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# $GABA_A$ agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic

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**Abstract**

The $GABA_A$ receptor system is implicated in a number of central nervous system (CNS) disorders, making $GABA_A$ receptor ligands interesting as potential therapeutic agents. Only a few different classes of structures are currently known as ligands for the GABA recognition site on the hetero-pentameric $GABA_A$ receptor complex, reflecting the very strict structural requirements for $GABA_A$ receptor recognition and activation. A large number of the compounds showing agonist activity at the $GABA_A$ receptor site are structurally derived from the $GABA_A$ agonists muscimol, THIP (Gaboxadol), or isoguvacine, which we developed at the initial stage of the project. Using recombinant $GABA_A$ receptors, functional selectivity has been shown for a number of compounds, including THIP, showing subunit-dependent potency and maximal response. The pharmacological and clinical activities of THIP probably reflect its potent effects at extrasynaptic $GABA_A$ receptors insensitive to benzodiazepines and containing $\alpha_4\beta_3\delta$ subunits. The results of ongoing clinical studies on the effect of the partial $GABA_A$ agonist THIP on human sleep pattern show that the functional consequences of a directly acting agonist are distinctly different from those seen after administration of $GABA_A$ receptor modulators, such as benzodiazepines. In the light of the interest in partial $GABA_A$ receptor agonists as potential therapeutics, structure-activity studies of a number of analogues of 4-PIOL, a low-efficacy partial $GABA_A$ agonist derived from THIP, have been performed. In this connection, a series of $GABA_A$ ligands has been developed showing pharmacological profiles ranging from low-efficacy partial $GABA_A$ agonist activity to selective antagonist effect.


*Keywords:* $GABA_A$ receptor; Agonist; Partial agonist; Functional selectivity; THIP (Gaboxadol); 4-PIOL; Muscimol; Clinical studies; Novel hypnotic effects; Non-opioid analgesia

## 1. GABA receptors: multiplicity, structure and function

The discovery of GABA in the early fifties and the identification of the alkaloid bicuculline [1] and its quaternized analogue bicuculline methochloride (BMC) [2] as competitive GABA antagonists in central nervous system (CNS) tissues initiated the pharmacological characterization of GABA receptors. The subsequent design of isoguvacine, 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, Gaboxadol) [3] (Fig. 1) and piperidine-4-sulphonic acid (P4S) [4] as a novel class of specific GABA agonists further stimulated studies of the pharmacology of the GABA receptors.

The GABA analogue baclofen did, however, disturb the picture of a uniform class of GABA receptors. Baclofen, which was designed as a lipophilic analogue of GABA capable of penetrating the blood-brain barrier (BBB), is an antispastic agent, but its GABA agonistic effect could not be antagonized by BMC. In the early eighties, Bowery and co-workers demonstrated that baclofen, or rather (*R*)-(−)-baclofen, was selectively recognized as an agonist by a distinct sub-population of GABA receptors, which was named $GABA_B$ receptors [5]. Cloning of the $GABA_BR1$ and $GABA_BR2$ receptors and the identification of functional heterodimeric $GABA_B$ receptors have greatly stimulated $GABA_B$ receptor research [6,7]. The ‘‘classical’’

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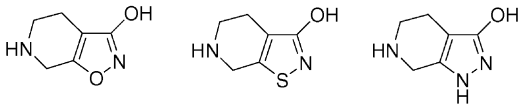


Fig. 1. Structures of the $GABA_A$ agonist/partial agonist THIP, the $GABA_A$ partial agonist/antagonist Thio-THIP, and the $GABA_C$ antagonist Aza-THIP.

BMC-sensitive GABA receptors were designated $GABA_A$ receptors. This receptor classification represents an important step in the development of the pharmacology of GABA.

During this period, the exploration of the GABA receptors was dramatically intensified by the observation that the binding site for the benzodiazepines (BZDs) [8,9] was associated with the $GABA_A$ receptors. After the cloning of a large number of $GABA_A$ receptor subunits, this area of the pharmacology of GABA continues to be in a state of very rapid development [10].

In connection with the design of conformationally restricted analogues of GABA, another "disturber of the peace" appeared on the GABA scene, namely *cis*-4-aminobut-2-enoic acid (CACA). This compound and the structurally related GABA analogue, *cis*-2-aminomethylcyclopropanecarboxylic acid (CAMP) are GABA-like neuronal depressants, which are not sensitive to BMC, and they bind to a class of GABA receptor sites, which neither recognize isoguvacine nor (*R*)-(−)-baclofen. The phosphinic acid analogue of isoguvacine, TPMPA [11] and, more recently, 4,5,6,7-tetrahydropyrazolo[5,4-*c*]pyridin-3-ol (Aza-THIP) [12] (Fig. 1) have been shown to be selective antagonists at $GABA_C$ receptors [13]. These receptors have been named $GABA_C$ receptors or non-$GABA_A$, non-$GABA_B$ (NANB) receptors for GABA [11,13].

The $GABA_A$ receptor complex is a pentameric structure formed by co-assembly of subunits from seven different classes ($\alpha_{1-6}$, $\beta_{1-3}$, $\gamma_{1-3}$, $\delta$, $\varepsilon$, $\theta$, $\rho_{1-3}$). The five subunits, which in native receptors usually are constituted by two $\alpha$, two $\beta$ and a $\gamma$, $\delta$ or $\varepsilon$ subunit, are situated in a circular array surrounding a central chloride-permeable pore [14].

Several studies have demonstrated the involvement of GABA and $GABA_A$ receptors in diseases like seizures, depression, anxiety and sleep disorders [14,15]. GABA and other directly acting $GABA_A$ receptor agonists (GABA-mimetics) bind specifically to a recognition site located at the interface between an $\alpha$ and a $\beta$ subunit [16–18] whereas the classical BZDs, such as diazepam, flunitrazepam, as well as the novel BZD ligands with a non-benzodiazepine structure as for example zaleplon, zolpidem, zopiclone and indiplon, bind to an allosteric site located at the interface between an $\alpha$ and a $\gamma$ subunit [16,18,19].

The quantitatively most predominant $GABA_A$ receptor subunit combination throughout the brain is comprised of $\alpha_1\beta_{2/3}\gamma_2$ subunits, primarily synaptically located [20–23]. However, recently, much attention has been focused on the less abundant but ostensibly functionally important receptor subtypes with a more restricted location and specialized function. Thus, the primarily extrasynaptically located $\alpha_4\beta_3\delta$ receptors, which are found in high concentrations in hippocampus and thalamus but also in neocortex [22,24], seem to play a key role in sleep. Results from our ongoing research projects have suggested that these receptors are the main target for THIP (Gaboxadol), which at present is subjected to clinical studies as a hypnotic.

Whereas Gaboxadol behaves as a partial agonist ($E_{max}$ = 70%) with a fairly low potency ($EC_{50}$ = 238 μM) at $\alpha_1\beta_3\gamma_{2S}$ receptors expressed in *Xenopus* oocytes [17,25], it acts as a highly potent ($EC_{50}$ = 1.3 μM) full agonist in the rat cortical wedge preparation [26]. Based on a series of in vitro and in vivo pharmacological studies [27,28] it is hypothesized that Gaboxadol and the BZDs interact with two distinct receptor populations: the benzodiazepines with synaptically located $\alpha_1\beta_3\gamma_{2S}$ receptors and Gaboxadol with extrasynaptically located $\alpha_4\beta_3\delta$ receptors. Hence, the effects exerted at these separate populations will sum in an additive rather than a supra-additive manner. A functional selectivity for $\alpha_4\beta_3\delta$ receptors at which Gaboxadol acts as a highly potent agonist with an $E_{max}$ of 165%, also explains that apparent potent and efficacious behaviour of Gaboxadol in the rat cortical wedge preparation.

$\alpha_4\beta_x\delta$ receptors have proven difficult to express in recombinant system and therefore only a few studies investigating the pharmacology of this receptor construct have been published. However, recently a novel murine L(tk) cell line stably expressing $\alpha_4\beta_3\delta$ receptors has been extensively used to study the basic pharmacology of this receptor subtype [29,30]. Other groups have published results from more specific studies in *Xenopus* oocytes expressing $\alpha_4\beta_{2/3}\delta$ receptors [31,32]. More recently, a broad spectrum of agonists have been found to behave as full and partial agonists in $\alpha_{1-6}\beta_x\gamma_2$ receptors expressed in *Xenopus* oocytes [17,25,33].

## 2. $GABA_A$ agonists

The basically inhibitory nature of the central GABA neurotransmission prompted the design and development of different structural types of GABA agonists. The 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 [34,35].

Muscimol, a constituent of the mushroom *Amanita muscaria*, has been extensively used as a lead for the design of different classes of GABA analogues (Fig. 2). The 3-isoxazolol carboxyl group bioisostere of muscimol

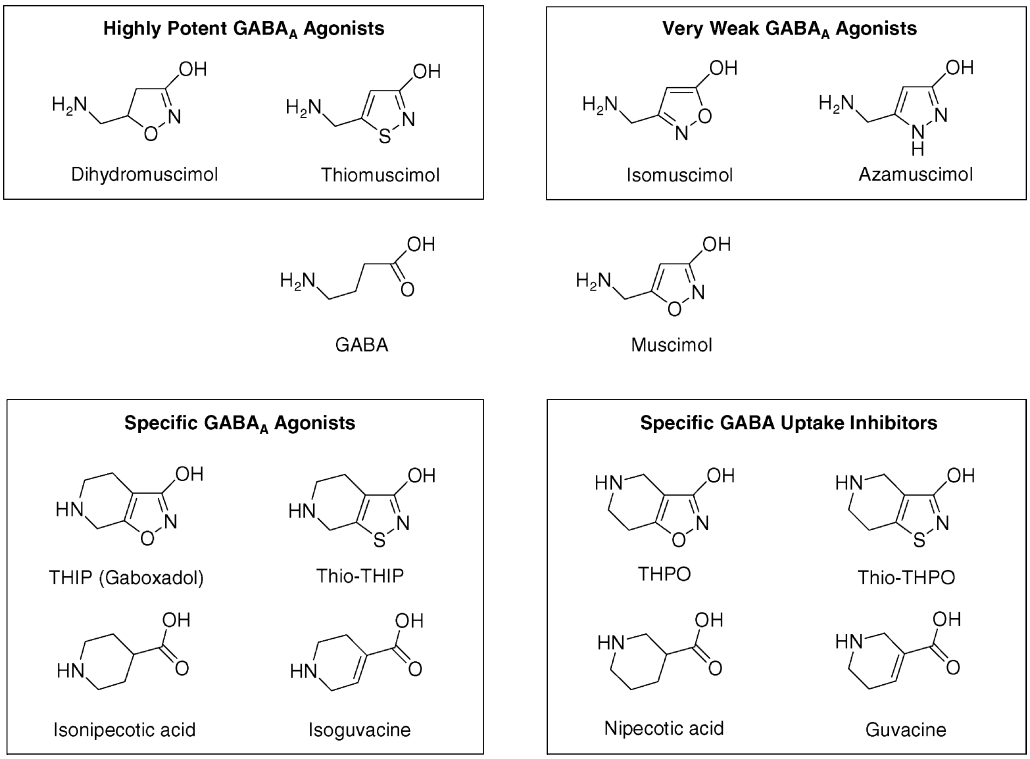


Fig. 2. Comparison of the structures of some $GABA_A$ agonists and GABA uptake inhibitors.

can be replaced by a 3-isothiazolol or 3-hydroxyisoxazoline group to give thiomuscimol and dihydromuscimol, respectively, without significant loss of $GABA_A$ receptor agonism [36]. (*S*)-Dihydromuscimol is the most potent $GABA_A$ agonist so far described [37]. The structurally related muscimol analogues, isomuscimol and azamuscimol, on the other hand are virtually inactive, emphasizing the very strict structural constraints imposed on agonist molecules by the $GABA_A$ receptors [36].

The conversion of muscimol into THIP (Gaboxadol) (Figs. 2 and 3) [3] and the isomeric compound 4,5,6,7-tetrahydroisoxazole[4,5-*c*]pyridin-3-ol (THPO) effectively separated $GABA_A$ receptor and GABA uptake affinity, THIP being a specific $GABA_A$ agonist and THPO a GABA uptake inhibitor (Fig. 2) [38].

Using THIP as a lead, a series of specific monoheterocyclic $GABA_A$ agonists, including isoguvacine and isonipecotic acid, was developed (Fig. 2) [3,36]. Whereas Thio-THIP (Fig. 1) shows $GABA_A$ agonist effects on cat spinal neurons [39], recent studies using human brain recombinant $GABA_A$ receptors have disclosed very low-efficacy partial agonism/antagonism of Thio-THIP [40].

In light of the structural similarity of THIP and Thio-THIP (Fig. 1) the markedly different pharmacology of these compounds is noteworthy and emphasizes the strict structural requirements of $GABA_A$ receptors. The $pK_a$ values of THIP (4.4; 8.5) and Thio-THIP (6.1; 8.5) [39] are different, and a significant fraction of the molecules of the latter compound must contain a non-ionized 3-isothiazolol 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 may have to be considered in order to explain their different potency and efficacy at $GABA_A$ receptors.

A series of cyclic amino acids derived from THPO, including nipecotic acid [38] and guvacine [41], was developed as GABA uptake inhibitors. Whereas nipecotic acid and guvacine potently inhibit neuronal as well as glial GABA uptake [42], THPO interacts selectively with the latter uptake system. Thio-THPO is slightly weaker than THPO as an inhibitor of GABA uptake [39].

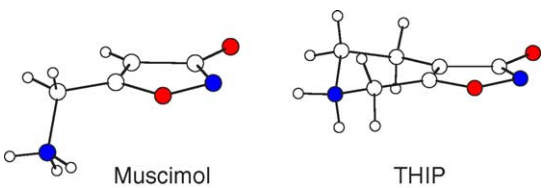


Fig. 3. The structures of muscimol and THIP as determined by X-ray crystallographic analyses (from The Chemical Record 2002;2: 419–30).

## 3. Partial $GABA_A$ agonists

Under clinical conditions where stimulation of the $GABA_A$ receptor system may be relevant, partial agonists displaying a relatively high efficacy may be particularly useful. The level of efficacy needed may be dependent on

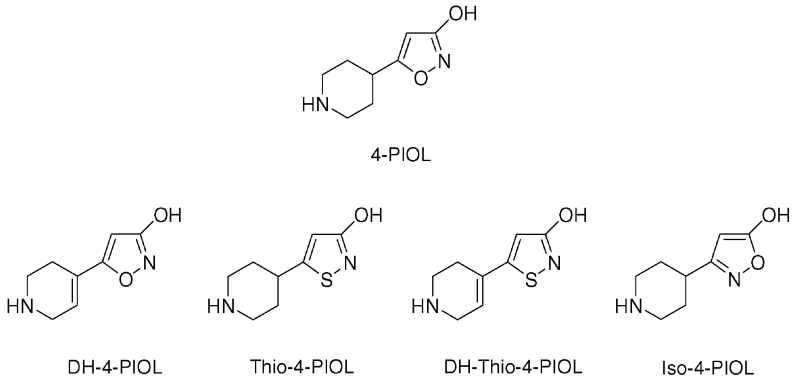


Fig. 4. Structures of the low-efficacy partial $GABA_A$ agonist, 4-PIOL, and a number of analogues.

the disease in question. The potent analgesic effects of THIP (Gaboxadol) (see later section) seem to indicate that this relatively high level of efficacy [43,44] is close to optimal with respect to treatment of pain.

Analogously, very low-efficacy $GABA_A$ agonists showing predominant antagonist profiles may have clinical interest in conditions where a reduction in $GABA_A$ receptor activity may be needed. Such compounds may produce sufficient $GABA_A$ receptor activity to avoid side effects.

The non-fused THIP analogue, 5-(4-piperidyl)isoxazol-3-ol (4-PIOL) (Fig. 4), is a low affinity $GABA_A$ agonist [45], which has been characterised as a partial $GABA_A$ agonist using functional patch-clamp techniques on cultured cerebral cortical and hippocampal neurones [46,47]. In cortical neurones, the action of 4-PIOL was compared with those of the $GABA_A$ agonist isoguvacine and the $GABA_A$ antagonist BMC [46]. Based on these studies, it is concluded that 4-PIOL is a low-efficacy partial agonist showing a predominant $GABA_A$ antagonist profile, being about 30-fold weaker than BMC as an antagonist at the $GABA_A$ receptors. Recently, 4-PIOL has been proposed to possess some subtype-specific characteristics [48]. On recombinant $GABA_A$ receptors of the $\alpha_1\beta_2\gamma_2$ subtype expressed in HEK-293 cells, 4-PIOL acted as a weak agonist, whereas it was devoid of activity in the $\alpha_6\beta_2\gamma_2$ receptor subtype.

## 4. 4-PIOL analogues as $GABA_A$ antagonists

Introduction of alkyl groups into the 4-position of the 3-isoxazolol ring of muscimol and THIP severely inhibits interaction with the $GABA_A$ receptor recognition site as illustrated in Fig. 5. Thus, 4-Me-muscimol is three to four orders of magnitude weaker than muscimol as an inhibitor of $GABA_A$ receptor binding, whereas 4-Et-muscimol [49], and also 4-Me-THIP [50] are inactive. In contrast, the $GABA_A$ recognition site tolerates introduction of alkyl groups into the 4-position of the 3-isoxazolol ring of 4-PIOL [51]. These structure-activity relationships indicate that the binding modes of the $GABA_A$ agonists, muscimol and THIP and in particular of the low-efficacy partial agonist 4-PIOL are different.

There is strong evidence that an arginine residue at the $GABA_A$ receptor recognition site is directly involved in the binding of the anionic part of the receptor ligand [52]. Based on this observation, a hypothesis has been proposed concerning the binding modes of the bioactive conformations of muscimol and 4-PIOL [51] as illustrated in Fig. 6. In these binding modes, the two 3-isoxazolol rings do not overlap. This means that the 4-position of the 3-isoxazolol ring in muscimol does not correspond to the 4-position in the 3-isoxazolol ring of 4-PIOL during interaction of muscimol and 4-PIOL with the receptor recognition site.

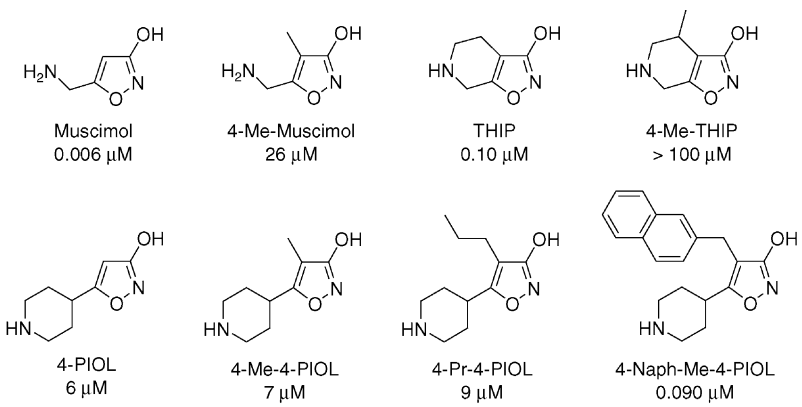


Fig. 5. $GABA_A$ agonist binding data ($IC_{50}$, μM) for muscimol, THIP, 4-PIOL and some analogues of these $GABA_A$ agonist ligands.

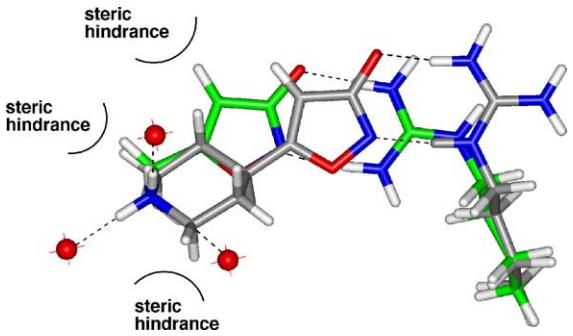


Fig. 6. A pharmacophore model for $GABA_A$ receptor agonists showing the proposed binding modes of muscimol (green bonds) and 4-PIOL (light grey bonds) and their interactions with two different conformations of an arginine residue. The red spheres indicate sites to which the ammonium group in muscimol interacts via hydrogen bonds (from The Chemical Record 2002;2: 419–30).

A number of analogues of 4-PIOL have been synthesized with substituents in the 4-position of the 3-isoxazolol ring in order to further investigate the steric tolerance of this position [53]. The results from these studies are exemplified in Fig. 5. Substitution of the 4-position with alkyl or benzyl groups resulted in affinity and potencies comparable with those of 4-PIOL. Interestingly, however, introduction of more bulky groups such as diphenylalkyl and naphthylalkyl groups, as exemplified by the 2-naphthylmethyl analogue, 4-Naph-Me-4-PIOL are not only tolerated but resulted in a marked increase both in affinity and potency.

Using whole-cell patch-clamp techniques on cultured cerebral cortical neurones in the electrophysiological testing, the pharmacology of the 4-PIOL analogues in the absence or presence of the specific $GABA_A$ receptor agonist isoguvacine was studied [53]. The results demonstrated that the structural modifications led to a change in the pharmacological profile of the compounds from moderately potent low-efficacy partial $GABA_A$ receptor agonist activity to potent and selective antagonist effect. The 2-naphthylmethyl and the 4-biphenylmethyl analogues (Fig. 7) showed antagonist potency comparable with or markedly higher than that of the standard $GABA_A$ antagonist gabazine.

These structure-activity studies seem to support the proposed hypothesis concerning the distinct binding mode of 4-PIOL, implying that the 4-position in 4-PIOL does not correspond to the 4-position in muscimol (Figs. 5 and 6). Thus, a cavity of considerable binding capacity seems to exist at the 4-PIOL recognition site of the $GABA_A$ receptor.

Molecular modeling studies of the two high affinity compounds containing a 2-naphthylmethyl and a 3,3-diphenylpropyl substituent, and the less active 1-naphthylmethyl and 4-biphenylmethyl analogues, indicate that this proposed binding cavity may be exploited in two directions (Fig. 7). In both of these positions an aromatic ring seems to be highly favourable for the receptor affinity [53].

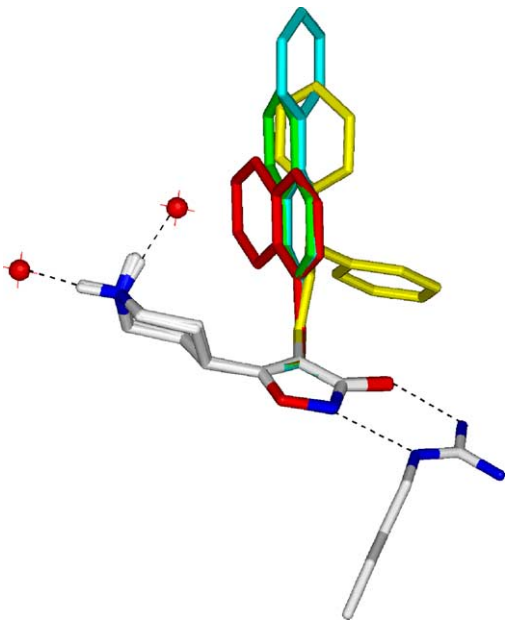

Fig. 7. Proposed bioactive conformations for the high affinity 1-naphtylmethyl, 2-naphthylmethyl, 3,3-diphenylpropyl, and 4-biphenylmethyl analogues of 4-PIOL (from The Chemical Record 2002;2: 419–30).

As mentioned earlier, the GABA binding site in the $GABA_A$ receptor is assumed to be located at the interface between α and β subunits [54]. It has been speculated that the $GABA_A$ antagonists bind to and stabilize a distinct inactive receptor conformation. In case of the 4-arylalkyl substituted 4-PIOL analogues it may be speculated that the large cavity accommodating the 4-substituent is located in the space between these subunits. $GABA_A$ receptors belong to the same superfamily as the nicotinic acetylcholine receptors. It has been proposed that the mechanism for ligand-induced channel opening in nicotinic acetylcholine receptors involves rotations of the subunits in the ligand binding domain [55]. Assuming that the $GABA_A$ receptors utilize a similar mechanism for channel opening, large substituents may interfere with the channel opening resulting in antagonistic effects of the compounds [53].

## 5. Behavioural and clinical effects of the partial $GABA_A$ agonist THIP (Gaboxadol)

### 5.1. 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 [34,56]. The demonstration of potent antinociceptive effects of the specific and metabolically stable partial $GABA_A$ agonist THIP (Gaboxadol) in different animal models and the potent analgesic effects of Gaboxadol in man greatly stimulated studies in this area of pain research. Gaboxadol-induced analgesic effects were shown to be insensi-

tive to the opioid antagonist naloxone indicating that these effects are not mediated by the opioid receptors [57].

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

In cancer patients and also in patients with chronic anxiety [58] the desired effects of Gaboxadol were accompanied by side effects, notably sedation, nausea, and in a few cases euphoria. The side effects of Gaboxadol have, however, been described as mild and similar in quality to those of other GABA-mimetics [58]. This combination of analgesic and anxiolytic effects of Gaboxadol obviously has therapeutic prospects.

The neuronal and synaptic mechanisms underlying Gaboxadol- and, in general, GABA-induced analgesia are still only incompletely understood. The insensitivity of Gaboxadol-induced analgesia to naloxone has been consistently demonstrated. 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 neurones 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 opioid drug addicts [56].

### 5.2. THIP (Gaboxadol) and sleep disorders

GABAergic compounds acting at the barbiturate site, the neurosteroid site, or the BZD site of the $GABA_A$ receptor complex have been used as hypnotics for years. Most of these hypnotics interact with all subtypes of receptors. However, as $\alpha_4$ and $\delta$ containing receptors do not bind BZDs, effect of BZDs at these receptor subtypes is absent [59]. It is generally agreed that the sedative effect of BZDs is mediated primarily via $\alpha_1$ containing receptors, whereas side effects related to amnesia may be mediated by $\alpha_5$, primarily located in the hippocampal region [60]. However, short-acting $\alpha_1$ selective BZD ligands like zaleplon, zopiclone and zolpidem do produce memory impairment and hangover effects [61], suggesting that even an $\alpha_1$ selective compound with a very short half life may produce side effects. The reason for this side effect profile may well be a consequence of the high degree of GABA receptor activation caused by the positive $GABA_A$ receptor modulator. The massive activation of GABA receptors will influence several other systems, ultimately resulting in a general acute modification of the overall function of the CNS. Electroencephalographic (EEG) measurements during sleep support this hyphotesis. A normal sleep pattern involves a complex variation of different degrees of sleep, ranging from light sleep via deeper sleep stages to the dream-associated rapid eye movement (REM) stage of sleep. Present understanding of sleep quality and the relation to EEG patterns is still limited, however, not only the duration of REM sleep but also the transitions between the different sleep stages are important [61]. The effect of BZDs at the sleep micro architecture is not limited to the onset of REM sleep, which is delayed.

In contrast to the observations with BZDs, barbiturates and neurosteroids, a series of studies have shown that this perturbation of the sleep micro architecture may not arise with compounds acting directly at the $GABA_A$ recognition site [62,63]. In this respect, Gaboxadol is an interesting compound. Gaboxadol appears to improve the quality of sleep, as measured using behavioural parameters. The compound shows no effect on the onset of REM sleep, as measured using EEG [62,63]. Furthermore, studies in elderly patients, suffering from significant reduction in non-REM sleep, showed that Gaboxadol was able to normalize the sleep pattern [64]. Following dosing with Gaboxadol, patients did not experience the hangovers or impaired attention as reported for BZDs. Similar results have been obtained with muscimol, with the GABA uptake inhibitor Tiagabine [65], and with the glia-selective GABA uptake inhibitor, THPO (Fig. 2) [66], strongly suggesting that the functional consequences of a direct acting agonist or enhanced synaptic GABA concentration are different from those seen with $GABA_A$ receptor modulators.

## 6. Conclusion

Molecular biology studies have revealed a high degree of structural heterogeneity of the $GABA_A$ receptors. Development of subtype selective or specific compounds is of key importance for the understanding of the physiological and pathological roles of different GABA receptor subtypes and may lead to valuable therapeutic agents. Studies along these lines have, so far, been complicated by the lack of information about the topography of the recognition site(s) of the $GABA_A$ receptor complex. In the absence of a crystal structure of the $GABA_A$ receptor complex, 3D-pharmacophore models, based on the analysis of known receptor ligands, have been useful tools in the design of new ligands. Recombinant receptors play a key role in the pharmacological characterization of ligands for the $GABA_A$ receptor. However, problems regarding subunit composition of native receptors, are far from being fully elucidated. Furthermore, the subtypes of receptors

involved in different disorders are still largely unknown. Although the functional consequences of modifications of subunit compositions, so far, is unpredictable, it has been shown that functional selectivity is obtainable for a number of $GABA_A$ agonists. An important aspect is the selection of subunit combinations relevant for the prediction of in vivo activity.

Neither full $GABA_A$ agonists nor antagonists may be useful therapeutic agents. Whereas the high-efficacy partial $GABA_A$ agonist Gaboxadol shows very potent non-opioid analgesic effects and a novel type of hypnotic effects in the human clinic, it seems likely that partial $GABA_A$ agonists showing lower levels of efficacy such as 4-PIOL analogues, may have therapeutic interest in certain CNS disorders such as schizophrenia. Information about the basic mechanism of receptor–ligand interaction resulting in partial agonism is, however, still not available, making design of new partial $GABA_A$ agonists on a rational basis difficult.

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## References

[1] Curtis DR, Duggan AW, Felix D, Johnston GAR. Bicuculline and central inhibition. Nature 1970;226:1222–4.
[2] Johnston GAR, Beart PM, Curtis DR, Game CJA, McCulloch RM, MacLachlan RM. Bicuculline methochloride as a GABA antagonist. Nature New Biol 1972;240:219–20.
[3] Krogsgaard-Larsen P, Johnston GAR, Lodge D, Curtis DR. A new class of GABA agonist. Nature 1977;268:53–5.
[4] Krogsgaard-Larsen P, Snowman A, Lummis SC, Olsen RW. Characterization of the binding of the GABA agonist [$^3$H]piperidine-4-sulfonic acid to bovine brain synaptic membranes. J Neurochem 1981;37:401–9.
[5] Bowery NG, Hill DR, Hudson AL, Doble A, Middlemiss DN, Shaw J, et al. (−)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 1980;283:92–4.
[6] Martin SC, Russek SJ, Farb DH. Molecular identification of the human $GABA_B$R2: cell surface expression and coupling to adenylyl cyclase in the absence of $GABA_B$R1. Mol Cell Neurosci 1999;13:180–91.
[7] Möhler H, Fritschy J-M. $GABA_B$ receptors make it to the top–as dimers. Trends Pharmacol Sci 1999;20:87–9.
[8] Möhler H, Okada T. Benzodiazepine receptors: demonstration in the central nervous system. Science 1977;198:849–51.
[9] Squires RF, Braestrup C. Benzodiazepine receptors in rat brain. Nature 1977;266:732–4.
[10] Egebjerg J, Schousboe A, Krogsgaard-Larsen P. Glutamate and GABA Receptors and Transporters: Structure, Function and Pharmacology. London: Taylor & Francis; 2002.
[11] Murata Y, Woodward RM, Miledi R, Overman LE. The first selective antagonist for a $GABA_C$ receptor. Bioorg Med Chem Lett 1996;6:2073–6.
[12] Krehan D, Frølund B, Ebert B, Nielsen B, Krogsgaard-Larsen P, Johnston GAR, et al. Aza-THIP and related analogues of THIP as $GABA_C$ antagonists. Bioorg Med Chem 2003;11:4891–6.
[13] Johnston GAR. $GABA_A$ receptor pharmacology. Pharmacol Ther 1996;69:173–98.
[14] Sieghart W, Fuchs K, Tretter V, Ebert V, Jechlinger M, Höger H, et al. Structure and subunit composition of $GABA_A$ receptors. Neurochem Int 1999;34:379–85.
[15] Thomsen C, Ebert B. Modulators of the $GABA_A$ receptor complex: novel therapeutic aspects. In: Egebjerg J, Schousboe A, Krogsgaard-Larsen P, editors. Glutamate and GABA Receptors and Transporters. London: Taylor & Francis; 2002. p. 407–27.
[16] Amin J, Weiss DS. $GABA_A$ receptor needs two homologous domains of the β-subunit for activation by GABA but not by pentobarbital. Nature 1993;366:565–9.
[17] Ebert B, Wafford KA, Whiting PJ, Krogsgaard-Larsen P, Kemp JA. Molecular pharmacology of gamma-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different α, β, and γ receptor subunit combinations. Mol Pharmacol 1994;46:957–63.
[18] Amin J, Brooks-Kayal A, Weiss DS. Two tyrosine residues on the α subunit are crucial for benzodiazepine binding and allosteric modulation of gamma-aminobutyric acid A receptors. Mol Pharmacol 1997;51:833–41.
[19] Duncalfe LL, Carpenter MR, Smillie LB, Martin IL, Dunn SM. The major site of photoaffinity labeling of the gamma-aminobutyric acid type A receptor by [$^3$H]flunitrazepam is histidine 102 of the α subunit. J Biol Chem 1996;271:9209–14.
[20] Fritschy JM, Möhler H. $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. J Comp Neurol 1995;359:154–94.
[21] Nusser Z, Roberts JD, Baude A, Richards JG, Somogyi P. Relative densities of synaptic and extrasynaptic $GABA_A$ receptors on cerebellar granule cells as determined by a quantitative immunogold method. J Neurosci 1995;15:2948–60.
[22] Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G. $GABA_A$ receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 2000;101:815–50.
[23] Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. J Neurosci 1992;12:1040–62.
[24] Sur C, Farrar SJ, Kerby J, Whiting PJ, Atack JR, Mckernan RM. Preferential coassembly of $\alpha_4$ and δ subunits of the gammaaminobutyric acidA receptor in rat thalamus. Mol Pharmacol 1999;56:110–5.
[25] Ebert B, Thompson SA, Saounatsou K, Mckernan R, Krogsgaard-Larsen P, Wafford KA. Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human gamma-aminobutyric acid type A receptors. Mol Pharmacol 1997;52:1150–6.
[26] Ebert B, Stórustovu S, Mortensen M, Frølund B. Characterization of $GABA_A$ receptor ligands in the rat cortical wedge preparation: evidence for action at extrasynaptic receptors? Br J Pharmacol 2002;137:1–8.
[27] Stórustovu S, Ebert B. Gaboxadol: in vitro interaction studies with benzodiazepines and ethanol suggest functional selectivity. Eur J Pharmacol 2003;467:49–56.
[28] Voss J, Sánchez C, Michelsen S, Ebert B. Rotarod studies in the rat of the $GABA_A$ receptor agonist Gaboxadol: lack of ethanol potentiation and benzodiazepine cross-tolerance. Eur J Pharmacol 2003;482:215–22.
[29] Adkins CE, Pillai GV, Kerby J, Bonnert TP, Haldon C, Mckernan RM, et al. α, 4β, 3δ $GABA_A$ receptors characterized by fluorescence resonance energy transfer-derived measurements of membrane potential. J Biol Chem 2001;276:38934–9.

[30] Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human α, 4β, 3δ $GABA_A$ receptors. Br J Pharmacol 2002;136:965–74.
[31] Sundström-Poromaa I, Smith DH, Gong QH, Sabado TN, Li X, Light A, et al. Hormonally regulated α, 4β, 3δ $GABA_A$ receptors are a target for alcohol. Nat Neurosci 2002;5:721–2.
[32] Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances α, 4β, 3δ and α, 6β, 3δ γ-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci USA 2003;100: 15218–23.
[33] Ebert B, Mortensen M, Thompson SA, Kehler J, Wafford KA, Krogsgaard-Larsen P. Bioisosteric determinants for subtype selectivity of ligands for heteromeric $GABA_A$ receptors. Bioorg Med Chem Lett 2001;11:1573–7.
[34] Krogsgaard-Larsen P, Frølund B, Kristiansen U, Frydenvang K, Ebert B. $GABA_A$ and $GABA_B$ receptor agonists, partial agonists, antagonists and modulators: design and therapeutic prospects. Eur J Pharm Sci 1997;5:355–84.
[35] Krogsgaard-Larsen P, Frølund B, Jørgensen FS, Schousboe A. $GABA_A$ receptor agonists, partial agonists and antagonists. Design and therapeutic prospects. J Med Chem 1994;37:2489–505.
[36] Krogsgaard-Larsen P, Hjeds H, Curtis DR, Lodge D, Johnston GAR. Dihydromuscimol, thiomuscimol and related heterocyclic compounds as GABA analogues. J Neurochem 1979;32:1717–24.
[37] Krogsgaard-Larsen P, Nielsen L, Falch E, Curtis DR. GABA agonists. Resolution, absolute stereochemistry, and enantioselectivity of (*S*)-(+)- and (*R*)-(−)-dihydromuscimol. J Med Chem 1985;28:1612–7.
[38] Krogsgaard-Larsen P, Johnston GAR. Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. J Neurochem 1975;25:797–802.
[39] Krogsgaard-Larsen P, Mikkelsen H, Jacobsen P, Falch E, Curtis DR, Peet MJ, et al. 4,5,6,7-Tetrahydroisothiazolo[5,4-*c*]pyridin-3-ol and related analogues of THIP. Synthesis and biological activity. J Med Chem 1983;26:895–900.
[40] Brehm L, Ebert B, Kristiansen U, Wafford KA, Kemp JA, Krogsgaard-Larsen P. Structure and pharmacology of 4,5,6,7-tetrahydroisothiazolo[5,4-c]pyridin-3-ol (Thio-THIP), an agonist/antagonist at $GABA_A$ receptors. Eur J Med Chem 1997;32:357–63.
[41] Johnston GAR, Krogsgaard-Larsen P, Stephanson A. Betel nut constituents as inhibitors of γ-aminobutyric acid uptake. Nature 1975;258:627–8.
[42] Schousboe A, Thorbek P, Hertz L, Krogsgaard-Larsen P. Effects of GABA analogues of restricted conformation on GABA transport in astrocytes and brain cortex slices and on GABA Receptor Binding. J Neurochem 1979;33:181–9.
[43] Maksay G. Thermodynamics of γ-aminobutyric acid type A receptor binding differentiate agonists from antagonists. Mol Pharmacol 1994;46:386–90.
[44] Krogsgaard-Larsen P, Hjeds H, Falch E, Jørgensen FS, Nielsen L. Recent advances in GABA agonists, antagonists and uptake inhibitors: structure-activity relationships and therapeutic potential. Adv Drug Res 1988;17:381–456.
[45] Mortensen M, Kristiansen U, Ebert B, Frølund B, Krogsgaard-Larsen P, Smart TG. Activation of single heteromeric $GABA_A$ receptor ion channels by full and partial agonists. J Physiol 2004;557. 2:393–417.
[46] Kristiansen U, Lambert JDC, Falch E, Krogsgaard-Larsen P. Electrophysiological studies of the $GABA_A$ receptor ligand, 4-PIOL, on cultured hippocampal neurons. Br J Pharmacol 1991;104:85–90.
[47] Frølund B, Kristiansen U, Brehm L, Hansen AB, Krogsgaard-Larsen P, Falch E. Partial $GABA_A$ receptor agonists. Synthesis and in vitro pharmacology of a series of nonannulated analogs of 4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol (THIP). J Med Chem 1995;38: 3287–96.
[48] Rabe H, Picard R, Uusi-Oukari M, Hevers W, Luddens H, Korpi ER. Coupling between agonist and chloride ionophore sites of the $GABA_A$ receptor: agonist/antagonist efficacy of 4-PIOL. Eur J Pharmacol 2000;409:233–42.
[49] Krogsgaard-Larsen P, Johnston GAR. Structure-activity studies on the inhibition of GABA binding to rat brain membranes by muscimol and related compounds. J Neurochem 1978;30:1377–82.
[50] Haefliger W, Révész L, Maurer R, Römer D, Büscher H-H. Analgesic GABA agonists. Synthesis and structure-activity studies on analogues and derivatives of muscimol and THIP. Eur J Med Chem Chim Ther 1984;19:149–56.
[51] Frølund B, Tagmose L, Liljefors T, Stensbøl TB, Engblom C, Kristiansen U, et al. A novel class of potent 3-isoxazolol $GABA_A$ antagonists: design, synthesis and pharmacology. J Med Chem 2000;43:4930–3.
[52] Westh-Hansen SE, Witt MR, Dekermendjian K, Liljefors T, Rasmussen PB, Nielsen M. Arginine residue 120 of the human $GABA_A$ receptor $\alpha_1$ subunit is essential for GABA binding and chloride ion current gating. NeuroReport 1999;10:2417–21.
[53] Frølund B, Jørgensen AT, Tagmose L, Stensbøl TB, Vestergaard HT, Engblom C, et al. Novel class of potent 4-arylalkyl substituted 3-isoxazolol $GABA_A$ antagonists: synthesis, pharmacology, and molecular modeling. J Med Chem 2002;45:2454–68.
[54] Smith GB, Olsen RW. Functional domains of $GABA_A$ receptors. Trends Pharmacol Sci 1995;16:162–8.
[55] Unwin N. Acetylcholine receptor channel imaged in the open state. Nature 1995;373:37–43.
[56] DeFeudis FV. GABA agonists and analgesia. Drug News Perspect 1989;2:172–3.
[57] Zorn SH, Enna SJ. The GABA agonist THIP attenuates antinociception in the mouse by modifying central cholinergic transmission. Neuropharmacology 1987;26:433–7.
[58] Hoehn-Saric R. Effects of THIP on chronic anxiety. Psychopharmacology 1983;80:338–41.
[59] Wafford KA, Thompson SA, Thomas D, Sikela J, Wilcox AS, Whiting PJ. Functional characterization of human γ-aminobutyric $acid_A$ receptors containing the $\alpha_4$ subunit. Mol Pharmacol 1996;50:670–8.
[60] Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, et al. Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. Nature 1999;401:796–800.
[61] Landolt HP, Gillin JC. $GABA_A$ $\alpha_1$ receptors: involvement in sleep regulation and potential of selective agonists in the treatment of insomnia. CNS Drugs 2000;13:185–99.
[62] Lancel M, Cronlein TA, Faulhaber J. Role of $GABA_A$ receptors in sleep regulation. Differential effects of muscimol and midazolam on sleep in rats. Neuropsychopharmacology 1996;15:63–74.
[63] Lancel M, Faulhaber J. The $GABA_A$ agonist THIP (Gaboxadol) increases non-REM sleep and enhances delta activity in the rat. NeuroReport 1996;7:2241–5.
[64] Lancel M, Wetter TC, Steiger A, Mathias S. Effect of the $GABA_A$ agonist Gaboxadol on nocturnal sleep and hormone secretion in healthy elderly subjects. Am J Physiol Endocrinol Metab 2001;281:E130–7.
[65] Lancel M, Faulhaber J, Deisz RA. Effect of the GABA uptake inhibitor tiagabine on sleep and EEG power spectra in the rat. Br J Pharmacol 1998;123:1471–7.
[66] Juhász G, Kékesi KA, Emri Zs, Ujszászi J, Krogsgaard-Larsen P, Schousboe A. Sleep promoting effect of a putative glial γ-aminobutyric acid uptake blocker applied in the thalamus of cats. Eur J Pharmacol 1991;209:131–3.