Ionotropic GABA Receptors as Therapeutic Targets for Memory and Sleep Disorders

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Contents
1. Introduction 13
2. Ionotropic GABA receptors 14
  2.1. Molecular composition of ionotropic receptors 15
  2.2. GABA_A and GABA_C receptor pharmacology 15
  2.3. Modulators of ionotropic GABA receptors 16
3. Ionotropic GABA receptors and sleep disorders 16
4. Ionotropic GABA receptors and memory disorders 19
5. Conclusion 21
References 21

1. INTRODUCTION

The chemical diversity of agents acting on receptors for γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain, is substantial and increasing. This rich diversity offers both challenges and opportunities for medicinal chemists [1]. GABA produces neuronal inhibition by acting on two major types of receptors: ionotropic receptors that are ligand-gated ion channels (GABA_A and GABA_C receptors) [1], and metabotropic receptors that are G-protein coupled receptors (GABA_B receptors) that act via second messengers [2]. The ionotropic GABA_A and GABA_C receptors belong to the nicotinicoid superfamily of ligand-gated ion channels that includes nicotinic acetylcholine, strychnine-sensitive glycine and 5HT_3 receptors [3]. Although GABA_C receptors are sometimes classified as subtypes of GABA_A receptors, they differ in their ability to form endogenous heteromeric and homomeric receptors respectively, and in their physiological and pharmacological properties [4].

There is also a significant diversity of ionotropic GABA receptor subtypes composed of different protein subunits. The discovery of subtype specific agents is a major challenge in the continuing development of ionotropic GABA receptor pharmacology. Leads for the discovery of new chemical entities that selectively influence ionotropic GABA receptors come from using recombinant receptors of known subunit composition and the use of genetically modified mice [5]. This has been elegantly demonstrated in...
mice with mutant α1 subunits that show the normal anxiolytic responses to benzodiazepines but not the sedative effects. Mice with mutant α2 subunits show the sedative but not the anxiolytic effects of benzodiazepines. Positive allosteric modulators of GABA_A receptor function that are selective for α1 subunits show non-anxiolytic sedative properties, e.g. 1 zolpidem [6]. While sufficiently selective α2 subunit agents are yet to be developed there are non-sedating anxiolytics, e.g. 2, 7,8,9,10-tetrahydro-3-phenyl-6-(2-pyridinyl-methoxy)-7,10-ethano-1,2,4-triazolo[3,4-a]phthalazine that show selectivity for α2, α3 and α5 subunit containing receptors over α1 [7].

GABA, as the major inhibitory neurotransmitter, is involved, directly or indirectly, in many disorders of brain function. The major disorders for which ionotropic GABA receptors represent important therapeutic targets include anxiety, depression, epilepsy, schizophrenia, sleep and memory disorders. Thus, therapeutic agents acting selectively on subtypes of ionotropic GABA receptors are much sought after. This review is concerned with ionotropic GABA_A and GABA_C receptors as therapeutic targets in particular for memory and sleep disorders. It is known from the actions of relatively non-selective agents that stimulating the function of ionotropic GABA receptors can enhance sleep while relatively non-selective agents that reduce the function of ionotropic GABA receptors can enhance aspects of memory. While classical benzodiazepines such as diazepam promote sleep by enhancing the function of GABA_A receptors that contain a γ2 subunit, they do have some adverse effects on memory. The challenge is to discover more selective agents that influence only sleep and not memory, and vice versa.

There are many reviews on aspects of GABA_A receptors including GABA_A receptor subtypes [8–12], drug interactions [13], specific agonists and partial agonists [14], receptor recycling and regulation [15], novel modulators [16], medicinal chemistry [1,17], and analysis of GABA_A receptors through mouse genetics [5]. GABA_C receptors as therapeutic targets have been the subject of a recent review [18].

2. IONOTROPIC GABA RECEPTORS

Structurally, GABA_A and GABA_C receptors are similar to other members of the nicotinicoid superfamily of ligand-gated ion channels, consisting of five protein subunits arranged around a central pore that constitutes the actual ion channel [1]. Each subunit has a large extracellular N-terminal domain which incorporates part of the agonist/antagonist binding site, followed by three membrane spanning domains.
(M1–3), an intracellular loop of variable length and a fourth membrane spanning domain (M4), with the C-terminal end being extracellular. Each subunit arranges itself such that the second membrane-spanning domain (M2) forms the wall of the channel pore and the overall charge of the domain determines whether the channel conducts anions or cations. Both GABA<sub>A</sub> and GABA<sub>C</sub> receptors are GABA-gated chloride ion channels causing inhibition of neuronal firing, with GABA<sub>A</sub> receptors being heteromeric, i.e., made up of different subunits (e.g. α1, β2 and γ2 subunits) and GABA<sub>C</sub> receptors being in general homomeric (e.g. made up exclusively of ρ1 subunits). The cytoplasmic loop, between the third and fourth transmembrane domains (M3 and M4), is believed to be the target for protein kinases, required for subcellular targeting and membrane clustering of the receptor.

2.1. Molecular composition of ionotropic receptors

There are 16 different subunits comprising the GABA<sub>A</sub> receptor family: α1–6, β1–3, γ1–3, δ, ε, and θ. In addition, there are splice variants of many of these subunits. If all of these subunits could co-assemble to form functional pentameric receptors the total number of GABA<sub>A</sub> receptors would be very large. Even if the combinations were restricted to those containing two α two β and one other subunit, then more than 2000 different GABA<sub>A</sub> receptors could exist. In fact, studies of native GABA<sub>A</sub> receptors suggest that there may be fewer than 20 widely occurring GABA<sub>A</sub> receptor subtype combinations, with the major combinations being α/β2/3γ2, α3β3γ2 and α2β3γ2 [8].

The molecular components of the GABA<sub>C</sub> receptors are the ρ-subunits. To date, two subunits (ρ1 and ρ2) have been cloned from human, while in rat three subunits (ρ1–3) have been cloned. There is a high degree of sequence homology (≥92%) shared between human and rat ρ-subunits, while 60–74% sequence homology is exhibited between the various ρ-subunits [18]. The subunits form functional homomeric receptors (formed from ρ1, ρ2 or ρ3 subunits) [19] or pseudo-heteromeric receptors (formed from a combination of ρ1 and ρ2 subunits, or ρ2 and ρ3 subunits) [19,20]. Neither ρ1 or ρ2 subunits assemble with α or β subunits of the GABA<sub>A</sub> receptor and, thus, are generally not regarded as part of the GABA<sub>A</sub> receptor family [21], although coassembly of mutated GABA<sub>C</sub> ρ1 subunits with GABA<sub>A</sub> γ2S, glycine α1 and glycine α2 subunits was demonstrated in vitro [22] and ρ1 with γ2 subunits in white perch retina [23], suggesting that heteromeric assembly can exist.

While GABA<sub>A</sub> receptors are found throughout the central nervous system, GABA<sub>C</sub> receptors have a more restricted distribution, having been found in the retina, hippocampus, spinal cord, pituitary and gut [24–28]. Their role may include visual processing, regulation of sleep-waking rhythms, pain perception, memory, learning, regulation of hormones and neuroendocrine gastrointestinal secretion [18].

2.2. GABA<sub>A</sub> and GABA<sub>C</sub> receptor pharmacology

GABA<sub>A</sub> receptors are defined pharmacologically by their inhibition by the alkaloid bicuculline. In addition, muscimol 3 and THIP 4 (gaboxadol, 4,5,6,7-tetrahydroisoxazolo
(5,4-c)pyridin-3-ol) are widely used as selective GABA$_A$ receptor agonists [1]. However, they also have potent actions on GABA$_C$ receptors, which means that interpretation of studies with these agents should be treated with some caution [18]. No ‘selective’ GABA$_A$ receptor agonist is known that does not have significant action on either GABA$_B$ and/or GABA$_C$ receptors. For example, muscimol 3, a conformationally restricted analog of GABA in which a hydroxyisoxazole moiety replaces the carboxyl group of GABA, is more potent at GABA$_C$ receptors than at GABA$_A$ receptors [29].

GABA$_C$ receptors are not inhibited by bicuculline nor activated by the GABA$_B$ receptor agonist (−)-baclofen [1]. Instead, these receptors are selectively activated by CACA 5 (cis-4-amino-crotonic acid; (Z)-4-amino-2-butenoic acid) and (+)-CAMP 6 ((1S,2R)-2-(aminomethyl)cyclopropanecarboxylic acid) and inhibited by TPMPA 7 ((1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid) [1,30,31].

2.3. Modulators of ionotropic GABA receptors

Allosteric modulators of GABA receptors are usually considered to be promising drug leads for two reasons. Firstly, the allosteric sites have greater diversity between receptor subtypes in amino acid sequence than on the GABA recognition sites (orthosteric sites). Secondly, they require the presence of the endogenous ligand to produce an effect and so are thought to be somewhat more ‘gentle’ drugs compared to ligands acting at the orthosteric site [32]. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at GABA$_A$ receptors. Although there is a significant chemical diversity of positive allosteric modulators acting at GABA$_A$ receptors [1], allosteric modulators of GABA$_C$ receptors are relatively unknown. A number of early SAR studies of GABA$_C$ receptors reported that GABA$_A$ receptor modulators including benzodiazepine and steroids were inactive at these receptors [33,34]. However, such compounds were only tested at concentrations active at GABA$_A$ receptors, that is, at nM concentrations. More recent studies found that higher doses (μM concentrations) of certain steroids could indeed modulate this receptor [35].

3. IONOTROPIC GABA RECEPTORS AND SLEEP DISORDERS

The treatment of insomnia is regarded as a developing market for agents acting on GABA$_A$ and possibly GABA$_C$ receptors. GABA systems are known to play an important
role in sleep and positive allosteric modulators of GABA_A receptors are widely used to promote restful sleep [36].

The brain lipid oleamide (8, Z-9-octadecenamide) accumulates in the CSF of sleep-derived cats suggesting that it may be an endogenous sleep-inducing factor. Oleamide enhances the effect of GABA on rat cultured cortical neurons with an EC_{50} of 15 μM [37]. In studies on recombinant GABA_A receptors expressed in Xenopus oocytes, oleamide enhanced the effects of GABA only at those receptors containing a γ2 subunit that are susceptible to positive modulation by benzodiazepines. However, its enhancing action was not sensitive to the specific benzodiazepine antagonist flumazenil [37,38].

![Chemical structure of oleamide](image)

Oleamide is inactive in β3 knockout mice and a mutation in β3 GABA_A receptor subunits has been described in a patient with chronic insomnia [39]. Oleamide and related compounds are being intensively investigated for use in sleep therapy [40]. Indiplon 9 acts in a similarly selective manner to oleamide and is in clinical trials for the treatment of insomnia [41,42].

Also in clinical trials for the treatment of sleep disorders is THIP 4, a directly acting GABA_A receptor partial agonist that interacts with a GABA_A receptor population that is insensitive to benzodiazepines, zolpidem, zaleplon and indiplon [43]. THIP is a moderately potent GABA_C receptor antagonist [29]. Unlike GABA, THIP passes the blood–brain barrier on systemic administration and is a potent analgesic [44]. The side effects of THIP (including sedation, dizziness, and blurred vision) meant that it had too low a therapeutic index to be therapeutically useful as an analgesic [45,46] but it is being investigated for sleep therapy. Interestingly, THIP-induced analgesia is not sensitive to bicuculline suggesting that GABA_A receptors are not involved [47]. Instead, the GABA_C receptor antagonist action of THIP may contribute to its analgesic action [18] as the analgesic action of THIP in rats is blocked by subconvulsant doses of picrotoxinin [48], a potent GABA_C receptor antagonist in addition to its well known action as a GABA_A receptor antagonist [18].

THIP produces slow wave sleep and reduces spindling activity in non-rapid eye movement sleep in humans [49]. It does appear that receptors other than the classical benzodiazepine-sensitive, bicuculline-sensitive GABA_A receptors are involved in the effects of THIP on both pain perception and sleep. Benzodiazepine-sensitive GABA_A receptors do not appear to be involved in the effects of THIP on sleep patterns [49]. The binding of THIP to rat brain membranes, unlike that of GABA and muscimol, is not stimulated by diazepam [50]. THIP was devoid of the anticonvulsant and
antiepileptogenic effects shown by diazepam and alphaxalone in pentamethylenetetrazole-kindled mice [51].

Clinical studies with THIP have indicated that sleep quality improving effects are obtained at plasma concentrations of the order of 1 μM [49]. THIP shows considerable variation in potency on recombinant receptors: THIP acts on α1β3γ2S recombinant GABA_A receptors expressed in oocytes as a partial agonist (EC_{50} 350 μM) and as a more potent agonist on α5β3γ3 (EC_{50} 40 μM) and α5β3γ3 (EC_{50} 29 μM) recombinant receptors [52]. On α4β3γ2 recombinant receptors THIP acts as a partial agonist (EC_{50} 102 μM) and on α4β3δ as a ‘superagonist’ (EC_{50} 6 μM) [53]. On recombinant GABA_C receptors THIP acts as an antagonist (K_b 32 μM for ρ1 [29] and 10 μM for ρ3 receptors [54]). On this basis, α4β3δ GABA_A and ρ3 GABA_C receptors are the most likely GABA receptors to respond to clinically relevant 1 μM plasma concentrations of THIP. In addition, the GABA_C receptor antagonist TPMPA 7 has been used to probe the involvement of GABA_C receptors in sleep-waking behavior in rats on intraventricular administration [55]. TPMPA enhanced both active and quiet wakefulness and decreased total slow wave sleep and paradoxical sleep. GABA_C receptors are also involved in sleep-waking regulation. It was concluded that since the sensitivity of GABA_C receptors to GABA is much higher than that of GABA_A and GABA_B receptors, GABA_C receptors modulators could be potential medications acting at low doses with fewer side effects [55].

Many herbal preparations are used to promote sleep. Their active ingredients include flavonoids and terpenoids known to modulate GABA_A receptor function. For example, chamomile tea contains the flavonoid apigenin, 10 R=H. Apigenin is known to have sedative effects in rats [56]. The effects of apigenin on GABA_A receptors are complex and involve both flumazenil-sensitive and flumazenil-insensitive components. Apigenin has been shown to inhibit the activation of recombinant α1β1γ2S GABA_A receptors in a flumazenil-insensitive manner and to have a similar effect on ρ1 GABA_C receptors [57]. Other studies on recombinant α1β2γ2L GABA_A receptors describe an inhibitory effect of apigenin on GABA responses and, in addition, describe an enhancement of the diazepam-induced positive allosteric modulation of GABA responses by apigenin [58]. Such a second order modulation by apigenin of benzodiazepine modulation of the activation by GABA of GABA_A receptors may indicate that apigenin needs to work through an endogenous benzodiazepine system to produce sedation in a flumazenil-sensitive manner. 6-Methylapigenin, 10 R=CH_3, has been isolated from Valeriana wallichii, a known sedative herb, and may be a more potent positive modulator of GABA_A receptors than apigenin [59].

Extracts of valerian (Valeriana officinalis) are widely used to reduce the latency of sleep onset, the depth of sleep and the perception of well-being. These extracts contain a variety of agents, including the sesquiterpenoid valerinic acid 11, that act on GABA_A receptors [60]. The monoterpenoid (+)-borneol 12, found in high concentrations in extracts of valerian, is a flumazenil-insensitive positive allosteric modulator of recombinant GABA_A receptors of low affinity but very high efficacy.
producing a 12-fold enhancement of the action of 10 μM GABA at a concentration of 450 μM [58,61].

4. IONOTROPIC GABA RECEPTORS AND MEMORY DISORDERS

GABA<sub>A</sub> receptor α5-subunits are likely to be involved in aspects of memory. Less than 5% of GABA<sub>A</sub> receptors in the brain are thought to contain α5-subunits. They are localized mainly to the hippocampus where they may play a key role in cognitive processes by controlling a component of synaptic transmission in the CA1. Mice lacking the α5 gene show improved performance in the Morris water maze model of spatial learning, whereas the performance in non-hippocampal-dependent learning and in anxiety tasks were unaltered in comparison with wild-type controls [62]. Novel selective α5 negative allosteric modulators, e.g. 6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one 13, that enhance spatial learning but lack the convulsant or proconvulsant activity associated with non-selective GABA<sub>A</sub> receptor negative allosteric modulators have been developed [63].

bis(7)-Tacrine (14, N,N'-bis(1,2,3,4-tetrahydro-9-acridinyl)-1,7-heptanediamine) is a potential Alzheimer’s disease drug on the basis of its superior acetylcholinesterase inhibition and memory-enhancing potency relative to tacrine. It is a potent competitive GABA<sub>A</sub> receptor antagonist (IC<sub>50</sub> 6 μM), some 18 times more potent than tacrine on these receptors, suggesting that its GABA<sub>A</sub> receptor antagonist activity may contribute to its memory-enhancing properties [64].

The sesquiterpene trilactone bilobalide 15 is one of the active constituents of the 50:1 <i>Ginkgo biloba</i> leaf extract widely used to enhance memory and learning. Bilobalide was
found to antagonize the direct action of GABA on recombinant $\alpha 1\beta 2\gamma 2L$ GABA$_A$ receptors expressed in *Xenopus* oocytes [65,66]. Bilobalide is unusual in being a GABA$_A$ receptor antagonist that lacks overt convulsant properties; indeed it is an anticonvulsant [67,68]. As with 13, the $\alpha 5$ subunit preferring negative allosteric modulator, the lack of convulsant action in an agent that reduces GABA action may be important for enhancement of memory and learning. The lack of convulsant action of bilobalide may result from subunit selectivity, but this has yet to be established. The structurally related ginkgolides act similarly to bilobalide as negative allosteric modulators of GABA$_A$ receptor function [69]. The biflavone amentoflavone 16 is negative allosteric modulator of GABA$_A$ receptor function [70]; it is found in *Ginkgo biloba* but is removed from the extracts used to enhance memory and learning.

GABA$_C$ receptors have also been associated with learning and memory. (3-Aminopropyl)butylphosphinic acid (17, CGP36742), a moderately potent antagonist at the GABA$_B$ receptor ($IC_{50} = 38 \mu M$), was found to also antagonize GABA$_C$ receptors. CGP36742 had memory-enhancing properties [71] and it was proposed that the memory enhancing effects of CGP36742 result from activity at GABA$_C$ receptors. The hypothesis that GABA$_C$ receptors enhanced memory was confirmed by the fact that TPMPA 7 was able to improve memory in rats and chicks [72]. ($\pm$)-cis-(3-Aminocyclopentyl)butylphosphonic acid 18, a GABA$_C$ receptor antagonist that lacks GABA$_B$ receptor activity, has been patented as a memory enhancing agent [73].

Resveratrol 19 is a moderately potent GABA$_C$ receptor non-competitive antagonist ($IC_{50} 72 \mu M$) [58]. It is an important constituent of red wine and has been patented for the treatment of mild cognitive disorders [74]. These studies provide some evidence that GABA$_C$ receptors play a role in memory.
Preparations of sage have been used in herbal medicine to assist memory [75] and an extract of *Salvia lavandulaefolia* (Spanish sage) has been shown to enhance memory in healthy young volunteers [76]. Hispidulin, 6-methoxyapigenin \( \text{R}=\text{OCH}_3 \), isolated from sage, has been shown to be a potent positive allosteric modulator of GABA\(_A\) receptors [77]. Sage extracts are known to contain a variety of flavonoids (including apigenin, hispidulin and linarin) and terpenoids (including galdosol, miltirone, carnosic acid and carbosol) that are known to enhance the function of ionotropic GABA receptors. Possibly the most interesting terpenoid in extracts of sage is \( \alpha \)-thujone 20 a known GABA\(_A\) receptor antagonist [78]. The levels of \( \alpha \)-thujone in individual sage plants are known to vary considerably [75]. The antagonistic effects of \( \alpha \)-thujone on GABA\(_A\) receptors may be an important component of the memory enhancing properties of sage extracts, while the constituents that enhance the functioning of GABA\(_A\) receptors, such as hispidulin, are more likely to be involved in sedative actions [79].

5. CONCLUSION

Agents acting on GABA\(_A\) or GABA\(_C\) receptors have widespread therapeutic potential including therapy of sleep and memory disorders. The discovery of selective ionotropic GABA receptor agents as modulators acting on the allosteric sites or as agonists, partial agonists or antagonists acting on the orthosteric, site is a major challenge in the continuing development of therapeutic agents acting on specific subtypes of GABA\(_A\) or GABA\(_C\) receptors.

REFERENCES


