Chapter 6

Antagonists of Ionotropic Receptors for the Inhibitory Neurotransmitter GABA: Therapeutic Indications

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Abstract

Agents that antagonize the action of GABA on ionotropic receptors are widely used to probe the function of this neurotransmitter. Three such agents are in common use: bicuculline, gabazine, and picrotoxinin. These three agents produce convulsions on systemic administration but act in significantly different ways. Bicuculline is a competitive antagonist of $GABA_A$ receptors. Gabazine is also a competitive antagonist of $GABA_A$ receptors, interacting with different residues on the receptors. Picrotoxinin is a noncompetitive antagonist acting on the chloride channel of $GABA_A$ and several other ionotropic CYS-loop receptors including glycine, $GABA_C$, and $5\text{-}HT_3$ receptors. Many other structurally diverse agents are now known to act as GABA receptor antagonists, providing opportunities for the discovery of agents with selectivity for the myriad of ionotropic GABA receptors. TPMPA is a selective antagonist for $GABA_C$ receptors, which are insensitive to bicuculline. Like TPMPA, many antagonists of ionotropic GABA receptors are not convulsants, indicating that there is still much to be learnt about GABA function in the brain from the study of such agents and their possible therapeutic uses. The most recently discovered $GABA_A$ receptor nonconvulsive antagonist is S44819, which is subtype selective for α5-containing receptors, and is arousing much interest in relation to cognition.

Keywords: antagonists, GABA receptors, bicuculline, gabazine, picrotoxinin

1. Introduction

"Advantages of an Antagonist" headed the Nature editorial in 1970 on the paper reporting the antagonist action of the convulsant alkaloid bicuculline on receptors for the neurotransmitter GABA in the cat spinal cord [1, 2]. The editorial predicted "With this tool it should now be possible to map fairly rapidly the distribution of GABA-inhibitory synapses in the CNS,

and to determine whether they are as numerous and widely distributed as the relatively high GABA content of the tissue would suggest." Indeed, interest in GABA antagonists continues today, with more than 120 publications per year containing the terms "bicuculline" and "GABA" since 1970 [3]. GABA-inhibitory synapses are widely distributed in the CNS with GABA being released by up to 40% of neurons in many brain regions [4]. Specific GABA receptor antagonists have been described as "essential tools of physiological and pharmacological elucidation of the different types of GABA receptor inhibition" [5].

GABA receptors can be divided into two major types based on their mechanism of action dating from the studies by David Hill and Norman Bowery in 1981 on the binding of the GABA analog baclofen to rat brain membranes [6]. They described a receptor that "differs from the classical GABA site as it is unaffected by recognized GABA antagonists such as bicuculline." They went on to state "We propose to designate the classical site as the $GABA_A$ and the novel site as the $GABA_B$ receptor." We now know that $GABA_A$ receptors are ionotropic receptors and that $GABA_B$ receptors are metabotropic. This perspective on GABA receptor antagonists is limited to mammalian ionotropic receptors.

Ionotropic GABA receptors are ligand-gated ion channels, where binding of GABA necessitates a change in conformation, which leads to opening of the ion channel. The ion channel is permeable to chloride, and increased conductance of this anion stabilizes the membrane potential, thereby reducing excitatory depolarization of the postsynaptic membrane. On the other hand, metabotropic GABA receptors are G-protein-coupled receptors, where GABA binding activates a variety of second messengers that lead to closing of cation channels to prevent sodium and calcium entry and opening of potassium channels to permit potassium efflux. The net effect is a reduction in excitability of the pre- or postsynaptic cell.

Ionotropic GABA receptors are part of the CYS-loop group of receptors that include glycine and $5\text{-}HT_3$ receptors. Ionotropic GABA receptors may be divided into two classes based on their sensitivity to antagonists. $GABA_A$ receptors may be antagonized selectively by bicuculline, while $GABA_C$ receptors are antagonized selectively by TPMPA ((1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid) and are insensitive to bicuculline [7]. It turns out that these two classes differ in several other respects. While $GABA_C$ receptors are relatively simple homomeric pentameric receptors, $GABA_A$ receptors are complex heteromeric pentameric receptors consisting of a variety of protein subunits, resulting in different possible combinations and thus a myriad of receptor subtypes. The structural complexity of the $GABA_A$ receptors further supports a range of allosteric binding sites which are binding targets for endogenous and exogenous allosteric modulators of these receptors. For example, receptors containing α_1, α_2, α_3, or α_5 subunits along with a γ_{2L} subunit permit high-affinity benzodiazepine binding [8]. Barbiturates are known to bind to an allosteric site on all $GABA_A$ receptor subtypes [9]. As another example, receptors containing δ subunits are targets for endogenous neurosteroids and ethanol [10–12]. Development of subtype-selective agonists, antagonists, and modulators of the ionotropic GABA receptors is imperative to the provision of valuable experimental tools for elucidation of the distribution and various functions of these $GABA_A$ receptor subtypes.

Antagonism of ionotropic GABA receptors may result from three distinct mechanisms: competitive antagonism where the binding site for the drug may overlap with the GABA-binding

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site, i.e., the orthosteric site; negative allosteric modulation where the drug binds to a site distinct from the orthosteric site to reduce the affinity of the agonist; and noncompetitive antagonism where the drug binds to a site on the chloride channel to reduce chloride permeability or channel opening by GABA [13]. In this perspective, we consider examples of all three types of antagonism of the ionotropic GABA receptors.

2. Picrotoxin, a channel blocker of ionotropic GABA receptors

The first reported GABA receptor antagonist was picrotoxin, a convulsant plant product, a combination of the Greek words "picros" (bitter) and "toxicon" (poison). It is a 50:50 mixture of picrotoxinin (**Figure 1**) and picrotin with picrotoxinin being the more active component as a GABA receptor antagonist. Early reports showed that picrotoxin antagonized the action of GABA at invertebrate inhibitory synapses and that it reduced presynaptic inhibition in the spinal cord [14]. In 1968, Davidoff and Aprison showed that picrotoxin antagonized the inhibitory

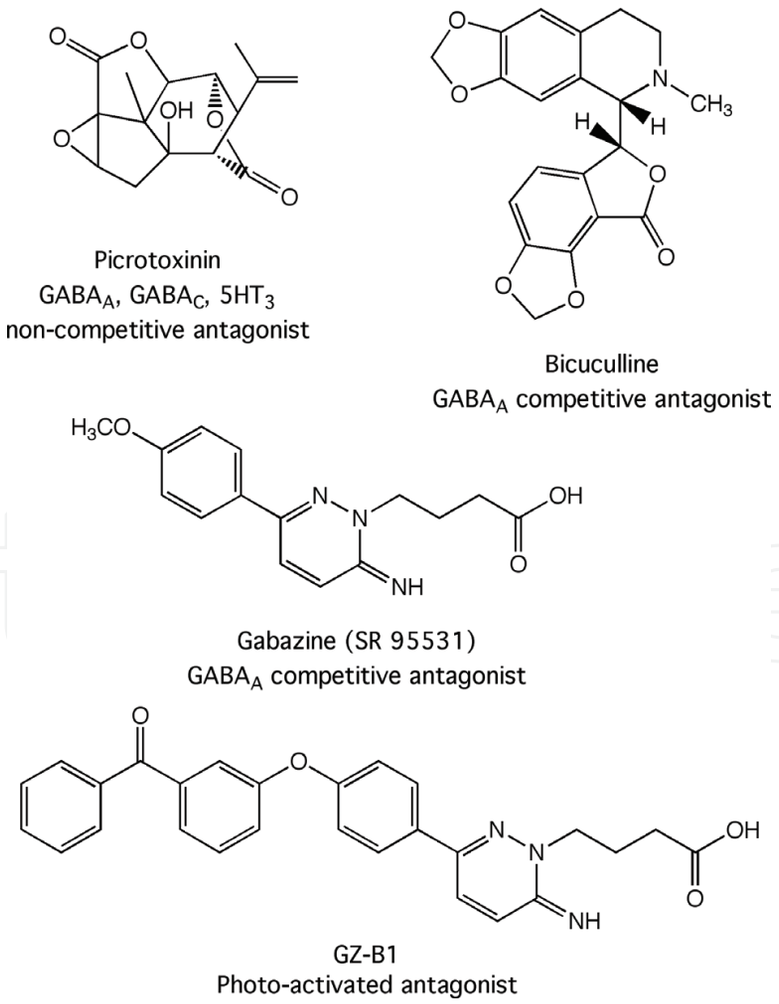

Figure 1. GABA$_A$ receptor antagonists that are convulsants.

action of glycine on spinal neurones [15]. Curtis et al. [16] reported antagonist action against both glycine and GABA, but the results were inconsistent due to the lack of ionization using the microiontophoresis method of drug administration.

These technical difficulties were eventually overcome using recombinant receptors with bath application of picrotoxin showing it to be a mixed/noncompetitive $GABA_A$ receptor antagonist [17]. But picrotoxin was also shown to be an antagonist of other CYS-loop receptors, including glycine, $GABA_C$, and 5-HT_3 receptors [18]. Thus, the utility of picrotoxin (and also picrotoxinin) as an experimental tool is mitigated by its lack of selectivity for $GABA_A$ receptors. It also has no therapeutic potential owing to its potent convulsant effects.

Many terpenoids related to picrotoxinin are convulsants acting on ionotropic receptors for GABA and glycine [19]. Of particular interest is tutin that occurs in the berries and flowers of the indigenous New Zealand tutu plant, *Coriaria arborea*, and has been credited with convulsing a circus elephant that consumed the berries [20]. Toxic honey was also produced from bees that collected nectar from the flowers.

3. Bicuculline, a competitive antagonist of $GABA_A$ receptors

The discovery of bicuculline as a selective antagonist of what became known as $GABA_A$ receptors arose out of a systematic study of convulsant alkaloids [3]. It was well known that the most widely understood convulsant alkaloid, strychnine, antagonized the inhibitory action of glycine without influencing the inhibitory action of GABA [21]. Indeed, most convulsant alkaloids turned out to be glycine receptor antagonists with the important exception of bicuculline (**Figure 1**), an alkaloid from *Dicentra cucullaria* [22].

While bicuculline is selective for $GABA_A$ receptors, having little effect on $GABA_B$, $GABA_C$, glycine, and 5-HT_3 receptors, its action is largely independent of $GABA_A$ subunit composition [17, 23]. Selectivity for $GABA_A$ receptors makes bicuculline a powerful experimental tool but without any therapeutic potential owing to the nonselective nature of binding to all $GABA_A$ receptor subtypes, causing profound convulsive effects. Bicuculline binds at the orthosteric site to stabilize the receptor in a closed state. It is three times the size of GABA and thus is able to bind to sites on the receptor that GABA cannot reach [3]. Bicuculline acts as a competitive antagonist in which it competitively inhibits GABA agonist binding to $GABA_A$ receptors, and GABA competitively inhibits bicuculline binding [24]. Single channel studies show that by competing with GABA for its binding site, bicuculline acts to reduce both chloride channel open time and opening frequency [25].

At physiological pH, bicuculline is slowly converted to bicucine, a much less active convulsant [26]. This transformation is slowly reversed at acidic pH. Thus, bicuculline solutions should always be freshly prepared in order to preserve maximum convulsant potency. Quaternary salts of bicuculline, such as bicuculline methiodide ("N-methyl bicuculline") or methochloride, are much more stable than bicuculline, are more water soluble, and are of similar potency as GABA receptor antagonists, but they do not cross the blood-brain barrier

on systemic administration [27, 28]. The quaternary salts differ in their pharmacology to bicuculline itself in that they are much less selective. It is not always clear in publications whether the investigators use bicuculline or a quaternary salt [3]. The quaternary salts have significant actions on nicotinic receptors, calcium-activated potassium channels, and acetylcholinesterase [29–31]. Thus, while ensuring chemical stability of bicuculline, the quaternary salts may be less effective tools owing to their reduced binding specificity for $GABA_A$ receptors. Subject to these considerations, bicuculline and its quaternary salts continue to be used extensively as $GABA_A$ receptor antagonists in experimentation.

Extensive structure-activity studies have been carried out on bicuculline with little improvement on potency, selectivity, or stability [3]. Investigations of bicuculline analogs devoid of the phenyl ring fused to the lactone moiety have yielded positive allosteric modulators. These analogs do not bind to the orthosteric binding site on $GABA_A$ receptors. Instead, they bind to the high-affinity benzodiazepine site on $GABA_A$ receptor subtypes containing subunit combinations described above and show subtype selectivity that differs from that shown by benzodiazepines [32].

Bicuculline has been shown to improve special memory in the rat hippocampus [33].

4. Gabazine, a competitive antagonist of $GABA_A$ receptors

Gabazine (also known as SR 95531, **Figure 1**) resulted from a study of arylaminopyridazine analogs of GABA. It was found to be a relatively specific, potent, and competitive antagonist of $GABA_A$ receptors [34]. Although both are functionally competitive inhibitors, gabazine and bicuculline also interact with other residues on $GABA_A$ receptors [35, 36]. Neither gabazine nor bicuculline compete for the binding at the barbiturate or neurosteroid binding sites on $GABA_A$ receptors. It is suggested that both antagonists act "as allosteric inhibitors of channel opening for the $GABA_A$ receptor after binding to the GABA-binding site" [36]. Gabazine has little activity at $GABA_C$ receptors [37]. At binary β3δ recombinant $GABA_A$ receptors, gabazine antagonized GABA currents, whereas bicuculline activated these receptors [38]. Thus, while functioning as competitive antagonists for $GABA_A$ receptors, gabazine and bicuculline clearly interact with different residues on $GABA_A$ receptors.

Structural analogs of gabazine have identified more potent agents [39]. Gabazine analogs incorporating photoactive groups, such as GZ-B1 (**Figure 1**), have been developed as photoactivated antagonists of $GABA_A$ receptors [40]. These antagonists provide dynamic tools for visualizing $GABA_A$ receptors, permitting a novel means of investigating receptor location, function, and trafficking [40].

5. TPMPA and related compounds, competitive antagonists of $GABA_C$ receptors

$GABA_C$ receptors, also known as GABA-ρ and $GABA_A$-ρ receptors, have distinctive distribution and pharmacological properties to $GABA_A$ receptors, making them particularly interesting [41].

They are CYS-loop ligand-gated ion channels with a similar pentameric structure to $GABA_A$ receptors but are not so widely distributed. They are homomeric rather than heteromeric and therefore much simpler receptors. These properties make them important drug targets [42].

TPMPA ((1,2,5,6-Tetrahydropyridin-4-yl)methylphosphinic acid, **Figure 2**) was the first selective $GABA_C$ receptor antagonist to be synthesized [43, 44]. Other $GABA_C$ receptor antagonists include the bicyclic GABA analog, THIP (Gaboxadol, 4,5,6,7-**t**etra**h**ydro**i**soxazolo(5,4-c)**p**yridin-3-ol, **Figure 2**), which is a moderately potent antagonist at the $GABA_C$ receptors, yet a potent agonist at $GABA_A$ receptor receptors [45]. Aza-THIP (1H,4H,5H,6H,7H-pyrazolo[3,4-c]pyridin-3-ol) is inactive at $GABA_A$ receptors but shows moderately potent antagonism at $GABA_C$ receptors. Phosphinic, phosphonic, and seleninic analogs of isonipecotic acid have also been shown to act as selective $GABA_C$ receptor antagonists [46], as have amide and hydroxamate analogs of 4-aminocyclopent-1-enecarboxylic acid [47].

Unlike many $GABA_A$ receptor antagonists, TPMPA is not a convulsant, consistent with many instances that $GABA_A$ and $GABA_C$ receptors have been shown to mediate opposing functions, for example, on excitability [48] and in memory formation [49].

TPMPA and other $GABA_C$ receptor antagonists have been used to demonstrate the important role of $GABA_C$ receptors in many aspects of vision [50–53]. TPMPA was shown to inhibit

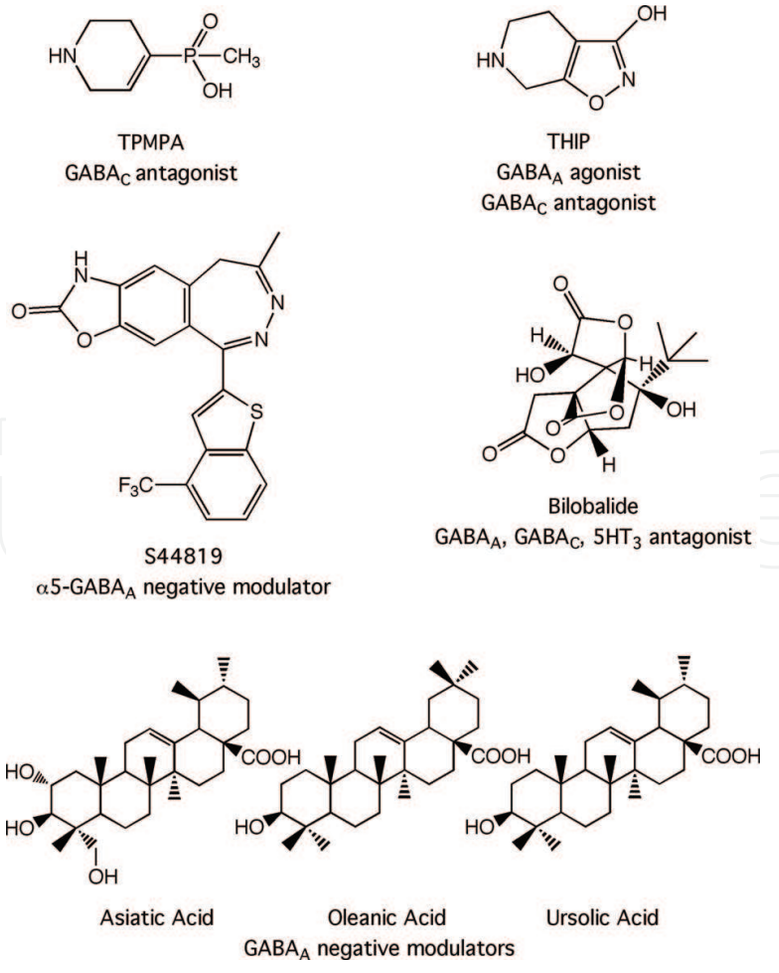

Figure 2. $GABA_A$ receptor antagonists that are not convulsants.

form-deprivation myopia [51], while (3-aminopropyl)-n-butylphosphinic acid (CGP36742 or SGS742) was found to inhibit the development of myopia in chicks [50]. Thus, TPMPA and related $GABA_C$ receptor antagonists have been suggested for the treatment of myopia, administered intravitreally, orally, and ophthalmically [52]. Indeed, $GABA_C$ receptor antagonists have been patented for the treatment of myopia [52].

$GABA_C$ receptor antagonists have also been useful experimental tools for demonstrating a role for these receptors in learning and memory [49, 50, 54]. TPMPA and the structural analog P4MPA ((piperidin-4-yl)methylphosphinic acid) were shown to enhance short-term memory in a bead discrimination task following injection into the multimodal association forebrain area in chicks [49]. Injection of bicuculline caused the opposite effect. TPMPA has also been used to demonstrate a role for $GABA_C$ receptors in fear learning and memory in rats [54]. Rats administered TPMPA via bilateral cannulae injections into the lateral amygdala showed reduced freezing in a foot shock conditioned fear task. This reduction in fear learning and memory is likely mediated by presynaptically located $GABA_C$ receptors in the lateral amygdala [54].

Arising out of studies on the orally active $GABA_{(B/C)}$ receptor antagonist (3-aminopropyl)-n-butylphosphinic acid (CGP36742 or SGS742) [55], *cis*- and *trans*-(3-aminocyclopentanyl) butylphosphinic acid were found to be selective potent $GABA_C$ receptor antagonists that enhanced learning and memory in rats in the Morris water maze task [50].

Based on the structure of the selective $GABA_C$ receptor antagonist (*S*)-4-ACPBPA [(4-aminocyclopenten-1-yl)-butylphosphinic acid], a series of fluorescent ligands were produced linking fluorophores to the parent compound [56]. One of these fluorescent ligands, (*S*)-4-ACPBPA-C5-BODIPY, showed moderately potent antagonism for $GABA_C$ receptors with greater than 100 times selectivity for these receptors over $GABA_A$ receptors. (*S*)-4-ACPBPA-C5-BODIPY thus provides a valuable molecular probe for the role of $GABA_C$ receptors in physiological and pathological processes [56].

6. Bilobalide, a nonconvulsant channel blocker

Bilobalide (**Figure 2**) and a series of terpenoids known as ginkgolides isolated from *Ginkgo biloba* are structurally related to picrotoxinin and are relatively potent $GABA_A$ and $GABA_C$ receptor antagonists [57], but they also act on glycine and $5\text{-}HT_3$ receptors [58, 59].

Unlike picrotoxin, bilobalide is an anticonvulsant. This may be due to its potent action on $GABA_C$ receptors [57]. Bilobalide also has differing effects to those of picrotoxin on the modulation of $GABA_A$ receptors by structurally different modulators [60], suggesting a different binding profile to picrotoxin to negatively modulate the $GABA_A$ receptors.

Thus, there are $GABA_A$ receptor antagonists that act as channel blockers and negative modulators that do not produce convulsions in vivo. Explanation of this apparent paradox includes selective actions on $GABA_A$ receptor subtypes, reduction of glutamate release from presynaptic terminals via presynaptic receptors and effects on GABA metabolism, together with actions on non-GABAergic systems.

Owing to their unique characteristics, bilobalide and other natural terpenoids from *Gingko biloba* are being investigated as cognitive enhancers via their effects on the GABAergic system [61]. Bilobalide has been shown to improve cognition in cognitive- and memory-impaired animals in a variety of animal models [62–65]. As a result to its nonconvulsant effects, bilobalide is a superior candidate for therapeutic use in memory impairment related to dementia and other neurological disorders compared with other $GABA_A$ receptor antagonists like picrotoxin which are pro-convulsive. Natural terpenoids from *Gingko biloba*, such as bilobalide, are being investigated in the treatment of neurological disorders via their effects on the GABAergic system [61].

The plant-derived triterpenoids, asiatic, oleanolic, and ursolic acids (**Figure 2**), are negative modulators of $GABA_A$ receptor activation acting in vivo as anxiolytics, anticonvulsants, and antidepressants in animal models [66, 67].

7. S44819, an α5-selective competitive antagonist

The α-β subunit interface has been highlighted as a novel target for subtype-selective drugs [68]. An example of a novel drug that targets this binding site and that is attracting considerable current attention as a new therapeutic agent is S44819 (Egis-13,529, 8-Methyl-5-[4-(trifluoromethyl)-1-benzothiophen-2-yl]-1,9-dihydro-2H-[1,3]oxazolo[4,5-h][2,3]benzodiazepin-2-one, **Figure 2**), a novel oxazolo-2,3-benzodiazepine derivative, which selectively inhibits $GABA_A$ receptors that contain the α5-subunit [69, 70].

S44819 appears to act as a competitive antagonist at the orthosteric site at the α-β subunit interface of $GABA_A$ receptors containing only α5 subunits. Thus, S44819 is a competitive antagonist, unlike other α5-subunit selective drugs that act as negative allosteric modulators by binding to the benzodiazepine recognition site between at the α5-γ2 subunits [71, 72]. Agents that are selective for α5 subunit-containing $GABA_A$ receptors enhance cognitive performance in a variety of animal models without sedative or pro-convulsive effects [73]. SR44819 has been shown in healthy young humans to be orally active, reaching the cerebral cortex on oral administration where it increases cortical excitability [74], acting on extrasynaptic receptors to reduce tonic inhibition. Consequently, clinical trials are now underway.

8. Other GABA receptor antagonists

It has not been possible to cover all known GABA receptor antagonists in this perspective. Other important classes of antagonists include sulfated neurosteroids [75] and agents derived from 4-PIOL (5-(4-piperidyl)isoxazol-3-ol) [5]. Of particular interest is DPP-4-PIOL (4-(3,3-diphenylpropyl)-5-(4-piperidyl)-3-isoxazolol hydrobromide) that selectively antagonizes tonic over phasic GABAergic currents in the hippocampus, suggesting a degree of substrate specificity [76].

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Salicylidene salicylhydrazide has been reported as a potent antagonist of $GABA_A$ receptors containing the β1 subunit using a high-throughput screen [77]. It was suggested that salicylidene salicylhydrazide is interacting at a previously unidentified site on the β1 subunit, but this does not appear to have been followed up after the initial publication in 2004.

The most potent $GABA_A$ receptor antagonist is the convulsant steroid derivative RU5135, being some 500 times more potent than bicuculline [78]. It acts as a competitive antagonist, sharing a common site of action with bicuculline. However it lacks specificity, as it is also a glycine receptor antagonist sharing a common site of action with strychnine [79].

9. Conclusion

There is still widespread interest in GABA receptor antagonists after many years of investigation. Reflecting on the use of GABA receptor antagonists in the last 10 years, citation counts via the Web of Science for publications citing GABA together with a GABA antagonist in the title or abstract are as follows: bicuculline 1203, picrotoxin or picrotoxinin 564, gabazine or SR 95531 290, TPMPA 48, and bilobalide 14. Thus far, there are only four publications directly related to the effects of S44819 on cognition.

Nonconvulsant antagonists of ionotropic GABA receptors have considerable therapeutic potential in the treatment of cognitive problems, myopia, and other CNS disorders. Such antagonists may be useful in the treatment of Down syndrome [62]. The myriad of possible subtypes of ionotropic GABA receptors in the CNS as a result of different combinations of protein subunits make the search for more subtype-specific agents highly desirable. The high-throughput analysis of ionotropic GABA receptor subtypes should result in the discovery of novel subtype-specific agonists, antagonists, and modulators that have therapeutic potential [80]. Clearly, we are going to hear a lot more about S44819 and other yet to be discovered ionotropic GABA receptor antagonists that act selectively on ionotropic GABA receptor subtypes.

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