Biochemical Pharmacology 82 (2011) 1521–1527

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

What we have learned from crystal structures of proteins to receptor function

J.-L. Reymond [a], Ruud van Deursen [a], D. Bertrand [b,*]

[a] Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, 3012 Berne, Switzerland
[b] HiQScreen, 15 rue de l'Athénée, 1206 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 17 June 2011
Accepted 6 July 2011
Available online 20 July 2011

Keywords:
Ligand gated channel
Structure
Function
Pharmacology

ABSTRACT

The activity of ligand gated channels is crucial for proper brain function and dysfunction of a single receptor subtype have led to neurological impairments ranging from benign to major diseases such as epilepsy, startle diseases, etc. Molecular biology and crystallography allowed the characterization at the atomic scale of the first four transmembrane ligand gated channels and of proteins sharing a high degree of homology with the neurotransmitter-binding domain.

Gaining an adequate knowledge of the structural features of the ligand binding pocket led to the possibilities of developing virtual screening based approaches and probing in silico the docking of very large numbers of molecules. Development of new computing tools further extended such possibilities and rendered possible the screening of the chemical universe database GDB-11, which contains all possible organic molecules up to 11 atoms of C, N, O and F. In the case of the nicotinic acetylcholine receptors molecules identified using such screening methods were synthesized and characterized in binding assays and their pose determined in crystal structure with the acetylcholine binding protein. However, in spite of these thorough approaches, functional studies revealed that these molecules had a greater affinity for the pore domain of the channel and acted as open channel blocker rather than binding site antagonist.

In this work, we discuss the potential and current limitations of how progresses made with the crystal structures of ligand gated channels, or ligand binding proteins, can be used in combination with virtual screening and functional assays, to identify novel compounds.

1. Introduction

Gaining a better understanding of how the brain works and how to design new therapeutic strategies to compensate for central nervous system dysfunction depends, in large part, on deciphering ligand gated receptor properties. These highly specialized integral membrane proteins have the key features of binding with high affinity neurotransmitters released by nerve cells and transducing such detection by the fast opening of an ionic pore causing the signaling to the nerve cells.

Ligand gated channels can be divided in two major classes with the cationic channels causing an influx of cations and the depolarization of the neuron and anionic channels able to flux anions when they are activated. With the progress in molecular biology, cloning and sequencing emerged a first glimpse of the architecture of the receptors as illustrated in the upper panel of Fig. 1. These diagrams illustrate that ligand gated channels display different structural features and that subunits composing the receptor complexes present two, three or four transmembrane domains. Receptors result from the assembly of multiple subunits that, in the simplest form, are homomeric (assembly of identical subunits) or heteromeric (assembly of two or more distinct subunits). Similar to other integral membrane proteins ligand gated channels are rather difficult to crystallize and knowledge of their structure function relationship has been inferred by many approaches including high-resolution electron microscopy, biophysical measurements, site directed mutagenesis, etc. [1–6]. The most recent results obtained by crystal structures are summarized in the lower panel of Fig. 1. These data illustrate the receptor assembly, complexity and the large extracellular domain where the neurotransmitter is binding.

The aim of this work is to discuss in the light of examples what we have learned from the crystal structures of proteins resembling ligand gated channel segments and the translation of such knowledge into structure activity relationships (SAR) of drug-like

Abbreviations: P_2X, ligand gated purinergic receptors; GluR, glutamate receptors comprising AMPA, kainate and NMDA receptors; nAChR, nicotinic acetylcholine receptors; $GABA_A$, γ-aminobutyric acid receptors; $5HT_3$, 5-hydroxy triptamine (serotonin) receptors; GlyR, glycine receptors; GluR-Cl, invertebrate glutamate sensitive chloride gated channels; AChBP, acetylcholine binding protein; VS, virtual screening; GOLD, genetic optimization for ligand docking; SAR, structure activity relationship; PDB, protein data bank (http://www.pdb.org/pdb/home/home.do); GDB, global data base.

* Corresponding author. Tel.: +41 22 347 26 22; fax: +41 22 347 26 23.
E-mail addresses: daniel.bertrand@hiqscreen.com, daniel.bertrand@unige.ch (D. Bertrand).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.07.061

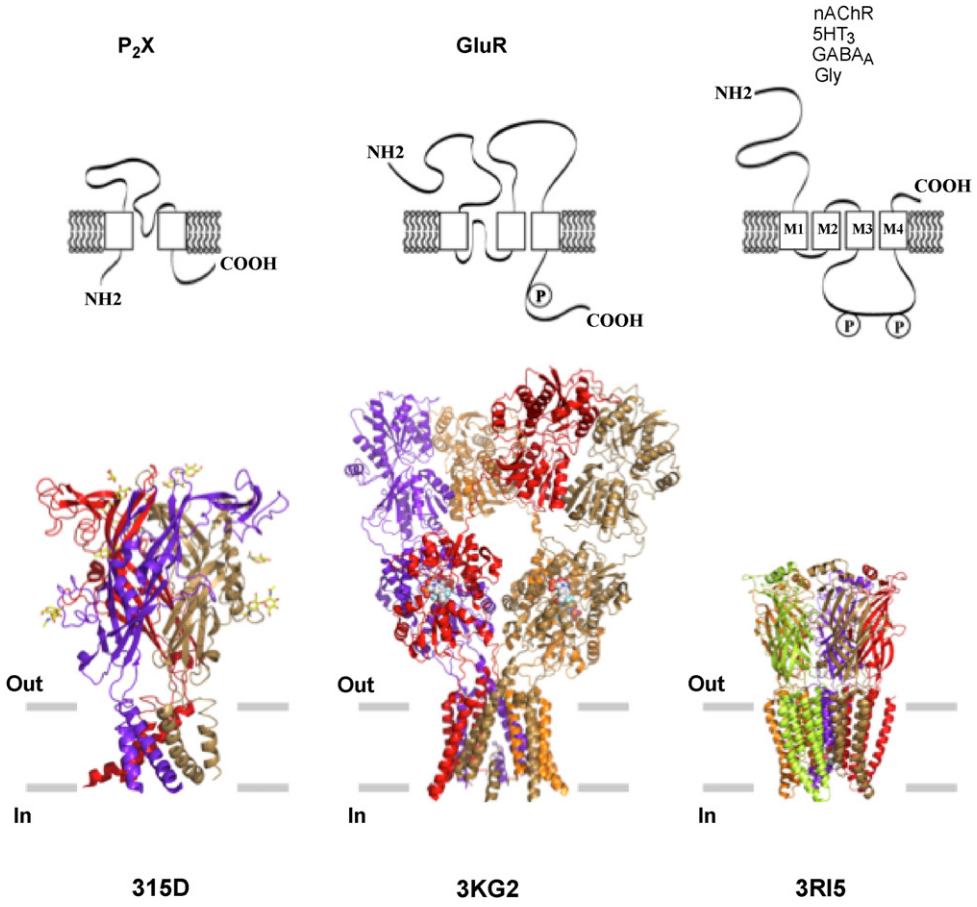

Fig. 1. Topology of ligand gated channels. The schematic representation of the structural features of ligand gated channels deduced from the protein sequences of the P_2X, GluR, nAChR, $5HT_3$, $GABA_A$ and glycine receptors are shown in the upper panel. Receptors are represented with the extracellular domain up and P's indicate putative phosphorylation sites. 3D structures determined by crystallography were redrawn from the protein data bank (PDB, references are indicated below each structure) in the lower part of the figure. References for the structure of the P_2X receptors (PDB, 3I5D [7]), for the glutamate receptors (PDB, 3K2G [8]) and for the nAChR, $5HT_3$, $GABA_A$, glycine and ionotropic invertebrate glutamate receptors (PDB, 3RI5 [2]). Note the large extracellular domain at which the neurotransmitter is binding. Gray lines symbolize the phospholipids of the membrane.

small molecule ligands. In the interest of conciseness, we shall focus our discussion on the nicotinic acetylcholine receptors which represent a broad family structure and for which more than one thousand molecules have been evaluated.

2. Probing receptor binding and function

Measuring binding of labeled active molecules is one of the approaches often chosen to examine pharmacological and biophysical properties of integral membrane proteins. Comparison of data obtained with binding experiments to those obtained with electrophysiological measurements, however, reveals major differences. For example, binding of nicotine, or other specific ligands, such as epibatidine, varenicline, etc. at the nicotinic acetylcholine receptors yields binding affinities in the picomolar (pM) or nanomolar (nM) range [9]. Electrophysiological experiments carried out on native or recombinant receptors reveal apparent affinities in the micromolar range (μM) [10].

To understand the origin of such large differences in affinities it is important to examine the conditions used for binding and electrophysiological experiments. Typical binding experiments are mainly performed on isolated membrane preparations with incubation of a labeled molecule that is subsequently used for the quantification of the receptors. A first difference lies in the fact that electrophysiological experiments are only probing the receptors expressed at the cell surface whereas binding experiments are probing all membrane bound proteins extracted from the cells. In this respect it must be recalled that in recombinant expression, up to 80% of the receptors are localized on internal membranes, and might therefore display different properties than receptors expressed at the cyptoplasmic membrane [11]. Another, and probably more significant difference, resides in the exposure time of the active molecule. To appreciate the importance of exposure time the fundamental principles of ligand gated channels must be examined with the interconversion between multiple states. In a minimal scheme, ligand gated channels are described by three interconvertible states, the resting (closed) state, the active (open) state and the desensitized (closed) state. In absence of ligand, most of the receptors are in the resting (closed) state and a fraction might be in the desensitized (closed) state. Exposure to the ligand stabilizes the receptor in the active (open) state and triggers the ion flux through the membrane. However, after prolonged exposure, receptors are progressively stabilized in the desensitized (closed) state. It is important to recall that the desensitized state of the muscle receptors display a higher affinity than the active state [12].

As binding experiments are conducted in most cases at equilibrium they are probing the ligand affinity for the desensitized state. On the contrary, determination of the EC_{50}, which is the concentration of ligand required to activate half the receptors is probing the affinity of the active state. This implies that binding experiments cannot measure receptor occupancy of the active state and are unlikely to correlate with receptor activation. An alternative functional measurement, using electrophysiology, is to

examine receptor desensitization. In this case, experiments are designed to evaluate how a sustained exposure to a ligand causes receptor desensitization. Importantly, the desensitization IC_{50} values (half inhibition) caused by prolonged exposure to the ligand are in close agreement with values obtained from binding.

3. Allosteric modulators and crystal structure

The nicotinic acetylcholine receptor has been used for a long time as a prototypic of allosteric protein [13]. In the allosteric model, receptors can have different conformations and the presence of the agonist preferentially stabilizes one or more conformation [14]. In the case of the nicotinic acetylcholine receptor, brief exposure to the agonist is thought to stabilize the receptor in the active (open) conformation, triggering the ion flux and modification of the cell membrane potential. Sustained exposure to the agonist stabilizes the receptor in a desensitized (closed) state causing a rapid decline of the inward current. Binding of a positive allosteric modulator on the receptor is expected to reduce the energy barrier between the resting (closed) state and the active (open) state. The functional outcome would be an increase of the apparent affinity, increase in the slope (Hill coefficient) of the concentration activation curve and increase in the maximal agonist evoked response. The finding in 1998 that ivermectin potentiates α7 nAChRs and increases its apparent affinity together with the slope of the curve supported these theoretical predictions [15]. Ivermectin is a large molecule initially characterized as an anthelmintic and shown to potentiate invertebrate glutamate activated chloride currents [16]. The observation that a point mutation (V251T) in the upper part of the second transmembrane domain of the α7 nAChR abolishes ivermectin potentiation suggested a possible role of this transmembrane domain (TM2) in ivermectin binding. The specificity of this mutation was further supported by the fact that the L247T, another point mutation, in the lower part of the second transmembrane domain of α7, caused no alteration of ivermectin potentiation. Additional site directed mutagenesis studies suggested that ivermectin might bind between TM1, TM4 and TM3 [17]. Interestingly enough, avermectin, a closely related molecule to ivermectin, was found to potentiate the P_2X4 receptor and shown to interact with the transmembrane domain, suggesting a conserved mechanism even across structurally distinct ligand gated channels [18].

The recent report of a crystal structure of the glutamate activated chloride channel from c-elegans brought our understanding one step further [2]. Namely, these authors provided direct evidence that ivermectin interacts with the transmembrane domain of this cys-loop and four transmembrane domain receptor which shares a high degree of homology with the nicotinic acetylcholine receptors. Moreover, the location of the ivermectin binding site in the c-elegans glutamate channel is in juxtaposition with the identified V251 T of the α7 nAChRs, but differs from the model based on computer simulation [17]. Although differences might exist between the c-elegans glutamate channel and the mammalian α7 nAChRs, these data highlight that while site directed mutagenesis or microchimera provide hypotheses about the possible binding site of a molecule, crystal structure reveals the precise localization and interaction of the molecule within the receptor complex.

4. Computer-aided drug design of ion channel modulators

Computer-aided drug design uses existing information on a drug target to design or select compounds, usually small organic molecules, that should be submitted for activity testing to find active ligands against that target [19–21]. This so-called virtual screening (VS) may be guided by the chemical structures of already known active ligands (ligand-based VS) [22], by information on the crystallographic structure of the protein target (structure-based VS) [23], or by both approaches simultaneously. Enormous progress on this method has been made in recent years by capitalizing on the availability of large computing power at low cost, and the development of improved algorithms, which properly interpret both ligand-derived and target-derived structural information. These include better ligand-similarity scoring functions based on substructure analysis [24] or on 3D-shape comparisons [25] as well as improved docking programs [23].

Recent progress in VS has been greatly facilitated by publicly available sets of known bioactive compounds such as those collected in the DUD (directory of useful decoys) dataset [26], or in public access databases such as PubChem [27], Chemspider [28], and Chembl [29], the latter being a very detailed source of curated bioactivity data. These datasets enable validation of virtual screening schemes. Numerous reports have shown that both ligand-based and target-based VS methods are quite efficient but with success rate largely correlated to the target properties. Typically protein targets whose binding pocket undergoes conformational changes upon ligand binding, such as kinases, perform very poorly using structure-based (docking) approaches, and also have difficulties with ligand-based methods. On the other hand proteins with more rigid binding pockets are better suited for computer-aided drug design. In general it should be noted that ligand-based VS based on simple substructure analyses of the ligands performs quite well [30–32]. VS is tested experimentally by selecting compounds for activity evaluation. In these "real life" applications, the percentages of active compounds are usually in the range of 2–20%, which is much better than what can be expected by random screening (0–0.1%) [33].

Decades of pharmaceutical research have been devoted to the development of ion channel agonists and modulators and eventually drugs against various neurological disorders. This has led to the synthesis of hundreds of ion channel interacting molecules, several of which have entered clinical trials and even reached the market [34]. Compound development was guided mostly by analoguing of known ligands such as the neurotransmitters themselves or related natural products [35] using classical medicinal chemistry approaches, with an unspecified but probably very limited use of computer-aided drug design. Examples of nAChR modulators of pharmacological interest include the natural products nicotine, epibatidine, cytisine, and related synthetic analogs such as sazetidine A, tropisetron, and varenicline (Fig. 2).

The availability of high-resolution structures of various ion channels with bound ligands has provided a testing ground to apply computer-aided drug design to search for new ion channel modulators. Two examples of structure-based drug design approaches for nAChR ligands were recently reported which used docking to the orthosteric site as seen in the AChBP bound with the agonists carbamoyl choline or nicotine [36]. These structures displayed the closed conformation of loop C characteristic for the agonist mode of binding as compared to the more open conformation observed with antagonists, as illustrated for the case of structures of *Aplysia* AChBP complexes with nicotinic agonists and antagonists (Fig. 3) [37]. The report of identical binding modes for nicotine in several different structures of AChBPs supports the idea that this binding pocket may be used for structure-based drug design for nAChR ligands. It is noteworthy that a similarly more compact pocket with bound agonist and a gradually more open pocket with bound partial agonist and antagonist is also observed in the AMPA receptor, another important ion channel [38].

In a recent study by Ulens et al. [39] an entirely structure-based approach was followed to discover new AChBP ligands. A

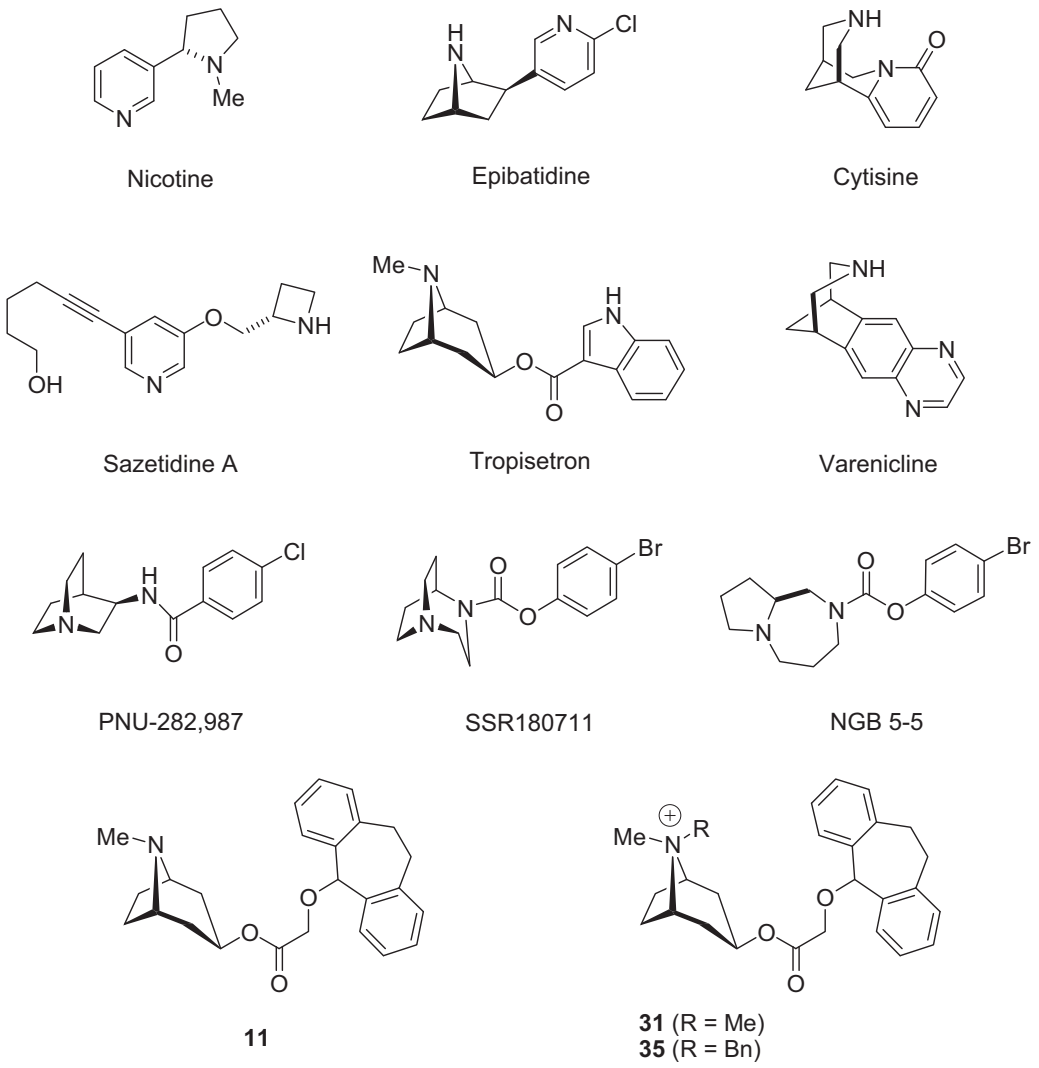

Fig. 2. Examples of molecules active at the nAChRs.

proprietary database of more than 5000 compounds was evaluated by docking to three different AChBP structures using GOLD as a docking program [40]. In each case the 50 strongest docking compounds (top 1%) were inspected visually and compounds displaying interaction modes characteristic of known agonists were selected. In total 25 ligands were tested for displacement of radiolabled α-bungarotoxin in a binding assay with Ls-AChBP, and about half of them showed a signal as strong as that of nicotine.

The authors focused on one of the hits, which was a tropisetron analog with an unusual side-chain, compound 11 (Fig. 2). Testing of further analogs of this initial hit led them to two secondary hits 31 and 35 with this same scaffold which were characterized in more detail (Fig. 3). These two compounds showed binding as strong as nicotine to the α7-nAChR, and no binding to the α4β2 nAChR when tested for displacement of epibatidine. The crystal structure of Ls-AChBP was solved with both bound ligands revealing the expected occupancy of the orthosteric site, however with a strong displacement of the C-loop rather characteristic of competitive antagonists. In-silico mutagenesis of residue Met-116 in the Ls-AChBP binding site to Gln-116 or Phe-116 correctly predicted binding compatibility with the human α7-nAChR (Gln-116) but a steric clash preventing binding for the α4β2-nAChR (Phe-116).

Electrophysiological measurement in *Xenopus* Oocytes showed that both ligands acted as inhibitors of ACh, however the inhibition mode was non-competitive, which suggested that the ligands acted by direct blockade of the ion channel rather than by occupying the orthosteric site as observed in the crystal structure. The very different ligand concentrations in the electrophysiology (micromolar) versus crystallization conditions (millimolar) might explain these apparently contradictory observations. From the ligand-based design perspective, an antagonist effect including blockade of the ion channel itself is not entirely surprising given that 31 and 35 were relatively large quaternary ammonium cations and the tricyclic structure reminiscent of carbamazepine or MK-801 that have been shown to inhibit α4β2 nAChRs in a non-competitive manner [41,42].

In a related approach reported by Garcia-Delgado et al. [43], a *de novo* ligand design strategy was chosen in which VS was used to guide the synthesis of test compounds. The authors designed virtual analogs of the known α7 nAChR ligands PNU-282,987 [44] and SSR180711 [45] by diversifying their common bicyclic diamine scaffold using analogs retrieved from the chemical universe database GDB-11, which lists all possible organic molecules up to 11 atoms of C, N, O and F [46–48]. This database has proven to be a reliable resource for designing new ligands in three related reports concerning the NMDA-receptor and the

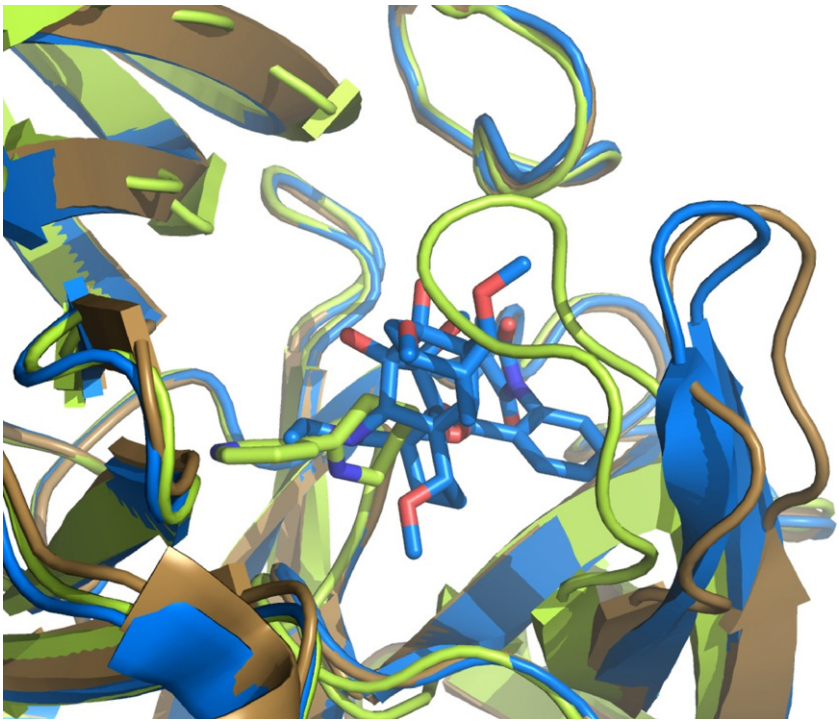

Fig. 3. Movement of the C-loop observed in structures of Aplysia AChBP. The apo-state (in absence of ligand) (brown, 2BYN) and with the antagonist MLA bound (blue, 2BYR) are in an open conformation, whereas the loop closes around the bound agonist anabaseine (green, 2WNL) [37].

glutamate transporter GLT-1 [49–51]. A library of over 500,000 virtual analogs of PNU-282,987 and SSR180711 was evaluated by high-throughput docking to the orthosteric site of AChBP in the agonist-bound conformation. Visual inspection of the top 1000 scoring compounds (top 0.2%) from two different docking programs (Autodock [52] and Glide [53]) was used to select compounds for synthesis, and 38 compounds derived from 9 different diamine scaffolds were successfully obtained.

The synthesized compounds were evaluated by electrophysiology for their effect on the human α7-nAChR, and 6 of them (16%) showed more than 70% inhibition in the acetylcholine evoked current. Upon detailed characterization of four of them, only compound NGB 5-5 (Fig. 3) proved to be a pure competitive ligand to acetylcholine, while two other ligands showed mixed inhibition and one exhibited only non-competitive inhibition. Despite the fact of being designed as close structural analogs of two known agonists, none of the tested ligand showed agonistic activity at the α7-nAChR.

Both of these two recent computer-aided drug design attempts at the nicotinic receptors used docking to an agonist-bound conformation of the orthosteric site of AChBP as selection criteria. Yet only antagonists were identified from the screening, this despite of the fact that significant ligand-based selection criteria were also employed that were designed to yield agonist-like compounds. In the first case selection was guided by visual inspection and focused on scaffold resembling known ligands, and in the second case the ligands were designed as structural analogs of known agonists. Both studies performed control docking runs showing that known agonists were placed in the binding pocket in the crystallographically observed pose with good docking score. This implies that the protein template chosen and the docking approach were sound. Both studies also found at least one ligand binding in the expected orthosteric site, however as an antagonist. This suggests that the features necessary for agonist action were not sufficiently well described by the protein structure, such that the docking score selection did not favor agonist binding. A ligand-based approach focusing on the specific distinction between agonists and antagonists might be better suited to approach the computer-aided design of nicotinic agonists in the future.

5. Conclusion and outlook

Tremendous progresses in our understanding of ligand gated channels made during the past twenty years brought us from structural hypothesis to crystal structures of these important integral membrane proteins. Gaining knowledge of the three-dimensional structure of ligand gated channels, together with the amino acids forming the ligand binding site or those bordering the ionic pore further increased the power of virtual screening. These attempts revealed, however, that in spite of important progress that has been made so far, the complexity of ligand gated channels remains the limiting factor in computer-aided approaches. One of the striking features of ion channels is their complex conformational behaviour with transitions between open and closed states. This is exemplified by the nAChR, which together with the AChBP provide a well-studied prototypical case of such channels. Difficulties encountered in the first approaches presented herein illustrate that knowledge of the binding of a molecule in the protein structure remains insufficient for the prediction of the physiological outcome.

Atomic resolution of the protein structure in different states (open and close) can be expected to provide further insights about changes in conformations and how they correlate with binding of a ligand in the extracellular domain. Today, designing drugs for ion channels based on structural and functional information remains a challenging task in computer-aided drug design methods.

Resolution of crystal structures, such as the glutamate activated chloride channel of c-elegans, brings extremely valuable information for our understanding of the receptor

function. For example, while mutagenesis experiments and computer simulations suggested that ivermectin binds in the transmembrane domain these data remained subject to hypothesis. Getting a high resolution of crystal structure allows one to address this question with new strategies and shall certainly lead to the discovery of additionally active molecules. In this respect, it is important to recall that observation of the potentiation of the α7 nAChR by ivermectin stimulated additional experiments and the discovery of a series of different allosteric modulators that are expected to widen the therapeutic approaches [54]. In this respect it should be recalled that benzodiazpines, which are allosteric modulators at the $GABA_A$ receptors, had a broad impact on clinical treatments.

As our knowledge of the protein structure is advancing together with the development of refined computer tools and increasingly powerful computers new methods are emerging that are paving the way to new and more efficient therapeutic drug design. In spite of these advances, in vitro and in vivo experimental testing remain necessary for the final confirmation of structurally deduced active chemicals.

Acknowledgments

This work was supported financially by the University of Berne and the Swiss National Science Foundation to J.-L. R. and by the EEC grant Neurocypres to DB.

References

[1] Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4A resolution. J Mol Biol 2005;346:967–89.
[2] Hibbs RE, Gouaux E. Principles of activation and permeation in an anion-selective Cys-loop receptor. Nature 2011.
[3] Bertrand D, Galzi JL, Devillers-Thiéry A, Bertrand S, Changeux JP. Stratification of the channel domain in neurotransmitter receptors. Curr Opin Cell Biol 1993;5:688–93.
[4] Devillers-Thiéry A, Galzi JL, Eiselé JL, Bertrand S, Bertrand D, Changeux JP. Functional architecture of the nicotinic acetylcholine receptor: a prototype of ligand-gated ion channels. J Membr Biol 1993;136:97–112.
[5] Laube B, Hirai H, Sturgess M, Betz H, Kuhse J. Molecular determinants of agonist discrimination by NMDA receptor subunits: analysis of the glutamate binding site on the NR2B subunit. Neuron 1997;18:493–503.
[6] North RA. Molecular physiology of P2X receptors. Physiol Rev 2002;82: 1013–67.
[7] Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. Nature 2009;460:592–8.
[8] Sobolevsky AI, Rosconi MP, Gouaux E. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. Nature 2009;462: 745–56.
[9] Gopalakrishnan M, Monteggia LM, Anderson DJ, Molinari EJ, Piattoni-Kaplan M, Donnelly-Roberts D, et al. Stable expression, pharmacologic properties and regulation of the human neuronal nicotinic acetylcholine alpha 4 beta 2 receptor. J Pharmacol Exp Ther 1996;276:289–97.
[10] Buisson B, Gopalakrishnan M, Arneric SP, Sullivan JP, Bertrand D. Human alpha4beta2 neuronal nicotinic acetylcholine receptor in HEK 293 cells: a patch-clamp study. J Neurosci 1996;16:7880–91.
[11] Vallejo YF, Buisson B, Bertrand D, Green WN. Chronic nicotine exposure upregulates nicotinic receptors by a novel mechanism. J Neurosci 2005;25: 5563–72.
[12] Changeux JP. The TiPS lecture. The nicotinic acetylcholine receptor: an allosteric protein prototype of ligand-gated ion channels. Trends Pharmacol Sci 1990;11:485–92.
[13] Changeux JP, Edelstein SJ. Allosteric mechanisms of signal transduction. Science 2005;308:1424–8.
[14] Hogg RC, Bertrand D. Partial agonists as therapeutic agents at neuronal nicotinic acetylcholine receptors. Biochem Pharmacol 2007;73:459–68.
[15] Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP, et al. Ivermectin: a positive allosteric effector of the alpha7 neuronal nicotinic acetylcholine receptor. Mol Pharmacol 1998;53:283–94.
[16] Cully DF, Vassilatis DK, Liu KK, Paress PS, Van der Ploeg LH, Schaeffer JM, et al. Cloning of an avermectin-sensitive glutamate-gated chloride channel from Caenorhabditis elegans. Nature 1994;371:707–11.
[17] Collins T, Millar NS. Nicotinic acetylcholine receptor transmembrane mutations convert ivermectin from a positive to a negative allosteric modulator. Mol Pharmacol 2010;78:198–204.
[18] Silberberg SD, Li M, Swartz KJ. Ivermectin interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. Neuron 2007;54:263–74.
[19] Schneider G, Fechner U. Computer-based de novo design of drug-like molecules. Nat Rev Drug Discov 2005;4:649–63.
[20] Klebe G. Virtual ligand screening: strategies, perspectives and limitations. Drug Discov Today 2006;11:580–94.
[21] Reymond JL, Van Deursen R, Blum LC, Ruddigkeit L. Chemical space as a source for new drugs. Med Chem Commun 2010;1:30–8.
[22] Geppert H, Vogt M, Bajorath J. Current trends in ligand-based virtual screening: molecular representations, data mining methods, new application areas, and performance evaluation. J Chem Inform Model 2010;50: 205–16.
[23] Kolb P, Ferreira RS, Irwin JJ, Shoichet BK. Docking and chemoinformatic screens for new ligands and targets. Curr Opin Biotechnol 2009;20:429–36.
[24] Rogers D, Hahn M. Extended-connectivity fingerprints. J Chem Inform Model 2010;50:742–54.
[25] Nicholls A, McGaughey GB, Sheridan RP, Good AC, Warren G, Mathieu M, et al. Molecular shape and medicinal chemistry: a perspective. J Med Chem 2010;53:3862–86.
[26] Huang N, Shoichet BK, Irwin JJ. Benchmarking sets for molecular docking. J Med Chem 2006;49:6789–801.
[27] Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH. PubChem: a public information system for analyzing bioactivities of small molecules. Nucl Acids Res 2009;37:W623–33.
[28] Williams AJ. Public chemical compound databases. Curr Opin Drug Discov Dev 2008;11:393–404.
[29] Overington J. ChEMBL. An interview with John Overington, team leader, chemogenomics at the European Bioinformatics Institute Outstation of the European Molecular Biology Laboratory (EMBL-EBI). Interview by Wendy A. Warr. J Comput Aid Mol Des 2009;23:195–8.
[30] Bender A, Jenkins JL, Scheiber J, Sukuru SC, Glick M, Davies JW. How similar are similarity searching methods? A principal component analysis of molecular descriptor space. J Chem Inform Model 2009;49:108–19.
[31] van Deursen R, Blum LC, Reymond JL. A searchable map of PubChem. J Chem Inform Model 2010;50:1924–34.
[32] van Deursen R, Blum LC, Reymond JL. Visualisation of the chemical space of fragments, lead-like and drug-like molecules in PubChem. J Comput Aided Mol Des 2011.
[33] Koeppen H. Virtual screening—what does it give us? Curr Opin Drug Discov Dev 2009;12:397–407.
[34] Cassels BK, Bermudez I, Dajas F, Abin-Carriquiry JA, Wonnacott S. From ligand design to therapeutic efficacy: the challenge for nicotinic receptor research. Drug Discov Today 2005;10:1657–65.
[35] Daly JW. Nicotinic agonists, antagonists, and modulators from natural sources. Cell Mol Neurobiol 2005;25:513–52.
[36] Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK. Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. Neuron 2004;41:907–14.
[37] Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y. Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J 2005;24:3635–46.
[38] Armstrong N, Gouaux E. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. Neuron 2000;28:165–81.
[39] Ulens C, Akdemir A, Jongejan A, van Elk R, Bertrand S, Perrakis A, et al. Use of acetylcholine binding protein in the search for novel alpha7 nicotinic receptor ligands. In silico docking, pharmacological screening, and X-ray analysis. J Med Chem 2009;52:2372–83.
[40] Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. J Mol Biol 1997;267:727–48.
[41] Picard F, Bertrand S, Steinlein OK, Bertrand D. Mutated nicotinic receptors responsible for autosomal dominant nocturnal frontal lobe epilepsy are more sensitive to carbamazepine. Epilepsia 1999;40:1198–209.
[42] Buisson B, Bertrand D. Open-channel blockers at the human alpha4beta2 neuronal nicotinic acetylcholine receptor. Mol Pharmacol 1998;53:555–63.
[43] Garcia-Delgado N, Bertrand S, Nguyen KT, van Deursen R, Bertrand D, Reymond J-L. Exploring a7-nicotinic receptor ligand diversity by scaffold enumeration from the chemical universe database GDB. ACS Med Chem Lett 2010;1:422–6.
[44] Bodnar AL, Cortes-Burgos LA, Cook KK, Dinh DM, Groppi VE, Hajos M, et al. Discovery and structure-activity relationship of quinuclidine benzamides as agonists of alpha7 nicotinic acetylcholine receptors. J Med Chem 2005;48: 905–8.
[45] Biton B, Bergis OE, Galli F, Nedelec A, Lochead AW, Jegham S, et al. SSR180711, a novel selective alpha7 nicotinic receptor partial agonist: (1) binding and functional profile. Neuropsychopharmacology 2007;32:1–16.
[46] Blum LC, Reymond JL. 970 million druglike small molecules for virtual screening in the chemical universe database GDB-13. J Am Chem Soc 2009;131:8732–3.
[47] Fink T, Reymond JL. Virtual exploration of the chemical universe up to 11 atoms of C, N, O, F: assembly of 26.4 million structures (110.9 million stereoisomers) and analysis for new ring systems, stereochemistry, physicochemical properties, compound classes, and drug discovery. J Chem Inform Model 2007;47:342–53.

[48] Fink T, Bruggesser H, Reymond JL. Virtual exploration of the small-molecule chemical universe below 160 daltons. Angew Chem Int Ed Engl 2005;44: 1504–8.
[49] Luethi E, Nguyen KT, Burzle M, Blum LC, Suzuki Y, Hediger M, et al. Identification of selective norbornane-type aspartate analogue inhibitors of the glutamate transporter 1 (GLT-1) from the chemical universe generated database (GDB). J Med Chem 2010;53:7236–50.
[50] Nguyen KT, Luethi E, Syed S, Urwyler S, Bertrand S, Bertrand D, et al. 3-(aminomethyl)piperazine-2,5-dione as a novel NMDA glycine site inhibitor from the chemical universe database GDB. Bioorg Med Chem Lett 2009;19:3832–5.
[51] Nguyen KT, Syed S, Urwyler S, Bertrand S, Bertrand D, Reymond JL. Discovery of NMDA glycine site inhibitors from the chemical universe database GDB. ChemMedChem 2008;3:1520–4.
[52] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. AJ Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998;19:1639–62.
[53] Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, et al. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. J Med Chem 2004;47:1739–49.
[54] Bertrand D, Gopalakrishnan M. Allosteric modulation of nicotinic acetylcholine receptors. Biochem Pharmacol 2007;74:1155–63.