# Extrasynaptic site of action for γ-hydroxybutyrate

**S. J. Enna**[1]

*Department of Molecular and Integrative Physiology and Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical School, Kansas City, KS 66160*

Advances in pharmacology often result from identifying the mechanism of action of drugs found empirically to be biologically active (1). γ-Hydroxybutyric acid (GHB), also known as Fantasy, is a centrally active agent that has been used clinically and recreationally for nearly half a century. Because of its history of abuse, its availability is tightly regulated, although it is prescribed for the treatment of alcoholism and narcolepsy. Depending on the dose, GHB displays a myriad of effects on CNS function. Despite decades of research, significant questions remain about its mechanism of action. In PNAS, Absalom et al. (2) identify a distinct population of extrasynaptic $GABA_A$ receptors as a target for GHB, providing unique insights into the molecular target for this agent.

## GHB and GABA

An involvement of GABAergic systems in the actions of GHB has been suspected for some time because it is structurally similar to, and a metabolite of, GABA (3). High- and low-affinity [$^3$H]GHB binding sites are present in rat and human brain (4, 5). Because [$^3$H]GHB binding is not inhibited by GABA or various isosteric $GABA_A$ receptor ligands, it appears not to attach to the GABA recognition site on this pentameric, ligand-gated ion channel receptor. Rather, because [$^3$H]GHB binding is influenced by chloride ions and allosteric $GABA_A$ receptor ligands, it has been speculated that its site of action may be a component of the $GABA_A$ receptor chloride ion channel (6).

γ-Hydroxybutyrate–induced neuronal hyperpolarization is blocked by antagonists for metabotropic, heterodimeric $GABA_B$ receptors (7). Studies with recombinant $GABA_B$ receptors indicate that GHB is a weak, isosteric $GABA_B$ receptor agonist (8), as does the discovery that the hypolocomotion and hypothermia caused by GHB are absent in $GABA_B$ receptor-deficient mice (9). However, there is no direct association between [$^3$H]GHB binding or the binding of [$^3$H]NCS-382, a GHB receptor antagonist (10–12), and $GABA_B$ receptors because the attachment of these radioligands is unaffected in the brains of transgenic mice lacking functional $GABA_B$ receptors. In addition, [$^3$H]GHB binding is undetectable in $GABA_B$ receptor-transfected HEK 293 cells (9, 13). Moreover, there is a lack of concordance between the sensitivity of the $GABA_B$ receptor to GHB and the affinity of the [$^3$H]GHB binding sites, with millimolar concentrations of GHB required to activate $GABA_B$ receptors in vitro (7, 8), whereas the $K_d$ values for the high- and low-affinity [$^3$H]GHB binding sites are in the nanomolar and micromolar ranges, respectively (5). These results suggest that the interaction of GHB with $GABA_B$ receptors mediates responses to high doses but contributes little to the effects observed with lower, more clinically relevant doses.

**At least some of the pharmacological effects of GHB are due to its activation of extrasynaptic $GABA_A$ receptors.**

Alternatively, these results might indicate that the [$^3$H]GHB binding site is of no physiological or pharmacological consequence. This appears unlikely, however, because in vivo studies with NCS-382 suggest that it interacts with a functional receptor; that is, NCS-382 administration diminishes the sedative and cataleptic effects of GHB and blocks the enhancing effects on the spontaneous firing rate of cortical neurons at low doses of GHB but not the depressant effects seen at higher doses (14, 15). Because NCS-382 administration blocks neither GHB-induced ataxia nor its depressant effects on locomotor activity, learned and unlearned behavior, and operant responses (16, 17), these actions are probably mediated by GHB activation of $GABA_B$ receptors.

Efforts to characterize the GHB binding site led to the identification of two parent clones (18). When expressed in CHO cells, one of these, C12K32, displays NCS-382–sensitive high- and low-affinity [$^3$H]GHB binding sites. Characterization of the binding component indicates a G protein-coupled site that is unassociated with either $GABA_A$ or $GABA_B$ receptors.

## GHB and Extrasynaptic Receptors

The study by Absalom et al. (2) provides evidence directly identifying an extrasynaptic $GABA_A$ receptor subtype as the functional component of the [$^3$H]GHB binding site, confirming earlier suggestions that were based on indirect findings (19–21). The work by Absalom et al. (2) is made possible by the synthesis of [$^{125}$I]4-hydroxy-4-[4-(2-iodobenzyloxy)phenyl]butanoate ([$^{125}$I]BnOPh-GHB), a photoaffinity label for the GHB binding site (22–24). In a previous study, these investigators demonstrated that this radioligand labels a single, high-affinity (7-nM $K_d$) site in rat brain membranes that displays a regional distribution identical to [$^3$H]NCS-382 binding (24). Although attachment to the [$^{125}$I]BnOPh-GHB binding site is inhibited by GHB, GABA is inactive. For the study in PNAS, these investigators subjected the [$^{125}$I]BnOPh-GHB binding protein to proteolysis. The resultant bands represent a variety of $GABA_A$ receptor subunits. A comprehensive functional screen of human recombinant $GABA_A$ receptor subtypes in *Xenopus* oocytes revealed that the most GHB-responsive site contains $\alpha_4$/δ- and $\beta_1$-subunits. They report that the $\alpha_4$/δ-subunit combination is responsible for GHB efficacy, and the $\beta_1$-subunit for potency. The $EC_{50}$ for GHB is 140 nM. It is concluded that GHB is a partial agonist at this site because its maximum efficacy at this recombinant receptor is 74% of that displayed by GABA. Results from pharmacological and α-subunit mutation experiments demonstrate that NCS-382 and GHB bind to the interface of the β-α combination at a site that overlaps with but is not identical to the $GABA_A$ receptor recognition site. The finding of a significant reduction in the $B_{max}$ for [$^3$H]NCS-382 binding in the brains of $\alpha_4$- but not δ-subunit KO mice provides further evidence that the $\alpha_4$-subunit is a molecular target for GHB. The authors propose that $\alpha_4$-containing $GABA_A$ receptors, in particular $\alpha_4\beta_1\delta$, represent the primary site of action for GHB. The fact that the functional response to GHB requires the presence of a δ-subunit, which is associated with only a minor fraction of $GABA_A$ receptors, but the binding site is located at the β-α interface, which is present in a large number of $GABA_A$ receptors, explains why some [$^3$H]NCS-382 binding is present after

Author contributions: S.J.E. wrote the paper.

The author declares no conflict of interest.

See companion article on page 13404.

E-mail: senna@kumc.edu.

deletion of $\alpha_4$-subunits. An alternative explanation is that this residual [$^3$H]NCS-382 binding represents attachment to other molecularly, and possibly functionally, distinct GHB receptors. Among these might be the G protein-coupled GHB site reported by others (18). Nonetheless, the findings of Absalom et al. (2) provide direct evidence that at least some of the pharmacological effects of GHB are due to its activation of extrasynaptic $GABA_A$ receptors.

Synaptic $GABA_A$ receptors mediate fast phasic inhibition, have a lower affinity for GABA than the extrasynaptic sites, and are responsible for tonic inhibition. Because of their higher affinity for GABA, extrasynaptic receptors are continuously activated by the small amounts of endogenous GABA present in the local environment. Whereas the most abundant synaptic receptor is composed of α-, β-, and γ-subunits, the extrasynaptic site contains $\alpha_4$- or $\alpha_6$-, β-, and δ-subunits (25). Extrasynaptic $GABA_A$ receptors are targets for steroids, ethanol, general anesthetics, convulsants, hypnotics, and analgesics (26). The rigid GABA analog 4,5,6,7-tetrahydroisoxazolo (5,4-c)pyridine-3-(-ol) (THIP) is an example of an extrasynaptic $GABA_A$ receptor agonist (27). Like GHB, THIP activates αβδ-containing $GABA_A$ receptors and displays a range of interesting CNS effects.

The discovery that the effects of GHB are due to an interaction with extrasynaptic $\alpha_4\beta_1\delta$ $GABA_A$ receptors opens the way to designing more selective agents for this site. The synthesis of such compounds could lead to the development of a new class of drugs for the treatment of anxiety, pain, insomnia, epilepsy, and other conditions associated with GABAergic dysfunction.


1. Enna SJ, Williams M (2009) Challenges in the search for drugs to treat central nervous system disorders. *J Pharmacol Exp Ther* 329:404–411.
2. Absalom N, et al. (2012) α4βδ $GABA_A$ receptors are high-affinity targets for γ-hydroxybutyric acid (GHB). *Proc Natl Acad Sci USA* 109:13404–13409.
3. Snead OC, 3rd, Furner R, Liu CC (1989) In vivo conversion of gamma-aminobutyric acid and 1,4-butanediol to gamma-hydroxybutyric acid in rat brain. Studies using stable isotopes. *Biochem Pharmacol* 38:4375–4380.
4. Benavides J, et al. (1982) High affinity binding sites for gamma-hydroxybutyric acid in rat brain. *Life Sci* 30: 953–961.
5. Snead OC, 3rd, Liu CC (1984) Gamma-hydroxybutyric acid binding sites in rat and human brain synaptosomal membranes. *Biochem Pharmacol* 33:2587–2590.
6. Snead OC, 3rd, Nichols AC (1987) Gamma-hydroxybutyric acid binding sites: Evidence for coupling to a chloride anion channel. *Neuropharmacology* 26:1519–1523.
7. Xie X, Smart TG (1992) Gamma-hydroxybutyrate hyperpolarizes hippocampal neurones by activating GABAB receptors. *Eur J Pharmacol* 212:291–294.
8. Lingenhoehl K, et al. (1999) Gamma-hydroxybutyrate is a weak agonist at recombinant GABA(B) receptors. *Neuropharmacology* 38:1667–1673.
9. Kaupmann K, et al. (2003) Specific gamma-hydroxybutyrate-binding sites but loss of pharmacological effects of gamma-hydroxybutyrate in GABA(B)(1)-deficient mice. *Eur J Neurosci* 18:2722–2730.
10. Maitre M, et al. (1990) A specific gamma-hydroxybutyrate receptor ligand possesses both antagonistic and anticonvulsant properties. *J Pharmacol Exp Ther* 255: 657–663.
11. Mehta AK, Muschaweck NM, Maeda DY, Coop A, Ticku MK (2001) Binding characteristics of the gamma-hydroxybutyric acid receptor antagonist [(3) H](2E)-(5-hydroxy-5,7,8,9-tetrahydro-6H-benzo[a][7] annulen-6-ylidene) ethanoic acid in the rat brain. *J Pharmacol Exp Ther* 299:1148–1153.
12. Castelli MP, et al. (2002) Stereoselectivity of NCS-382 binding to gamma-hydroxybutyrate receptor in the rat brain. *Eur J Pharmacol* 446:1–5.
13. Wu Y, et al. (2004) Gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acidB receptor (GABABR) binding sites are distinctive from one another: Molecular evidence. *Neuropharmacology* 47:1146–1156.
14. Schmidt C, et al. (1991) Anti-sedative and anti-cataleptic properties of NCS-382, a gamma-hydroxybutyrate receptor antagonist. *Eur J Pharmacol* 203:393–397.
15. Godbout R, Jelenic P, Labrie C, Schmitt M, Bourguignon JJ (1995) Effect of gamma-hydroxybutyrate and its antagonist NCS-382 on spontaneous cell firing in the prefrontal cortex of the rat. *Brain Res* 673(1):157–160.
16. Cook CD, Aceto MD, Coop A, Beardsley PM (2002) Effects of the putative antagonist NCS382 on the behavioral pharmacological actions of gammahydroxybutyrate in mice. *Psychopharmacology (Berl)* 160(1):99–106.
17. Castelli MP, Pibiri F, Carboni G, Piras AP (2004) A review of pharmacology of NCS-382, a putative antagonist of gamma-hydroxybutyric acid (GHB) receptor. *CNS Drug Rev* 10:243–260.
18. Andriamampandry C, et al. (2007) Cloning and functional characterization of a gamma-hydroxybutyrate receptor identified in the human brain. *FASEB J* 21: 885–895.
19. Follesa P, et al. (2003) Gamma-hydroxybutyric acid and diazepam antagonize a rapid increase in GABA(A) receptors alpha(4) subunit mRNA abundance induced by ethanol withdrawal in cerebellar granule cells. *Mol Pharmacol* 63:896–907.
20. Cope DW, et al. (2009) Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat Med* 15:1392–1398.
21. Nasrallah FA, Maher AD, Hanrahan JR, Balcar VJ, Rae CD (2010) γ-Hydroxybutyrate and the GABAergic footprint: A metabolomic approach to unpicking the actions of GHB. *J Neurochem* 115:58–67.
22. Høg S, et al. (2008) Novel high-affinity and selective biaromatic 4-substituted γ-hydroxybutyric acid (GHB) analogues as GHB ligands: Design, synthesis, and binding studies. *J Med Chem* 51:8088–8095.
23. Sabbatini P, et al. (2010) Design, synthesis, and in vitro pharmacology of new radiolabeled gamma-hydroxybutyric acid analogues including photolabile analogues with irreversible binding to the high-affinity gamma-hydroxybutyric acid binding sites. *J Med Chem* 53:6506–6510.
24. Wellendorph P, et al. (2010) Novel radioiodinated γ-hydroxybutyric acid analogues for radiolabeling and Photolinking of high-affinity γ-hydroxybutyric acid binding sites. *J Pharmacol Exp Ther* 335:458–464.
25. Möhler H (2007) *The GABA Receptors*, eds Enna SJ, Möhler H (Humana Press, Clifton, NJ), pp 23–39.
26. Belelli D, et al. (2009) Extrasynaptic GABAA receptors: Form, pharmacology, and function. *J Neurosci* 29: 12757–12763.
27. Meera P, Wallner M, Otis TS (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol* 106:2057–2064.