QSAR and Molecular Modeling Studies of Baclofen Analogues as $GABA_B$ Agonists. Insights into the Role of the Aromatic Moiety in $GABA_B$ Binding and Activation

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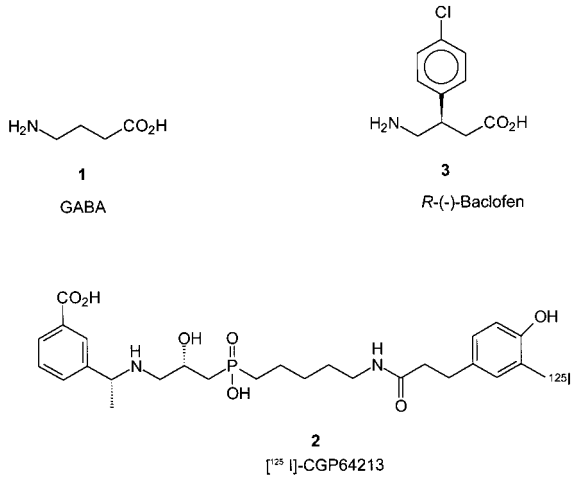
An integrated QSAR/molecular modeling study is carried out on a series of baclofen analogues with the aim of addressing the role of their aromatic moiety in $GABA_B$ receptor binding and activation. The strong correlation found between electronic descriptors (HOMO and LUMO orbital energies) and the biological activity expressed as receptor binding is discussed also on the basis of available experimental mutagenesis data and of the results obtained from homology modeling of $GABA_B$ receptor. In particular, it can be inferred from the QSAR analysis that the ability of being involved in aromatic−aromatic π interaction is the distinctive feature of the *p*-chlorophenyl moiety of baclofen. This conclusion is confirmed by homology modeling and docking studies which indicate that the *p*-chlorophenyl moiety of baclofen is disposed into a pocket formed by Tyr366 and Tyr395. These results are discussed in terms of mechanism of $GABA_B$ activation promoted by baclofen or GABA.

Introduction

γ-Aminobutyric acid (GABA, **1**, Chart 1), the major inhibitory neurotransmitter in the central nervous system of mammalians, exerts its physiological actions through the interaction with three receptor subtypes, termed $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_C$ are ligand-gated ion channels permeable to anions and convey the fast synaptic transmission, whereas $GABA_B$ is a G-protein coupled receptor which modulates the synaptic transmission through intracellular effector systems. More specifically, $GABA_B$ is involved in the presynaptic inhibition of transmitter release and mediates the slow synaptic inhibition by increasing the potassium conductance responsible for long-lasting inhibitory postsynaptic potentials.[1]

Although the $GABA_B$ receptor was pharmacologically characterized more than 15 years ago,[2] the molecular structure of $GABA_B$ has remained elusive for many years, until a cDNA encoding a putative $GABA_B$ receptor was cloned in 1997.[3] The cloned $GABA_B$ receptor showed a significant similarity with metabotropic glutamate receptors (mGluRs), the Ca^{2+} sensing receptor, and putative pheromone receptors. Indeed, these receptors are now classified as members of family C of the G-protein coupled receptor (GPCR) superfamily, a family which bears no similarity with "classical" GPCRs. Although the protein product of the cDNA encoding the putative $GABA_B$ receptor had an overall profile comparable with the endogenous $GABA_B$ receptor, when expressed in heterologous systems the cloned $GABA_B$ receptor failed to efficiently couple effector systems and, hence, to produce functional activity. The search for a "missing factor" that hampered the expression of functional $GABA_B$ receptor ended with the discovery, in 1998, of a second cDNA encoding a $GABA_B$ receptor, termed $GABA_BR2$, which bears about 35% similarity with the former $GABA_BR1$ receptor.[4] Attempts to functionally express $GABA_BR2$ were unsuccessful, whereas coexpression of $GABA_BR1$ and $GABA_BR2$ in heterologous systems produced functionally active $GABA_B$ receptors, thus pointing out the $GABA_B$ receptor as the first example of a G-protein coupled receptor active as heterodimer.[5]

Chart 1



The widespread distribution of $GABA_B$ receptors in both the CNS and the periphery is a clear clue of their physiological and physiopathological importance. Indeed, several potential therapeutic applications are associated with pharmacological control of $GABA_B$ receptor; $GABA_B$ agonists, in particular, may be employed as antispastic agents, in respiratory diseases such as asthma, in the pharmacological control of cocaine addiction, in migraine, and in pain. $GABA_B$ antagonists, when employed at doses that do not induce convulsion, increase neutrophin expression in the CNS

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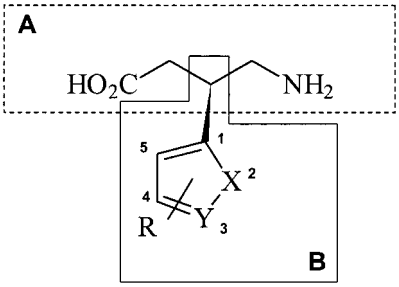
Table 1. Binding Data (IC_{50}, μM)[a]



code name	X	Y	R	*R*-(−)-[H^3]-baclofen binding
3 baclofen	CH=CH	CH	*p*-Cl	0.33
4	CH=CH	CH	*m*-Cl	20
5	CH=CH	CH	*o*-Cl	0.80
6	CH=CH	CH	H	>100
7	CH=CH	CH	*p*-OH	>100
8	CH=CH	CH	*p*-F	0.80
9	S	CH	H	9.72
10	S	CH	3-CH_3	1.34
11	S	CH	5-CH_3	124
12	S	CH	3-Br	1.86
13	S	CH	4-Br	39.1
14	S	CH	3-Cl	0.61
15	S	CH	4-Cl	45.3
16	CH	S	H	20.9
17	O	CH	H	>100
18	O	CH	3-CH_3	>100

[a] Taken from ref 9a.

and in the spinal cord and, thus, may have therapeutic relevance in neurodegenerative diseases.[6]

Several selective $GABA_BR$ ligands are nowadays available, either agonists or antagonists. Whereas $GABA_BR$ antagonists, such as [^{125}I]CGP64213 (**2**), with subnanomolar potency have been developed and used for radioligand binding studies,[7] *R*-(−)-γ-amino-β-(*p*-chlorophenyl)-butyric acid (Baclofen, **3**), introduced in 1973 in the therapy of muscle spasticity, is still the prototype of a selective $GABA_BR$ agonist.[8] Several baclofen analogues (Table 1) have been designed and synthesized over the last 15 years, but none of them has displayed pharmacological improvements over baclofen (**3**) in terms of potency and selectivity.[9]

Furthermore, although occasional studies[10] have enabled insights to be gained into the steric and electronic requirements for the binding of baclofen (**3**) and baclofen analogues (Table 1) to $GABA_BR$, the impact of structural features such as aromatic ring substitutions or conformational constraining on binding and selectivity has long remained unclarified.

A major contribution to the understanding of the structural basis of the interaction of $GABA_BR$ with its ligands was recently given by site-directed mutagenesis experiments and homology studies. Indeed, on the basis of the similarity with LIVBP, Pin et al. have constructed 3D models of the amino terminal domain (ATD) of $GABA_BR1$ and have used them to identify possible residues involved in GABA (**1**) binding.[11−13] All these residues are located in the cleft formed by the two globular domains of ATD. In the case of $GABA_BR1$, two residues of serine (Ser246 and Ser269) have been proposed to interact with the carboxylic moiety of GABA (**1**), and a residue of aspartate (Asp471) has been proposed to form a salt bridge with the GABA (**1**) ammonium group.[13] Interestingly, both the binding of GABA (**1**) and the GABA-mediated $GABA_BR$ activation require the presence of Ca^{2+}. Indeed, mutagenesis experiments suggest that calcium may act as a co-agonist of GABA (**1**), and a single residue, namely Ser269, has been shown to affect the binding of calcium.[12] Conversely, the binding of the potent and selective $GABA_BR$ agonist baclofen (**3**) is much less affected by the presence of calcium, thus raising the possibility that GABA (**1**) and baclofen (**3**) may adopt different modes of binding. Partially supporting this hypothesis is the recent finding of a single amino acid (Tyr366) that is crucial in determining the pharmacological profile of baclofen (**3**) while not affecting that of GABA (**1**), although a decrease in binding is observed.[13] It should also be mentioned that very recently another homology model of the closed form of the ligand binding domain has been reported by Bernard et al. on the basis of similarity with LIVBP.[21]

In an attempt to help clarify these issues and in the frame of a wider research project devoted to the understanding of the structural factors responsible for the binding of ligands to family C receptors,[14,15] we report here a computational study composed of two parts. In the first one, a series of baclofen analogues, available through the literature (Table 1), are analyzed by means of QSAR models, and the structural features responsible for activity will be identified. In the second one, those structural features will be projected into a 3D model of the ligand binding domain of $GABA_BR$ and discussed also in light of the residues object of mutagenesis analysis.

Methods

Quantitative Structure−Activity Relationship (QSAR) Studies. A training set of 16 baclofen analogues was collected from the literature (Table 1).[9] Compounds were chosen by taking into account their structural diversity on the aromatic moiety. To restrict the analysis only to the aromatic groups, the molecular decomposition−recomposition (MDR) approach was used, as previously described.[17] Briefly, the MDR technique assumes that a molecule can be decomposed in substructures such that each set of substructures can be analyzed in terms of QSAR and molecular modeling studies using the biological activity data of the parent compound. MDR analysis assumes that physicochemical changes in one substructure do not affect the properties of any other substructure in the reference compound.

All compounds defining the training set have the same general structure shown in Table 1. Two substructures for each compound can thus be defined (features A and B, Table 1). All the substructures defining feature B were collected and studied in terms of QSAR in order to investigate quantitatively the role played by the aromatic moiety in the interaction with the binding site of the receptor. In all the studied compounds, the above substructures are bound to the skeleton (feature A) by saturated, single carbon−carbon bonds. Thus, no complications about resonance/electronic effects should occur in the computation of electronic descriptors of the parent compounds by using its representative substructure. The correlation between the biological activity (dependent variable), expressed as the natural logarithm of the reciprocal of *R*-(−)-[H^3]-baclofen binding displacement (ln $1/IC_{50}$), and a series of molecular descriptors was quantitatively studied by means of partial least-squares analysis (PLS). The binding values of the inactive derivatives was set to 100 μM, in order to include them into the set and train the model with respect of inactivity. Also in agreement with a previous SAR study,[10b] three electronic descriptors (HOMO energy, LUMO energy, dipole moment),

two shape-dependent descriptors (molecular volume and molecular surface area), and one thermodynamic descriptor (ALOGP98) were used. HOMO and LUMO energies were calculated by using the AM1 method as implemented in MOPAC 6.0. The magnitude of dipole moment was calculated by utilizing partial atomic charges and atomic coordinates. Either AM1 derived partial atomic charges or partial atomic charges computed using the charge-equilibration method[18] were used. It was found that best results in terms of correlation were obtained by using charges derived from the charge-equilibration methods. ALOGP98 was calculated using the method described by Ghose.[19] Cross-validation protocol and randomization tests were used to determine the statistical significance of the analyses. The predictive index of the generated models is given by the cross-validated r^2 (r^2_{cv}, q^2). All the PLS analyses were performed on autoscaled variables. Outliers, if present, were removed, and new PLS analyses were carried out. All calculations were carried out on a SGI O2 R12000 machine using the QSAR module implemented in the Cerius2 software package distributed by Molecular Simulations Inc.

Structure-Based Approach: ATD GABA$_B$R1 Modeling. The 3D model of the ATD of GABA$_B$R1 was constructed on the basis of the similarity with the controller of the amidase operon (AmiC, PDB code: 1pea) proposed by us in the case of metabotropic glutamate receptors[15,16] and also used by Galvez and co-workers in the construction of the closed form of the ATD of GABA$_B$R1.[13] The alignment between the ATD of GABA$_B$R1 and AmiC was identical to that used by Galvez et al.[13] The end residues of the final model were capped by acetamide at the N-terminus and *N*-methylamide at the C-terminus. Ionizable residues localized on the solvent accessible surface were considered as charged. Ionizable residues localized in the cleft were considered charged since in the open conformation of the receptor they are exposed to the solvent. The GABA$_B$R1 ATD model was submitted to a cycle of 200 steps of steepest descents minimization with a fixed constraint on the coordinates of backbone atoms, followed by another 200 steps of steepest descents minimization with an high harmonic constraint on the backbone atoms and a fixed constraint on the end residues. After this cycle, it was submitted to a full minimization using the conjugate gradient method until a gradient convergence of 0.05 kcal/mol Å was reached.

Docking experiments were performed with the manual docking module of Insight II. In particular, several spatial orientations and conformations of ligands were evaluated in the binding site of GABA$_B$R1. Where possible, conformational space of residues interacting directly with ligands were explored using the rotamer library of residues as implemented in Insight II. Among the several dispositions of the ligands, the one endowed with the lowest interaction energy was taken and minimized using the conjugate gradient method until a gradient convergence of 0.05 kcal/mol Å was reached. For all of the above calculations the dielectric function was set as a radial dependent with a value of 1. The minimization protocols were carried out with the CHARMM force field. All computations were carried out on a SGI O2 R12000 workstation using the Insight II molecular modeling software package distributed by Molecular Simulations, Inc.

Results

QSAR Studies. All of the performed analyses and the obtained results are summarized in Table 2.

The first analysis (run 1, Table 2) was carried out with 16 compounds and six variables. The q^2 value was close to zero, but two outliers were immediately identified, namely compounds **4** and **10**. The two outliers were removed, and the analysis was carried out again (run 2, Table 2), yielding $q^2 = 0.749$, already statistically significant, with a three-component model. The two outliers are *m*-chlorophenyl-GABA (**4**) and 2-methylthienyl-GABA (**10**), respectively. There is no clear reasons why these two compounds are poorly predicted by the model. In particular, **4** is predicted much more active than it is, while **10** is predicted less active. While for compound **10** a poor calculation of the inductive effect of the methyl group on the thienyl ring cannot be ruled out, for compound **4** a specific, not series-dependent, effect should be considered. The back rotation of the PLS loadings into pseudo-regression coefficients gave the following QSAR equation (eq 1)

$$\ln(1/R\text{-}(-)\text{-}[H^3]\text{-baclofen binding}) = 2.957(\text{LUMO}) - 3.666(\text{HOMO}) + 1.024(\text{dipole-mag}) + 0.889(\text{ALOGP98}) - 0.023(\text{area}) - 0.035(\text{volume}) - 35.799 \quad (1)$$

where the high coefficients of both HOMO and LUMO orbital energies can be appreciated, while the effect of the molecular area and volume is negligible. On the other hand, it should also be noted that, within the series of baclofen analogues, orbital energies and shape-dependent parameters are highly correlated (Table 3), and thus the PLS analysis cannot properly discern the effective contribution of individual variables.

Given this warning, the PLS model was validated by means of some tests, including leave-many-out cross-validation and randomization tests. These results indicate the reliability of the model. It should be noted that in the similar analysis carried out by Pirard et al.,[10b] a precise role for the HOMO energies was put forward, albeit at a qualitative level. Our own results tend to quantitatively confirm Pirard's hypothesis on the role played by the energies of frontier orbitals. Less important seem to be steric factors which, encoded in our model by molecular area and molecular volume, have lower weights in the correlation analysis.

ATD GABA$_B$R1 Modeling. Given the same alignment procedure, the homology modeling approach re-

Table 2. PLS Loadings of the Descriptors Used in Different Analyses

descriptors	run 1	run 2[a]
dipole-mag	1.724	1.538
LUMO	0.274	0.699
HOMO	−0.109	−0.355
ALOGP98	0.223	0.553
volume	−0.054	0.030
area	0.241	0.015
r^2	0.530	0.921
q^2	0.027	0.749
outliers	2 (**4**, **10**)	

[a] This run was performed removing compounds **4** (PLS residual = −2.456) and **10** (PLS residual = 3.297) from the training set. The descriptor dipole-mag is the magnitude of the dipole moment. HOMO and LUMO are the energies of the molecular orbitals, localized on the aromatic rings, and computed at the AM1 level of theory. ALOGP is an estimation of the hydrophobic/hydrophilic balance, computed according to ref 19. Volume and area are the molecular volume and the molecular surface, respectively.

Table 3. Correlation Matrix of Descriptors

	LUMO	HOMO	dipole mag	ALOGP98	volume	area
LUMO	1.000	0.473	−0.791	−0.587	−0.535	−0.530
HOMO	0.473	1.000	−0.474	−0.708	−0.419	−0.366
dipole mag	−0.791	−0.474	1.000	0.646	0.576	0.561
ALOGP98	−0.587	−0.708	0.646	1.000	0.867	0.786
volume	−0.535	−0.419	0.576	0.867	1.000	0.979
area	−0.530	−0.366	0.561	0.786	0.979	1.000

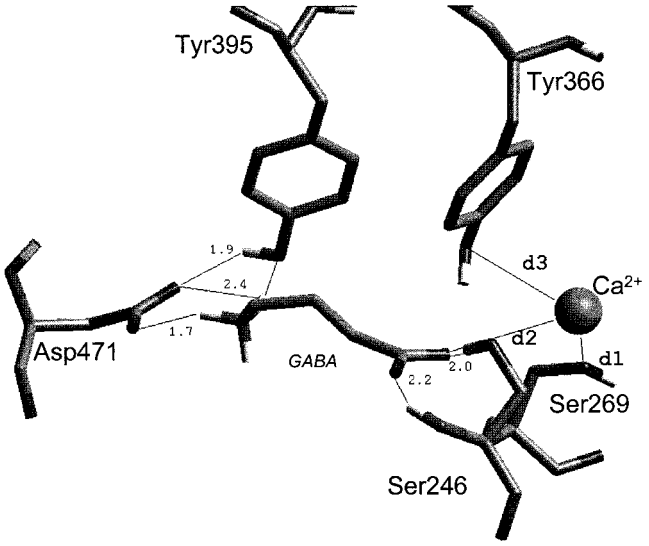
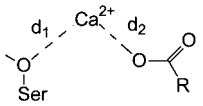


Figure 1. Docking of GABA and Ca^{2+} into the proposed binding pocket of the $GABA_BR1$ receptor. For d_1, d_2, and d_3 distances, see Table 4.

Table 4. Distribution Histogram of d_1 and d_2 Distances in Crystals Containing a Ca^{2+} Ion Interacting with an Aliphatic Hydroxyl Group and a Carboxylic Group[a]



dist	d_1	d_2
1.6	* 1	* 1
1.8	** 4	**
2	****** 26	****** 26
2.2	********** 84	********** 57
2.4	********** 150	********** 144
2.6	********** 219	********** 142
2.8	******** 46	********** 91
3	** 4	********** 36
3.2	**** 11	******** 30

[a] Data are taken from the RELIBASE server. Distances d_1 (2.95 Å) and d_2 (3.41 Å) from the docked complex of Ca^{2+} are in the border line indicating a limitation in force field in reproducing the exact geometry. Distance d_3 (3.38 Å) is not presented in crystals in a statistical significance (data not shown).

sulted in a model of the closed conformation of the amino terminal domain of $GABA_BR1$ very similar to that obtained by Galvez et al.[13] but significantly different from that reported by Bernard et al.[21] GABA (**1**) and baclofen (**3**) were docked into the putative binding pocket. Since the binding of GABA (**1**) requires the copresence of Ca^{2+},[12] these two species were docked simultaneously. The docking studies identified a low-energy disposition of GABA (**1**) and Ca^{2+} in which the carboxylic moiety of GABA (**1**) is involved in a hydrogen bond network with the hydroxy groups of Ser246 and Tyr366 and in a strong interaction with Ca^{2+}. The amino group of GABA (**1**) forms a salt bridge with Asp471 and a hydrogen bond with Tyr395 (Figure 1, see also ref 13). The Ca^{2+} ion is placed near Ser269, interacting with the carboxylic group of GABA (**1**) and the hydroxy group of Ser269 (Figure 1). A search of the PDB database disclosed a relevant number of Ca^{2+}−protein complexes characterized by a binding geometry similar to that we observe in the case of $GABA_BR1$ (Table 4).

It is worth noting that also the hydroxy group of Tyr366 lies near Ca^{2+}, and in principle, it can interact with the cation (Figure 1). However, this kind of interaction is not significantly present in crystallized Ca^{2+}−protein complexes (data not shown). The same docking procedure was carried out on baclofen (**3**), and a geometrical disposition of its scaffold similar to that of GABA (**1**) was identified. Analogously, hydrogen bonds can be identified between the carboxylic group of baclofen (**3**) and the hydroxy groups of Ser246 and Tyr366. The amino moiety of baclofen (**3**) is involved in an electrostatic enforced hydrogen bond with Asp471. Interestingly, the aromatic group of baclofen (**3**) is projected into a cavity formed by Tyr366 and Tyr395. The sandwich-like disposition adopted by the three aromatic rings highlights a possible $\pi-\pi$ stacking interaction among these groups (Figure 2).

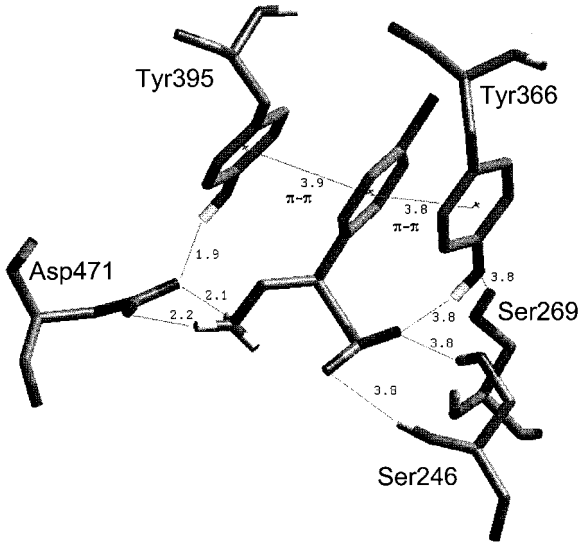


Figure 2. Docking of baclofen into the proposed binding pocket of $GABA_BR1$. The *p*-chlorophenyl aromatic ring is stacked between two tyrosine residues, namely Tyr 366 and Tyr 395.

Discussion

Our QSAR analysis indicates that electronic descriptors such as the energies of HOMO and LUMO orbitals of a series of baclofen analogues, localized on the aromatic moieties, are strongly correlated with receptor binding (Table 2). The specific role of the aromatic group of baclofen (**3**) in the receptor binding is further confirmed by our docking experiments. In the best binding disposition, the aromatic ring is placed in a pocket formed by the aromatic side chains of Tyr366 and Tyr395 (Figure 2). This interaction is a $\pi-\pi$ stacking described by the transfer of an electron from the highest occupied molecular orbital (HOMO) of the donating species (Tyr366 or Tyr395) to the lowest unoccupied molecular orbital (LUMO) of the accepting species [baclofen (**3**), Figure 3].

It should be mentioned that a hydrogen bond interaction was previously proposed by Galvez et al. between Tyr366 and baclofen (**3**).[13] The formation of this hydrogen bond is certainly possible, but its presence is unable to explain the lack or the poor potency of several other baclofen analogues that can be involved in the same interaction as well.

Previous structural studies indicated that $GABA_BRs$ belong to family C of GPCRs. As members of this family,

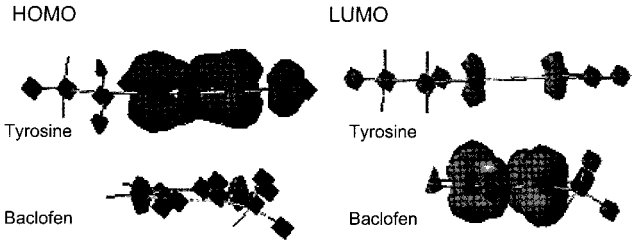


Figure 3. HOMO and LUMO orbitals (AM1 level of theory) for tyrosine and baclofen.

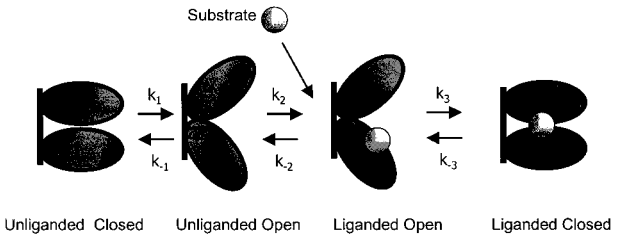


Figure 4. Hypothetical mechanism of ligand binding for $GABA_BR1$ based on periplasmic binding proteins.

the amino terminal domains (ATD) of $GABA_BRs$ are constituted of two globular domains (lobes) connected by a hinge region formed with two or three filaments. The two lobes are separated by a deep cleft in which the substrate is accommodated. The receptors exist in an equilibrium between an open form, where the two lobes are separated from each other, and a closed form, where the two lobes collapse each on the top of the other upon a movement of the hinge region. The substrate binds the open form, causing a shift of the equilibrium toward the functionally active closed form (Figure 4).

Studies aimed at clarifying the binding mode of GABA (**1**) and baclofen (**3**) to the ATD of $GABA_BR1$ suggested that both GABA (**1**) and baclofen (**3**) interact mainly by means of hydrogen bonds with Ser246 and Tyr366 and by means of an ionic interaction with Asp471. Moreover, it has also been proposed that GABA (**1**) and GABA-related agonists need the presence of calcium ion to enforce its $GABA_BR1$ binding and activation. A Ca^{2+} binding site has been localized by means of mutagenesis experiments on Ser269. Interestingly, baclofen (**3**) activates the receptor independently by the presence of Ca^{2+}. Our QSAR studies allow one to argue that the aromatic ring of baclofen (**3**), which is missing in GABA (**1**), may play the role of Ca^{2+} in the GABA receptor binding and activation. In this regard, it is worth noting that in our docking experiments Ca^{2+} affects the GABA binding by interacting with Ser269, the carboxylic moiety of GABA (**1**), and Tyr366 (Figure 1). Since Tyr366 is localized on the upper lobe of ATD (lobe II), we can delineate a scenario in which GABA (**1**) binds the open form of the receptor interacting with Ser246 and Asp471 (lobe I). Ca^{2+} enforces the GABA binding by interacting with Ser269 and the carboxylic moiety of GABA (**1**). Then, a perturbation of the receptor equilibrium is promoted, and a rapid shift of the ATD toward its functionally active closed form is observed, the latter being thermodynamically stabilized by means of interactions between GABA (**1**), Ca^{2+}, and residues of the upper lobe (lobe II) such as Tyr366 and Tyr395. A similar mechanism for receptor activation should also be adopted by baclofen (**3**). In this case, the presence of Ca^{2+} to promote a rapid shift of the equilibrium is not strictly required since the *p*-chlorophenyl aromatic ring should serve in this aim by interacting directly with Tyr366 and Tyr395. Aromatic−cation and aromatic−aromatic interactions are very well known in proteins[20] and contribute to both protein stability and ligand recognition. Should the mechanism we propose be proven, it would be an indication that these two kinds of interactions, although physically different (mainly electrostatic the aromatic−cation interactions, prevalently due to dispersive forces the aromatic−aromatic interactions), can be adopted by protein−ligand complexes to achieve the same functional effect.

Conclusion

Our QSAR studies report a pivotal role of the aromatic ring of baclofen (**3**) and baclofen-related agonists in the binding and activation of the ATD of $GABA_BR1$. On the basis of this observation, a putative model of the receptor activation of baclofen (**3**) has been drawn. The proposed model is in agreement with available experimental data and points out that the aromatic ring of baclofen (**3**) and related compounds may cover the same role covered by Ca^{2+} in the mechanism of receptor activation mediated by GABA (**1**) or GABA-related agonists.

Supporting Information Available: Statistics on PLS runs and coordinates of the complex between the ATD of $GABA_B$ and baclofen. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Bowery, N. G. $GABA_B$ receptor pharmacology. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 109−147.
(2) Bowery, N. G.; Hill, D. R.; Hudson, A. L.; Doble, A.; Middlemiss, D. N.; Shaw, J.; Turnbull, M. (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **1980**, 283, 92−4.
(3) Kaupmann, K.; Huggel, K.; Heid, J., Flor, P. J. Bischoff, S.; Mickel, S. J.; McMaster, G.; Angst, C.; Bittiger, H.; Froestl, W.; Bettler, B. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **1997**, *386*, 239−46.
(4) Bockaert, J.; Pin, J.-P. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* **1999**, *18*, 1723−9.
(5) Kaupmann, K.; Malitschek, B.; Schuler, V.; Heid, J.; Froestl, W.; Beck, P.; Mosbacher, J.; Bischoff, S.; Kulik, A.; Shigemoto, R.; Karschin, A.; Bettler, B. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* **1998**, *396*, 683−7. (b) Jones, K. A.; Borowsky, B.; Tamm, J. A.; Craig, D. A.; Durkin, M. M.; Dai, M.; Yao, W. J.; Johnson, M.; Gunwaldsen, C.; Huang, L. Y.; Tang, C.; Shen, Q.; Salon, J. A.; Morse, K.; Laz, T., Smith, K. E.; Nagarathnam, D.; Noble, S. A.; Branchek, T. A.; Gerald, C. GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* **1998** *396*, 674−9. (c) White, J. H.; Wise, A.; Main, M. J.; Green, A.; Fraser, N. J.; Disney, G. H.; Barnes, A. A.; Emson, P.; Foord, S. M.; Marshall, F. H. Heterodimerization is required for the formation of a functional GABA(B) receptor. *Nature* **1998**, *396*, 679−82.
(6) Malcangio, M.; Bowery, N. G. Possible therapeutic application of GABAB receptor agonists and antagonists. *Clin. Neuropharmacol.* **1995**, *18*, 285−3
(7) Froestl, W.; Bettler, B.; Bittiger, H.; Heid, J.; Kaupmann, K.; Mickel, S. J.; Strub, D. Ligands for the isolation of GABA(B) receptors. *Neuropharmacology* **1999**, *38*, 1641−6.
(8) Hill, D. R.; Bowery, N. G. 3H-baclofen and 3H-GABA bind to bicuculline-insensitive GABA B sites in rat brain. *Nature* **1981**, *290*, 149−52.
(9) Berthelot, P.; Vaccher, C.; Flouquet, N.; Debaert, M.; Luyckx, M.; Brunet, C. 3-Thienyl- and 3-furylaminobutyric acids. Synthesis and binding GABAB receptor studies. *J. Med. Chem.* **1991**, *34*, 2557−60. (b) Shuto, S.; Shibuya, N.; Yamada, S.; Ohkura, T.; Kimura, R.; Matsuda, A. Synthesis of conformationally restricted analogs of baclofen, a potent GABAB receptor agonist, by the introduction of a cyclopropane ring. *Chem. Pharm. Bull.* **1999,** *47*, 1188−92.

(10) Mann, A.; Boulanger, T.; Brandau, B.; Durant, F.; Evrard, G.; Heaulme, M.; Desaulles, E.; Wermuth, C. G. Synthesis and biochemical evaluation of baclofen analogues locked in the baclofen solid-state conformation. *J. Med. Chem*. **1991**, *34*, 1307−13. (b) Pirard, B., Carrupt, P. A.; Testa, B.; Tsai, R. S.; Berthelot, P.; Vaccher, C.; Debaert, M.; Durant, F. Structure-affinity relationships of baclofen and 3-heteroaromatic analogues. *Bioorg. Med. Chem*. **1995**, *3*, 1537−45. (c) Pirard, B.; Durant, F. Molecular modelling and conformational analysis of a GABAB antagonist. *J. Comput.-Aided Mol. Des*. **1996**, *10*, 31−40.

(11) Galvez, T.; Parmentier, M. L.; Joly, C.; Malitschek, B.; Kaupmann, K.; Kuhn, R.; Bittiger, H.; Froestl, W.; Bettler, B.; Pin, J.-P. Mutagenesis and modeling of the GABAB receptor extracellular domain support a Venus flytrap mechanism for ligand binding. *J. Biol. Chem*. **1999**, *274*, 13362−9.

(12) Galvez, T.; Urwyler, S.; Prezeau, L.; Mosbacher, J.; Joly, C.; Malitschek, B.; Heid, J.; Brabet, I.; Froestl, W.; Bettler, B.; Kaupmann, K.; Pin, J.-P. Ca(2+) requirement for high-affinity gamma-aminobutyric acid (GABA) binding at GABA(B) receptors: involvement of serine 269 of the GABA(B)R1 subunit. *Mol. Pharmacol*. **2000**, *57*, 419−26.

(13) Galvez, T. C.; Prezeau, L.; Milioti, G., Franek, M.; Joly, C., Froestl, W.; Bettler, B.; Bertrand, H. O.; Blahos, J.; Pin, J.-P. Mapping the agonist binding site of GABAB Type 1 subunit sheds light on the activation process of GABAB receptors. *J. Biol. Chem*. **2000**, 275, 411666−74.

(14) Costantino, G.; Macchiarulo, A.; Pellicciari, R Modeling of amino-terminal domains of group I metabotropic glutamate receptors: structural motifs affecting ligand selectivity. *J. Med. Chem*. **1999**, *42*, 5390−401.

(15) Costantino, G.; Macchiarulo, A.; Pellicciari, R. Homology model of the closed, functionally active, form of the amino terminal domain of mGluR1. *Bioorg. Med. Chem*. In press.

(16) Pellicciari, R.; Costantino, G.; Macchiarulo, A.; Bellocchi, D. Comparative Molecular Field Analysis and Molecular Modeling of $GABA_B$ Agonists. Towards a rationalization of comformational, steric and physicochemical requirements for $GABA_B$ activation. *$GABA_B$ Receptors*. The 8th Neuropharmacology Conference, 94, 1999.

(17) Cardozo, M. G.; Iimura, Y.; Sugimoto, H.; Yamanishi, Y.; Hopfinger, A. J. QSAR analyses of the substituted indanone and benzylpiperidine rings of a series of indanone-benzylpiperidine inhibitors of acetylcholinesterase. *J. Med. Chem*. **1992**, *35*, 584−9.

(18) Rappe, A. K.; Goddard, W. A., III. Charge Equilibration for Molecular Dynamics Simulation. *J. Phys. Chem*. **1991**, *95*, 3358−3363.

(19) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Phys. Chem. A* **1998**, *102*, 3762−3772.

(20) Burley, S. K.; Petsko, G. A. Aromatic−aromatic interaction: a mechanism of protein structure stabilization. *Science* **1985**, *229*, 23−8. (b) Gallivan JP, Dougherty DA. Cation-pi interactions in structural biology. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 9459−64.

(21) Bernard, P.; Guedin, D.; Hibert, M. Molecular modeling of the GABA/GABA(B) receptor complex. *J. Med. Chem*. **2001**, *44*, 27−35.

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