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Structural determinants for interaction of partial agonists with AChBP and neuronal 7 nicotinic ACh receptor

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 06 May 2009

Thank you very much for submitting your research manuscript for consideration to The EMBO Journal editorial office. The enclosed referee reports clearly appreciate the atomic insight into AChBP binding to various ligands that can, according to earlier work, be extended to Ach-receptor/ligand binding. All the comments indicate that a more cautious discussion of the actual reported data in relation to what had been shown before would increase the impact of the current study. Particularly, ref#1 requests incorporation of crystal packing and more careful reflection of the partial agonist activity, similar to ref#2 that asks to better emphasize the gathered results in the model and ref#3 also indicating some inconsistencies between results and conclusions that would need your careful attention. We therefore kindly invite you to submit a revised version that addresses all the concerns raised from our referee's. I also have to remind you that it is EMBO_J policy to allow a single round of revisions only, which means that the final decision depends entirely on the content of the ultimate version of your manuscript.

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

REFEREE REPORTS

Referee #1 (Remarks to the Author):

This paper describes four new X-ray structures of the acetylcholine binding protein (Aplysia AChBP) in complex with competitive ligands: anabaseine, the two anabaseine derivatives DMXBA and 4-OH-DMXBA, and tropisetron. Anabaseine acts as a full agonist on nAChRs and the three other compounds are partial agonists.

Comparative analysis of the way the four compounds bind in the ACh pocket reveals that, briefly:
Anabaseine binds to AChBP in a way similar to that of nicotine, with the notable exception that the capping loop C is in a rather "open" conformation in anabaseine complex, while it is in a "closed" conformation tightly interacting with the ligand in the nicotine complex. The weak contribution of loop C in anabaseine binding probably causes its weak affinity for AChBP.
DMXBA is an anabaseine derivative substituted with a benzylidene ring. The surprising observation for this compound is that it binds in two alternate and very different orientations inside the pentamer. In both orientations, the benzylidene ring protrudes from the binding site to interact with different regions of the plus and minus sides of the interface. In both cases, the loop C conformation is intermediate between the anabaseine "open" and nicotine "closed" conformations.
4-OH-DMXBA binds in only one of the orientations adopted by DMXBA, and is characterized by an important contribution of the loop F to ligand binding. However, loop C adopts two distinct conformations, with one almost "closed", and one much more "open".

This work thus provides a comprehensive set of data that reveals in details the way AChBP and nAChRs bind these important nicotinic ligands. Of major interest here is to understand the determinant of ligand specificity among the various nAChR subtypes, and this aspect is discussed in the manuscript, with the convincing conclusion that loop F, of variable sequence, plays a key role in the pharmacological specificity of anabaseine derivatives. This observation will be particularly useful for medical chemistry and for the design of specific ligands.

This work also reveals unexpected features of ligands binding, such as the occurrence of two distinct binding conformations for DMXBA, and two distinct conformations of the loop C in 4-OH-DMXBA. One can wonder whether the crystal packing may alter artificially the local shape of the binding pocket, especially concerning loop C. Therefore, the analysis of these structures requires a systematic analysis of the crystal packing, and of its potential contribution to the loop C conformation.

Last, it is claimed in the abstract that the data "suggest a molecular basis for partial agonist activity". It seems rather that the provided data complicate the picture. Indeed, the numerous structures of AChBP in complex with agonists and antagonists previously showed that agonist binding (versus antagonist) is associated with a "closed" (versus "open") conformation of the C loop. However, the present data show that the full agonist anabaseine is associated with an "open" C loop conformation, and that of partial agonists with various conformations of the C loop, including one which is rather "closed". Thus the data seriously contradict the dogma that agonist binding promote channel gating through a capping motion of the C loop in a "closed" conformation. This aspect should be explicitly discussed in the manuscript and stated in the abstract. Another model of partial agonist activity provided by this work is the duality in binding orientation of DMXBA, which appears to bind in both an agonist-like and antagonist-like conformation. While this idea is elegant, it remains highly speculative and it is unlikely that such a mechanism would apply to the numerous partial agonists known.

Overall, this paper presents a comprehensive set of data that deserves publication in the EMBO journal, providing that crystal packing is incorporated in the analysis, and that the discussion about partial agonist activity reflects the structural observations.

Referee #2 (Remarks to the Author):

This is an interesting and well-written manuscript describing novel crystal structures of the acetylcholine binding protein (AChBP) bound to a range of nicotinic ligands. The experimental data is of a very high standard.

An interesting novel finding is evidence that partial agonists to adopt multiple orientations within the AChBP binding site. The authors provide an interesting discussion of the possible relevance of this in explaining partial agonism.

It is also suggested that the new structural data supports previous studies concerning the influence of agonist and antagonist binding on the extent of closure of loop C (a cartoon depicting the extent of

loop C closure with an agonist, partial agonist and an antagonist is presented in Figure 8B). I was slightly concerned that the inclusion of this cartoon might be potentially misleading. The reason for this being that the data presented in THIS study does not fully support this model. In particular, this is because the only structure that is presented of AChBP with a full agonist (anabaseine), loop C is more open than in structures of AChBP with partial agonists bound. Perhaps this should be discussed in more detail.

Another issue that should perhaps be discussed in more detail (and may, perhaps, be connected to the previous point) is that, although the ligands examined are known to be either full or partial agonists on some nicotinic acetylcholine receptors, there is no evidence as to whether they should be considered full or partial "agonists" of the snail AChBP.

In discussing the lack of loop C closure in the anabaseine structure (on page 8), the authors appear to suggest that this may be a consequence of anabaseine binding to AChBP with relatively weak affinity. Are they suggesting that binding affinity might be as relevant to the degree of closure of loop C as the efficacy of the ligand? Perhaps this could be clarified.

Referee #3 (Remarks to the Author):

This is an important study that provides structural insight into the determinants of agonists and antagonists for nicotinic acetylcholine receptors. Specific comments:

1. The schematic representation of agonist and antagonist binding modes in Fig. 8 seems very similar to a model in a recent study [Yi et al, PNAS 105, 8280 (2008)]. It is of interest to comment on that study in light of the results reported here.

2. The authors conclude that loop C wraps tightly around a full agonist but is more open in the case of a partial agonist (see, e.g., Fig. 8). However, in the last paragraph on p. 9, the authors state that, for two partial agonists, "the loop C conformational position (Fig. 4) is intermediate between those observed for nicotine and epibatidine, and for anabaseine," all the last three being full agonists. Am I reading this incorrectly, or are the results and conclusion inconsistent?

3. In a number of places, the authors refer to agonist or antagonist AT some receptor. Consider changing AT to FOR.

1st Revision - authors' response 13 July 2009

Thank you for the editorial decision letter, the accompanying favorable comments of three reviewers and particularly for the expeditious reviews and decision. In fact, all of the reviewers comment on the atypical open position of loop C in AChBP bound with the full agonist anabaseine, compared with the position usually found in presence of other full agonists. Since we were also puzzled with this result, during the review process we worked at solving a new structure of this complex that would satisfactory respond to this issue. In fact, we have now found conditions where we have higher binding site occupancy by anabaseine, and issues regarding loop C closure with full agonists can be resolved. The low affinity of the complex and opening of the diihydropyridine ring presented a challenge.

We apologize for the delay in submitting the revised version of our manuscript that responds to all the issues raised in the reviews and have deposited the structural coordinates with the Protein Data Bank. Below are our responses to each of the three reviewers. These responses appear as *italicized blue inserts* below the referees' comments. Also, we have reread the manuscript and carefully revised it throughout to make it shorter, clearer and more appropriate for a general audience.

My co-authors and I look forward to learning of your decision.

Referee 1:

This paper describes four new X-ray structures of the acetylcholine binding protein (Aplysia AChBP) in complex with competitive ligands: anabaseine, the two anabaseine derivatives DMXBA and 4-OH-DMXBA, and tropisetron. Anabaseine acts as a full agonist on nAChRs and the three other compounds are partial agonists.

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This work thus provides a comprehensive set of data that reveals in details the way AChBP and nAChRs bind these important nicotinic ligands. Of major interest here is to understand the determinant of ligand specificity among the various nAChR subtypes, and this aspect is discussed in the manuscript, with the convincing conclusion that loop F, of variable sequence, plays a key role in the pharmacological specificity of anabaseine derivatives. This observation will be particularly useful for medical chemistry and for the design of specific ligands.

We thank the referee for the favorable comments and for noting the importance of these structures to the drug design community.

This work also reveals unexpected features of ligands binding, such as the occurrence of two distinct binding conformations for DMXBA, and two distinct conformations of the loop C in 4-OH-DMXBA. One can wonder whether the crystal packing may alter artificially the local shape of the binding pocket, especially concerning loop C. Therefore, the analysis of these structures requires a systematic analysis of the crystal packing, and of its potential contribution to the loop C conformation.

We agree with the reviewer, and have added in the Experimental section a systematic analysis of the possible contribution of loop C in crystal packing for all structures. This analysis shows that crystal contacts do not influence the position of the tip of loop C of the primary pentameric assembly.

Last, it is claimed in the abstract that the data "suggest a molecular basis for partial agonist activity". It seems rather that the provided data complicate the picture. Indeed, the numerous structures of AChBP in complex with agonists and antagonists previously showed that agonist binding (versus antagonist) is associated with a "closed" (versus "open") conformation of the C loop. However, the present data show that the full agonist anabaseine is associated with an "open" C loop conformation, and that of partial agonists with various conformations of the C loop, including one which is rather "closed". Thus the data seriously contradict the dogma that agonist binding promote channel gating through a capping motion of the C loop in a "closed" conformation. This aspect should be explicitly discussed in the manuscript and stated in the abstract. Another model of partial agonist activity provided by this work is the duality in binding orientation of DMXBA, which appears to bind in both an agonist-like and antagonist-like conformation. While this idea is elegant, it remains highly speculative and it is unlikely that such a mechanism would apply to the numerous partial agonists known.

We agree, and during the review process we were able to solve a new structure of an anabaseine-AChBP complex. This new structure, albeit at lower resolution than the other complexes despite the high quality of the data, has replaced the previous one. It shows a closed conformation of loop C in the two binding pockets with bound cyclic form of anabaseine, consistent with earlier structures of

AChBP in complex with full agonists. The text, figures and tables related to this complex have been modified accordingly.

Overall, this paper presents a comprehensive set of data that deserves publication in the EMBO journal, providing that crystal packing is incorporated in the analysis, and that the discussion about partial agonist activity reflects the structural observations.

We agree, and the manuscript has been revised in light of these criticisms

Referee 2:

This is an interesting and well-written manuscript describing novel crystal structures of the acetylcholine binding protein (AChBP) bound to a range of nicotinic ligands. The experimental data is of a very high standard.

We thank the referee for noting the quality of the data and impact of the study.

An interesting novel finding is evidence that partial agonists to adopt multiple orientations within the AChBP binding site. The authors provide an interesting discussion of the possible relevance of this in explaining partial agonism. It is also suggested that the new structural data supports previous studies concerning the influence of agonist and antagonist binding on the extent of closure of loop C (a cartoon depicting the extent of loop C closure with an agonist, partial agonist and an antagonist is presented in Figure 8B). I was slightly concerned that the inclusion of this cartoon might be potentially misleading. The reason for this being that the data presented in THIS study does not fully support this model. In particular, this is because the only structure that is presented of AChBP with a full agonist (anabaseine), loop C is more open than in structures of AChBP with partial agonists bound. Perhaps this should be discussed in more detail.

As explained in our reply to referee 1 (cf. above), a structure of an anabaseine-AChBP complex has been solved during the review process and has been included in the revised manuscript. This structure, albeit at lower resolution despite the high quality of the data, shows a closed conformation of loop C in the two binding pockets with bound cyclic form of anabaseine, largely consistent with earlier structures of AChBP in complex with full agonists.
Another issue that should perhaps be discussed in more detail (and may, perhaps, be connected to the previous point) is that, although the ligands examined are known to be either full or partial agonists on some nicotinic acetylcholine receptors, there is no evidence as to whether they should be considered full or partial "agonists" of the snail AChBP.

We have clearly stated that AChBP is a surrogate of the extracellular domain of the nicotinic receptor and lacks a linkage to the trans-membrane spans forming the channel. Hence we can only describe the conformations of the AChBP complex when agonists and antagonisms of receptor function are considered. Our most suitable comparison is with α7 *who also is a homomeric pentamer.*

In discussing the lack of loop C closure in the anabaseine structure (on page 8), the authors appear to suggest that this may be a consequence of anabaseine binding to AChBP with relatively weak affinity. Are they suggesting that binding affinity might be as relevant to the degree of closure of loop C as the efficacy of the ligand? Perhaps this could be clarified.

This confusing statement has been clarified with the new structure of the anabaseine-AChBP complex.

Referee 3:

This is an important study that provides structural insight into the determinants of agonists and antagonists for nicotinic acetylcholine receptors. Specific comments:
1. The schematic representation of agonist and antagonist binding modes in Fig. 8 seems very similar to a model in a recent study [Yi et al, PNAS 105, 8280 (2008)]. It is of interest to comment on that study in light of the results reported here.

We do not see the connection with this theoretical model since we have only emphasized the difference in loop C position between full and partial agonists. The reference cited by this referee refers to rotational motion of subunits. Fluctuations of loop tips near the membrane is difficult to correlate with the binding protein since it lacks a linkage to the transmembrane spans forming the channel. Moreover, in the mentioned manuscript we did not find a schematic representation similar as the one we show in Fig. 7.

2. The authors conclude that loop C wraps tightly around a full agonist but is more open in the case of a partial agonist (see, e.g., Fig. 8). However, in the last paragraph on p. 9, the authors state that, for two partial agonists, "the loop C conformational position (Fig. 4) is intermediate between those observed for nicotine and epibatidine, and for anabaseine," all the last three being full agonists. Am I reading this incorrectly, or are the results and conclusion inconsistent ?

As explained in our replies to referees 2 and 3 (cf. above), a new structure of the anabaseine-AChBP complex has been solved during the review process and included. The new structure, albeit at lower resolution despite the high quality of the data, shows a closed conformation of loop C in the two binding pockets with bound cyclic form of anabaseine, consistent with earlier structures of AChBP in complex with full agonists. The conclusion of our manuscript has been modified accordingly.

3. In a number of places, the authors refer to agonist or antagonist AT some receptor. Consider changing AT to FOR.

An AOL search using Google retrieved 2,460,000 hits for "agonist at", 2,480,000 hits for "agonist for", and 2,470,000 hits for "agonist to", and several references cited in our manuscript use "at", e.g., de Fiebre et al 1995; Hogg & Bertrand 2007; Machu et al 2001). However, and although one of us strongly requested that "at" be used, to please this referee we substituted "for" where required.

