BJP British Journal of Pharmacology

DOI:10.1111/bph.12127
www.brjpharmacol.org

REVIEW

Advantages of an antagonist: bicuculline and other GABA antagonists

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Keywords
GABA receptors; antagonists; bicuculline; gabazine; picrotoxin

Received
11 December 2012
Revised
21 January 2013
Accepted
31 January 2013

The convulsant alkaloid bicuculline continues to be investigated more than 40 years after the first publication of its action as an antagonist of receptors for the inhibitory neurotransmitter GABA. This historical perspective highlights key aspects of the discovery of bicuculline as a GABA antagonist and the sustained interest in this and other GABA antagonists. The exciting advances in the molecular biology, pharmacology and physiology of GABA receptors provide a continuing stimulus for the discovery of new antagonists with increasing selectivity for the myriad of GABA receptor subclasses. Interesting GABA antagonists not structurally related to bicuculline include gabazine, salicylidene salicylhydrazide, RU5135 and 4-(3-biphenyl-5-(4-piperidyl)-3-isoxazole. Bicuculline became the benchmark antagonist for what became known as $GABA_A$ receptors, but not all ionotropic GABA receptors are susceptible to bicuculline. In addition, not all $GABA_A$ receptor antagonists are convulsants. Thus there are still surprises in store as the study of GABA receptors evolves.

Abbreviations
gabazine, SR 95531, 4-[6-imino-3-(4-methoxyphenyl)pyridazin-1-yl] butanoic acid; RU5135, 3α-hydroxy-16-imino-17-aza-5β-androstan-11-one; 4-PIOL, 5-(4-piperidyl)-3-isoxazolol

Introduction

The editorial accompanying our 1970 Nature paper on the GABA receptor antagonist action of the convulsant alkaloid bicuculline (Curtis *et al.*, 1970b) was entitled 'Advantages of an Antagonist' (Editorial, 1970). This was a very enthusiastic editorial beginning with 'Students of nervous transmission should be stimulated to a new burst of enthusiasm' and ending with '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.' Interest in the GABA antagonist action of bicuculline has been remarkably sustained with an average of more than 120 publications per year since 1970 containing the terms 'GABA' and 'bicuculline' in their title or abstract according to the American Chemical Society's Chemical Abstracts and related databases as accessed via SciFinder. Only picrotoxin/picrotoxinin, averaging 70 publications per year since 1970, and gabazine, averaging 19 publications per year since it was introduced in 1986, rival bicuculline as relatively widely used $GABA_A$ receptor antagonists.

Thanks to the pioneering work of Gene Roberts and others by 1970 (Roberts *et al.*, 1970) there had been much speculation about the possible function of GABA as an inhibitory transmitter in the CNS based on its distribution, metabolism and release from nervous tissue and the observation that GABA hyperpolarized neurons in a manner similar to synaptic inhibition. It was known that the convulsant alkaloid strychnine was able to antagonize synaptic inhibition in the spinal cord without influencing the inhibitory action of GABA. This puzzling observation was explained by the important findings by Werman *et al.*, (1967) that the simple amino acid glycine was likely to be an inhibitory transmitter in the spinal cord and our subsequent finding that strychnine antagonized the inhibitory action of glycine but not that of GABA (Curtis *et al.*, 1967). This work was a good example of the advantages of a selective antagonist.

The search was then on for a similarly selective antagonist of the inhibitory action of GABA that did not influence that of glycine. An obvious place to start was known convulsants. In the late 1960s, there were no readily available electronic databases; one had to hunt through paper versions of Chemical Abstracts, Index Medicus and the like. The Merck Index

actually proved to be the most useful in providing leads that could then be followed up in the original literature. A pattern emerged that many alkaloids containing the tetrahydroisoquinoline nucleus were convulsants. As we acquired these alkaloids from our chemist colleagues and chemical suppliers and tested them *in vivo* on neurons in the cat spinal cord, it became clear that most were glycine antagonists like strychnine without effect on the action of GABA.

Bicuculline as a GABA receptor antagonist

On 17 March 1970 (St Patrick's Day), David Curtis, Arthur Duggan, Dominik Felix and I, first tested bicuculline in the spinal cord of a cat under pentobarbitone anaesthesia. By 19 May, we had submitted a manuscript, 'GABA, bicuculline and central inhibition', to Nature that was published on 27 June (Curtis *et al.*, 1970b). An example of the selective antagonist action of bicuculline on the action of GABA is shown in Figure 1 where bicuculline reduced the effect of GABA without influencing the action of glycine.

The Nature issue of 14 November contained three papers on bicuculline and GABA: Hugh McLennan's findings on the antagonist action of bicuculline on GABA responses in crayfish (McLennan, 1970), an article by Godfraind *et al.* (Godfraind *et al.*, 1970) on the 'Doubtful value of bicuculline as a specific antagonist of GABA' and our response to the latter 'Bicuculline and central GABA receptors' (Curtis *et al.*, 1970a). Godfraind *et al.* concluded 'Whatever the mechanism of its convulsant action, we find it difficult to see how bicuculline could be useful for critical tests of GABA-mediated inhibition'. Their negative findings have never been satisfactorily explained but caused us to undertake elemental and spectroscopic analyses to confirm the chemical identity of the substance we had tested (Curtis *et al.*, 1970a). It seems unlikely that the 32 neurons that they examined in four cats had predominantly bicuculline-insensitive GABA receptors, later classified as $GABA_B$ receptors. More likely, the chemical instability of aqueous solutions of bicuculline was the problem. Their microelectrodes were filled with their bicuculline solutions 2–3 days before testing. While they were able to clearly show the convulsant properties of these test solutions taken from their microelectrodes, the potency appeared to be much reduced; they quoted pronounced convulsions being observed on systemic administration of bicuculline (5 µmole). This works out at around 1.8 mg kg^{-1} whereas many investigators have reported convulsions at 0.1 mg kg^{-1}.

Chemical instability of bicuculline

Many years earlier, Welch and Henderson (Welch and Henderson, 1934) had noted the slow conversion of bicuculline in solution to bicucine, a much less active convulsant (Figure 2). The chemical instability of bicuculline was further investigated by Olsen *et al.*, (1975). The conversion of bicuculline to bicucine involved the opening of a lactone ring and this could be readily monitored by UV spectroscopy. This conversion took place most readily at physiological pH. Bicuculline was relatively more stable at acidic pH as used in microelectrode studies. Bicucine was slowly converted to bicuculline at acidic pH. Quaternary salts of bicuculline, for example, bicuculline methiodide ('N-methyl bicuculline') (Pong and Graham, 1972) or methochloride (Johnston *et al.*, 1972), are much more stable than bicuculline, more water soluble and of similar potency as GABA antagonists but they do not appear to cross the blood brain barrier on systemic administration. Injected intracisternally, bicuculline methiodide was a more potent convulsant than bicuculline (Pong and Graham, 1972). While the quaternary salts are much easier to use than bicuculline, which exists as a protonated cation at physiological pH, care should be taken when using them, as they appear to be much less selective than bicuculline with respect to interactions with other systems (Seutin and Johnson, 1999). Bicuculline and its quaternary salts are agents with different pharmacological effects and sometimes it is not clear as to which agent was used in a particular study;

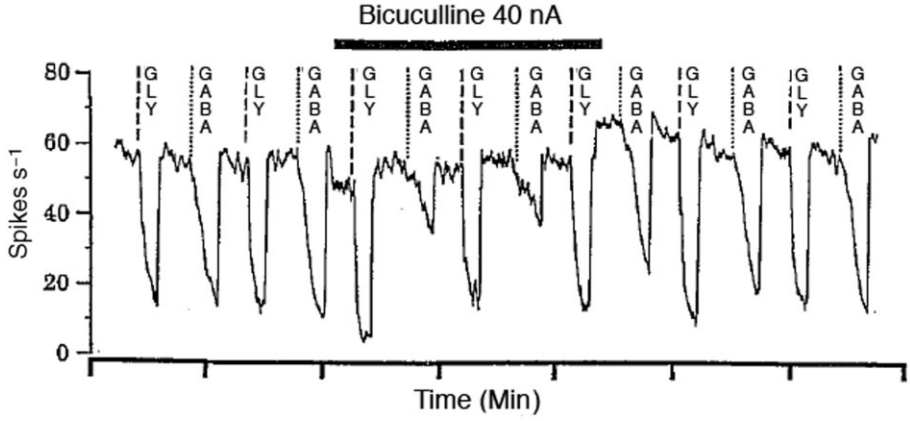

Figure 1

Differential effect of bicuculline, 40 nA ejection current, on the inhibition of a Renshaw cell by glycine (5 nA) and GABA (8 nA) in the spinal cord of a cat anaesthetized by pentobarbitone. Drugs were administered extracellularly from a multibarreled micropipette via electrophoresis. The firing frequency of the cell was maintained at around 60 spikes per second by the continuous electrophoretic ejection of DL-homocysteic acid (15 nA).

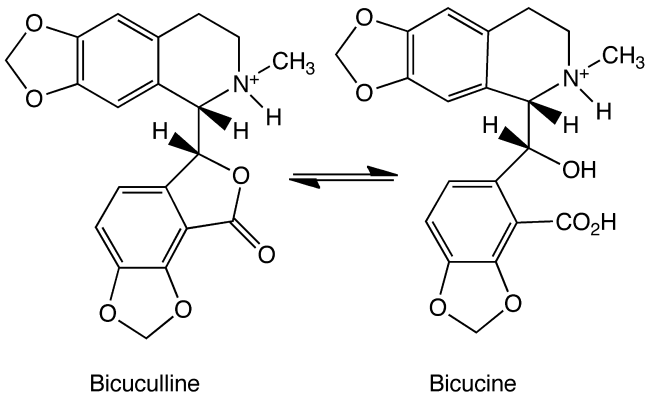

Figure 2
Structure of the protonated forms of the active GABA receptor antagonist bicuculline and the inactive bicucine that may be interconverted dependent on pH with bicucine favoured at physiological pH.

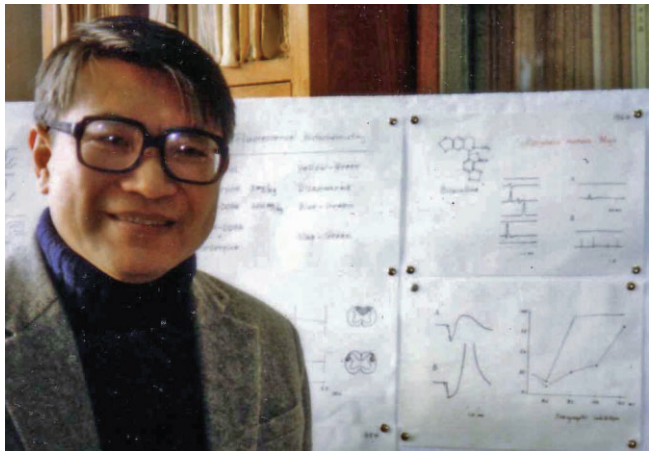

Figure 3
Dr Kang Tsou in Shanghai in 1984 with his original records showing bicuculline influencing synaptic inhibition.

sometimes a study appears to be about bicuculline whereas in fact it involves a quaternary salt (Ueno *et al.*, 1997).

Earlier work on bicuculline

After publication of our work on bicuculline as a GABA receptor antagonist, we learnt of studies in the 1960s by Dr Kang Tsou in the Department of Pharmacology of the Shanghai Institute of Materica Medica. He had found that bicuculline was a potent convulsant when applied to the cerebral cortex and that it also reduced strychnine-insensitive inhibition of spinal reflexes. He presented these results at a Congress of the Chinese Society for Physiological Sciences in 1964 and submitted a full-length paper to Acta Physiologica Sinica in 1965 that was never published due to the suspension of the publication of all Chinese scientific journals during the Cultural Revolution. He was eventually able to publish on the CNS effects of bicuculline in 1976 in a new Chinese language journal, Diseases of the Nervous System. I was delighted to meet Dr Tsou in Shanghai in 1984 during a visit of Australian pharmacologists to China (Johnston, 1985) and to discuss his work on bicuculline (Figure 3). I saw some of his original records that clearly supported his original claims (Tsou, 1987). An excellent article on his career 'Lost in the cultural revolution' was published in 1994 (Hoffman, 1994).

GABA as a neurotransmitter

The advantage of bicuculline as a GABA receptor antagonist quickly became apparent as we started to investigate the influence of bicuculline on strychnine-insensitive synaptic inhibition. In our initial 1970 Nature paper (Curtis *et al.*, 1970b), we showed that bicuculline reduces strychnine-insensitive inhibition of pyramidal cells in the cerebral cortex and Purkinje cells in the cerebellum to 'provide for the first time decisive pharmacological evidence that, of the amino acids present in the mammalian nervous system, GABA is most likely to be the actual transmitter at these inhibitory synapses. The use of bicuculline and related substances will be of considerable assistance in studying other synapses for which GABA has been proposed as a possible transmitter.' We subsequently showed that bicuculline reduced presynaptic inhibition of spinal reflexes and the accompanying primary afferent depolarization (Curtis *et al.*, 1971).

GABA receptor subtypes

The division of GABA receptors into $GABA_A$ and $GABA_B$ subtypes dates from the studies of David Hill and Norman Bowery in 1981 on the binding of baclofen and GABA to rat brain membranes (Hill and Bowery, 1981). 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.' $GABA_A$ receptors were ionotropic receptors that gate chloride channels, while $GABA_B$ receptors were G-protein coupled receptors. These receptors could be differentiated on the basis of agonist and antagonist selectivity. $GABA_A$ receptors were antagonized by bicuculline and insensitive to baclofen, whereas $GABA_B$ receptors were activated by baclofen and insensitive to bicuculline. In 1984, we described GABA receptors in rat cerebellum that were insensitive to both bicuculline and baclofen that became known as $GABA_C$ receptors (Drew *et al.*, 1984; Chebib and Johnston, 2000). Subsequent molecular biological and neuropharmacological studies showed that $GABA_C$ receptors were homomeric ionotropic GABA receptors made up of ρ-subunits, that had been first cloned from the retina, and having a distinct pharmacological agonist and antagonist profile (Bormann and Feigenspan, 1995; Johnston, 1996). More recently, the International Union of Basic and Clinical Pharmacology recommended that $GABA_C$ receptors were a subtype of $GABA_A$

receptors and should be classified as $GABA_A$ receptors (Olsen and Sieghart, 2008, see also Alexander *et al.*, 2011). It was 'especially recommended that the name $GABA_C$ receptor should not be used as the sole name for the ρ-receptors in an article including, especially, the title and abstract'. This recommendation has not met with general acceptance with investigators actually working on $GABA_C$ receptors (including me), particularly those working in the retina, where there is a very interesting interaction between $GABA_A$ and $GABA_C$ receptors (Lukasiewicz and Shields, 1998; Bormann and Feigenspan, 2001). Based on data in SciFinder, more than 80 publications in major journals including the British Journal of Pharmacology (Gasulla *et al.*, 2012) since 2009 have contained the term $GABA_C$ in their titles or abstracts.

Bicuculline-sensitive GABA receptors are part of the superfamily of Cys-loop pentameric ligand-gated ion channel receptors that include nicotinic acetylcholine, glycine and $5HT_3$ receptors (Chebib and Johnston, 2000). A variety of $GABA_A$ protein subunits have been described including α1–6, β1–3, γ1–3, δ, ε, θ and π that can combine to provide a large multiplicity of $GABA_A$ receptor subtypes with a diversity of function and pharmacology (Olsen and Sieghart, 2009). The combinatorial capability of heteromeric ligand-gated ion channels to afford a large variety of functional receptors by different combinations of a relatively small number of protein subunits rivals the wide variety of G-protein coupled receptors produced from a large number of protein subunits widely quoted as being the major drug targets for CNS drug development. Both ligand-gated and G-protein coupled receptors offer a seemingly bewildering array of drug targets.

Functional studies on recombinant and native $GABA_A$ receptors have indicated a lack of dependence on subunit composition for the antagonist action of bicuculline (Krishek *et al.*, 1996). $GABA_A$ receptor subtypes that contain the α6 subunit are less sensitive to bicuculline, while $GABA_C$ receptors containing ρ subunits are insensitive to bicuculline (Ng *et al.*, 2011).

While the potency of agonists and modulators of $GABA_A$ receptors varies with subunit composition, the potency of most antagonists is largely independent of receptors subunit composition (Ebert *et al.*, 1997). Whereas, antagonists show comparable potencies in binding and functional studies, the potency of agonists in binding studies are generally two to three orders of magnitude higher than the agonist potencies measured electrophysiologically. These findings indicate that the $GABA_A$ receptor mechanisms transducing binding into physiological response, rather than the binding *per se*, is more dependent on the receptor subunit composition (Ebert *et al.*, 1997). Studies on the differential sensitivity of three inbred strain of mice to bicuculline suggest that genetic factors influence the convulsant action of bicuculline (Freund *et al.*, 1987).

How does bicuculline act on $GABA_A$ receptors?

Bicuculline acts as a competitive antagonist at $GABA_A$ receptors in that it competitively inhibits GABA binding to these receptors and, in turn, GABA competitively inhibits bicuculline binding (Andrews and Johnston, 1979). This evidence is supported by functional studies. Single channel studies have shown that bicuculline reduces GABA activated conductance by reducing both channel open times and opening frequency (Macdonald *et al.*, 1989). Studies indicate that bicuculline binds at the orthosteric site, interacting with additional sites to GABA resulting in stabilization of the receptor in a closed state and in this sense bicuculline has been described as an allosteric inhibitor (Ueno *et al.*, 1997). Given that bicuculline is some three times the molecular size of GABA, it is not unexpected that bicuculline could bind to sites to which GABA could not reach outside the orthosteric site.

Key aspects of structure-activity studies on bicuculline as a $GABA_A$ antagonist are summarized in Figure 4 (Johnston, 1991). The cationic nitrogen and the lactone/ester function are important for interaction with the orthosteric site, while the two aromatic rings with methylenedioxy or methoxy

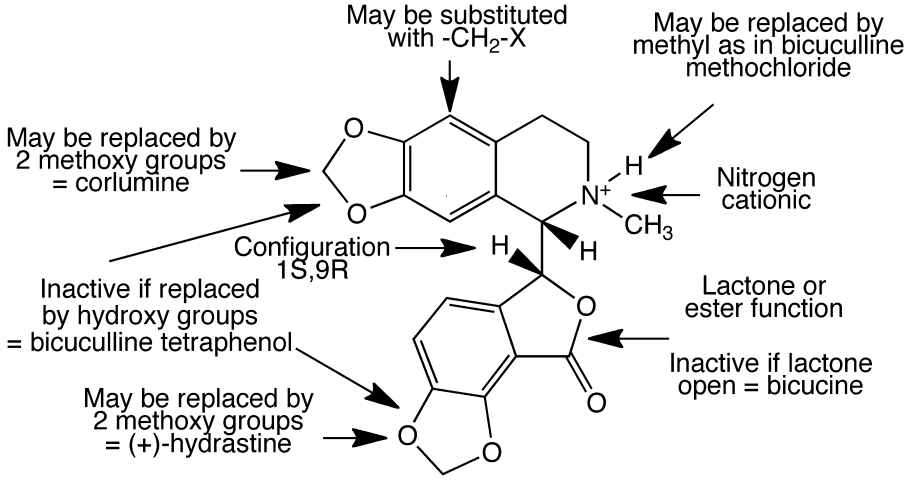

Figure 4

Summary of structure-activity studies on bicuculline and related compounds showing important aspects that contribute to the GABA receptors antagonist properties.

groups are considered important for interaction with the additional sites that contribute to antagonist action.

Why does bicuculline lack activity at some ionotropic GABA receptors?

Zhang and colleagues have studied the structural determinants for bicuculline antagonist activity using individual or combined mutations of nine residues in ρ1 $GABA_C$ and α1β2γ2 $GABA_A$ recombinant receptors (Zhang *et al.*, 2008). They showed that the bicuculline insensitivity of the ρ1 $GABA_C$ receptor was mainly determined by Tyr106, Phe138 and Phe240 residues. They also studied gabazine insensitivity that was highly dependent on Tyr102, Tyr106 and Phe138, consistent with differences between bicuculline and gabazine as $GABA_A$ receptor competitive antagonists. Mutation of Tyr157 to Ser in the β2 subunit of α1βγ2 L rat $GABA_A$ recombinant receptors resulted in bicuculline acting as a weak agonist (Ueno *et al.*, 1997). Mutations in the α1 subunit of α1β2γ2 $GABA_A$ recombinant receptors showed that the substitution in the N-terminal region of the extracellular domain of Phe64 by Leu decreased the affinities of bicuculline methiodide and gabazine by 60- to 200-fold, suggesting 'close functional and structural association of the α-subunit with the agonist/antagonist binding site' (Sigel *et al.*, 1992).

Other actions of bicuculline

Bicuculline and more particularly its quaternary salts have significant actions other than as $GABA_A$ receptors antagonists (Seutin and Johnson, 1999). While the actions of these agents on nicotinic acetylcholine receptors can be readily distinguished from their effects on $GABA_A$ receptors, they reflect the structural similarities between nicotinic and $GABA_A$ receptors, for example, (+)-tubocurarine acts on both but is more potent on nicotinic receptors (Hill *et al.*, 1973). Bicuculline quaternary salts and (+)-tubocurarine also act on SK (small conductance) calcium-activated potassium channels. Detailed studies on the antagonism of recombinant nicotinic acetylcholine receptors by bicuculline led to the suggestion that 'bicuculline may serve as a lead molecule to design new anticholinergic substances' (Demuro *et al.*, 2001). Bicuculline quaternary salts also act as inhibitors of acetylcholinesterase (Breuker and Johnston, 1975). GABA-metabolising enzymes are not influenced by bicuculline (Beart and Johnston, 1972).

Picrotoxin and picrotoxinin

Reports in the late 1950s that picrotoxin blocked the action of GABA at invertebrate inhibitory synapses and the observations in the early 1960s by Eccles and his colleagues (1963) of the reduction of the presynaptic inhibition of monosynaptic reflexes by this convulsant suggested that GABA was a transmitter in the mammalian spinal cord. We experienced difficulties in demonstrating that picrotoxin or its active component picrotoxinin (Figure 5, picrotoxin is a 1:1 mixture of picrotoxinin and relatively inactive picrotin) influenced the effect of GABA on spinal neurones using microelectrophoresis. Because picrotoxinin is poorly soluble and not ionized in water, local concentrations achieved by attempted microelectrophoretic administration were presumably too low. We were unable to reliably repeat the demonstration by others (Davidoff and Aprison, 1968) that microelectrophoretically administered picrotoxin consistently blocked the action of glycine on spinal interneurons (Curtis *et al.*, 1969). Frustration and confusion over our results regarding picrotoxinin led us to search for agents that could reliably distinguish between the effects of GABA and glycine such as we had already demonstrated with strychnine (Curtis *et al.*, 1967).

Using recombinant receptors, bath application of picrotoxin was subsequently shown to be a mixed/non-competitive $GABA_A$ receptor antagonist, in contrast, to the competitive antagonist action of bicuculline (Krishek *et al.*, 1996). In addition to its action as a $GABA_A$ receptor antagonist, picrotoxinin is now known to act upon $GABA_C$, glycine and $5HT_3$ receptors. Picrotoxin is considered to bind in the pore region of these ligand-gated ion channels (Thompson *et al.*, 2010).

Other $GABA_A$ receptor antagonists

A wide variety of $GABA_A$ receptor antagonists now exists. Four of the more interesting antagonists are discussed briefly below (Figure 5). Of these, gabazine is the most widely studied.

Gabazine, as SR 95531, was introduced as a specific, potent and competitive $GABA_A$ antagonist in 1986 as a result of the study of arylaminopyridazine derivatives of GABA (Heaulme *et al.*, 1986). While gabazine and bicuculline have similar actions as antagonists of the activation of $GABA_A$ receptors on hippocampal neurons by GABA, gabazine is much weaker against activation by pentobarbitone and etomidate (Uchida *et al.*, 1996). This and other findings suggests that GABA, pentobarbitone and etomidate act at non-identical sites on these neurons to open chloride ionophores and that these can be distinguished by gabazine and bicuculline (Ueno *et al.*, 1997). As noted above, gabazine and bicuculline interact with different residues on $GABA_A$ receptors (Zhang *et al.*, 2008). Structure-activity studies have been carried out on gabazine, identifying more potent analogues (Iqbal *et al.*, 2011).

Salicylidene salicylhydrazide (SCS) was identified as a potent inhibitor of the activation of $GABA_A$ receptors containing the β1 subunit using a high throughput screening assay (Thompson *et al.*, 2004). It produced a maximum inhibition of 56% with an IC50 of 32 nM. The presence of threonine 255 and isoleucine 308 within the β1 subunit and the lack of interaction with a range of $GABA_A$ receptor agents, including bicuculline and picrotoxin, suggest that SCS is interacting at a previously unidentified site.

RU5135, a convulsant steroid derivative, is one of the most potent $GABA_A$ antagonists, being some 500 times more potent than bicuculline in binding studies (Hunt and Clements-Jewery, 1981). In electrophysiological studies, it acted as a competitive antagonist with a pA2 of 8.31 and

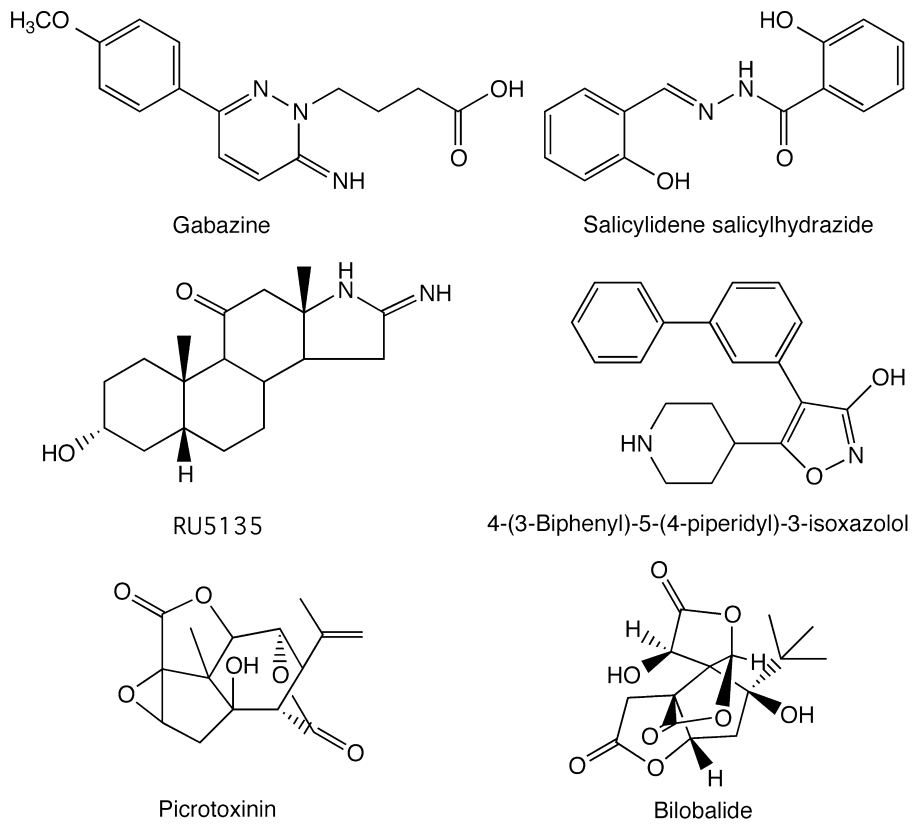

Figure 5
Structures of some $GABA_A$ receptors antagonists unrelated to bicuculline.

appeared to share a common site of action with bicuculline (Simmonds and Turner, 1985). However, it lacks specificity, as it is also a glycine antagonist sharing a common site of action with strychnine.

4-(3-Biphenyl)-5-(4-piperidyl)-3-isoxazolol (3-biphenyl-4-PIOL, 9 K in Frølund *et al.*, 2007) was derived from 4-PIOL, a low efficacy partial $GABA_A$ receptor agonist. It is a selective $GABA_A$ receptor antagonist acting in the low nM range. Studies on analogues of 4-PIOL substituted in the four-position of the isoxazole ring yielded $GABA_A$ receptor antagonists of increased potency, and there was a linear correlation between the lipophilicity of the four-subsitutent and antagonist activity, providing evidence for a hydrophobic binding pocket at the GABA recognition site (Frølund *et al.*, 2007).

Not all $GABA_A$ receptor antagonists are convulsants

The basic tenet for the successful search for bicuculline as a GABA receptor antagonist was that such an agent would be a convulsant on systemic administration, just as strychnine was a convulsant acting on glycine receptors. We and others have described a series of terpenoids structurally related to picrotoxinin, including bilobalide (Figure 4), from *Ginkgo biloba* that are relatively potent antagonists at ionotropic GABA receptors (Sasaki *et al.*, 1999b; Huang *et al.*, 2003; Ivic *et al.*, 2003). These agents also antagonize glycine and $5HT_3$ receptors (Hawthorne *et al.*, 2006; Thompson *et al.*, 2011), but they are not convulsants on systemic administration to mammals. Indeed, they act as anticonvulsants (Sasaki *et al.*, 1999a) and neuroprotectants (DeFeudis, 2002; Huang *et al.*, 2012). They appear to have diverse and competing actions on CNS neurotransmission including reducing the release of L-glutamate thus, reducing excitation (Johns *et al.*, 2002) and inhibiting GABA synthesis via inhibition of glutamate decarboxylase (Sasaki *et al.*, 1999a). The lack of convulsant actions of these terpenoids may be masked by their multiplicity of actions on a variety of neurotransmitters.

Advantages of an antagonist

Thus bicuculline became a useful tool for probing GABA-mediated synaptic inhibition. The subsequent discovery of bicuculline-insensitive GABA receptors, its instability and actions of bicuculline not related to GABA receptors meant that caution had to taken in interpreting results using bicuculline.

Until the discovery of bicuculline as a GABA receptor antagonist, along with many scientists, David Curtis did not believe that GABA was a neurotransmitter in the spinal cord.

This was based on the relative ubiquity of GABA's action as a neuronal depressant in the brain and spinal cord, and the difficulties in showing that its action could be blocked by picrotoxin. He writes in his autobiography (Curtis, 2006) about his participation in the May 1959 symposium 'Inhibition in the Nervous System and Gamma-Aminobutyric Acid' organized by Gene Roberts in Duarte, California: 'My paper dealt with the effects of GABA, L-GLUT, and related amino acids on spinal neurons, and my negative conclusions related to transmitter functions were unfortunately based on a faulty technique and incorrect assumptions.' Nonetheless, Curtis continued to investigate the function of GABA in the CNS. Spurred on by the discovery of strychnine as a glycine antagonist (Curtis *et al.*, 1967), Curtis actively encouraged what turned out to be a successful search for an equivalent GABA antagonist and with great enthusiasm demonstrated that this GABA antagonist was able to reduce the strychnine-insensitive postsynaptic inhibitions of Deiters' cells, Purkinje cells, pyramidal cells in the cerebral and hippocampal cortices and thalamocortical relay cells. Such studies provided substantive evidence for the role of GABA as an inhibitory neurotransmitter in the CNS.

David Curtis became a convert to the concept of GABA as an inhibitory neurotransmitter as a result of the breakthrough in the use of bicuculline as a GABA antagonist. This is reminiscent of his great mentor, Sir John Eccles, becoming a convert to the concept of chemical neurotransmission as a result of significant technological improvements in electrophysiological recordings. Interestingly, just as it is now known that electrical communication between neurones can take place, we now also know that GABA has many other functions including acting as a trophic factor to influence events such as proliferation, migration, differentiation, synapse maturation and cell death (Owens and Kriegstein, 2002). In mammals, GABA is found in many organs outside of the CNS where it serves various functions. GABA is involved in cell proliferation and migration, and may play a role in cancer. Recent evidence implicates GABA receptors in mucus overproduction in asthma acting on airway epithelial cells. GABA regulates insulin secretion from pancreatic β cells in concert with changes in glucose concentration and may be involved with type 1 diabetes (Braun *et al.*, 2010). Functional GABA receptors have also been described in T cells and macrophages (Tian *et al.*, 1999; Shiratsuchi *et al.*, 2009). Thus, in addition to neurotransmission in the CNS, GABA is involved in asthma, cancer, diabetes and the immune system (Hanrahan and Johnston, 2009). The discovery of agents (not only antagonists but also the full range of allosteric, full and partial agonists, and negative, neutralizing and positive modulators) that selectively influence the function of GABA in these systems remains a challenge for medicinal chemists and pharmacologists, since such molecules will have wide applicability in diverse clinical conditions.

Acknowledgements

The author is indebted to the many colleagues and students that contributed to the studies on bicuculline and related substances. He is grateful to Philip Beart, Jane Hanrahan and Ken Mewett for their constructive input into the writing of this review.

Conflict of interest

The author has no conflict of interest.

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