GABA of cerebral blood vessels[111] is of particular interest and may have major therapeutic implications. It is possible that this dilatation actually is mediated by ACh released via a $GABA_A$ receptor-regulated mechanism.[112] The effect of THIP (**5**) on cortical blood flow in humans has been used for diagnostic purposes as a new test for hemispheric dominance.[113] Thus, THIP decreases in a dose-dependent manner blood flow in nonactivated brain areas. This submaximal depression can be counteracted physiologically by the patient.[113]

The involvement of GABA in the endocrine pancreatic functions[105,114] is an area of growing therapeutic interest. Autoantibodies to glutamic acid decarboxylase

(GAD) appear to play an important role for the initiation of insulin dependent diabetes,[115] underlining the importance of the GABA system in pancreatic function. GABA is present in the endocrine part of the pancreas at concentrations comparable to those encounted in the CNS, and co-localizes with insulin in the pancreatic β-cells. GABA seems to mediate part of the inhibitory action of glucose on glucagon secretion by activating $GABA_A$ receptors in α_2 cells.[116] Thus, $GABA_A$ receptors probably are playing a key role in the feedback regulation of glucagon release, which seems to be an important mechanism in the hypersecretion of glucagon, frequently associated with diabetes. These $GABA_A$ receptors of as yet not disclosed subunit composition are potential targets for therapeutic GABA intervention.

There is a rapidly growing interest in the role of GABA as a transmitter in hearing mechanisms. This interest has been stimulated by the demonstration of a substantial, selective, and age-related loss of GABA in the central nucleus of the inferior colliculus (CIC) in rat.[117] There is immunocytochemical evidence for the existence of a GABAergic system in the guinea pig vestibule and of a role of GABA as a vestibular afferent transmitter.[118-120] Impairment of inhibitory GABAergic transmission in the CIC may contribute to abnormal auditory perception and processing in neural presbycusis.[117] These observations may lead to the identification of novel targets for GABAergic therapeutic intervention in different age-related diseases and conditions involving defective hearing.

In the PNS, $GABA_A$ agonists as well as antagonists are potential therapeutic agents. Whereas the latter class of GABAergic drugs may be rather difficult to use therapeutically in CNS diseases due to seizure potential (see later sections), $GABA_A$ antagonists being unable to penetrate the BBB may be of great therapeutic value in the periphery.

GABA in Analgesia and Anxiety

The involvement of central $GABA_A$ receptors in pain mechanisms and analgesia has been thoroughly studied, and the results have been discussed and reviewed.[82,83,89,121,122] The demonstration of potent antinociceptive effects of the specific and metabolically stable $GABA_A$ agonist THIP (**5**) in different animal models and the potent analgesic effects of THIP in humans greatly stimulated studies in this area of pain research. THIP-induced analgesic effects were shown to be insensitive to the opiate antagonist naloxone, indicating that these effects are not mediated by the opiate receptors. Quite surprisingly, THIP analgesia could not be reversed by bicuculline (**2**), which may reflect the involvement of a distinct class of $GABA_A$ receptors or, perhaps, a NANB-type of GABA receptor. On the other hand, THIP-induced analgesia could be reduced by atropine and potentiated by cholinergics such as physostigmine, reflecting as yet unclarified functional interactions between GABA and ACh neurons and, possibly, the central opiate systems rather than a direct action of THIP on muscarinic ACh receptors.

THIP and morphine are approximately equipotent as analgesics, although their relative potencies are dependent on the animal species and experimental models used. Acute injection of THIP potentiates morphine-induced analgesia, and chronic administration of THIP produces a certain degree of functional tolerance to its analgesic effects. In contrast to morphine, THIP does not cause respiratory depression. Clinical studies on postoperation patients, and patients with chronic pain of malignant origin have disclosed potent analgesic effects of THIP, in the latter group of patients at total doses of 5–30 mg (im) of THIP.

In these cancer patients and also in patients with chronic anxiety[123] the desired effects of THIP were accompanied by side effects, notably sedation, nausea, and in a few cases euphoria. The side effects of THIP have been described as mild and similar in quality to those of other GABA mimetics.[123]

It is assumed that the postsynaptic $GABA_A$ receptor complex is mediating the anxiolytic effects of the BZDs, and, consequently, it is of interest to see whether $GABA_A$ agonists have anxiolytic effects. Muscimol (**14**) has proved effective in conflict tests, though with a pharmacological profile different from that of diazepam, and in humans, muscimol in low doses was found to sedate and calm schizophrenic patients.[124] In a number of patients with chronic anxiety the effects of THIP were assessed on several measures of anxiety.[123] Although these effects were accompanied by side effects, the combination of analgesic and anxiolytic effects of THIP would seem to have therapeutic prospects.

The neuronal and synaptic mechanisms underlying THIP- and, in general, GABA-induced analgesia are still only incompletely understood. The insensitivity of THIP-induced analgesia to naloxone has been consistently demonstrated. Sensitivity of THIP analgesia to a serotonin agonist seems to indicate an interaction between central GABA and serotonin systems. GABA-induced analgesia does not seem to be mediated primarily by spinal $GABA_A$ receptors but rather by GABA mechanisms in the forebrain, and it appears also to involve neurons in the midbrain. The naloxone-insensitivity and apparent lack of dependence liability of $GABA_A$ agonist-mediated analgesia suggest that GABAergic drugs may play a role in future treatment of pain. Furthermore, it has been suggested that pharmacological manipulation of GABA mechanisms may have some relevance for future treatment of opiate drug addicts.

Other observations further emphasize the complexity of the role of GABA in pain mechanisms. Thus, THIP has been shown to inhibit its own analgesic action at higher doses producing a bell-shaped dose–response curve.[125] In addition, subconvulsant doses of bicuculline (**2**) were shown to increase the latency of licking in the hot plate test in mice, an effect which was not modified by naloxone or atropine but was antagonized by the $GABA_B$ antagonist, CGP 35348.[126] These latter observations suggest that more than one type of $GABA_A$ receptors, perhaps including autoreceptors (Figure 1), are involved in pain mechanisms and, furthermore, that interactions between $GABA_A$ and $GABA_B$ receptors are playing a role (see earlier section).

Curiously, the full $GABA_A$ agonist muscimol, the very efficacious partial $GABA_A$ agonist THIP, as well as the $GABA_A$ antagonist bicuculline show potent antinociceptive effects. It is possible that the side effects of THIP in patients somehow are associated with its high efficacy at $GABA_A$ receptors. If so, medicinal chemists are faced with the challenge of designing a series of $GABA_A$ agonists showing a broad spectrum of efficacies. In this

regard, thio-THIP (**19**) (Figure 5) and other compounds derived from THIP are of particular interest (see later section).

GABA in Neurological Disorders

There is an overwhelming amount of indirect evidence, derived from experimental models of epilepsy, supporting the view that pharmacological stimulation of the GABA neurotransmission may have therapeutic interest in epilepsy.[127-129] The anticonvulsant effects of THIP (**5**) and muscimol (**14**) have been compared in a variety of animal models. THIP typically is 2–5 times weaker than muscimol in suppressing seizure activities. In mice and in gerbils with genetically determined epilepsy, systemically administered THIP has proved very effective in suppressing seizure activity, and THIP is capable of reducing audiogenic seizures in DBA/2 mice. However, THIP failed to protect baboons with photosensitive epilepsy against photically induced myoclonic responses.

THIP has been subjected to a single-blind controlled trial in patients with epilepsy, in which THIP was added to the concomitant antiepileptic treatment. Under these conditions no significant effects of THIP were detected, although a trend was observed for lower seizure frequency during a period of submaximal doses of THIP.[130]

In light of these quite surprising effects of THIP in photosensitive baboons and the lack of clinical antiepileptic effects of this specific $GABA_A$ agonist, its effects on human brain glucose metabolism has been studied using positron emission tomography (PET) scanning techniques.[131,132] Due to the sedative effects of THIP observed in animals and patients,[89] the sleepiness and decrease of α-rhythms observed in the patients and normal volunteers involved in these PET studies were not unexpected. Accordingly, brain glucose hypometabolism was anticipated in these volunteers and epileptic patients receiving clinically relevant doses of THIP (0.2 mg/kg). Surprisingly and paradoxically, brain glucose metabolism increased globally, showing an average increase in grey matter of 17%.[132]

Dysfunctions of the central GABA neurotransmitter system(s) have also been associated with other neurological disorders such as Huntington's chorea[133] and tardive dyskinesia.[134,135] In Huntington's chorea there is a marked loss of GABA neurons, whereas no significant changes in numbers and binding characteristics of $GABA_A$ receptors could be detected.[136] Nevertheless, replacement therapies using the specific $GABA_A$ agonists muscimol (**14**) or THIP (**5**) did not significantly ameliorate the symptoms of choreic patients.[137] Similarly, THIP only marginally improved the symptoms of patients suffering from tardive dyskinesia.[138]

There are several possible explanations of these largely negative effects of THIP in the neurological disorders mentioned: (i) Disinhibitory neuronal mechanisms, converting inhibitory effects into functional excitation (see earlier section), may play a key role in the brain areas affected in these disorders; (ii) $GABA_A$ autoreceptors regulating GABA release may be more sensitive to the $GABA_A$ agonists studied than the hyperpolarizing postsynaptic $GABA_A$ receptors (Figure 1); (iii) The $GABA_A$ agonists studied may cause rapid and effective receptor desensitization after prolonged activation by systemically administered agonists;[139,140] (iv) The ϱ-like receptors found in retina,[39,40] where THIP shows antagonistic effects[40] may play a role in certain parts of the human brain.

These aspects open up the prospects of designing new types of therapeutic $GABA_A$ receptor ligands. Antagonists at postsynaptic $GABA_A$ receptors, notably those involved in disinhibitory mechanisms, may in principle have therapeutic interest, but selective antagonists at $GABA_A$ autoreceptors seem to have major interest. There is, however, an obvious need for partial $GABA_A$ agonists showing different levels of efficacy and, in addition, showing selectivity for $GABA_A$ receptors in brain areas of particular relevance for the disorders under discussion (see later sections).

GABA in Alzheimer's Disease

There is a well-documented loss of central ACh nerve terminals in certain brain areas of patients suffering from Alzheimer's disease.[141] Consequently, most efforts for a therapeutic treatment of this neurodegenerative disease have hitherto been focused on the processes and mechanisms at cholinergic synapses.

Central cholinergic neurons appear to be under inhibitory GABAergic control,[142,143] and consequently, the function of such neurons may be indirectly stimulated by blockade of the $GABA_A$ receptors involved in this regulation. These $GABA_A$ receptors may be located pre- or postsynaptically on ACh neurons (Figure 1). Therapies based on agents with antagonist actions at $GABA_A$ receptors, or at one of the modulatory sites of the $GABA_A$ receptor complex should, at least theoretically, be applicable in Alzheimer's disease. $GABA_A$ receptor antagonists, which in addition show low-efficacy $GABA_A$ agonist effects, might stimulate ACh release, and, thus, improve learning and memory in Alzheimer patients without causing convulsions.

The results of studies on different $GABA_A$ receptor ligands in animal models relevant to learning and memory seem to support such GABAergic therapeutic approaches in Alzheimer's disease. Thus, whereas administration of $GABA_A$ agonists impairs learning and memory in animals[144,145] via modulation of cholinergic pathways,[145] memory enhancement was observed after injection of the $GABA_A$ antagonist BMC (**3**).[144] Similarly, agonists and inverse agonists at the BZD site of the $GABA_A$ receptor complex impair and enhance, respectively, performance in learning and memory tasks.[146] Administration of THIP (**5**) to Alzheimer patients failed to significantly improve cognitive performance.[147]

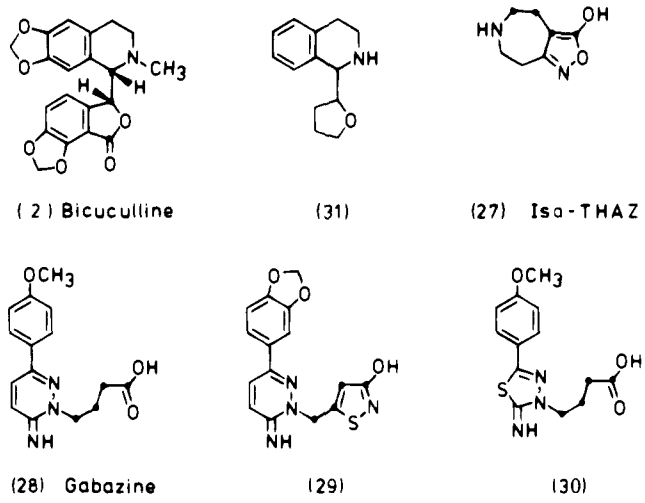
The lack of positive and, in particular, negative effects of THIP in Alzheimer patients is very interesting and may reflect that THIP, as mentioned earlier, is a rather efficacious partial $GABA_A$ agonist. These observations seem to support the view that low-efficacy partial $GABA_A$ agonists may be of clinical interest in Alzheimer's disease (see later section).

$GABA_A$ Antagonists—Recent Developments

Specific receptor antagonists are essential tools for studies of the physiological role and pharmacological importance of the particular receptors. The classical $GABA_A$ antagonists bicuculline (**2**) (Scheme 2) and BMC (**3**) (Figure 2) have played a key role in such studies on $GABA_A$ receptors.

In recent years, new structural classes of $GABA_A$ antagonists have been developed, and this line of GABA

Scheme 2



drug research has been stimulated by the growing interest in such compounds as potential therapeutic agents, at least in theory. Whereas the bicyclic 5-isoxazolol compound, Iso-THAZ (**27**) (Scheme 2), derived from THIP (**5**), is a moderately potent $GABA_A$ antagonist[148,149] a series of arylaminopyridazine analogues of GABA, notably Gabazine (**28**), show very potent and selective $GABA_A$ antagonist effects.[149-152] These compounds bind tightly to $GABA_A$ receptor sites, and tritiated Gabazine (**28**) is now used as a standard receptor ligand. Although **28** and related compounds containing a GABA structure element show convulsant-effects after systemic administration,[153] these zwitterionic compounds do not easily penetrate the BBB. Compound **29**, in which the GABA stucture element has been replaced by a thiomuscimol (**15**) unit, is the most potent $GABA_A$ antagonist in the arylaminopyridazine series.[153] This increased potency has been explained by the more pronounced lipophilic character of compound **29** as compared with the corresponding analogues of GABA.[153] Bioisosteric substitution of a 2-amino-1,3,4-thiadiazole unit for the 3-aminopyridazine part of **28** gives compound **30**, which also shows $GABA_A$ antagonistic properties though markedly weaker than those of **28**.[154]

$GABA_A$ autoreceptors (Figure 1), which regulate GABA release via a negative feedback mechanism, are interesting novel targets for GABAergic drug design. Although such autoreceptors basically are $GABA_A$ receptors, they have pharmacological characteristics markedly different from those of postsynaptic $GABA_A$ receptors.[155] Selective $GABA_A$ autoreceptor antagonists may function as positive modulators of GABA neurotransmission processes. Interestingly, compound **31**, which is a "peeled" analog of bicuculline (**2**), and a number of other related compounds are 2 orders of magnitude more potent as $GABA_A$ autoreceptor antagonists than as antagonists at postsynaptic $GABA_A$ receptors.[155,156] This particular class of GABA receptor antagonists have therapeutic potential in a number of CNS disorders (see earlier sections).

Partial $GABA_A$ Agonists—Pharmacology and Pharmacokinetics

Full $GABA_A$ agonists or antagonists may be rather difficult to introduce as drugs of practical clinical applicability, at least in diseases in the CNS (see earlier section). While the former class of compounds may induce rapid desensitization of the target receptors (see Figure 8) after constant activation by systemically administered agonist drugs, $GABA_A$ antagonists are potential anxiogenics, proconvulsants, or frank convulsants.

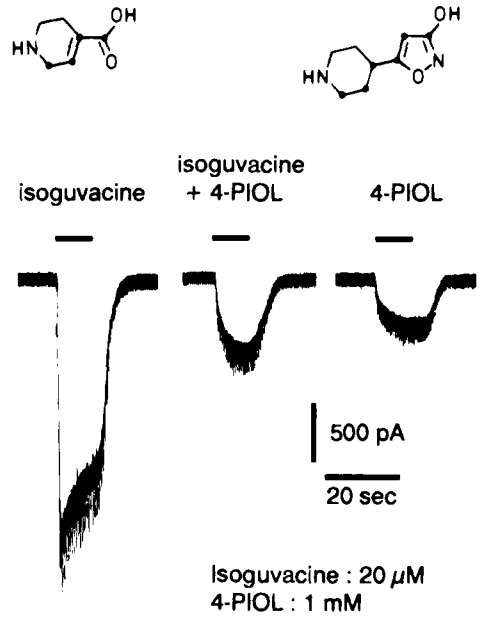


Figure 8. Whole-cell patch-clamp recording from a hippocampal neuron. Holding potential was −60 mV with −10 mV command potentials superimposed to monitor membrane conductance. Drugs were applied in the vicinity of the neuron by a multibarrel perfusion pipette. The response to isoguvacine (**4**) was reduced by simultaneous application of 4-PIOL (**33**) to a value slightly higher than the intrinsic agonist response to 4-PIOL alone. In contrast to the response to 20 μM isoguvacine alone, the responses with 4-PIOL present did not show desensitization.

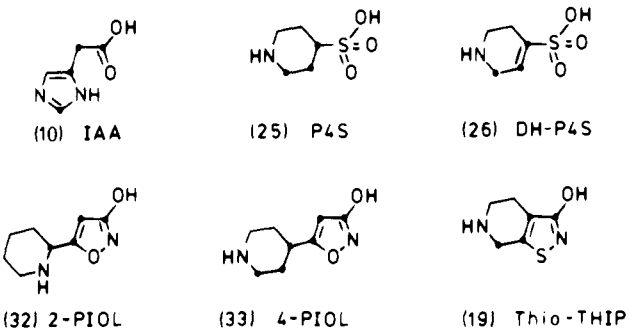
In clinical conditions where $GABA_A$ agonist therapies may be relevant, relatively efficacious agonist may be appropriate drugs, the levels of efficacy probably being dependent on the particular disease. The very potent analgesic effects of THIP (**5**) (see earlier section) seem to indicate that the relatively high level of efficacy of this partial $GABA_A$ agonist[83,157] is close to optimal with respect to pain treatment, although it may be postulated that a slightly less efficacious $GABA_A$ agonist may show fewer side effects than does THIP.

Analogously, very low-efficacy $GABA_A$ agonist showing predominant antagonist profiles may have clinical usefulness in certain diseases. Such compounds showing sufficient $GABA_A$ receptor agonism to avoid seizure side effects may, theoretically, be useful therapeutic agents in for example Alzheimer's disease or, quite paradoxically, in epileptic disorders (see earlier section).

The heterocyclic GABA bioisosteres IAA (**10**),[157,158] P4S (**25**), and DH-P4S (**26**) (Scheme 3) show the characteristics of partial $GABA_A$ agonists. Interestingly, the unsaturated compound **26** is about 1 order of magnitude less potent than P4S (**25**)[91] whereas the reverse relative potency is observed for the full agonists isoguvacine (**4**) and its saturated analogue isonipecotic acid (**18**) (Scheme 1), P4S and isoguvacine being approximately equipotent with GABA.[19,91]

Whereas the nonfused THIP (**5**) analog (*RS*)-5-(2-piperidyl)isoxazol-3-ol (2-PIOL, **32**) does not interact detectably with $GABA_A$ receptors, the 4-piperidyl analogue 4-PIOL (**33**) (Scheme 3 and Figure 7) is a moderately potent agonist at $GABA_A$ receptors in the

Scheme 3



cat spinal cord.[159,160] 4-PIOL did not, however, show significant stimulatory effects on BZD binding, but it antagonized dose-dependently muscimol (**14**)-induced stimulation of BZD binding in a manner similar to that of the $GABA_A$ antagonist BMC (**3**).[161]

Whole-cell patch-clamp recordings from cultured hippocampal neurons have been used to further characterize the action of 4-PIOL (**33**) (Figure 8).[162] The action of 4-PIOL was compared with those of the full $GABA_A$ agonist isoguvacine (**4**) and the $GABA_A$ antagonist bicuculline methobromide. The response to 4-PIOL was competitively antagonized by bicuculline methobromide (not illustrated). 4-PIOL was about 200 times less potent as an agonist than isoguvacine. The maximum response to 4-PIOL was only a small fraction of that to submaximal concentrations of isoguvacine, and 4-PIOL antagonized the response to isoguvacine (Figure 8) with a parallel shift to the right of the dose–response curve (not illustrated). On the basis of these studies it can be concluded that 4-PIOL is a low-efficacy partial $GABA_A$ agonist showing a predominant $GABA_A$ antagonist profile, being about 30 times weaker than bicuculline methobromide as a $GABA_A$ antagonist. Importantly, repeated administration of 4-PIOL did not cause significant desensitization of the $GABA_A$ receptors studied (Figure 8). Unfortunately, 4-PIOL does not show pharmacological effects after systemic administration.[161] In contrast to THIP (**5**), which penetrates the BBB very easily,[89] the protolytic properties of 4-PIOL do not allow this compound to pass the BBB.[161] 4-PIOL analogues more potent than 4-PIOL itself and having different pharmacokinetic properties are under development.

The ratio between the concentrations of ionized (zwitterionic) and un-ionized neutral amino acids (I/U ratio, zwitterionic constant) is a function of the difference between the pK_a I and II values.[84] A large difference between these two pK_a values is tantamount to high I/U ratios. Only very small concentrations of un-ionized compound will be present in solution, and the ability to penetrate the BBB will be correspondingly low. Whereas GABA (I/U ratio = 800 000) does not penetrate the BBB to any significant extent, muscimol (I/U ratio = 900) or THIP (I/U ratio = 1000) are better suited for this purpose in view of their low I/U ratios. A relatively large difference between the pK_a values of 4-PIOL (5.16; 10.19) and, consequently, a high I/U ratio (31 000) is consistent with the observation that 4-PIOL does not penetrate the BBB.

In an attempt to overcome this pharmacokinetic obstacle, and to shed further light on the relationship between structure and $GABA_A$ agonist efficacy of this class of heterocyclic GABAergic compounds, a number of 4-PIOL analogues have been synthesized and tested.[163] With a few notable exceptions, as mentioned above, these analogs or ring homologs showed negligible affinity for $GABA_A$ receptor sites, emphasizing the very strict structural requirements for binding to the $GABA_A$ receptors (Figure 7).

The low-efficacy partial agonism, and thus predominant antagonist effect, of 4-PIOL was expressed by $GABA_A$ receptors in hippocampal neurons[162] (Figure 8). As mentioned above, 4-PIOL did, however, show moderately potent $GABA_A$ agonist effects on cat spinal neurons after microelectrophoretic application.[160] Although these dissimilar effects were detected using different techniques, they indicate that spinal and supraspinal $GABA_A$ receptors, to some extent, show different pharmacological characteristics. These aspects in general and the particular pharmacological profile of 4-PIOL seem to have interesting therapeutic prospects.

A very similar but even more accentuated pharmacological profile has been demonstrated for thio-THIP (**19**). Whereas thio-THIP shows distinct $GABA_A$ agonist effects on cat spinal neurons[84] (Figure 5), recent studies using human brain recombinant $GABA_A$ receptors have disclosed that such receptors express low-efficacy partial agonism of thio-THIP.[164] The efficacy of this partial agonism is even lower than that shown by 4-PIOL (Figure 8) consistent with thio-THIP having predominantly antagonistic effects at this type of brain receptors.

In light of the structural similarity of THIP (**5**) and thio-THIP (**19**) (Scheme 1, Figure 9) the markedly different pharmacology of these compounds is noteworthy and emphasizes the very strict structural constraints imposed on ligands for $GABA_A$ receptors. The pK_a values of THIP (4.4; 8.5) and thio-THIP (6.1; 8.5)[84] are different, and a significant fraction of the molecules of the latter compound must contain a non-ionized 3-hydroxyisothiazole group at physiological pH. Furthermore, the different degree of charge delocalization of the zwitterionic forms of THIP and thio-THIP and other structural parameters of these two compounds, especially the bioisosteric 3-hydroxyisoxazole and 3-hydroxyisothiazole groups, may have to be considered in order to explain their different potency and efficacy at $GABA_A$ receptors.

Whereas 4-PIOL, which has an I/U ratio of 31 000, does not easily penetrate the BBB (see above), an I/U ratio of 16 for thio-THIP[84] suggests that this compound may readily enter the CNS after systemic administration. Behavioral studies on thio-THIP are currently awaiting the synthesis of sufficient quantities of compound.

Toward Rational Design of Specific $GABA_A$ Receptor Ligands

Molecular pharmacological studies using recombinant heteromeric $GABA_A$ receptors (see earlier section) on BZDs that already have major therapeutic importance should be accompanied by similar studies on ligands interacting directly with the $GABA_A$ receptor recognition site(s). Such studies have now been initiated, and the effects of thio-THIP (**19**) on recombinant $GABA_A$ receptors of well-defined subunit composition (see previous section) suggest that such studies may result in novel classes of $GABA_A$ receptor ligands. It may be

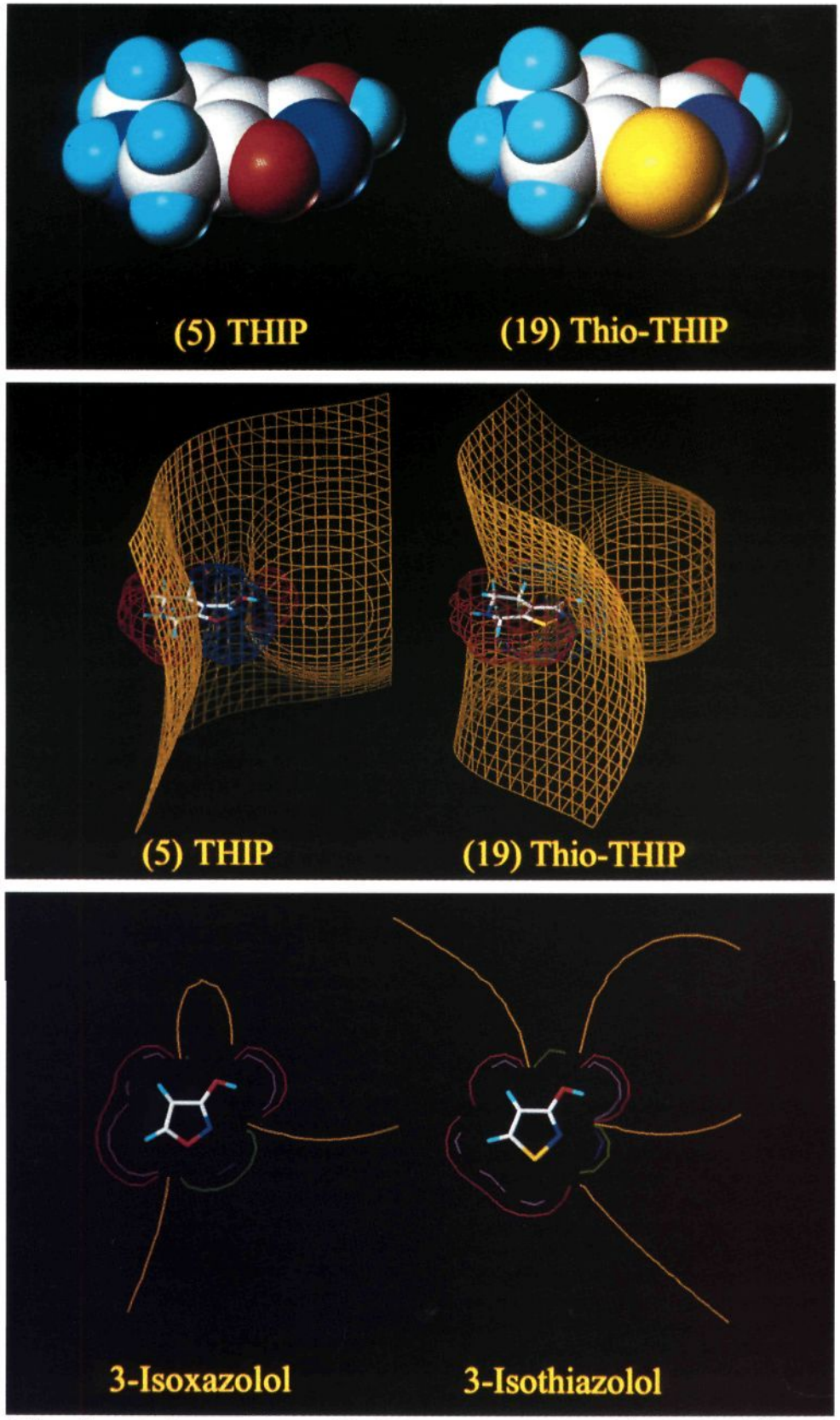
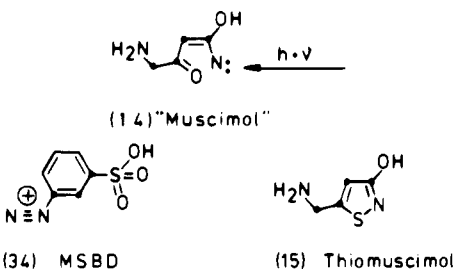


Figure 9. (Top) Three-dimensional representations of the $GABA_A$ agonists THIP (**5**) and thio-THIP (**19**), illustrating the structural similarity of the compounds. (Middle) Three-dimensional representations of the electrostatic potentials of the $GABA_A$ agonists THIP (**5**) and thio-THIP (**19**) illustrating the difference in electrostatic characteristics of the compounds. The electrostatic potentials are based on PM3 calculated charges and contoured at −5 (blue), 0 (yellow), and 5 kcal/mol (red). (Bottom) Two-dimensional representations of the electrostatic potentials of the bioisosteric ring systems 3-hydroxyisoxazole and 3-hydroxyisothiazole. The electrostatic potentials are based on PM3 calculated charges and contoured at −10 (blue), −5 (green), 0 (yellow), 5 (red), and 10 kcal/mol (magenta) in the plane of the ring systems.

Scheme 4



anticipated that research along these lines may represent the initial steps in the development of new types of antiepileptics, analgesics, and learning- and memory-improving drugs.

These aspects represent major drug design challenges. There is an urgent need for $GABA_A$ agonists, partial agonists, and antagonists with specific effects at physiologically relevant and pharmacologically important $GABA_A$ receptors of different subunit composition. The observations that THIP (**5**) binds selectively to a β-subunit of such receptors[165] and that affinity as well as relative efficacy of partial $GABA_A$ agonists is dependent on the receptor subunit composition[166] suggest that this is not an unrealistic objective.

In the $GABA_A$ receptor field there are many examples of design of specific receptor ligands following systematic stereochemical and bioisosteric approaches. Identification and topographical analysis of the $GABA_A$ recognition site(s) using molecular modeling and X-ray crystallography may allow rational design of new specific drugs in the future.

Identification of the $GABA_A$ recognition site(s) may be facilitated by the availability of agents capable of binding irreversibly to different amino acid residues at these sites. *m*-Benzenesulfonic acid diazonium chloride (MSBD, **34**) (Scheme 4) has been introduced as a compound capable of alkylating $GABA_A$ binding sites.[167] Muscimol (**14**) has been used with varying success as a photoaffinity label of $GABA_A$ receptor sites. It has been proposed that photochemical cleavage of the N—O bond converts muscimol into chemically reactive species at the receptor sites[168] (Scheme 4). More recently, thiomuscimol (**15**) (Scheme 1) has been shown to be an effective photolabel for the $GABA_A$ receptor.[169]

Molecular biology techniques have revealed a very high degree of heterogeneity of the $GABA_A$ receptors. The challenge for medicinal chemists is to further develop these observations into rational drug design projects and to develop receptor ligands, which show specificity and bioavailability at the level of isoreceptors.

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Arne Schousboe (D.Sc.) is professor of biochemistry and neurobiology at the Royal Danish School of Pharmacy. He is primarily interested in biosynthetic and metabolic aspects of GABA and glutamic acid and the receptors for these neurotransmitters. His expertise includes cell culture systems. Dr. Schousboe has established a number of projects on the biochemical and cellular pharmacology of GABA uptake inhibitors and ligands for GABA and excitatory amino acid receptors.

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