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3-Chloropropanoic acid (UMB66): a ligand for the gamma-hydroxybutyric acid receptor lacking a 4-hydroxyl group

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Abstract—Gamma-Hydroxybutyric acid (GHB) has gained in notoriety in recent years due to its association with sexual assaults. GHB is an endogenous ligand for GHB receptors, but its complete pharmacological mechanism of action in vivo remains unclear due to apparent GABAergic components. It has been proposed that the hydroxyl group in the 4-position acts as a hydrogen bond donor to the GHB receptor. Herein we show that 3-chloropropanoic acid possesses significant affinity for the GHB receptor, has no affinity for GABA receptors, and cannot undergo metabolism to GABAergic compounds. UMB66 is thus a selective agent for the study of GHB in vivo. These results, in combination with data from quantum mechanical calculations, suggest that the hydroxyl group of GHB actually acts as a hydrogen bond acceptor in contrast to the currently accepted model. This finding is anticipated to facilitate the rational design of novel agents with selectivity for GHB receptors that may be used to elucidate the mechanism of action of this common drug of abuse.

1. Introduction

Gamma-Hydroxybutyric acid (GHB) (**1**) (Fig. 1) is a naturally occurring neurotransmitter, and has been shown to interact with specific GHB receptors.[1] Interest in GHB has grown in recent years due to its increasing use as a recreational drug, its reported association with serious sexual assaults, and the corresponding increase in emergency room episodes.[2,3] GHB is known to cause sedation,[4] and produces a coma-like state when given at high doses.[5] Even though GHB has been known for many years, its mechanism of action remains elusive. It has been demonstrated that specific GHB binding sites (or receptors) are localized in various brain regions,[6] and that the purported antagonist [NCS-382 (**2**) (Fig. 1)][7] labels the same brain regions.[8] However, GHB is known to interact with $GABA_B$ receptors and it is rapidly metabolized to gamma-aminobutyric acid (GABA (**3**) Fig. 1),[1] which could result in actions at $GABA_A$ as well as $GABA_B$ receptors. It is thus not surprising that much of the behavioral pharmacology of GHB in many assays appears GABAergic in nature.[4,9] Our approach to developing tools to delineate the profile of GHB in vivo has focused on developing analogues of GHB which possess little or no affinity for $GABA_B$ receptors, but importantly also cannot be metabolized to GABAergic compounds.

Previous studies by others have led to the development of preliminary structure–activity relationships (SAR) for GHB.[10] The acid group of GHB, (**1**) as shown in Figure 1, is essential and it has been proposed that the 4-hydroxyl group is also required for activity, acting as a hydrogen bond donor to the receptor.[10] Due to the fact that GHB is metabolized to GABA (**2**) through oxidation of this alcohol,[11] we considered that its removal would eliminate any possibility of GABAergic metabolites. Although GHB binds to $GABA_B$ receptors, GABA does not bind to GHB receptors.[1] The current SAR model predicts that GABA should bind, as only a

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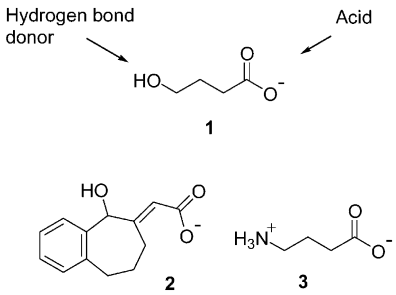

Figure 1. Physiologically relevant structures of GHB, NCS-382, GABA, and the currently accepted structural features required for recognition at GHB receptors.

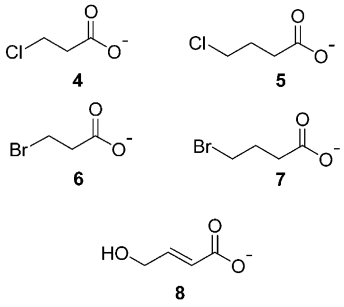

Figure 2. Structures of the halogenated GHB analogues and of *trans*-4-hydroxycrotonic acid.

hydrogen donor is required. On the basis of this apparent contradiction, we considered that the alcohol of GHB may be acting as a hydrogen bond acceptor, and that this would explain the lack of affinity of GABA at GHB receptors: GABA exists under physiological conditions as the protonated amine which is incapable of accepting a hydrogen bond. To investigate this hypothesis, we studied the halogenated analogues of GHB that are shown in Figure 2. The halogens were selected as they are capable of forming dipole–dipole interactions with a receptor hydrogen bond donor. In addition, the compounds would be anticipated to undergo metabolism to GABAergic compounds very slowly if at all.

2. Results

The four commercially available compounds (**4**–**7**) (Sigma-Aldrich) were studied through displacement assays as previously described,[8] and the results are presented in Tables 1 and 2.

GHB was previously shown to displace [^{3}H]GABA from both $GABA_A$ and $GABA_B$ receptors,[12] although

Table 1. IC_{50} values for GHB and the halogenated acids using [^{3}H] NCS-382 (16nM) as a radio ligand in rat cerebrocortical membranes

Compd	IC_{50} (μM)
(**1**) GHB[a]	25.0±1.8
(**4**) UMB66	76.7±15.2
(**5**)	>1 mM
(**6**)	135±81.9
(**7**)	403±123

[a] Data from ref 8.

Table 2. Inhibition of [^{3}H]GABA (10 nM) binding to the $GABA_A$ and $GABA_B$ receptors in rat membranes from cerebral cortex and cerebellum, respectively by GHB and halogenated acids at concentrations of 100 μM and 1 mM. Each value is mean±S.E.M. of at least three individual experiments performed in triplicate

Compd	Percentage inhibition of binding			
	$GABA_A$ receptors		$GABA_B$ receptors	
	100 μM	1 mM	100 μM	1 mM
(**1**) GHB[a]	30.3±5.0	35.5±3.7	22.7±4.4	41.1±3.1
(**4**) UMB66	0.7±0.1	2.0±0.1	5.1±1.6	4.7±1.0
(**6**)	5.6±0.1	12.8±0.1	11.1±2.1	10.3±2.1
(**7**)	6.6±0.1	0.1±0.1	24.2±3.2	36.8±0.7

[a] Data from ref 12.

the displacement from $GABA_A$ receptors appears non-dose related. 3-Chloropropanoic acid (**4**) possesses an affinity at GHB receptors displacing [^{3}H]NCS382 binding with an IC_{50} of 76 μM, which represents a value only three-fold lower than GHB. Importantly, this compound had no effect on the binding of [^{3}H]GABA at GABA receptors. Interestingly, 4-chlorobutyric acid (**5**) did not displace [^{3}H]NCS-382. The two brominated analogues [3-bromopropanic acid (**6**) and 4-bromobutyric acid (**7**)] had lower affinity at [^{3}H]NCS-382 labeled GHB sites and had very little affinity for GABA receptors.

To obtain meaningful SAR of GHB and compounds **4**–**7**, a computational study was performed to determine if the halogenated analogues of GHB could indeed act as hydrogen bond acceptors in a manner similar to that of GHB. As no 3D structure of the GHB receptor is available, we simply modeled the putative hydrogen bond donor on the receptor with a single water molecule. Ab initio calculations were then designed to test if GHB and the halogenated analogues would interact with the putative receptor hydrogen bond donor in a similar orientation with respect to the carboxylate of GHB. This was performed by calculating the distances (d) between the carboxylate carbon and the hydrogen atom of the model water as the water orbits around GHB or **4**–**7** as a function of the angle θ (Fig. 3). Calculations were performed with the Gaussian98 software[13] using the MP2/6-31G* level of the theory, which has been shown to yield satisfactory hydrogen bond distances.[14]

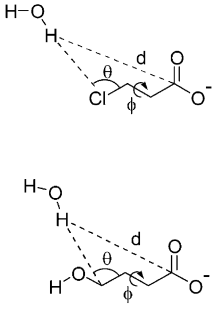

Figure 3. Schematic view of two complexes, where a water molecule is used to model a putative receptor hydrogen bond donor, illustrating the geometric parameters used for analysis.

Table 3. Calculated relative stabilities and geometric parameters of the conformers of each compound with respect to the E conformer using MP2/6-31G*. Negative energies correspond to increased stability

	1-E	1-SE	1-F	4-E	4-SE	5-E	5-SE
ΔE (kcal/mol)	0	−3.92	−15.7	0	3.51	0	−3.18
ΔE^{IPCM} (kcal/mol)	0	−5.79	−7.53	0	0.89	0	−2.78
d_{x-c} (Å)	5.001	4.500	3.184	4.199	3.213	5.363	4.802
ϕ (°)	173.0	65.9	49.2	180.0	60.0	173.0	−63.9
	6-E	6-SE		7-E	7-SE	8-E	8-SE
ΔE (kcal/mol)	0	3.61		0	−3.37	0	−2.25
ΔE^{IPCM} (kcal/mol)	0	1.41		0	−3.69	0	−2.78
d_{x-c} (Å)	4.365	3.298		5.511	4.961	4.911	4.810
ϕ (°)	180.0	60.0		172.7	−63.5	180.0[a]	−124.7[a]

Initial calculations focused on identification of stable conformations of GHB and compounds **4** through **8**. Three conformers of GHB were identified, which have been previously referred to as fully extended (E), semi-extended (SE) and folded (F).[15] The dihedral (ϕ) describing the rotation around the bond between carbons 2 and 3 is used to describe the conformations. Two conformers were identified for compounds **4** through **8**, as summarized in Table 3. The F conformer of GHB allows intramolecular hydrogen-bond formation between the carboxylate and hydroxyl and is the most stable in the gas phase. The E conformer is a local minimum in all compounds and is the least stable except with **4** and **6**. Solvation effects on the conformational energies were explored by doing isodensity surface polarized continuum model (IPCM)[16] single point energy calculations with a dielectric of 78.39 at the MP2/6-31G* level of theory on the optimized gas phase geometries, which changed the relative energies but not the qualitative order of stability of the conformers for all the compounds. Thus, for the most active compounds, GHB, **4** and **6** (Table 1) the most stable conformers are F, E and E respectively (Table 3). It is possible that IPCM overestimates the stability provided by the intramolecular hydrogen bond in the F conformer since it neglects explicit solute–solvent interactions by treating the solvent as a dielectric continuum. Notably, the F conformation is not a stable local minimum for **4** and **6** due to repulsion between the halogen atoms and the carboxylate. This suggests that the active conformation of GHB, **4** and **6** is either E or SE.

Supporting this is experimental data on *trans*-4-hydroxycrotonate (**8**), showing this compound to have activity similar to GHB.[10] Since **8** cannot assume a F conformation due to the double bond, either the E or the SE conformation must be the active conformer (data on *cis*-4-hydroxycrotonate is not available). Calculations on **8** (Table 3) show it to favor the SE conformer over the E conformer. Thus, it is evident that the active conformation is either the E or the SE conformer. However, energetic analysis based on the QM calculations is ambiguous as to the most stable of these two conformers when all four compounds are considered, especially since the addition of thermodynamic data could alter the relative energies when these are small as in **4** and **6**. Alternatively, the distance (d_{x-c}) between the carboxylate carbon and the X atom (where X=O, Cl, or Br) for the compounds may be compared. For GHB, **4**, **6** and **8** the d_{x-c} values are more similar for the E conformers (range: 4.2–5.0 Å), while for the SE conformers the distances range from 3.2–4.8 Å. Thus, the similarity of the d_{x-c} distances suggest that the E conformer may be the active conformation. Further studies were, therefore, performed with the E conformer.

Next, the calculations looked at the distance, d, of a putative receptor hydrogen bond donor, mimicked by a water molecule, relative to the carboxylate C for compounds **1**, **4**–**7**. In addition, binding energies were obtained for these complexes. For the scans between water and the compounds the relative orientation of the two molecules along with their internal geometries were fixed, with only the hydrogen bond distance optimized (Fig. 3). Figure 4 compares the water *H* to carboxylate C distances as a function of the angle θ. To allow a direct comparison, θ is defined as the angle between the H_{wat}-Cl-C_3 atoms (or H_{wat}-Br-C_3) in compounds **4** and **6** and between H_{wat}-C_4-C_3 for **1**, **5** and **7**. In the range where H-bond formation is most favorable for GHB (**1**) (θ = ~70–100°), the optimal distance (d) between the water hydrogen and the carboxylate carbon for **4** and **6** are similar to that of **1** while the distance for **5** and **7** are

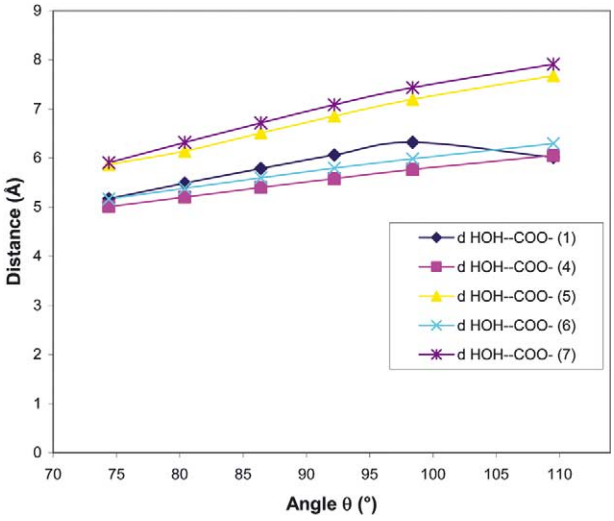

Figure 4. Distances (d) between the carboxylate carbon and the water hydrogen for the energetically optimal hydrogen-bond interactions.

longer. Although **4** and **6** are shorter than GHB (**1**) due to one less methylene carbon, the combined effect of longer carbon-halogen and hydrogen bonds in **4** and **6** with respect to **1** result in similar distances between the carboxylate carbon and the model hydrogen bond donor. The covalent bonds between carbon and O (**1**), Cl (**4**), and Br (**6**) are 1.442, 1.831 and 2.016 Å, respectively, while the hydrogen bonds lengths are ~2, ~2.5 and ~2.6 Å, respectively. The presence of the extra methyl group in **5** and **7**, and nearly identical covalent and hydrogen bond lengths as those in **4** and **6**, result in the longer distances (d) shown in Figure 3. These results indicate that **1**, **4** and **6** are capable of acting as hydrogen bond acceptors for a putative receptor donor with similar spatial relationships to the ligand carboxylate carbon.

GHB (**1**) binds strongest to the water when θ is approximately 90°, hydrogen bonding stronger than the halogenated compounds at angles less than ~95° (Table 4). At angles greater than ~95°, GHB is more restricted in its hydrogen bonding ability due to repulsive interactions between the water and the 4-hydroxyl hydrogen, which are not present in the halogenated compounds. Two geometries were chosen for further evaluation of the energies corresponding to the approximate angles where stronger interaction energies between the water and the halogenated compounds or GHB occurred, $\theta = 109.5°$ and 92.2°, respectively. These calculations used the more accurate cc-aug-pVDZ and cc-aug-VTZ[17] basis sets along with a correction for basis set superposition error (BSSE) using the counterpoise method.[18] Results for the higher basis sets (Table 4) shows GHB to be the strongest hydrogen bond acceptor based on the 92.2° result, followed by **4** and **6**, based on the 109.5° results. The energy differences between **4** and **6** are consistent with the IC_{50} values (Table 1), although the magnitude of the difference is most likely within the accuracy of the level of theory used.

3. Discussion

The fact that UMB66 (3-chloropropanoic acid, **4**) possesses affinity for GHB receptors only three-fold lower than GHB is not consistent with one current model[10] for ligand binding at these receptors. No hydroxyl is present in **4** to act as a hydrogen bond donor; only the halogen atom is present to form a dipole–dipole interaction with the receptor. This suggests that the hydroxyl of GHB acts as a hydrogen bond acceptor when binding to the GHB receptor. To test the hypothesis that **4** is acting as a hydrogen bond acceptor in a similar fashion to GHB, ab initio calculations were undertaken where a water molecule was used as a model for a hydrogen bond donor in the protein receptor site. It was shown that the optimal distance between the water hydrogen and the carboxylate carbon was similar for GHB (5.79 Å) and **4** (5.40 Å), but that the distance for **5** was significantly greater (6.51 Å). This analysis supports the assumption that the chlorine of **4** is acting as an acceptor in a similar fashion as GHB. The significantly longer distances with **5** indicates that, although the chlorine still acts as an acceptor, the length of the molecule disallows it to interact with the receptor in the same orientation as GHB, consistent with its significantly lower affinity (Table 1).

Acids **6** and **7** possess a bromine, and it might be expected that a weaker dipole–dipole bond would be responsible for the lower affinity at GHB receptors. MP2 calculations using larger basis sets are consistent with this, although the chlorinated species is only more favorable by a few tenths of a kcal/mol.

While the present study is limited to only a few compounds and the QM calculations are based on a simple model of a receptor hydrogen bond donor using gas phase electronic binding energies, it is evident from the complex geometries that both GHB and **4** can act as hydrogen bond acceptors in a similar fashion. These results support that the hydroxyl of GHB is acting as a hydrogen bond acceptor when bound to the GHB receptor. Further studies utilizing this finding are expected to lead to high affinity and selective ligands for GHB receptors as essential tools for delineating the pharmacological mechanism of action of GHB.

Table 4. Interaction energies between the water and compounds calculated at two different positions with a variety of basis sets using the MP2/6-31G* geometries[a]

	θ = 92.2°			θ = 109.5°		
	(1)	(4)	(6)	(1)	(4)	(6)
No corrections						
6-31G*	−6.79	−4.59	−6.61	−4.51	−4.98	−6.81
cc-aug-pVDZ	−5.85	−5.09	−5.28	−3.88	−5.20	−5.22
cc-aug-pVTZ	−5.98	−4.98	−5.47	−3.94	−5.22	−5.62
With BSSE correction						
6-31G*	−4.82	−3.32	−3.37	−2.59	−3.77	−4.01
cc-aug-pVDZ	−4.99	−4.10	−3.95	−3.13	−4.42	−4.17
cc-aug-pVTZ	−5.43	−4.47	−4.38	−3.49	−4.77	−4.59

[a] Energies in kcal/mol.

4. Conclusion

The relatively good affinity for GHB receptors, coupled with the lack of affinity at $GABA_A$ and $GABA_B$ receptors, and the fact that 3-chloropropanoic acid (UMB66, **4**) cannot be rapidly metabolized to GABA active compounds, means that this readily available compound may prove to be a useful ligand for further study of the GHB system free from the complications of GABAergic actions. In addition, the fact that **4** cannot act as a hydrogen donor strongly suggests that the current model of ligand binding to the GHB receptor is incomplete, and led to the development of a novel model based on the experimental binding studies and supported by QM calculations.

5. Experimental

The geometries of compounds **1**, **4**–**8** were first optimized in a variety of conformations by gradually changing

each dihedral of the carbon backbone and optimizing without any constraints using second order Møller-Plesset Perturbation (MP2) theory with the 6-31G*[19] basis set.

Using the optimized geometries of the E monomers, complexes were set up in which the water formed a linear hydrogen bond with the compounds (∠OH-X = 180° where X = Cl, Br, or O) and was in the plane defined by the XCC- atoms of **1** and **4–7** (Fig. 3). Since the actual orientation within the receptor site is not known, these restrictions upon the degrees of freedom were applied in order to facilitate comparison among the complexes in this simplified model.

The scan of the MP2/6-31G* electronic interaction energies was performed by gradually increasing the value of the angle (θ) between the water hydrogen and the compounds (Fig. 3) and allowing the hydrogen bond distance to optimize while the internal geometries of the monomers were kept rigid. While calculations were done over a larger range of values for θ, the reported range is between ~115 and ~70°. At angles smaller than 70° the strong interaction between the charged carboxylate oxygens and the water breaks the hydrogen bond as the water moves closer to those oxygens for all compounds while at larger angles the water hydrogen is repelled by the 4-hydroxyl hydrogen of **1**. Two orientations (θ = 92.2°, 109.5°) were chosen to further investigate the effect of basis set incompleteness upon the interaction energies. Single points energy calculations on the MP2/6-31G* optimized geometries were performed using the aug-cc-pVDZ and aug-cc-pVTZ basis sets.[17] Basis set superposition errors (BSSE) were calculated using Boys and Bernardi's counterpoise correction method.[18]

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