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α4β2 nACh Receptor pharmacophore models

Richard A. Glennon* and Małgorzata Dukat

Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, Richmond, VA 23298, USA

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Abstract—Progress towards the development of α4β2 nicotinic acetylcholinergic receptor pharmacophores is reviewed from the early Beers and Riech model to the newer vector models.

A pharmacophore is generally considered to be an ensemble of the *minimal* structural (e.g., atomic, electronic, distance and/or steric) features necessary to define a given pharmacological action. A pharmacophore might be based on functional activity. However, because activity typically represents a composite of pharmacodynamic, pharmacokinetic, and pharmacophoric factors, depending upon the system being studied, it might be expected that pharmacophore models should be more reliably based on binding data. With respect to the latter, an agent that possesses the correct pharmacophoric features need not bind with high affinity. That is, the agent might lack an affinity-enhancing moiety or, conversely, the molecule might possess some added molecular feature not tolerated by the receptor. In theory, it is possible to have multiple pharmacophores for a given pharmacological effect. That is, two series of agents might bind with a receptor in a competitive manner, and yet parallel structural changes do not necessarily result in parallel shifts in affinity. With this as background, we trace the development of pharmacophore models for nicotinic acetylcholinergic (nACh) receptors.

Nicotinic receptors are pentameric structures consisting of various subunits (α, β, γ, δ, ε). Isoforms of certain subunits are possible (e.g., α1–α10, β1–β4); hence, multiple types of nACh receptors are possible. The major population of nACh receptors in mammalian brain are, by far, the α4β2 receptors. Consequently, more is known about this population than any other nACh receptor type. Eventually, unique (and perhaps similar) pharmacophores will be formulated for each of the different nACh receptors but at this time, there is too little information to address any but the α4β2 receptors.

Early studies toward the development of nicotinic pharmacophore models have been reviewed.[1] But, the first useful model was that developed by Beers and Reich who suggested that nicotinic ligands require an onium group, and a hydrogen bond acceptor feature that interacts with a receptor-based hydrogen bond donor site.[2] Sheridan et al.[3] refined this model using a distance geometry approach and formulated a three-point pharmacophore (Fig. 1). The onium group N^+ was proposed to be situated 4.8 (±0.3)Å from a hydrogen bond acceptor (e.g., the pyridine nitrogen atom of nicotine-like agents, or the carbonyl oxygen of carbonyl-containing compounds); the hydrogen bond acceptor moiety was located 1.2 Å from aryl centroid C which was, in turn, 4.0 (±0.3)Å from N^+.

The Sheridan et al.,[3] pharmacophore model was based on four ligands and did not implicitly consider functional or binding data. At the time the model was proposed, there was a paucity of nicotinic ligands and little understanding of multiple types of nACh receptors.

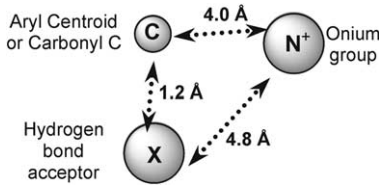

Figure 1. Nicotinic pharmacophore model proposed by Sheridan et al.[3] See text for more information.

* Corresponding author. Tel.: +1-804-828-8487; fax: +1-804-828-7404; e-mail: glennon@hsc.vcu.edu

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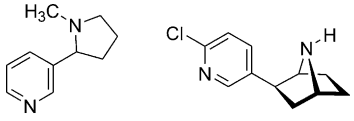
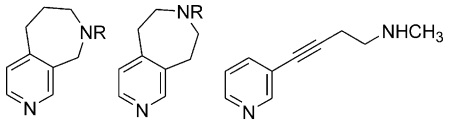
Glennon et al. raised the issue of whether the Sheridan et al.,[3] model was applicable to the binding of nicotine (**1**; K_i ca. 2 nM) and nicotine-like agents at α4β2 nACh receptors. While their investigation was underway, a novel high-affinity nACh receptor ligand, epibatidine (**2**; K_i ca. 0.05 nM),[4] was introduced by Daly et al.[5] It was found that a parabolic relationship existed between N–N distance (that is, the distance between features X and N^+ as shown in Fig. 1) and affinity, and that epibatidine seemed to possess the optimal N–N distance (that is, 5.5 Å).[6] Others later found that low energy barriers to rotation could result in N–N distances for epibatidine as short as 4.4 Å to as much as 5.7 Å.[7,8] Convincing arguments were made that a Sheridan distance was sufficient to account for the binding of epibatidine (reviewed[9]).



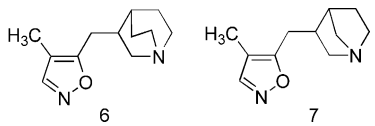
1 2

Yet, there were compounds that could not achieve this short distance but still displayed affinity for nACh receptors. For example, pyridoazepine **3**, with an NN distance of 4.6 Å displayed low affinity (K_i > 1000 nM when R = H or methyl); however, when the nitrogen substituent was relocated as in **4** (N–N = 5.5 Å), K_i = 47 nM when R = H.[9] Caldwell et al.,[10] reported that alkyne **5** binds with K_i = 58 nM. We have found that there are multiple low-energy conformers for **5** and that the lowest energy conformers possess a N–N distance of > 8 Å; the shortest achievable N–N distance is 5.8 Å (for a conformer that is within 2.5 kcal/mol of the lowest energy conformer). Hence, it seems unlikely that **4** and **5** bind in a manner consistent with the Sheridan distance. Nevertheless, these compounds lack the high affinity of epibatidine and nicotine. It was suggested that the 'long' versus 'short' distance controversy would not be resolved until high-affinity conformationally-constrained analogues were identified.[11]



3 4 5

Abbott investigators suggested a 'four-point' mode of binding where both the onium and hydrogen bond acceptor moieties, as well as the corresponding receptor-related features with which they interact, should be considered.[12] But, the model was never fully developed. At about the same time, several conformationally constrained high affinity nicotinic ligands were reported.[13]



6 7

The *Z* isomers of **6** (IC_{50} = 3.2 nM) and **7** (IC_{50} = 0.3 nM) bind with high affinity and possess N–N distances of about 5.6 Å.[13] However, rather than N–N distance, Olesen et al.[13] suggested that the distance between *Site point a* and *Site point b*, receptor-related features on vectors 2.9 Å in length from the onium group and hydrogen bond acceptor moieties, define binding (Fig. 2). This novel model seemingly accounted for the binding of 'short' and 'long' ligands.

Soon thereafter, these same investigators proposed an improved vector model (Fig. 3) in an attempt to define a three-point pharmacophore (for purpose of molecular alignment for QSAR studies).[14,15]

While seemingly the best available model to date, the initial vector model might be too permissive (i.e., agents can approach the *Site points* from different *z*-planes);[9] That is, there is little need for superimposition of specific molecular features for various agents and, consequently, the model would be difficult to apply to drug design. On the other hand, the improved vector model might be too restrictive in that, by virtue of introducing the aryl centroid, various pyridine-containing ligands would be required to interact in such a manner that their pyridine rings are tightly superimposed.[9] There is some evidence to support the latter. For example, parallel substituent changes in the aryl rings of nicotine and epibatidine result in parallel shifts in affinity;[16] similar findings have been reported for nicotine analogues and AMP (i.e., aminomethylpyridine **8**) analogues.[9] However, this is not the case for nicotine analogues and analogues of AXP (i.e., aminoethoxypyridine **9**, X=O), suggesting that they bind in a different manner.[17] Both the AMP and AXP analogues conform to the initial vector pharmacophore model, and do so by approaching the *Site points* from different planes.[9] To better account for molecular overlap, we modified the original vector model using the Abbott fourpoint concept; that is, we considered both nitrogen functions as well as both *Site points*. However, the potentially diminished importance of N–N distance was acknowledged by weighting

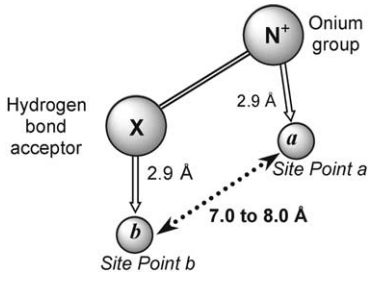


Figure 2. The original vector pharmacophore model as proposed by Olesen et al.[13]

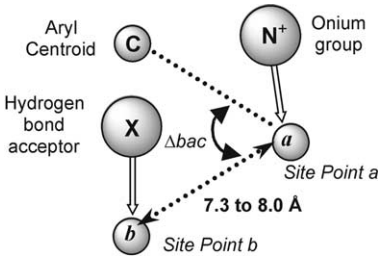
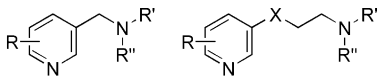


Figure 3. The improved vector model proposed by Olesen et al.[14,15]

the nitrogen atoms at only 10% the weight of the *Site points*. In this manner, the two types of molecules were forced into a closer *z*-plane fit (Fig. 4).

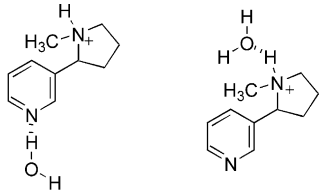


8 9

As can be seen from Figure 4, the two structure types are relatively well aligned. However, even with this modifcation the terminal amine substituents are pointed in somewhat different directions, as are potential substituents that might be incorporated into the pyridyl rings.

The vector models still fail to account for the binding of certain ligands. For example, AXP analogues (**9**, X=O) are accounted for by the vector model.[9] However, replacement of the ether oxygen atom by a methylene group to afford APP derivatives (aminopropylpyridines **9**, X=–CH_2–) results in compounds that lack affinity (K_i >10,000 nM) for nACh receptors but are, nevertheless, accommodated by the initial and modified vector models. Evidently, the oxygen atom of the AXP analogues is playing a role in binding that is not accounted for by any of the present models.

A possibility that has not yet been explored is that a molecule of water might hydrogen bond to the 'short' compounds converting them to 'long' compounds.[18] That is, water could intercede in the interaction of compounds with short N–N distances such that they might mimic the longer distance. The question becomes one of where water might participate. The hydrogen bond acceptor (e.g., see Fig. 1) might hydrogen bond to water (e.g., as with **10**) which, in turn, hydrogen bonds with the receptor. Alternatively, water might hydrogen bond with the 'onium' group (e.g., as with **11**). There is some support for this latter concept. For example, different terminal amine substituents on nornicotine, AMP analogues, and AXP analogues have different effects on affinity suggesting that they are in different environments (or are at least oriented in different directions).[6,9,17] Furthermore, quaternization of nicotine results only in slightly enhanced affinity[9] whereas, in contrast, quaternization of AXP-type compounds enhances their affinity by >50-fold.[19] It might not be expected that the quaternary amine analogue of nicotine would readily form a hydrogen bond with water whereas with the longer AXP analogues, water would not be necessary. Hence, the different results might be rationalized in this manner. This 'water-extension' concept will require further investigation.



10 11

Another issue that needs to be addressed is whether partial agonists or competitive antagonists bind in a manner similar to that of nicotine; that is, should pharmacophore models account for the binding of these agents as well as for agonists? Until relatively recently, there have been few such agents. One of the simplest examples in the nicotine series results from substitution at the 6-position. The (−)-isomers of nicotine, 6-methylnicotine and 6-ethylnicotine possess antinociceptive activity in the mouse tail-flick assay, whereas 6-*n*-propylnicotine and 6-*n*-butylnicotine lack such action but antagonize the actions of nicotine.[20] 5-Substituted analogues of nicotine also provide some interesting results. For example, 5-bromonicotine substituted for nicotine in a drug discrimination task, lacked antinociceptive actions in the tail-flick assay, and behaved as a partial agonist at α4β2 receptors expressed in frog oocytes and in an α4β2 $^{86}Rb^+$-efflux assay, whereas 5-methoxynicotine lacked antinociceptive properties but antagonized the antinociceptive actions of nicotine in the tail-flick assay.[21] Given the competitive nature of these agents in the binding assay, they might fit the same pharmacophore model. But, if they do, it might be important to identify those aspects of the model that are associated with antagonist or partial agonist action. For example, given that repeated exposure of a system to nicotinic agonists can result in desensitization, partial agonists might be a useful approach to developing agents with partial agonist action that would be less desensitizing. Knowledge of how specific pharmacophore-related structural features impact function would be useful for the design of novel partial agonists (or antagonists).

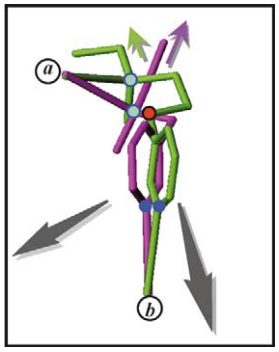


Figure 4. Superimposition of AMP (**8**) and AXP-type (**9**, X=O) compounds using the modified vector model with reduced weighting for the two nitrogen atoms.

With regard to pharmacophore models for α4β2 nACh receptor binding, the vector models are promising but still fail to explain some of the available data. To date, although novel agents have been identified that fit the vector models, the models themselves have not been used to design novel agents. Actually, it might be quite difficult to do so given the nature of the models as described above. The 'water-extension' concept is novel and requires further study. But the possibility still exists that more than one pharmacophore model might be required to define the binding of known nicotinic ligands. Once a reliable pharmacophore model(s) has been identified, it might lead to the development of novel ligands. Additional information might allow

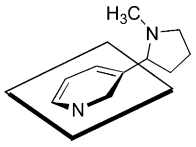


Figure 5. Barlow et al.,[22,23] '*point plus flat area*' concept for explaining binding at peripheral nACh receptors.

incorporation into these models of features that account for agonist versus partial agonist versus antagonist action. However, such ligands will in all likelihood lack selectivity. Additional binding (and functional) data will be required from other populations of nACh receptors so that pharmacophore models can be developed for the other receptor types. Only then can these models be used to develop novel and selective ligands. For example, Barlow et al.[22,23] have proposed a '*point plus flat area*' model (Fig. 5) that, while apparently not applicable to α4β2 binding,[9] could provide clues to binding of nicotinic ligands at peripheral nACh receptors.

Agents also have been identified that behave as noncompetitive nicotinic ligands and probably act at various allosteric nicotinic binding sites. In theory, it should be possible to develop pharmacophore models for these sites too. So, even with 50 years of history,[1] nicotinic pharmacophore models still require extensive work.

Acknowledgements

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