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# Agonist responses of (*R*)- and (*S*)-3-fluoro-γ-aminobutyric acids suggest an enantiomeric fold for GABA binding to $GABA_C$ receptors†

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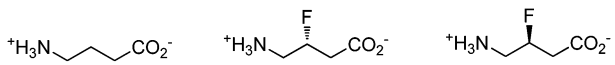
**The enantiomers of 3F-GABA were evaluated on $GABA_C$ receptors. Both enantiomers were agonists, with the (*R*)-enantiomer being an order of magnitude more potent. This result is consistent with a folded binding mode for GABA, a conclusion which suggests a different binding mode to that found in the related but pharmacologically distinct $GABA_A$ receptors.**

γ-Aminobutyric acid (GABA) **1** is the major inhibitory neurotransmitter in the central nervous system.[1] The GABA response is mediated by $GABA_A$, $GABA_B$ and $GABA_C$ receptors.[2,3]

$GABA_C$ receptors are composed of the *rho* subunits ($\rho_1$–$\rho_3$) and unlike $GABA_A$ receptors, they form homopentameric receptors.[4] $GABA_C$ receptors are highly expressed in the retina and in distinct anatomical areas in the central nervous system *e.g.* hippocampus and visual cortex. These receptors have distinct, pharmacological profiles compared to $GABA_A$ receptors being selectively activated by CACA (*cis*-aminocrotonic acid)[5] and the 5-substituted analogue of I-4AA (imidazole-4-acetic acid), 5-methyl-imidazole-4-acetic acid.[6]Also they are selectively blocked by TPMPA (1,2,5,6-tetrahydropyridine-4-yl-methyl-phosphinic acid),[7] (*S*)-/(*R*)-ACPBPA ((*S*)-/(*R*)-aminocyclopentenyl-butylphosphinic acids)[8] and 3-GOHP (3-(guanido)-1-oxo-1-hydroxy-phospholane).[9]

There is strong evidence suggesting all members of the Cys-loop family share a similar 3D structure.[10] Recently crystal structures of the related prokaryotic proton-gated ion channels were reported[11–14] and there is a lot of interest in developing homology models of $GABA_A$ and $GABA_C$ receptors using either the acetylcholine-binding protein (AChBP) crystal structure or the prokaryotic ion channels. The AChBP has been widely used to develop homology models of the $GABA_C$ receptor. The GABA binding site on these models have been deduced largely by extensive mutagenesis data and GABA analogue activities. Several independent studies have proposed different binding models.[15–18] Three of these models[15–17] propose specific binding modes for GABA but the models do not agree with each other both in the way GABA contacts key amino acid residues, and in their assessment of the conformation of the molecule on binding. One model[17] predicts an extended structure for GABA binding whereas two of the models[15,16] predict a similarly folded conformation, although they differ in the nature of the amino acid residues contacted by the neurotransmitter.




GABA **1** 3F-GABA (*R*)-**2** 3F-GABA (*S*)-**3**

We have previously explored GABA **1** binding conformations by comparing the relative activities of the enantiomers of 3-fluoro-γ-aminobutyric acid (3F-GABA) as a tool to probe the binding conformation of GABA **1** to $GABA_A$ receptors[19,20] and their binding to the GABA **1** metabolising enzyme, GABA transaminase.[21]

This approach recognises that when the C–F bond is placed beta to the ammonium group, there is a charge–dipole interaction which favours a close interaction between the C–F bond dipole and the charged ammonium group.[22] This interaction can provide upto 5.0 kcal $mol^{-1}$ stability.[23,24] Thus *gauche* conformations are favoured over *anti* conformations around C-3-C4 of **2**. Therefore if the three staggered conformations are considered for both enantiomers of 3F-GABA as illustrated in Fig. 1, then there are two disfavoured conformers where the fluorine and ammonium groups are *anti* rather than *gauche* to each other. The small steric impact of fluorine renders it a good hydrogen substitute, and therefore it does not substantially change the shape of the molecule relative to GABA, however the C–F bond has a strong electronic effect and the dipole influences conformation. The introduction of fluorine also generates a stereogenic centre, thus the (*R*)-**2** and (*S*)-**3** enantiomers can be compared to examine if there is a preferred chiral binding mode, not apparent with GABA **1** itself as it is a non chiral molecule. In our previous study with recombinant $GABA_A$ receptors there was no significant difference in the agonist response between the (*R*)-**2** and (*S*)-**3** enantiomers.[20] It was concluded

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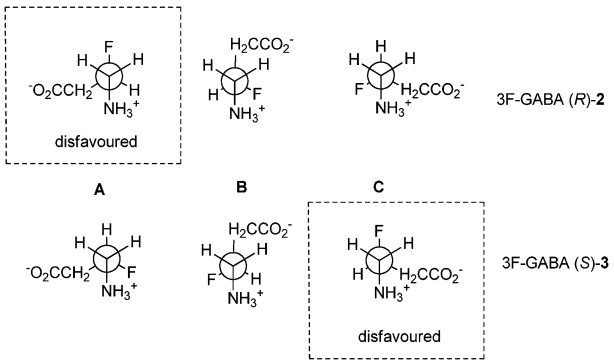


**Fig. 1** Staggered conformations **A**, **B** and **C** of 3F-GABA enantiomers (*R*)-**2** and (*S*)-**3** after rotation around the C-3-C4. *Gauche* C-F...$H_3N^+$-C interactions are stabilising whereas *anti*-relationship are significantly higher in energy and relatively disfavoured.

that GABA most likely bound the $GABA_A$ receptor in an extended zig-zag mode (conformer **B**) around C3–C4 as both enantiomers can easily adopt that conformation.[20] We now report the influence of these enantiomers on $GABA_C$ receptors. The enantiomers were assayed separately and in this case they induce different levels of response indicating a folded binding mode. The (*R*)-**2** and (*S*)-**3** were prepared as previously described.[20] Recombinant human $\rho_1$ and $\rho_2$ $GABA_C$ receptors were expressed using *Xenopus* oocytes and the two-electrode voltage clamp was used to measure the response. Molecular biology, oocyte preparation and data analysis have also been previously reported.[9]

At both $\rho_1$ and $\rho_2$ $GABA_C$ receptors, GABA showed concentration dependent activation, being 2 fold more potent on $\rho_2$ $GABA_C$ receptors (Fig. 2). Like GABA both (*R*)-**2** and (*S*)-**3** displayed agonist activity without any antagonist effects. Antagonist activity is common with other 2-substituted GABA analogues,[25] however it may be that the small fluorine atom renders these mimetics essentially identical to GABA in their space demand, and thus they do not contact additional amino acid residues at the binding site to elicit an antagonist response. Both of the enantiomers are weaker agonists than GABA itself (Fig. 2), by up to 20 fold for (*S*)-**3** and 10 fold for (*R*)-**2** (Table 1). This was also observed previously with $GABA_A$ receptors[20] and is probably due to the fluorine reducing the $pK_a$ of the amine (10.6 to 9.0), and thus weakening electrostatic interactions (*e.g.* H-bonding) to the surface of the receptors. This in turn suggests a folded binding mode (conformation **C**) for GABA **1** to the $GABA_C$ receptor. The data conflicts with one recent model[17] which suggests a linear extended structure for GABA binding to $GABA_C$. The two studies[15,16] that propose a folded conformation for GABA binding to $GABA_C$ receptors, also predict the same chiral sense for binding *i.e.* conformation **C** (Fig. 1) rather than conformation **A**, therefore this study comparing (*R*)-3F-GABA **2** and (*S*)-3F-GABA **3** reinforces that developing hypothesis.

The homology model of $\rho_1$ $GABA_C$ receptor was developed based on the X-ray crystal structure of the *Lymnae stagnalis* acetylcholine binding protein (AChBP)[26] by using the 'prime' suite in Maestro (see the Supporting Information for more details†). To delineate the key interactions responsible for differences in binding affinity, the structures of GABA **1**, (*R*)-3F-GABA **2** and (*S*)-3F-GABA **3**, were flexibly docked into the ligand-binding site of a $\rho_1$ $GABA_C$ homology model (Fig. 3). Both ligands docked in the more favourable *gauche* conformation,[19] with the dipole of the C–F bond pointing towards the positively charged amino group of the ligands. The acidic groups of the ligands were flanked between the guanidium groups of Arg104 and Arg158 forming a salt bridges and H-bonds with the hydroxy group of Thr244. The basic amine group oriented towards the acidic amino acid (Glu196), forming an H-bond. Additionally, the amino group of the ligands was shown to be making an H-bond with Tyr198 and possibly a cation-π interaction. Fig. 3 also shows intra-hydrogen bonding network between Tyr198, Ser168 and Arg104, which may be important for the shape and stability of the active site.

For both isomers the orientation of the C–F bond was examined for its interaction with other groups which may be stabilising or destabilising. There were no obvious short contacts to F in the case of the (*S*)-**3** isomer. In the case of the (*R*)-**2** isomer, the C–F bond is located 3.5 Å from a guanidinium hydrogen of Arg-104 (See Fig. 3B). This is beyond a reasonable H-bonding distance for organic bound fluorine,[27] and the C–F to H–N angle is rather acute, however this interaction may contribute additional stabilisation for that isomer.

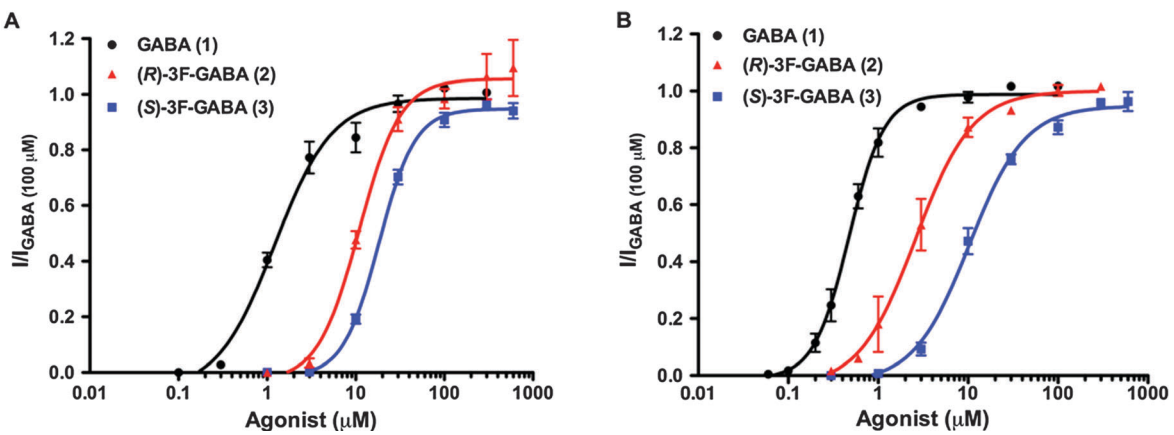


**Fig. 2** Concentration-response curves for GABA **1** (black dot), (*S*)-3F-GABA **3** (blue square), and (*R*)-3F-GABA **2** (red triangle) (A) at human $\rho_1$ $GABA_C$ receptors, and (B) at human $\rho_2$ $GABA_C$ receptors expressed in *Xenopus* oocytes. Data are the means ± SEM ($n$ = 3–4 oocytes).

**Table 1** Pharmacological evaluation of compounds at human $\rho_1$ and $\rho_2$ $GABA_C$ receptors expressed in *Xenopus* oocytes

| Compound | Human $GABA_C$ receptors $EC_{50}$[a] ± SEMs (95% CI) (μM) | |
|---|---|---|
| | $\rho_1$ | $\rho_2$ |
| GABA **1** | 0.81 ± 0.07 (0.74–0.88) | 0.48 ± 0.05 (0.43–0.53) |
| (*R*)-3F-GABA **2** | 11.10 ± 1.24 (9.86–12.34) | 2.92 ± 0.66 (2.26–3.58) |
| (*S*)-3F-GABA **3** | 18.72 ± 0.60 (18.12–19.32) | 10.76 ± 1.90 (8.86–12.66) |

[a] The concentration that activates 50% of maximum response. All data are the mean ± SEMs ($n$ = 3–4 oocytes).

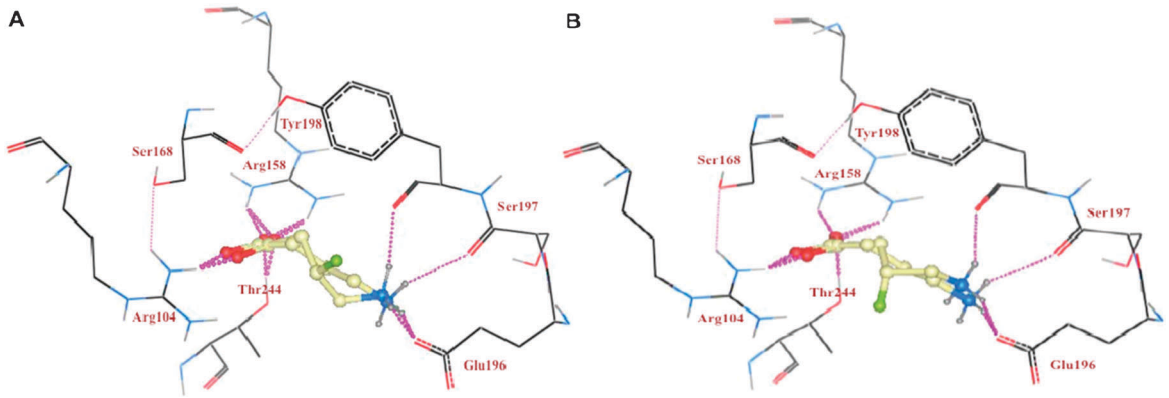


**Fig. 3** (A) GABA **1** and (*S*)-3F-GABA **3**, (B) GABA **1** and (*R*)-3F-GABA **2** docked into the $\rho_1$ $GABA_C$ receptor ligand binding site.

This study suggests a different mode of binding to that found in the related but pharmacologically distinct $GABA_A$ receptors (Fig. 4).[20] The deduced conformation for $GABA_A$ binding comes from our previous study[20] and other studies on conformationally constrained GABA analogues.[19,28,29]

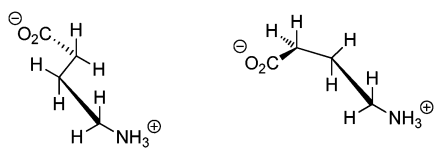


**Fig. 4** The prefered binding modes of the neurotransmitter GABA to $GABA_C$ and $GABA_A$ receptors as deduced by comparative binding of (*R*)- and (*S*)-3F-GABA isomers.

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