*Clinical and Experimental Pharmacology and Physiology, 1999, **26**, 937-940*

THE 'ABC' OF GABA RECEPTORS: A BRIEF REVIEW

Mary Chebib and Graham A R Johnston

Adrien Albert Laboratory of Medicinal Chemistry, Department of Pharmacology, The University of Sydney, Sydney, New South Wales, Australia

SUMMARY

1. In the mammalian central nervous system, GABA is the main inhibitory neurotransmitter. GABA is a highly flexible molecule and, thus, can exist in many low-energy conformations. Conformationally restricted analogues of GABA have been used to help identify three major GABA receptors, termed $GABA_A$, $GABA_B$ and $GABA_C$ receptors.

2. $GABA_A$ and $GABA_C$ receptors are members of a superfamily of transmitter-gated ion channels that include nicotinic acetylcholine, strychnine-sensitive glycine and 5HT3 receptors. $GABA_A$ receptors are hetero-oligomeric Cl^- channels that are selectively blocked by the alkaloid bicuculline and modulated by steroids, barbiturates and benzodiazepines. To date, 16 human $GABA_A$ receptor cDNA have been cloned.

3. $GABA_B$ receptors are seven transmembrane receptors that are coupled to G-proteins and activate second messenger systems and $Ca2^+$ and K^+ ion channels. To date, three $GABA_B$ receptor proteins have been cloned and these resemble metabotropic glutamate receptors. $GABA_B$ receptors are hetero-oligomeric receptors made up of a mixture of a combination of the subunits. These receptors are selectively activated by (-)-baclofen and CCGP27492 and are blocked by phaclofen, the phosphonic acid analogue of baclofen.

4. In contrast, $GABA_C$ receptors represent a relatively simple form of transmitter-gated Cl^-; channel made up of a single type of protein subunit. Two human $GABA_C$ receptor cDNA have been cloned. These receptors are not blocked by bicuculline nor are they modulated by steroids, barbiturates or benzodiazepines. Instead, $GABA_C$ receptors are selectively activated by the conformationally restricted analogues of GABA in the folded conformation cis-4-aminocrotonic acid and (1S,2R)-2-(aminomethyl)-1-carboxycyclopropane. (1,2,5,6-Tetrahydropyridine-4-yl)methylphosphinic acid, a methylphosphinic acid analogue of GABA in a partially folded conformation, is a selective antagonist at $GABA_C$ receptors.

Correspondence: Dr Mary Chebib, Adrien Albert Laboratory of Medicinal Chemistry, Department of Pharmacology, DO6, The University of Sydney, NSW 2006, Australia. E-mail: maryc@pharmacol.usyd.edu.au

Presented at the Australian Neuroscience Society Symposium on GABA and Glycine Receptors, Hobart, January/February 1999.

Received 4 February 1999; accepted 10 June 1999.

Key words: (3-aminopropyl) methylphosphinic acid; CGP44530; CGP70523; cis-4-aminocrotonic acid; GABA; $GABA_A$ receptors; $GABA_B$ receptors; $GABA_C$ receptors; (1,2,5,6-tetrahydropyridine-4-yl)methyl phosphinic acid; trans-4-aminocrotonic acid

INTRODUCTION

The inhibitory neurotransmitter GABA (Fig. 1, structure 1), activates three major classes of receptors, termed $GABA_A$, $GABA_B$ and $GABA_C$ receptors. These receptors have different characteristics: the $GABA_A$ and $GABA_C$

receptors are ionotropic, while the $GABA_B$ receptors are metabotropic.[1]

IONOTROPIC $GABA_A$ AND $GABA_C$ RECEPTORS

The $GABA_A$ and $GABA_C$ receptors are Cl^-; channels that mediate fast synaptic inhibition. Both the $GABA_A$ and $GABA_C$ receptors are members of a superfamily of transmitter-gated ion channels that includes the nicotinic acetylcholine, strychnine-sensitive glycine and 5HT3 receptors. These transmitter-gated ion channels are believed to be structurally very similar, composed of five subunits that arrange together to form an ion channel. Each subunit has four transmembrane domains.[2] When the channel forms, all five subunits arrange in such a way that their second transmembrane domains form the wall of the channel pore. Furthermore, there is a large intracellular loop between transmembrane domains three and four, which is believed to be the target for protein kinases and to be required for subcellular targetting and membrane clustering of the receptor.[2] It is in this region that the receptors can anchor to the cytoskeleton. Both the C- and N-terminal regions lie extracellular to the cell surface and part of the agonist/antagonist binding site lies within the N-terminal region.[2]

$GABA_A$ and $GABA_C$ receptors are biochemically, pharmacologically and physiologically different.[3,4] GABA is an order of magnitude less potent at $GABA_A$ than $GABA_C$ receptors. $GABA_A$ receptors are selectively blocked by the alkaloid bicuculline and are modulated by benzodiazepines, steroids and barbiturates.[1,3,4] $GABA_C$ receptors are not blocked by bicuculline, nor are they modulated by benzodiazepines, steroids or barbiturates.[3,4] Instead, $GABA_C$ receptors are activated by Z-4-aminobut-2-enoic acid (cis-aminocrotonic acid (CACA); Fig. 1, structure 2)[3,4] and (1S,2R)-(+)-2-(aminomethyl)-cyclopropane-1-carboxylic acid ((1S,2R)-(+)-CAMP; Fig. 1, structure 3; RK Duke et al., unpubl. obs., 1998)[5] and are selectively blocked by (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA; Fig. 1, structure 4).[6,7] Very little is known about chemical modulators for the $GABA_C$ receptors.

To date, there are 16 human $GABA_A$ receptor subunits (α1-6, β1-4, γ1-4, δ, ε)[1,8] and two human $GABA_C$ receptor subunits (ρ1 and ρ2)[9-11] that have been cloned. There is approximately 30% sequence identity between the subunits and approximately 70% sequence identity between subunit subtypes.[12] $GABA_A$ receptors are hetero-oligomeric, made up of a mixture of α1-6, β1-4, γ1-4, δ and ε subunits. Consequently, an enormous array of combinations may exist for these receptor subtypes. However, for a fully functional $GABA_A$ receptor, it appears that an α, β and one other subunit type, such as γ, δ or [small element of], are required.[1] In contrast, $GABA_C$ receptors are homo-oligomeric, made up of either ρ1 or ρ2 subunits, although there is increasing evidence that these receptors may be hetero-oligomeric, made up of a combination of the ρ subunits.[13] The ρ subunits do not assemble with α and β subunits to form a receptor.[14,15] Thus, the composition of $GABA_C$ receptors is different to $GABA_A$ receptors.

Single channel electrophysiological studies using outside-out patches from rat retinal bipolar cells showed that $GABA_C$ receptors conducted less current than $GABA_A$ receptors.[3,4] When activated, $GABA_C$ receptors had a longer channel opening time and desensitized less readily with maintained agonist application.[3,4] Recently, different associated proteins have been cloned that link these receptors to the cytoskeleton.[16,17] $GABA_A$ receptors are linked via the γ2-subunit to the cytoskeleton by $GABA_A$ receptor-associated proteins (GABARAP),[16] while $GABA_C$ receptors are linked via the ρ1-subunit to the cytoskeleton by microtubule-associated protein 1B (MAP-1B).[17] The fact that two different proteins associate with the $GABA_A$ and $GABA_C$ receptors allows these receptors to exist and function separately.

METABOTROPIC $GABA_B$ RECEPTORS

$GABA_B$ receptors are seven transmembrane receptors that activate the second messenger systems phospholipase C and adenylate cyclase and activate K^+ and Ca^{2+} ion channels via G-coupled proteins.[18] These receptors are selectively activated by (R)-(-)-baclofen and the phosphinic acid analogue of GABA (3-aminopropyl)phosphinic acid (CGP27492). The phosphonic and sulphonic acid analogues of

(R)-(-)-baclofen, phaclofen and saclofen, respectively, selectively antagonize these receptors.[18]

$GABA_B$ receptors produce slow, prolonged inhibitory signals and function to modulate the release of neurotransmitters. To date, three subunits have been cloned and are termed $GABA_{BR1a}$, $GABA_{BR1b}$ and $GABA_BR2$.[19-21] These subunits resemble metabotropic glutamate receptors.[19] When the $GABA_{BR1a}$ or $GABA_{BR1b}$ subunit is combined with the $GABA_{BR2}$ subunit, fully functional $GABA_B$ receptors are formed when these subunits are expressed in Xenopus oocytes or mammalian cell expression systems.[20-23] Double immunoprecipitation studies have shown that these subunit combinations also exist in vivo as either dimers or multimers.[20-23] Therefore, $GABA_B$ receptors are hetero-oligomeric receptors, made up of a $GABA_{BR1a}$ or $GABA_{BR1b}$ subunit and a $GABA_{BR2}$ subunit.

CONFORMATIONALLY RESTRICTED ANALOGUES OF GABA

GABA is a highly flexible molecule; therefore, it can attain many low-energy conformations that bind to the different GABA receptors. E-4-Aminobut-2-enoic acid (trans-4-aminocrotonic acid (TACA); Fig. 1, structure 5) and CACA are two conformationally restricted analogues of GABA: TACA is in an extended conformation, while CACA is in a folded conformation.

$GABA_C$ receptors were first proposed when a series of conformationally restricted analogues of GABA, including TACA and CACA, were tested for their effects in depressing the firing of spinal interneurons of the cat.[24] All three compounds depressed the firing of spinal interneurons. However, it was only the effects of GABA and TACA that were blocked by bicuculline. Bicuculline did not block the effects of CACA. As CACA had no effect on glycine receptors, it was proposed that CACA activated a GABA receptor that was insensitive to bicuculline, while TACA activated a GABA receptor that was sensitive to bicuculline. This novel GABA receptor was further distinguished from $GABA_B$ receptors by the fact that CACA also depressed the firing of Renshaw cells in the cat, while baclofen did not have any effect.[24] Therefore, CACA activated a GABA receptor that was insensitive to both bicuculline and baclofen. This receptor was termed the $GABA_C$ receptor.[25]

The effects of GABA, TACA and CACA were further investigated using recombinant $GABA_A$ and $GABA_C$ receptor subunit mRNA expressed in Xenopus oocytes.[26] ^{3}H-CGP27492 binding to rat cortical membranes was used to determine the activity at $GABA_B$ receptors.[27] These studies further showed that CACA, the restricted analogue of GABA in the folded conformation, selectively activated $GABA_C$ receptors, while TACA, the restricted analogue of GABA in the extended conformation, was not selective. However, we cannot make a direct quantitative comparison with the $GABA_B$ receptors because the effects on $GABA_B$ receptors were assessed using a radioligand binding assay system, while effects on $GABA_A$ and $GABA_C$ receptors were assessed using functional assay systems.

EFFECTS OF MUSCIMOL AND THIP ON $GABA_A$ AND $GABA_C$ RECEPTORS

The compounds muscimol (Fig. 1, structure 6) and its conformationally restricted analogue 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP; Fig. 1, structure 7), act at both $GABA_A$ and $GABA_C$ receptors. Muscimol can exist in two low-energy conformations: one conformation is extended, while the other is partially folded. In the case of THIP, there are also two low-energy conformations. However, both of these exist in the partially folded conformation. The only difference between the two conformations is the orientation of the nitrogen: in one conformation the nitrogen is below and, in the other, the nitrogen is above the plane made by THIP.

At $GABA_A$ receptors, muscimol is an agonist while THIP is a partial agonist.[26,28] At $GABA_C$ receptors, muscimol is a partial agonist[26,29] while THIP is an antagonist.[29] Therefore, THIP can be used to pharmacologically distinguish these receptors. Muscimol and THIP are six- and two-fold more potent, respectively, at $GABA_C$ than $GABA_A$ receptors.[29] Because the pharmacology of muscimol is similar at both $GABA_A$ and $GABA_C$ receptors, it cannot be used to pharmacologically distinguish $GABA_A$ and $GABA_C$ receptors. Therefore, further work into developing selective $GABA_A$ receptor agonists is

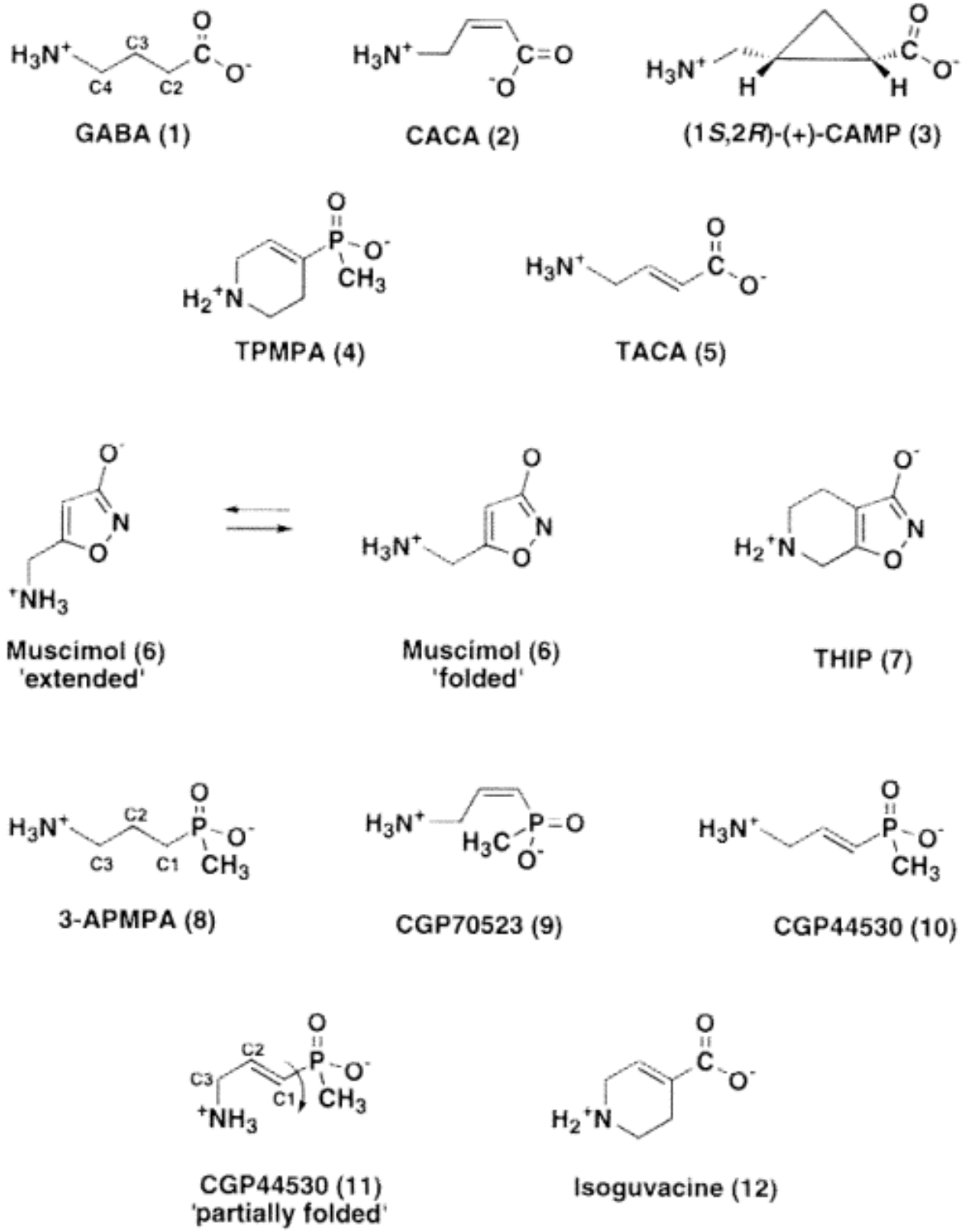

Fig 1. The structures of some GABA analogues. Numbers in parentheses indicates the structure numbers referred to in the text.

required. In the meantime, one needs to exert caution when using muscimol and THIP as pharmacological tools to characterize $GABA_A$ receptors.

CGP35024, CGP44530 and CGP70523 were tested on $GABA_A$, $GABA_B$ and $GABA_C$ receptors. Compounds were tested on the $GABA_A$ receptor subtype, the α1β2γ2 expressed in Xenopus oocytes and assessed using two electrode voltage clamp methodology (M Chebib et al., unpubl. obs., 1998). On $GABA_C$ receptors, compounds were tested using the ρ1 mRNA expressed in Xenopus oocytes and assessed using two electrode voltage clamp methodology.[30] On $GABA_B$ receptors, compounds were tested using [3H]-CGP27492 binding at rat cortical membranes.[27] CGP35024, CGP44530 and CGP70523 had little effect on $GABA_A$ receptors, activated $GABA_B$ receptors and blocked $GABA_C$ receptors. At $GABA_C$ receptors, CGP44530 and CGP70523 were both weaker than CGP35024. These compounds were selective for $GABA_C$ receptors when compared with $GABA_A$ receptors but were not selective when compared with $GABA_B$ receptors. However, we cannot make a direct quantitative comparison of CGP35024, CGP44530 and CGP75023 with $GABA_B$ receptors because the effects on $GABA_B$ receptors were assessed using a radioligand binding assay system. Now that functional $GABA_B$ receptors can be expressed in Xenopus oocytes and other cell expression systems,[20-23] this comparison can be achieved.

One reason for the apparently reduced selectivity of CGP44530 and CGP70523 for the $GABA_C$ receptors may be that these compounds are more flexible when compared with TACA and CACA.[30] With CGP44530 and CGP70523, there is less restricted rotation in the methylphosphinic acid group than with the carboxylic acid groups of TACA and CACA. If restricted analogues of GABA in the folded conformation selectively bind to $GABA_C$ receptors, then CGP44530, for example, binds in a partially folded conformation. This leaves the bond between the phosphorous and C1 carbon free to rotate (Fig. 1, structure 11). Therefore, it is difficult to determine the space where the methylphosphinic acid group resides when binding to the receptor.

Isoguvacine (Fig. 1, structure 12) is a conformationally restricted analogue of TACA in a partially folded conformation. It is a weak partial agonist at $GABA_C$ receptors [29,31] and an agonist at $GABA_A$ receptors.[29] Replacing the carboxylic acid group of isoguvacine with a methylphosphinic acid group results in the compound TPMPA. This compound is a weak antagonist at $GABA_A$ receptors and a weak agonist at $GABA_B$ receptors.[6,7] However, it is a highly potent and selective $GABA_C$ receptor antagonist.[6,7,31]

In conclusion, reducing the number of conformations that GABA can attain helps determine which conformation(s) bind to the different GABA receptors. Conformationally restricted analogues of GABA in the folded conformation, such as the carboxylic acid derivatives CACA and (1S,2R)-(+)-CAMP, selectively activate $GABA_C$ receptors, while conformationally restricted analogues of GABA in the partially folded conformation, such as the methylphosphinic acid derivative TPMPA, selectively block $GABA_C$ receptors.

ACKNOWLEDGEMENTS

We thank Dr Wolfgang Froestl (Novartis, Switzerland) for providing samples of CGP35024, CGP44530 and CGP70523, Dr Ken Mewett (Department of Pharmacology, The University of Sydney) for the synthesis of TPMPA, TACA and CACA, Dr Rujee Duke (Department of Pharmacology, The University of Sydney) for the resolving the enantiomers of CAMP and the National Health and Medical Research Council of Australia and Circadian Technologies Ltd for financial support.

REFERENCES

1. Johnston GAR. $GABA_A$ receptor pharmacology. Pharmacol. Ther. 1996; 69: 173-98.
2. Macdonald RL, Olsen RW. $GABA_A$ receptor channels. Annu. Rev. Neurosci. 1994; 17: 569-602.
3. Johnston GAR. $GABA_C$ receptors: Relatively simple transmitter-gated ion-channels? Trends Pharmacol. Sci. 1996; 17: 319-23.

4. Bormann J, Feigenspan A. $GABA_C$ receptors. Trends Neurosci. 1995; 18: 515-19.
5. Duke RD, Allan RD, Chebib M, Greenwood JR, Johnston GAR. Resolution and conformational analysis of diastereoisomeric esters of cis- and trans-2-(aminomethyl)-1-carboxycyclopropanes. Tetrahedron Asymm. 1998; 9: 2533-48.
6. Murata Y, Woodward RM, Miledi R, Overman LE. The first selective antagonist for a $GABA_C$ receptor. Bioorg. Med. Chem. Lett. 1996; 6: 2071-6.
7. Ragozzino D, Woodward RM, Murata F, Eusebi F, Overman LE, Miledi R. Design and in vitro pharmacology for a selective γ-aminobutyric acidC receptor antagonist. Mol. Pharmacol. 1996; 50: 1024-30.
8. Davies PA, Hanna MC, Hales TG, Kirkness EF. Insensitivity to anaesthetic agents conferred by class of $GABA_A$ receptor subunit. Nature 1997; 385: 820-3.
9. Cutting GR, Lu L, O'Hara B et al. Cloning of the GABAρ1 cDNA: A novel GABA subunit highly expressed in the retina. Proc. Natl Acad. Sci. USA 1991; 88: 2673-7.
10. Cutting GR, Curristin S, Zoghbi H, O'Hara B, Seldin MF, Uhl GR. Identification of a putative γ-aminobutyric acid (GABA) receptor subunit ρ2 cDNA and colocalization of the genes encoding ρ2 and ρ1 to human chromosome 6q14-q21 and mouse chromosome 4. Genomics 1992; 12: 801-6.
11. Wang T-L, Guggino WB, Cutting GR. A novel γ-aminobutyric acid receptor subunit (ρ2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in Xenopus oocytes. J. Neurosci. 1994; 14: 6524-31.
12. Johnston GAR, Chebib M, Duke RK, Mewett KN, Mitrovic AD, Vandenberg RJ. Medicinal chemistry and molecular pharmacology of GABA receptors and glutamate transporters: Complementary structure-activity relationships. Drug Dev. Res. 1999; 46: 255-60.
13. Zhang DX, Pan ZH, Zhang XH, Brieau AD, Lipton SA. Cloning of gamma-aminobutyric acid type C receptor subunit in rat retina with a methionine residue critical for picrotoxinin channel block. Proc. Natl Acad. Sci. USA 1995; 92: 11 756-60.
14. Hackam AS, Wang TL, Guggino WB, Cutting GR. A 100 amino acid region in the GABA ρ1 subunit confers robust homo-oligomeric expression. Neuroreport 1997; 8: 1425-30.
15. Hackam AS, Wang TL, Guggino WB, Cutting GR. The N-terminal domain of human GABA receptor ρ1 subunits contains signals for homo-oligomeric and hetero-oligomeric interaction. J. Biol. Chem. 1997; 272: 13 750-7.
16. Wang H, Bedford F, Brandon N, Moss S, Olsen R. $GABA_A$-receptor-associated protein links $GABA_A$ receptors and cytoskeleton. Nature 1999; 397: 69-72.
17. Hanley J, Koulen P, Bedford F, Gorden-Weeks P, Moss S. The protein MAP-1B links $GABA_C$ receptors to the cytoskeleton at retinal synapses. Nature 1999; 397: 66-9.
18. Kerr DIB, Ong J. $GABA_B$ receptors. J. Pharmacol. Ther. 1995; 67: 187-46.
19. Kaupmann K, Huggel K, Heids J et al. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. Nature 1997; 386: 239-46.
20. Kaupmann K, Malitschek B, Schuler V et al. $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 1998; 396: 683-7.
21. White J, Wise A, Main M et al. Heterodimerization for the formation of a functional $GABA_B$ receptor. Nature 1998; 396: 679-82.
22. Jones K, Borowsky B, Tamm J et al. $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_{BR1}$ and $GABA_{BR2}$. Nature 1998; 396: 674-8.
23. Kuner R, Kohr G, Grunwald S, Eisenhardt G, Bach A, Kornau H-C. Role of heteromer formation in $GABA_B$ receptor function. Science 1999; 283: 74-7.
24. Johnston GAR, Curtis DR, Beart PM, Game CJA, McCulluch RM, Twitchin B. Cis- and trans-4-Aminocrotonic acid as GABA analogues of restricted conformation. J. Neurochem. 1975; 24: 157-60.
25. Drew CA, Johnston GAR, Weatherby RP. Bicuculline-insensitive GABA receptors:

Studies on the binding of (-)-baclofen to rat cerebellar membranes. Neurosci. Lett. 1984; 52: 317-21.

26. Kusama T, Spivak CE, Whiting P, Dawson VL, Schaeffer JC, Uhl GR. Pharmacology of GABA ρ1 and GABA α/β receptors expressed in Xenopus oocytes and COS cells. Br. J. Pharmacol. 1993; 109: 200-6.
27. Froestl W, Mickel S, Hall R et al. Phosphinic acid analogues of GABA. 1. New potent and selective $GABA_B$ agonists. J. Med. Chem. 1995; 38: 3297-312.
28. Krogsgaard-Larsen P, Frölund B, Jorgensen FS, Schousboe A. $GABA_A$ receptor agonists, partial agonists and antagonists. Design and therapeutic prospects. J. Med. Chem. 1994; 37: 2489-505.
29. Woodward RM, Polenzani L, Miledi R. Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in Xenopus oocytes. II. Pharmacology of γ-aminobutyric acidA and γ-aminobutyric acidB receptor agonists and antagonists. Mol. Pharmacol. 1993; 43: 609-25.
30. Chebib M, Vandenberg RJ, Froestl W, Johnston GAR. Unsaturated phosphinic analogues of γ-aminobutyric acid as $GABA_C$ receptor antagonists. Eur. J. Pharmacol. 1997; 329: 223-9.
31. Chebib M, Mewett KN, Johnston GAR. $GABA_C$ receptor antagonists differentiate between human ρ1 and ρ2 receptors expressed in Xenopus oocytes. Eur. J. Pharmacol. 1998; 357: 227-34.