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OVERVIEW

Muscimol as an Ionotropic GABA Receptor Agonist

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Abstract Muscimol, a psychoactive isoxazole from *Amanita muscaria* and related mushrooms, has proved to be a remarkably selective agonist at ionotropic receptors for the inhibitory neurotransmitter GABA. This historic overview highlights the discovery and development of muscimol and related compounds as a GABA agonist by Danish and Australian neurochemists. Muscimol is widely used as a ligand to probe GABA receptors and was the lead compound in the development of a range of GABAergic agents including nipecotic acid, tiagabine, 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, (Gaboxadol®) and 4-PIOL.

Keywords GABA · Muscimol · GABA receptors · GABA transporters

Introduction

Mushroom of the genus *Amanita* contain numerous compounds of pharmacological interest including muscarine that gave the name to muscarinic acetylcholine receptors [1]. The isoxazole muscimol (also known as agarine, pantherine and pyroibotenic acid) was isolated from *Amanita muscaria* by several groups in the early 1960 s. These mushrooms are psychoactive and have a rich history [2] and muscimol is considered to contribute significantly to their behavioural effects [3]. The publication of the structures of muscimol and ibotenic acid (Fig. 1) by Professor Eugster in Zürich [4] showed their clear structural resemblance to the mammalian neurotransmitters GABA and glutamic acid, respectively. In Australia, we obtained samples of muscimol and ibotenic acid from Professor Eugster and showed that they indeed acted like GABA and glutamic acid when applied to spinal neurones in anaesthetised cats [5]. Muscimol was as potent as GABA as a depressant of the firing of spinal neurones and this action was not antagonised by strychnine, an antagonist of the spinal inhibitory neurotransmitter glycine [6]. We stated "with respect to its central action, muscimol is apparently a "GABA-like" amino acid as anticipated on structural grounds." When the GABA antagonist bicuculline became available we showed that muscimol was also antagonised by this agent [7]. Interest in the GABA related actions of muscimol continue to this day with 91 publications in 2013 containing the terms 'GABA' and 'muscimol' in their title or abstract according to the American Chemical Society's Chemical Abstracts and related data bases as accessed via SciFinder with an average of more than 78 publications per year since 1971. PubMed has more than 5800 hits for muscimol.

Muscimol, 5-aminomethyl-isoxazol-3-ol, is a conformationally restricted analogue of GABA in which the 3-hydroxyisoxazole moiety acts as 'a masked carboxyl group'. As far as certain GABA receptors are concerned, it looks like the carboxyl group of GABA, while other macromolecules ignore it. The pKa values for GABA (4.0 and 10.7) are similar to those for muscimol (4.8 and 8.4). The conformational restriction comes both from the incorporation of a double bond into the GABA backbone and the incorporation of the backbone into the isoxazole ring structure [8].

Lotte Brehm, Hans Hjeds and Povl Krogsgaard-Larsen at the Royal Danish School of Pharmacy in Copenhagen then reported the crystal structure of muscimol showing

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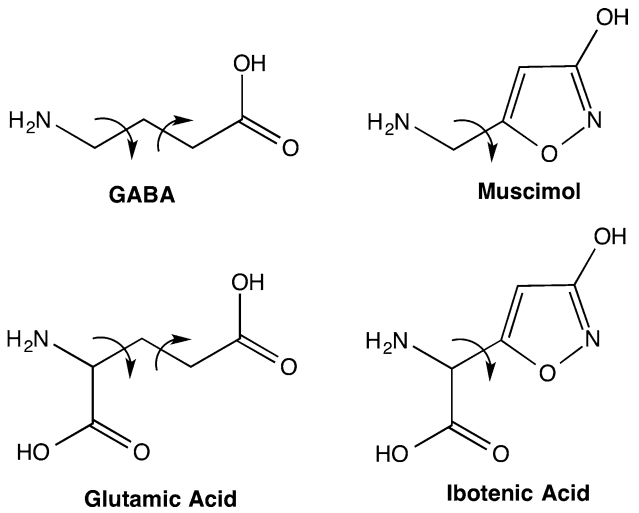

Fig. 1 Structures of the neurotransmitters GABA and glutamic acid and of their analogues extracted from *Amanita muscaria* mushrooms

its similarity with GABA [9]. In February 1974, Povl Krogsgaard-Larsen from the Royal Danish School of Pharmacy joined David Curtis and colleagues at the John Curtin School of Medical Research in Canberra as an Honorary Fellow. This first of four visits to Australia (later visits in 1977 to Canberra, 1981 and 1987 to Canberra and Sydney) began a highly successful and productive collaboration that resulted in 29 papers co-authored by colleagues in Australia and Denmark from 1975 to 2003 [10]. In 1974 Krogsgaard-Larsen brought with him to Canberra a large number of muscimol analogues that we tested on cat spinal neurones and rat brain slices. These investigations formed the basis for an extensive structure–activity study on conformationally restricted analogues of GABA as leads for new agents [11]. I was a Visiting Fellow at the Royal Danish School of Pharmacy in 1975, extending the collaboration that continues to this day between our younger colleagues in Australia and Denmark. In 2007 I was awarded an Honorary Doctorate in Pharmaceutical Science by the University of Copenhagen in recognition of my work in promoting research links between that university and Australian scientists. Most recently Petrine Wellendorf spent a year's study leave in Sydney, Mary Chebib visited Copenhagen and Thomas Balle took up a Senior Lectureship in Pharmacy in Sydney.

Selectivity of muscimol for ionotropic GABA receptors

There are two types of GABA receptors found in the central nervous system based on their mechanism of action. Ionotropic receptors are GABA gated chloride ion channels while metabotropic receptors are G-protein coupled receptors [12]. On the basis of their pharmacology, ionotropic receptors may be divided into two subclasses. $GABA_A$ receptors are antagonised by bicuculline while $GABA_C$ receptors are antagonised by TPMPA ((1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid). I have recently reviewed the discovery of bicuculline as a selective GABA antagonist [13]. Metabotropic GABA receptors are classified as $GABA_B$ receptors that are activated by baclofen and are insensitive to bicuculline and TPMPA.

Muscimol was the first selective conformationally restricted GABA agonist to define structure–activity relationships at bicuculline-sensitive $GABA_A$ receptors. It was followed by 4-aminotetrolic acid [14] and the cis and trans isomers of 4-aminocrotic acid [15] eventually leading to the discovery of bicuculline-insensitive $GABA_C$ receptors [16]. Tritiated muscimol is widely used to study ionotropic GABA receptors [17].

We now know that muscimol is a potent agonist at $GABA_A$ receptors, a potent partial agonist at $GABA_C$ receptors and inactive at $GABA_B$ receptors. Unlike bicuculline and TPMPA, it does not distinguish between $GABA_A$ and $GABA_C$ receptors. It is a weak inhibitor/substrate of GABA uptake and not a substrate for GABA transaminase [18–21].

Thus it can be seen that muscimol has a much more restrictive interaction in the nervous system than does GABA due to the isoxazole ring structure. Muscimol is widely used as a selective $GABA_A$ agonist, but, in fact, it has a more potent action as a partial agonist at $GABA_C$ receptors. Thus, interpretation of the effects of muscimol solely on the basis of the involvement of $GABA_A$ receptors in whole tissues is not justified unless the effects of muscimol are shown to be completely blocked by the $GABA_A$ antagonist bicuculline and insensitive to TPMPA.

The action of muscimol on different subunit combinations of $GABA_A$ receptors is relatively uniform [22] except for $\alpha4\beta3\delta$ receptors that are considered to represent extrasynaptic GABA receptors [23, 24]. Muscimol appeared to act as a super agonist on these receptors with increased efficacy when compared with GABA, producing some 120-140 % of the maximal efficacy of GABA. This observation has been explained by reduced desensitisation of the extrasynaptic receptors.

There is little evidence that muscimol binds to sites other than ionotropic GABA receptors and GABA transporters. Most investigators have found similar binding densities for muscimol and GABA to brain membranes. DeFeudis [25] however found more binding sites for tritiated muscimol than for tritiated GABA suggesting that muscimol bound to sites other than GABA. This finding may be the result of the presence of both high- and low-affinity agonist sites, where the high-affinity sites are conformational variants of the low-affinity sites [26]. Muscimol might favour a different ratio of high-affinity, low-capacity to low-affinity, high-capacity receptors for GABA under the conditions of the binding study.

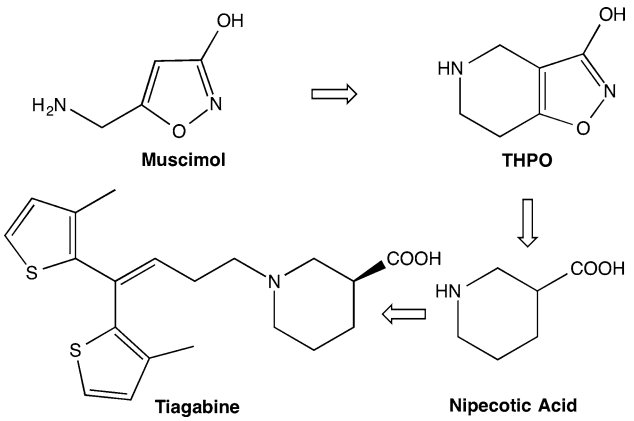

Fig. 2 Structures showing the development from muscimol of agents interacting with GABA uptakes systems

Molecular modelling studies of GABA, muscimol and bicuculline support the concept of these three agents acting at the orthosteric site at $GABA_A$ receptors in varying active conformations [27]. Most recently, Thomas Balle and colleagues in Denmark [28] have suggested that, unlike GABA, muscimol binds in concert with a water molecule to allow muscimol to bind in a low energy conformation. It is likely that these agents bind differently to different $GABA_A$ receptor subtypes [29].

The development of therapeutically useful GABA uptake inhibitors

Arising out of the studies on GABA uptake was our first new lead, nipecotic acid (Fig. 2). Four muscimol analogues were found to be inhibitors of the uptake of GABA into rat brain slices [30]. I had shown earlier that muscimol was a weak inhibitor of GABA uptake [19] so it was not a surprise that some muscimol analogues showed uptake activity. Of particular interest was 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol (THPO), a bicyclic analogue (Fig. 2), which was shown to be the most potent. 'Reverse engineering' of this analogue by replacing the hydroxyisoxazole moiety with a carboxylic acid lead to nipecotic acid, a potent competitive inhibitor of GABA uptake [30].

Structural studies using X-ray crystallography and NMR showed the similarities between nipecotic acid and partially folded conformations of GABA as it is taken up in rat brain slices [31]. We showed that nipecotic acid was a high affinity substrate for the GABA uptake system in rat brain slices [32] while muscimol was a weak substrate [20]. Nipecotic acid proved to be a stereoselective inhibitor of GABA uptake, with R(-)-nipecotic acid being considerably more potent than the S(+)-isomer [33], acting as an alternative substrate for GABA rather than a true inhibitor [32]. R(-)-Nipecotic acid was a much more potent inhibitor of the uptake of GABA

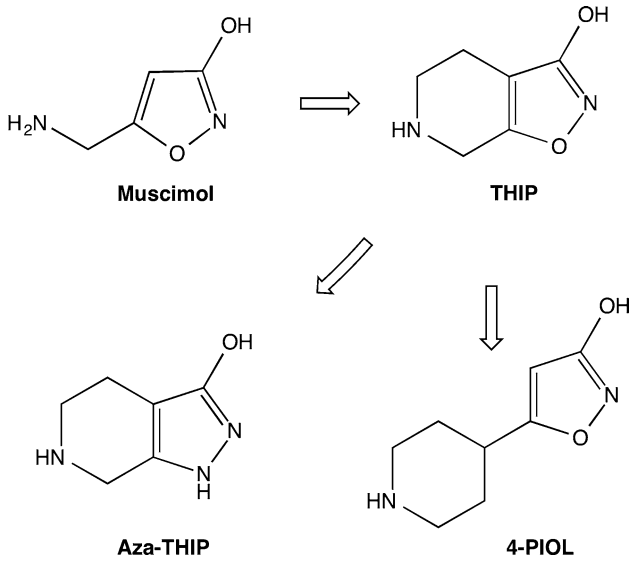

Fig. 3 Structures showing the development from muscimol of agents interacting with ionotropic GABA receptors

than of β-alanine in rat brain slices suggesting that it was selective for neuronal rather than glial GABA uptake systems [33]. THPO, in contrast to nipecotic acid, was not a substrate for GABA transporters and preferentially inhibited glial GABA uptake. It was more potent than nipecotic acid at increasing extracellular GABA levels when infused into rat thalamus [34]. Analogues of THPO have been investigated as inhibitors of glial GABA uptake systems [35].

Using nipecotic acid as a lead compound, much more potent inhibitors of GABA uptake bearing a diarylbutenyl substituent on the nitrogen atom of nipecotic acid were developed [36]. These new compounds were true inhibitors of GABA uptake rather than alternate substrates and they influenced nervous system function upon systemic administration by acting as anticonvulsants. Of these and related compounds, tiagabine (Gabitril®) is used clinically as an anticonvulsant. It was discovered at Novo Nordisk in Denmark in 1988 by a team of chemists and pharmacologists under the general direction of Claus Bræstrup [37]. Tiagabine is a highly selective inhibitor of the GAT1 subtype of GABA transporter, as are SK&F 89976-A, CI-966, and NNC-711 [38]. The development of therapeutically useful GABA uptake inhibitors based on nipecotic acid continues, e.g. in studies carried out by Arne Schousboe and his colleagues in Copenhagen [39]. A more detailed discussion of this topic may be found in an accompanying review [40].

Muscimol, THIP and the development of ionotropic GABA receptor agonists

4,5,6,7-Tetrahydroisoxazolo(5,4-c)pyridin-3-ol, Gaboxadol® (THIP), an isomer of the GABA uptake inhibitor

THPO, proved to be of particular interest (Fig. 3). This bicyclic agent is a conformationally restrained analogue of muscimol and thus might be expected to have more selective properties. Together with Povl Krogsgaard-Larsen, we showed that THIP was a new class of $GABA_A$ agonist [41].

Like muscimol, THIP was shown to be a super agonist at extrasynaptic $GABA_A$ receptors [24]. Although less potent that muscimol (EC50 0.20 μM) and GABA (EC50 0.35 μM) at α4β3δ $GABA_A$ receptors, THIP (EC50 13 μM) was more efficacious (Emax 224 %) than either muscimol (Emax 120 %) or GABA (98 %). The super agonist behaviour of THIP on these receptors was due to an increase in the duration of channel openings and in their frequency, resulting in longer burst durations [24]. At synaptic α1β3γ2 $GABA_A$ receptors THIP was a partial agonist (EC50 107 μM, Emax 85 %) compared to muscimol and GABA (EC50 0.92 and 3.4 μM. Emax 101 and 100 % respectively [24].

Unlike muscimol, THIP was an antagonist (IC50 25 μM) at ρ1 $GABA_C$ receptors [21, 42]. Of a series of analogues, aza-THIP (4,5,6,7-tetrahydropyrazolo[5,4-c]pyridin-3-ol, Fig. 3) showed similar antagonism (Ki 31 μM) at ρ1 $GABA_C$ receptors but was inactive at 100 μM at α1β2γ2 $GABA_A$ receptors [42]. Also unlike the binding of muscimol and GABA, the binding of THIP to rat brain membranes was not stimulated by diazepam [43]. These and other observations illustrate the differences between muscimol and THIP with respect to ionotropic GABA receptors.

THIP proved to be an interesting non-opioid analgesic and a novel type of hypnotic agent but a variety of problems have prevented its clinical development [44]. The effects of THIP on sleep resembled those reported earlier for muscimol and were dissimilar from those induced by benzodiazepine modulators of $GABA_A$ receptors [45].

4-PIOL – probing the orthosteric binding site of $GABA_A$ receptors

A series of nonannulated analogues of THIP have been developed by Bente Frølund and her colleagues. 4-PIOL (5-(4-piperidyl)isoxazol-3-ol, Fig 3) is a low-efficacy partial $GABA_A$ agonist [46]. Many analogues of 4-PIOL show potent antagonist actions at $GABA_A$ receptors, with the 2-naphthylmethyl and the 3,3-diphenylpropyl analogues being as potent as gabazine as antagonists [47]. As with THIP, 4-PIOL and its analogues show differences in activity at synaptic and extrasynaptic $GABA_A$ receptors with thio-4-PIOL showing partial agonist responses up to 30 % of GABA on extrasynaptic receptors with little partial agonist response (0-4 % of GABA) at synaptic receptors [48].

Extending these studies from 3-hydroxyisoxazole derivatives, as in muscimol, THIP and 4-PIOL, to a variety of heterocyclic carboxylic acid isosteres has provided the means to probe the orthosteric binding site of $GABA_A$ receptors [49]. A network of interactions between the analogues and the binding pocket leave no room for substituents and underline the limited space in the orthosteric binding site when in the agonist conformation, thus explaining the low activity of highly substituted derivatives [49]. It is hoped that this approach will lead to the discovery of further subtype-specific $GABA_A$ agonists.

Conclusion

The discovery of muscimol as a selective agonist at ionotropic GABA receptors and as a GABA uptake inhibitor has opened up the way to the discovery of an ever-increasing array of muscimol analogues with increasingly selective profiles. Danish and Australian neurochemists continue to play an important role in these investigations. Povl Krogsgaard-Larsen made many valuable contributions to this research both in Denmark and Australia.

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References

1. Wieland T (1968) Poisonous principles of mushrooms of the genus Amanita. Science 159:946–952
2. Wasson RG (1968) Soma: Divine Mushroom of Immortality. Harcourt Brace Jovanovich, New York
3. DeFeudis FV (1980) Physiological and behavioral studies with muscimol. Neurochem Res 5:1047–1068
4. Eugster CH, Takemoto T (1967) Zür Nomenclature der neuen Verbindungen aus Amanita-Arten. Helv Chim Acta 50:126–127
5. Johnston GAR, Curtis DR, De Groat WC, Duggan AW (1968) Central actions of ibotenic acid and muscimol. Biochem Pharmacol 17:2488–2489
6. Curtis DR, Hösli L, Johnston GAR (1967) Inhibition of spinal neurons by glycine. Nature 215:1502–1503
7. Curtis DR, Duggan AW, Felix D, Johnston GAR (1971) Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. Brain Res 32:69–96
8. Johnston GAR, GABA chemistry: Analogs of GABA as therapeutic and investigational agents, in GABA In The Nervous System: The View At Fifty Years, D.L. Martin and R. Olsen, Editors. 2000, Lippincott Williams and Wilkins: Philadelphia. p. 65-80.
9. Brehm L, Hjeds H, Krogsgaard-Larsen P (1972) The structre of muscimol, a GABA analogue of restricted conformation. Acta Chem Scand 26:1298–1299
10. Curtis DR (2006) David R. Curtis Autobiography. The History of Neuroscience in Autobiography 5:171–225
11. Krogsgaard-Larsen P, Johnston GAR, Curtis DR, Game CJA, McCulloch RM (1975) Structure and biological activity of a

series of conformationally restricted analogues of GABA. J Neurochem 25:803–809
12. Chebib M, Johnston GAR (2000) GABA-activated ligand gated ion channels: medical chemistry and molecular biology. J Med Chem 43:1427–1447
13. Johnston GAR (2013) Advantages of an antagonist: bicuculline and other GABA antagonists. Br J Pharmacol 169:328–336
14. Beart PM, Curtis DR, Johnston GAR (1971) 4-Aminotetrolic acid: new conformational-restricted analogue of γ-aminobutyric acid. Nature - New Biology 234:80–81
15. Johnston GAR, Curtis DR, Beart PM, Game CJA, McCulloch RM, Twitchin B (1975) Cis- and trans-4-aminocrotonic acid as GABA analogues of restricted conformation. J Neurochem 24: 157–160
16. Johnston GAR, Chebib M, Hanrahan JR, Mewett KN (2010) Neurochemicals for the investigation of $GABA_C$ receptors. Neurochem Res 35:1970–1977
17. Snodgrass SR (1978) Use of ^{3}H-muscimol for GABA receptor studies. Nature 273:392–394
18. Krogsgaard-Larsen P, Johnston GAR (1978) Structure-activity studies on the inhibition of GABA binding to rat brain membranes by muscimol and related compounds. J Neurochem 30:1377–1382
19. Johnston GAR (1971) Muscimol and the uptake of γ-aminobutyric acid by rat brain slices. Psychopharmacologia 22:230–233
20. Johnston GAR, Kennedy SM, Lodge D (1978) Muscimol uptake, release and binding in rat brain slices. J Neurochem 31:1519–1523
21. Woodward RM, Polenzani L, Miledi R (1993) Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes. II. Pharmacology of γ-aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ receptor agonists and antagonists. Mol Pharmacol 43:609–625
22. Ebert B, Thompson SA, Saounatsou K, McKernan R, Krogsgaard-Larsen P, Wafford KA (1997) Differences in agonist/antagonist binding affinity and receptor transduction using recombinant human γ-aminobutyric acid type A receptors. Mol Pharmacol 52:1150–1156
23. Storustovu SI, Ebert B (2006) Pharmacological characterization of agonists at delta-containing $GABA_A$ receptors: Functional selectivity for extrasynaptic receptors is dependent on the absence of gamma2. J Pharmacol Exp Ther 316:1351–1359
24. Mortensen M, Ebert B, Wafford K, Smart TG (2010) Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. J Physiol 588:1251–1268
25. DeFeudis FV (1980) Binding stuides with muscimol; Relation to synaptic γ-aminobutrate receptors. Neuroscience 5:675–688
26. Baur R, Sigel E (2003) On high- and low-affinity agonist sites in $GABA_A$ receptors. J Neurochem 87:325–332
27. Andrews PR, Johnston GAR (1979) Conformational analysis of muscimol, a GABA agonist. J Theor Biol 79:263–273
28. Bergmann R, Kongsbak K, Sorensen PL, Sander T, Balle T (2013) A unified model of the GABA(A) receptor comprising agonist and benzodiazepine binding sites. PLoS ONE [Electronic Resource] 8:e52323
29. Karim N, Wellendorph P, Absalom N, Bang LH, Jensen ML, Hansen MM, Lee HJ, Johnston GAR, Hanrahan JR, Chebib M (2012) Low nanomolar GABA effects at extrasynaptic alpha4beta1/beta3delta $GABA_A$ receptor subtypes indicate a different binding mode for GABA at these receptors. Biochem Pharmacol 84:549–557
30. Krogsgaard-Larsen P, Johnston GAR (1975) Inhibition of GABA uptake in rat brain slices by nipecotic acid, various isoxazoles and related compounds. J Neurochem 25:797–802
31. Brehm L, Krogsgaard-Larsen P, Johnston GAR, Schaumburg K (1976) X-Ray crystallographic and proton magnetic resonance spectorscopic investigations of nipecotic acid, a potent inhibitor of γ-aminobutyric acid (GABA) uptake. Acta Chem. Scand. B 30:542–548
32. Johnston GAR, Stephanson AL, Twitchin B (1976) Uptake and release of nipecotic acid by rat brain slices. J Neurochem 26:83–87
33. Johnston GAR, Krogsgaard-Larsen P, Stephanson AL, Twitchin B (1976) Inhibition of the uptake of GABA and related amino acids in rat brain slices by the optical isomers of nipecotic acid. J Neurochem 26:1029–1032
34. Juhasz G, Kekesi KA, Nyitral G, Dobolyl A, Krogsgaard-Larsen P, Schousboe A (1997) Differential effects of nipecotic acid and 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol on extracellular γ-aminobutyrate levels in rat thalamus. Eur J Pharmacol 331: 139–144
35. Falch E, Perregaard J, Frølund B, Sokilde B, Buur A, Hansen LM, Frydenvang K, Brehm L, Bolvig T, Larsson OM, Sanchez C, White HS, Schousboe A, Krogsgaard LP (1999) Selective inhibitors of glial GABA uptake: synthesis, absolute stereochemistry, and pharmacology of the enantiomers of 3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (exo-THPO) and analogues. J Med Chem 42:5402–5414
36. Falch E, Krogsgaard-Larsen P (1989) GABA uptake inhibitors containing mono- and diarylmethoxyalkyl N-subsitutents. Drug Design & Delivery 4:205–215
37. Andersen KE, Braestrup C, Gronwald FC, Jorgensen AS, Nielsen EB, Sonnewald U, Sorensen PO, Suzdak PD, Knutsen LJ (1993) The synthesis of novel GABA uptake inhibitors. 1. Elucidation of the structure-activity studies leading to the choice of (R)-1-[4,4-bis(3-methyl)-3-thienyl-3-butenyl]-3-piperidinecarboxylic acid (tiagabine) as an anticonvulsant drug candidate. J Med Chem 36:1716–1725
38. Borden LA, Murali Dhar TG, Smith KE, Weinshank RL, Branchek TA, Gluchowki C (1964) Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. Eur J Pharmacol 269:219–224
39. Schousboe A, Madsen KK, White HS (2011) GABA transport inhibitors and seizure protection: the past and future. Future Medicinal Chemistry 3:183–187
40. Schousboe A, Madsen KK, Barker-Haliski ML, and White HS (2014) The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters. Neurochemical Research: in press this issue
41. Krogsgaard-Larsen P, Johnston GAR, Lodge D, Curtis DR (1977) A new class of GABA agonist. Nature 268:53–55
42. Krehan D, Frølund B, Ebert B, Nielsen B, Krogsgaard-Larsen P, Johnston GAR, Chebib M (2003) Aza-THIP and related analogues of THIP as $GABA_C$ antagonists. Bioorganic and Medicinal Chemistry 11:4891–4896
43. Skerritt JH, Johnston GAR (1983) Diazepam stimulates the binding of GABA and muscimol but not THIP to rat brain membranes. Neurosci Lett 38:315–320
44. Krogsgaard-Larsen P, Frolund B, Liljefors T, Ebert B (2004) $GABA_A$ agonists and partial agonists: THIP (Gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. Biochem Pharmacol 68:1573–1580
45. Lancel M, Faulhaber J (1996) The GABA(a) agonist THIP (gaboxadol) increases non-rem sleep and enhances delta activity in the rat. NeuroReport 7:2241–2245
46. Frølund B, Kristiansen U, Brehm L, Hansen AB, Krogsgaard-Larsen P, Falch E (1995) Partial $GABA_A$ receptor agonists. Synthesis and in vitro pharmacology of a series of nonannulated analogs of 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol. J Med Chem 38:3287–3296
47. Frølund B, Tagmose L, Jorgensen AT, Kristiansen U, Stensbol TB, Liljefors T, Krogsgaard-Larsen P (2003) Design and

synthesis of a new series of 4-alkylated 3-isoxazolol GABA A antagonists. Eur J Med Chem 38:447–449

48. Hoestgaard-Jensen K, O'Connor RM, Dalby NO, Simonsen C, Finger BC, Golubeva A, Hammer H, Bergmann ML, Kristiansen U, Krogsgaard-Larsen P, Brauner-Osborne H, Ebert B, Frølund B, Cryan JF, Jensen AA (2013) The orthosteric $GABA_A$ receptor ligand Thio-4-PIOL displays distinctly different functional properties at synaptic and extrasynaptic receptors. Br J Pharmacol 170:919–932
49. 49. Peterson JG, Bergmann R, Krogsgaard-Larsen P, Balle T, and Frølund B (2014) Probing the orthosteric binding site of $GABA_A$ receptors with heterocyclic GABA carboxylic acid bioisosteres. Neurochemical Research:in press