View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by RERO DOC Digital Library

Eur Biophys J (2004) 33: 247–254
DOI 10.1007/s00249-003-0380-1

ARTICLE

P. T. F. Williamson · B. H. Meier · A. Watts

Structural and functional studies of the nicotinic acetylcholine receptor by solid-state NMR

Received: 1 September 2003 / Revised: 17 November 2003 / Accepted: 20 November 2003 / Published online: 22 January 2004
© EBSA 2004

Abstract Over the last seven years, solid-state NMR has been widely employed to study structural and functional aspects of the nicotinic acetylcholine receptor. These studies have provided detailed structural information relating to both the ligand binding site and the transmembrane domain of the receptor. Studies of the ligand binding domain have elucidated the nature and the orientation of the pharmacophores responsible for the binding of the agonist acetylcholine within the agonist binding site. Analyses of small transmembrane fragments derived from the nicotinic acetylcholine receptor have also revealed the secondary structure and the orientation of these transmembrane domains. These experiments have expanded our understanding of the channel's structural properties and are providing an insight into how they might be modulated by the surrounding lipid environment. In this article we review the advances in solid-state NMR applied to the nicotinic acetylcholine receptor and compare the results with recent electron diffraction and X-ray crystallographic studies.

Keywords Integral membrane proteins · Magic angle sample spinning · Nicotinic acetylcholine receptor · Oriented samples · Solid-state NMR

Presented at the Biophysical Society Meeting on "Ion channels – from structure to disease" held in May 2003, Rennes, France

P. T. F. Williamson (✉) · B. H. Meier
Laboratorium für Physikalische Chemie,
ETH-Hönggerberg, 8093 Zurich, Switzerland
E-mail: piwi@nmr.phys.chem.ethz.ch
Tel.: +41-1-6324402
Fax: +41-1-6321021

A. Watts
Biomembrane Structure Unit, Biochemistry Department,
University of Oxford, Oxford, OX1 3QU, UK

Introduction

The nicotinic acetylcholine receptors are a family of integral membrane ion channels that bind two molecules of acetylcholine to mediate synaptic transmission in the nervous system. They represent one of the best characterized members of this family, which includes the γ-aminobutyric acid, glycine and 5-hydroxytryptamine receptors. This in part is due to the extensive biochemical and biophysical studies that have been performed over the last 30 years (Changeux and Edelstein 1998). The muscle-type nAcChoR is found both at the neuromuscular junction and in a related synapse in the electric organ of *Torpedo*. The muscle nAcChoR consists of five glycosylated subunits ($\alpha_2\beta\gamma\delta$) with a total molecular mass of 280 kDa. Each subunit is composed of a large N-terminal extracellular domain of ~200 amino acids followed by four hydrophobic transmembrane domains of 20–30 residues in length (M1–M4) linked by hydrophilic loops of variable length and then ends with a C-terminal extracellular domain (Barrantes 1998). Although a number of experimental approaches have been applied to the nicotinic acetylcholine receptor, perhaps the most comprehensive structural studies so far are a series of electron diffraction studies performed by Unwin and co-workers (Miyazawa et al. 2003; Unwin 2000; Unwin et al. 2002). These have enabled the overall morphology of the receptor to be mapped at a resolution of 4 Å in the resting (Miyazawa et al. 2003), active (Unwin 1995) and desensitized states (Toyoshima et al. 1988), and reveal a barrel-like structure approximately 120 Å long and 70–80 Å wide with a channel-type structure through the middle. These data have been complemented by an X-ray study of an acetylcholine binding protein homologous to the synaptic portion of the nicotinic acetylcholine receptor (Brejc et al. 2001). Taken together, these data have provided a structural basis with which we can begin to unravel the molecular events associated with binding of the agonist to its binding sites on the receptor and the subsequent channel

activation. The overall structure of the receptor being known, a number of specific questions can be addressed using solid-state NMR, which is providing high-resolution structural and dynamic information regarding the functional aspects of the nicotinic acetylcholine receptor.

In contrast to X-ray crystallography and solution state NMR, where structural studies of membrane proteins require their removal from the lipid bilayer, solid-state NMR and electron diffraction share the advantage of enabling the protein to be studied in its native environment, namely the lipid bilayer. This is particularly important for membrane proteins, as their function can strongly depend on the local lipid environment (Lee 2003). For example, changes in local lipid composition have been shown to modulate the channel gating behaviour of the nicotinic acetylcholine receptor (Barrantes 1993). Additionally, the lipid/protein interface has been identified as the site of interaction for a range of important pharmaceuticals, including a range of general anaesthetics (Campagna et al. 2003; Miller 2002). Using recent developments in solid-state NMR, it has proved possible to extract structural and dynamic information from integral membrane proteins whilst they are resident in the lipid bilayer under near physiological conditions. The goal of this brief article is to review how these recent advances have been applied to the nicotinic acetylcholine receptor and to highlight how the results obtained have furthered our understanding of this system.

Solid-state NMR methodology

In solution NMR the spectra are typically dominated by isotropic contributions to the spectra, including the chemical shift and the scalar *J*-coupling. This phenomenon is due to rapid tumbling of the molecules in solution, which averages out anisotropic interactions. For studies of membrane proteins, whether surface associated or inserted into biological membranes, these isotropic tumbling conditions are rarely met and thus anisotropic components appear in the spectra, resulting in individual resonance lines for each molecular orientation in the sample, leading to a significant drop in both resolution and sensitivity. These anisotropic components include contributions from the chemical-shielding, dipolar and in some cases nuclear quadrupolar interactions. In principle, the anisotropic interactions which lead to a broadening of the spectral features contain information about the structure and dynamics within the system. Recent developments in solid-state NMR methodology have focused on how to simplify these spectra sufficiently such that the information contained in both the isotropic and anisotropic interactions can be exploited to obtain structural and functional information from ''solid'' systems. Two main techniques have been used for the study of biological membranes. First, the macroscopic orientation of the biological membrane with respect to the magnetic field, and second, the rapid rotation of the sample within the magnetic field about an angle of 54.7°, mechanically reintroducing the averaging in a manner similar to that observed in solution. Both of these techniques simplify the spectra such that site-specific resolution becomes possible.

Using samples prepared from macroscopically aligned membranes, it is possible to obtain orientational constraints with respect to the magnetic field. Under such conditions, the orientation of the anisotropic interaction, be it dipolar coupling (e.g. ^{1}H–^{15}N), chemical shielding anisotropy (e.g. ^{15}N, ^{13}C) or quadrupolar coupling (e.g. ^{2}H), is encoded in the resonance frequency of the observed line. As the orientation of these anisotropic interactions is frequently known with respect to functional groups within the molecule under study, the position of the resonance lines in the spectra can be used to determine the orientation of a particular functional group with respect to the magnetic field and to the macroscopic order in the sample, typically the membrane normal. The drawback of this technique is the necessity to orient the samples within the magnetic field; however, many techniques have become available, including the orientation of lipid bilayers on glass plates using methods such as pressure and temperature annealing (Ge et al. 1994), smearing and dehydration (Auge et al. 1997; Jarrell et al. 1987) and centrifugation (Grobner et al. 1997). In addition, other techniques which permit magnetic alignment of lipid bilayers in the magnetic field have been developed (Ottiger and Bax 1999; Sanders and Schwonek 1992).

In contrast, rotation of the sample around an axis tilted at 54.7° with respect to the magnetic field, so-called magic angle sample spinning (MAS), results in a partial averaging of the anisotropic interactions which observe a $1/2(3\cos^2\theta - 1)$ dependence, including the chemical shielding anisotropy, dipolar couplings and in part the quadrupolar interaction. When the frequency of sample spinning greatly exceeds the magnitude of the anisotropic interaction, the resulting spectrum retains primarily information from the isotropic interactions present in the system, including the isotropic chemical shift and *J*-coupling. Although MAS greatly improves the resolution in the spectra, the information contained in the anisotropic interactions is lost. Many of the recent developments in the field of MAS NMR have focused on the development of pulse sequences, which permit the selective reintroduction of these interactions, such that the structural and dynamic information can be extracted (for review see Williamson et al. 2003). In particular, the reintroduction of the dipolar couplings between labelled sites has enabled both the distance between sites within proteins and ligand to be probed and the relative orientation of the sites to be determined. More recently, sample spinning techniques have been combined with oriented samples, offering the possibility to obtain high-resolution spectra where the orientation of anisotropic interactions can be determined with respect to the macroscopic alignment in the sample (Glaubitz and Watts 1998; Sizun and Bechinger 2002; Zandomeneghi et al. 2003a, 2003b).

In contrast to solution NMR, where detection of protons is frequently the method of choice, solid-state NMR experiments have so far largely been restricted to the observation of low gamma nuclei such as ^{2}H, ^{15}N and ^{13}C, as the strong dipolar couplings present in solid samples restrict the available resolution in the proton spectra. Isotopic substitution (^{2}H, ^{13}C and ^{15}N) is mandatory in solid-state NMR studies of membrane proteins, aiding in sensitivity, assignment and spectral simplification. Therefore the development of these solid-state NMR methods has relied on the development of synthetic or expression strategies for the labelling of individual membrane components. Some recent examples will be given below, showing how these solid-state NMR methods have been used to complement electron diffraction and biochemical/biophysical studies of the nicotinic acetylcholine receptor.

Ligand binding domain of the nicotinic acetylcholine receptor

Various solution NMR experiments have been applied to the study of the ligand binding domain of the nicotinic acetylcholine receptor. These have focused either on the expression of the extra-membraneous component of the receptor and subsequent structural analysis by conventional solution state NMR techniques (Grant et al. 1999; Yao et al. 2002) or on the structural analysis of small fragments of the extracellular domain upon binding to a range of toxins (Moise et al. 2002) or antibodies (Skok et al. 2001). With recent advances in both oriented and MAS NMR, it has been possible to extend these studies to the ligand binding domain of the intact nicotinic acetylcholine receptor whilst resident in the lipid bilayer. Utilizing the selective observation of agonist, acetylcholine, labelled with either ^{2}H or ^{13}C, it was possible to probe both its conformation and dynamics within the binding site and to report on the local electrostatic environment.

Dynamic and structural information has been obtained for a deuterated analogue of acetylcholine, bromoacetylcholine, whilst resident in the receptor binding site (Williamson et al. 2001a). The selective reactivity of bromoacetylcholine for residues α-Cys192/193 (Silman and Karlin 1969) has permitted the introduction of this labelled agonist into the receptor binding site. Deuterium labels in the quaternary ammonium group were employed as non-perturbing probes of both the conformation and dynamics in the ligand binding site. The lineshape in deuterium spectra are sensitive to motions that are occurring on the microsecond timescale and thus deuterium NMR spectroscopy has been extensively used to study dynamics occurring in biological membranes (Seelig 1977). In particular, it has been shown that for model compounds containing deuterated quaternary ammonium groups the lineshapes are dominated by rotation of both the methyl groups and the whole quaternary ammonium group (Penner et al. 1995).

An analysis of the deuterium lineshape of bromoacetylcholine as a function of temperature permitted the dynamics of the ligand within the agonist binding site to be probed. The relatively high temperature where motions of both the methyl groups and the entire quaternary ammonium moiety were hindered suggests a high degree of complementarity exists between the quaternary ammonium group and the agonist binding site. This further supports findings from structure–activity studies, where even small modifications of the quaternary ammonium group of the agonist, acetylcholine, lead to a dramatic drop in its affinity for the receptor binding site (Michelson and Zeimal 1973).

To further investigate the orientation of acetylcholine with respect to the membrane normal, nicotinic acetylcholine receptor membranes labelled with deuterated bromoacetylcholine were macroscopically aligned on glass plates using isopotential spin dry ultracentrifugation (Grobner et al. 1997). The deuterium spectra were subsequently measured at different tilt angles (Fig. 1A/B). Numerical simulations of this series of spectra permitted the orientation of the quaternary ammonium group of the bromoacetylcholine to be deduced with respect to the membrane normal, revealing an angle of 42° (Fig. 1C) (Williamson et al. 2001a). Notably, in this orientation, the van de Waals surface of the acetylcholine is highly complementary to the cavities proposed by the electron diffraction structure of the intact receptor (Unwin 2000), indicating that the acetylcholine is well constrained within the binding site. Additional analysis of the proposed acetylcholine binding site in the crystal structure of the acetylcholine binding protein (Brejc et al. 2001) also indicates that such a ligand orientation would lead to favourable contacts between ligand and receptor.

These studies have been complemented by cross-polarization (CP) MAS studies of the native ligand, acetylcholine, bound to the nicotinic acetylcholine receptor. The synthesis of ^{13}C-labelled acetylcholine allowed selective observation of the acetylcholine whilst bound to the nicotinic acetylcholine receptor. Upon binding to the receptor, the restricted mobility observed by the sample precludes the effective averaging of dipolar couplings between protons and ^{13}C atoms within the bound ligand. Through the application of CP, a technique which mediates the transfer of magnetization between heteronuclear spins by means of the heteronuclear dipolar couplings in the solid state, it is possible to selectively observe the population of acetylcholine motionally restricted in the binding site, effectively suppressing the signal arising from the acetylcholine in excess in free solution. Using such a technique to selectively observe only the bound ligand, it is possible to assign resonances arising from the acetylcholine in the receptor binding site. Notably, under such conditions the resonance assigned to the methyl groups attached to the quaternary ammonium have been observed to shift upfield by 1.6 ppm (Fig. 2A/B). These perturbations can

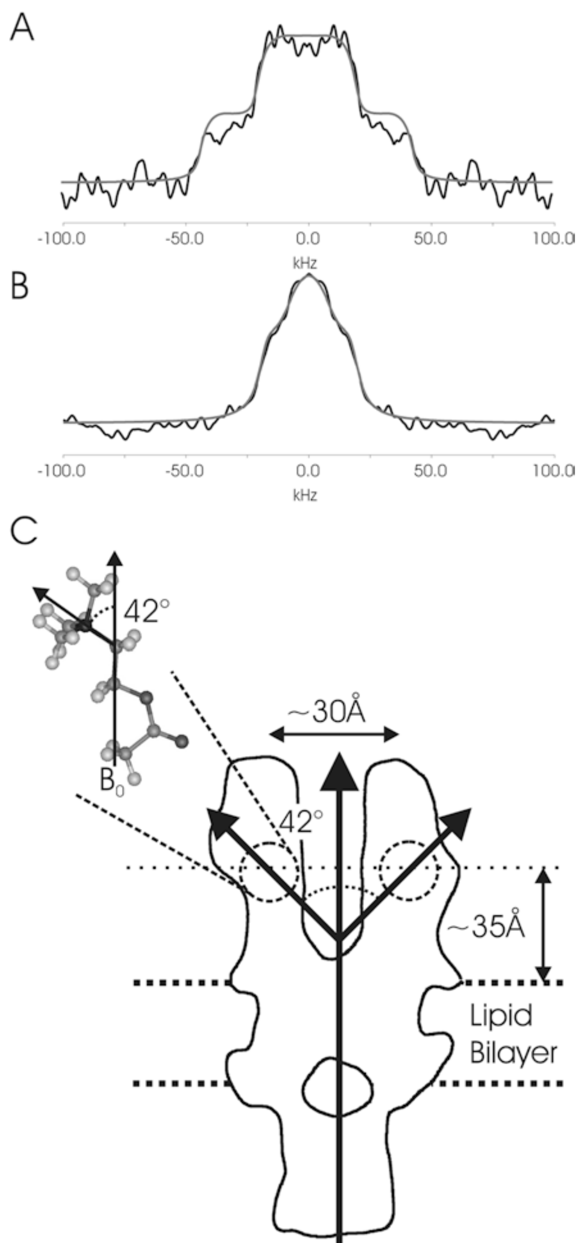

Fig. 1 ^{2}H spectra of the deuterium-labelled bromoacetylcholine bound to oriented nicotinic acetylcholine receptors oriented at 0° (**A**) and 90° (**B**) with respect to the magnetic field, together with simulated spectra. (**C**) Diagram showing the orientation of the ligand, bromoacetylcholine, with respect to the overall morphology of the receptor membrane. The quaternary ammonium group is oriented at $42 \pm 5°$ with respect to the membrane normal

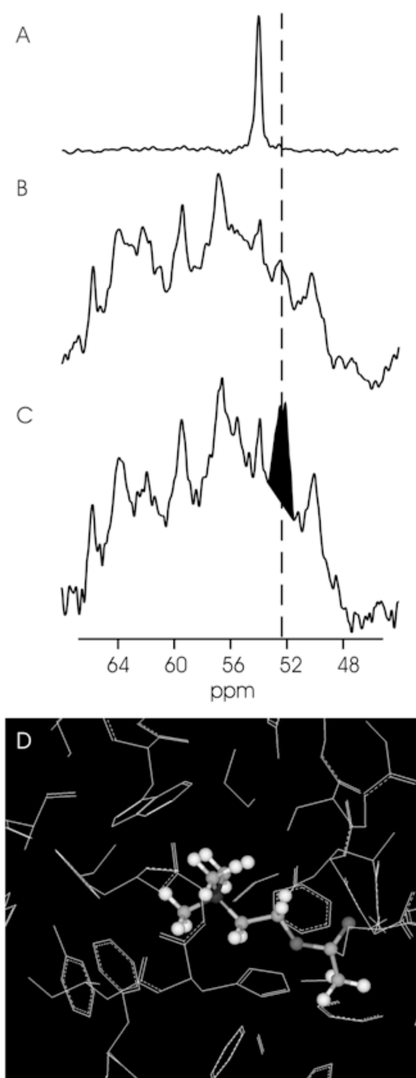

Fig. 2 Perturbation in chemical shift upon the binding of ^{13}C-labelled acetylcholine to the nicotinic acetylcholine receptor. Proton-decoupled ^{13}C spectra showing the excess labelled acetylcholine in solution (**A**). The CP-MAS spectrum of the acetylcholine receptor-enriched membranes in the absence (**B**) and presence (**C**) of ^{13}C-labelled acetylcholine showing the selective observation of the bound ligand. The upfield perturbation in chemical shift is indicative of the close proximity of the aromatic groups to the labelled site. The perturbation observed in the chemical shift is similar to that predicted upon the binding of acetylcholine to the acetylcholine esterase ((**D**) structure from pdb file 2ACE), a protein which possesses a similar aromatic-rich domain responsible for the binding of acetylcholine (adapted from Williamson et al. 1998)

be interpreted and provide information regarding the electrostatic environment within the receptor binding site (Williamson et al. 1998). These perturbations in chemical shifts were analysed using empirical chemical shift calculations on crystal structures of acetylcholine binding proteins (Fig. 2C). Such an analysis suggests that the upfield perturbations observed in the resonance line arise from the close proximity of the quaternary ammonium group to an aromatic group within the binding site, giving rise to ring current effects which lead to the observed shift (Williamson et al. 1998). Such observations are consistent with the relatively high density of aromatic residues located in close proximity to the acetylcholine binding site proposed in the crystal structure of the acetylcholine binding protein (Brejc et al. 2001). These results support earlier findings which suggest that cation–π interactions are crucial for the binding of acetylcholine to the receptor (Beene et al. 2002; Dougherty 1996).

Transmembrane domain of the nicotinic acetylcholine receptor

The recently published structural model of the transmembrane domain from electron diffraction studies (Miyazawa et al. 2003) shows a predominantly helical transmembrane domain. This is readily reconciled with much of the data arising from chemical labelling (Blanton et al. 1998b) and optical spectroscopic studies (Corbin et al. 1998). Prior to this analysis, several solid-state NMR groups had undertaken NMR studies of the transmembrane domain of the nicotinic acetylcholine receptor. Whilst the solid-state NMR methodology does not yet permit a full structural determination of the whole transmembrane domain, valuable insights into the structure of the individual transmembrane domains and their interactions with the lipid bilayer can be obtained.

The M2 transmembrane domain (TMD) of the nicotinic acetylcholine receptors is perhaps the best characterized of the four transmembrane domains. Extensive site-directed mutagenesis experiments combined with patch-clamp electrophysiology and photoaffinity labelling with non-competitive channel blockers have all indicated that the M2-TMD lines the channel lumen, the data being consistent with an α-helical conformation. These biochemical/biophysical studies have been complemented by a series of solution and solid-state NMR measurements of a peptide identical to that of the M2-TMD of the δ-subunit from *Torpedo marmorata* (Opella et al. 1999). Solution NMR techniques have been applied to the M2-TMD reconstituted into detergent micelles composed of dodecylphosphocholine (DPC). Under these conditions the M2-TMD is solvated in the detergents and has a rotational correlation time sufficiently short to permit the application of solution NMR techniques. These studies in a micellar environment indicate that the M2-TMD adopts a linear structure, with only slight evidence of curvature and no evidence of kinks in the secondary structure (Opella et al. 1999), in agreement with studies performed on the M2-TMD in organic solvents (Pashkov et al. 1999). These studies were complemented by studies of ^{15}N-labelled M2-TMD reconstituted into DMPC bilayers. By ordering the DMPC bilayers containing the M2-TMD onto glass plates, the spectra observed depend on the orientation of a particular interaction with respect to the magnetic field and thus to the bilayer normal. Therefore, in the CP ^{15}N spectrum the resonance line positions depend upon the orientation of the individual nitrogen atoms with respect to the membrane normal. To improve spectral resolution, two-dimensional PISEMA spectra were acquired, resulting in a single resonance line per residue which correlates the orientation of the chemical shielding anisotropy with the ^{1}H–^{15}N dipolar coupling (Fig. 3A) (Opella et al. 1999). Such spectra contain characteristic patterns, so-called PISA wheels (Mesleh et al. 2003; Nevzorov and Opella 2003). In favourable cases, an analysis of these patterns permits both an assignment of the resonances to an amide nitrogen in the peptide backbone and the orientation of the group with respect to the magnetic field, and hence the bilayer normal, to be determined. Such an analysis reveals that the M2-TMD is indeed helical, as indicated in the electron diffractions studies, immobile in the membrane and tilted at 12° with respect to the membrane normal, and that polar residues line the N-terminal, pore-forming, side of the helix (Mesleh et al. 2003; Opella et al. 1999). Using these constraints with the data obtained from the solution NMR studies, a model of the transmembrane channel formed from five M2-TMDs with a diameter of 3 Å at its narrowest and 8.6 Å at its widest has been proposed (Fig. 3B) (Opella et al. 1999). The channel is lined with the residues Glu1, Ser4, Ser8, Val15, Leu18 and Gln22, in agreement with the electron diffraction, mutagenesis, chemical labelling and cysteine scanning accessibility studies performed on the intact receptor (Akabas et al. 1994; Colquhoun and Sakmann 1998; Galzi 1992; Lena and Changeux 1997; Miyazawa et al. 2003; Oiki et al. 1990).

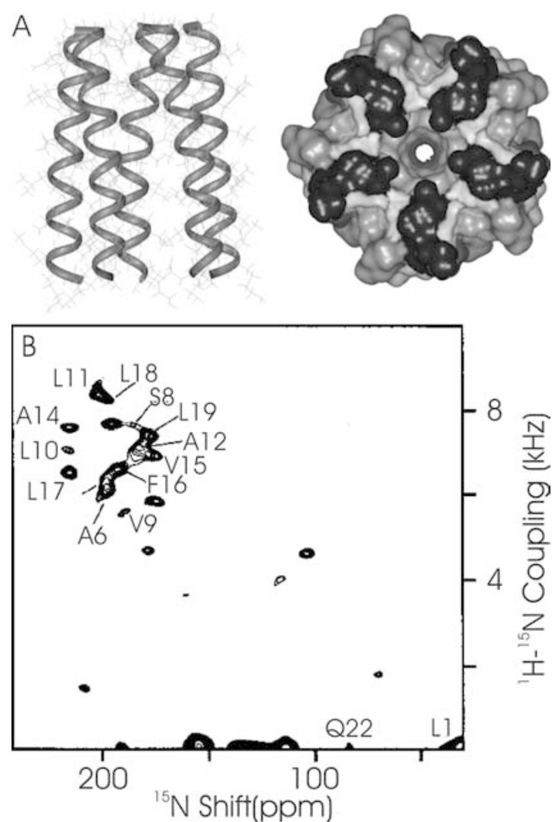

Fig. 3 PISEMA spectra (**B**) of the M2-TMD reconstituted into oriented lipid bilayers showing the correlation between the ^{15}N chemical shift anisotropy and the ^{15}N–^{1}H heteronuclear dipolar couplings. Following assignment of the resonance, each peak provides the orientation of a particular peptide plane with respect to the membrane normal (reproduced with permission from Opella et al. 1999). On the basis of these measurements, a model of a pore composed of the M2-TMD can be generated (**A**) which is in agree in agreement with the solid-state NMR constraints and predicts accurately the observed electrophysiology data (pdb accession no. 1EQ8)

On the basis of chemical labelling studies, it has been proposed that another domain, namely the M1-TMD, is accessible to the lipid environment (Blanton and Cohen 1994). However, in contrast to other transmembrane domains of the nicotinic acetylcholine receptor, the labelling pattern was not directly compatible with the formation of an α-helical conformation, and whilst some studies have observed labelling with hydrophobic channel blockers (Karlin et al. 1986), others show the M1-TMD is exposed to the lipid bilayer (Blanton and Cohen 1994; Blanton et al. 1998a). The ambiguity surrounding the secondary structure adopted by the M1-TMD was further compounded by circular dichroism (CD) and Fourier transform infrared (FTIR) spectra of M1-TMD membrane fragments which indicated a high percentage of non-helical structure (Corbin et al. 1998). To date, the only NMR studies performed on the M1-TMD of the nicotinic acetylcholine receptor have been solid-state NMR studies aimed at elucidating its secondary structure and determining how its structure is modulated by its lipid environment (de Planque and Separovic 2001; de Planque et al. 2003). Through the application of MAS-NMR, it was possible to determine the isotropic chemical shifts for several labelled sites within the peptide backbone. The resonance frequencies for ^{13}C atoms located in the peptide backbone have been shown to be sensitive to the secondary structure adopted by the peptide. Comparison of the isotropic shifts observed with previously published values for backbone chemical shifts obtained for each type of amino acid in an α-helix and β-sheet (Wishart and Sykes 1994) enables the backbone conformation of the peptide to be predicted. These studies showed, in agreement with other spectroscopic studies, that although significant portions of the M1-TMD adopt an α-helical conformation when reconstituted into a lipid bilayer, the presence of a proline located in the middle of the TMD significantly disrupts the α-helical secondary structure. Furthermore, conformational studies as a function of the lipid environment suggest that the degree of helicity in this region strongly depends on the lipid environment (de Planque and Separovic 2001; de Planque et al. 2003). Such an observation, together with the observed labelling from both hydrophobic and hydrophilic probes (Blanton and Cohen 1994; Karlin et al. 1986), suggests that the conformational flexibility around the proline in the M1-TMD may be important for the modulation of channel gating by the lipid environment and by other molecules which partition into the lipid bilayer and modulate receptor function, such as general anaesthetics.

On the basis of chemical labelling with hydrophobic probes, the two remaining transmembrane domains, M3 and M4, have been suggested to predominantly adopt a helical structure with a significant portion exposed to the lipids. The only NMR studies performed so far on the M3-TMD are solution studies of the alpha subunit (residues 277–301) from *Torpedo californica* (Lugovskoy et al. 1998). These indicate a helical conformation in chloroform/methanol/0.1 M $LiClO_4$ solution, in agreement with both the electron diffraction structure (Miyazawa et al. 2003) and photo-labelling studies performed on the intact receptor (Blanton and Cohen 1994). To date, no studies have been performed that ascertain the orientation of the M3-TMD with respect to the bilayer normal. No solution structures were performed for the M4-TMD; however, solid-state NMR experiments have been performed on a synthetic peptide corresponding to the M4-TMD of *Torpedo californica*. Through the selective introduction of NMR-sensitive isotopes at sites along the peptide, both the secondary structure and the orientation of the peptide with respect to the membrane normal have been probed. In contrast to studies of the M2-TMD, where the orientation the peptide was studied on lipid membranes oriented on glass plates, orientational studies of the M4-TMD were performed in magnetically aligned media. Using the appropriate mixture of the dialkyl lipids DoMPC and DoHPC, it was possible to exploit the negative diamagnetic susceptibility of the lipid present in the sample to form small bilayered discs, which align with the membrane normal perpendicular to the magnetic field (Williamson et al. 2001b). MAS spectra of the samples permitted the resolution and assignment of both ^{13}C and ^{15}N labels (Fig. 4A) in the peptide backbone of the M4-TMD whilst reconstituted into the lipid bilayer. Analysis of the observed chemical shifts in a manner analogous to that described for the M1-TMD confirmed a α-helical conformation for the M4-TMD, in agreement with CD studies performed on the same system (Williamson et al. 2001b) and with other chemical labelling studies (Barrantes et al. 2000). Static spectra (Fig. 4B) of the M4-TMD reconstituted into bicelles permitted the orientation of the peptide to be calculated. In contrast to the M2-TMD, significant dynamics were observed in the system which leads to an averaging of the anisotropic interactions observed in the solid-state NMR spectra of the oriented sample. An analysis of the observed averaging permitted the orientation of the anisotropic interaction to be ascertained with respect to the axis of motional averaging. The analysis of each of the individual labelled sites indicated that the M4-TMD adopts an α-helical conformation, which is not distorted in the region where NMR labels were incorporated. Furthermore, analysis of the oriented lineshapes indicated that the axis of motional averaging was inclined by no more than 15° with respect to the membrane normal (Williamson et al. 2001b) (Fig. 4C). This is in good agreement with that observed in the latest model of the transmembrane domain from electron diffraction studies of the intact nicotinic acetylcholine receptor (Miyazawa et al. 2003).

Outlook

With the arrival of structural models of the nicotinic acetylcholine receptors, we are in a position to rationalize many of the results arising from the extensive

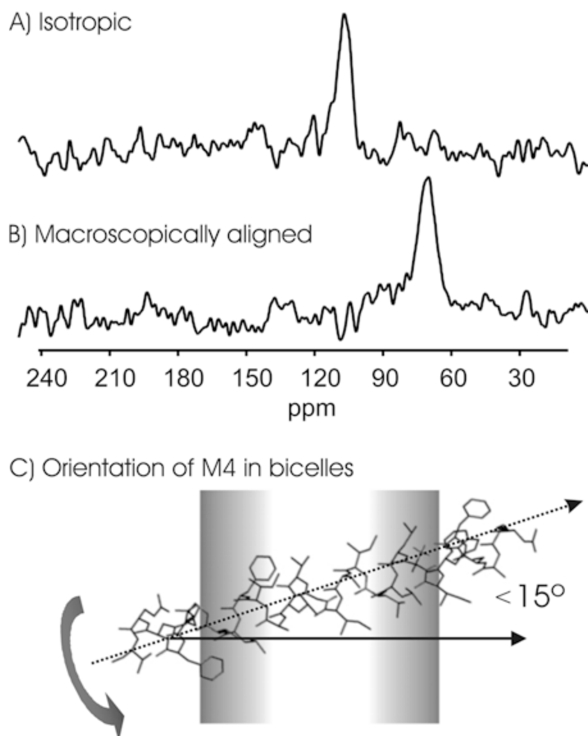

Fig. 4 The ^{15}N CP-MAS spectrum of the M4-TMD showing the isotropic chemical shift for the backbone labelled site. **A** The ^{15}N CP static spectrum of the M4-TMD reconstituted into lipid bicelles showing the change in lineshape upon the orientation of the peptide within the magnetic field. **B** Analysis of the change in resonance position enables the orientation of the peptide to be determined. Proposed orientation and conformation of the M4-TMD within the lipid bilayer (**C**)

biophysical and biochemical studies performed on the receptor. In this brief article we have attempted to correlate the observations made by solid-state NMR techniques with other biophysical/biochemical methods as well as with diffraction studies. The application of solid-state NMR techniques has enabled the environment within ligand binding site to be probed and identification of key pharmacophores responsible for the interaction of the agonist, acetylcholine, with the receptor binding site to be ascertained. It is envisaged that these techniques can be readily extended to provide conformational information at an atomic resolution about the structure of other small pharmacologically relevant molecules whilst bound to membrane receptors. Solid-state NMR studies have aided in refining the structure of the transmembrane domains of the receptor, supporting data from other biophysical and diffraction studies. However, most importantly, the development of these techniques will allow us to study how the changes in the lipid environment affect the structure of these transmembrane domains, and will enable us to correlate these structural changes to the receptor function.

Acknowledgements S.J. Opella, F. Separovic and M.R.R. de Planque are gratefully acknowledged for helpful discussions on their latest studies on the transmembrane domain of the nicotinic acetylcholine receptor.

References

Akabas MH, Kaufmann C, Archdeacon P, Karlin A (1994) Identification of acetylcholine receptor channel lining residues of the entire M2 segment of the alpha-subunit. Neuron 13:919–927

Auge S, Mazarguil H, Tropis M, Milon A (1997) Preparation of oriented lipid bilayers on ultrathin polymers for solid state NMR analyses of peptide-membrane interactions. J Magn Reson 124:455–458

Barrantes FJ (1993) Structural and functional crosstalk between acetylcholine receptor and its membrane environment. Mol Neurobiol 6:463–482

Barrantes FJ (1998) The nicotinic acetylcholine receptor: current views and future trends. Springer, Berlin Heidelberg New York

Barrantes FJ, Antollini SS, Blanton MP, Prieto M (2000) Topography of nicotinic acetylcholine receptor membrane-embedded domains. J Biol Chem 275:37333–37339

Beene DL, Brandt GS, Zhong WG, Zacharias NM, Lester HA, Dougherty DA (2002) Cation-pi interactions in ligand recognition by serotonergic (5HT3A) and nicotinic acetylcholine receptors: the anomalous binding of nicotine. Biochemistry 31:10262–10269

Blanton MP, Cohen JB (1994) Identifying the lipid-protein interface of the *Torpedo* nicotinic acetylcholine receptor—secondary structure implications. Biochemistry 33:2859–2872

Blanton MP, Dangott LJ, Raja SK, Lala AK, Cohen JB (1998a) Probing the structure of the nicotinic acetylcholine receptor ion channel with the uncharged photoactivable compound H-3 diazofluorene. J Biol Chem 273:8659–8668

Blanton MP, McCardy EA, Huggins A, Parikh D (1998b) Probing the structure of the nicotinic acetylcholine receptor with the hydrophobic photoreactive probes I-125 TID-BE and I-125 TIDPC/16. Biochemistry 37:14545–14555

Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, Smit AB, Sixma TK (2001) Crystal structure of an acetylcholine binding protein reveals the ligand binding domain of nicotinic receptors. Nature 411:269–276

Campagna JA, Miller KW, Forman SA (2003) Mechanisms of action of inhaled anesthetics. New Engl J Med 348:2110–2124

Changeux JP, Edelstein SJ (1998) Allosteric receptors after 30 years. Neuron 21:959–980

Colquhoun D, Sakmann B (1998) From muscle endplate to brain synapse: a short history of synapses and agonist activated channels. Neuron 20:381–387

Corbin J, Methot N, Wang HH, Baenziger JE, Blanton MP (1998) Secondary structure analysis of individual transmembrane segments of the nicotinic acetylcholine receptor by circular dichroism and Fourier transform infrared spectroscopy. J Biol Chem 273:771–777

de Planque MRR, Separovic F (2001) Interaction of anaesthetics with transmembrane nAChR segments: a solid state NMR study. Biophys J 80:1963

de Planque MRR, Rijkers DTS, Separovic F (2003) Conformation of the M1 transmembrane segment of the nicotinic acetylcholine receptor in a membrane environment. Biophys J 84:1116

Dougherty DA (1996) Cation-pi interactions in chemistry and biology: a new view of benzene, Phe, Tyr and Trp. Science 271:163–168

Galzi JL (1992) Mutations in the channel domain of the neuronal nicotinic acetylcholine receptor convert ion selectivity from cationic to anionic. Nature 359:500–505

Ge MT, Budil DE, Freed JH (1994) ESR studies of spin-labelled membranes aligned by isopotential spin-dry ultracentrifugation—lipid protein interactions. Biophys J 67:2326–2344

Glaubitz C, Watts A (1998) Magic angle-oriented sample spinning (MAOSS): a new approach toward biomembrane studies. J Magn Reson 130:305–316

Grant MA, Gentile LN, Shi QL, Pellegrini M, Hawrot E (1999) Expression and spectroscopic analysis of soluble nicotinic acetylcholine receptor fragments derived from the extracellular domain of the alpha-subunit. Biochemistry 38:10730–10742

Grobner G, Taylor A, Williamson PTF, Choi G, Glaubitz C, Watts JA, deGrip WJ, Watts A (1997) Macroscopic orientation of natural and model membranes for structural studies. Anal Biochem 254:132–138

Jarrell HC, Jovall PA, Giziewicz JB, Turner LA, Smith ICP (1987) Determination of conformational properties of glycolipid headgroups by H-2 NMR of oriented multibilayers. Biochemistry 26:1805–1811

Karlin A, Kao PN, Dipaola M (1986) Molecular pharmacology of the nicotinic acetylcholine receptor. Trends Pharmacol Sci 7:304–308

Lee AG (2003) Lipid-protein interactions in biological membranes: a structural perspective. Biochim Biophys Acta 1612:1–40

Lena C, Changeux JP (1997) Pathological mutations of nicotinic receptors and nicotine based therapies for brain disorders. Curr Opin Neurobiol 7:674–682

Lugovskoy AA, Maslennikov IV, Utkin YN, Tsetlin VI, Cohen JB, Arseniev A (1998) Spatial structure of the M3 transmembrane segment of the nicotinic acetylcholine receptor alpha-subunit. Eur J Biochem 255:455–461

Mesleh MF, Lee S, Veglia G, Thiriot DS, Marassi FM, Opella SJ (2003) Dipolar waves map the structure and topology of helices in membrane proteins. J Am Chem Soc 125:8928–8935

Michelson MJ, Zeimal EJ (1973) Acetylcholine: an approach to molecular mechanism and action. Pergamon, Oxford, p 82

Miller KW (2002) The nature of sites of general anaesthetic action. Br J Anaesth 89:17–21

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. Nature 423:949–955

Moise L, Piserchio A, Basus VJ, Hawrot E (2002) NMR structural analysis of alpha bungarotoxin and its complex with the principle alpha-neurotoxin-binding sequence on the alpha-7 subunit of the neuronal nicotinic acetylcholine receptor. J Biol Chem 277:12406–12417

Nevzorov AA, Opella SJ (2003) Structural fitting of PISEMA spectra of aligned proteins. J Magn Reson 160:33–39

Oiki S, Madison V, Montal M (1990) Bundles of amphipathic transmembrane alpha-helices as a structural motif for ion-conducting proteins: studies on sodium channels and acetylcholine receptors. Proteins 8:226–236

Opella SJ, Marassi FM, Gesell JJ, Valente AP, Kim Y, Oblatt-Montal M, Montal M (1999) Structures of the M2 channel-lining segments from nicotinic acetylcholine and NMDA receptors by NMR spectroscopy. Nat Struct Biol 6:374–379

Ottiger M, Bax A (1999) Bicelle-based liquid crystals for NMR measurement of dipolar couplings at acidic and basic pH values. J Biomol NMR 13:187–191

Pashkov VS, Maslennikov IV, Tchikin LD, Efremov RG, Ivanov VT, Arseniev A (1999) Spatial structure of the M2 transmembrane domain of the nicotinic acetylcholine receptor alpha subunit. FEBS Lett 457:117–121

Penner GH, Zhao BY, Jeffrey KR (1995) Molecular dynamics in the solid trimethylamine-borane complex—a deuterium NMR study. Z Naturforsch A 50:81–89

Sanders CR, Schwonek JP (1992) Characterization of magnetically orientable bilayers in mixtures of dihexanoylphosphatidylcholine and dimyristoylphosphatidylcholine by solid state NMR. Biochemistry 31:8898–8905

Seelig J (1977) Deuterium magnetic resonance: theory and application to lipid membranes. Q Rev Biophys 10:355–418

Silman I, Karlin A (1969) Acetylcholine receptor—covalent attachment of depolarizing groups at active site. Science 164:1420–1421

Sizun C, Bechinger B (2002) Bilayer sample for fast or slow magic angle oriented sample spinning solid-state NMR spectroscopy. J Am Chem Soc 124:1146–1147

Skok M, Lykhmus E, Bobrovnik S, Tzarto S, Tsouloufis T, Vanderesse R, Coutrot F, Cung MT, Marraud M, Krikorian D, Sakarellos M (2001) Structure of epitopes recognized by the antibodies to alpha(181–192) peptides of the neuronal nicotinic acetylcholine receptors: extrapolation to the structure of the acetylcholine binding site. J Neuroimmunol 121:59–66

Toyoshima C, Kubalek E, Unwin N (1988) Structure of the acetylcholine receptor in the resting and desensitized states. Biophys J 53:A492–A492

Unwin N (1995) Acetylcholine receptor channel imaged in the open state. Nature 373:37–43

Unwin N (2000) The Croonian lecture 2000. Nicotinic acetylcholine receptor and the structural basis of fast synaptic transmission. Philos Trans R Soc London Ser B 355:1813–1829

Unwin N, Miyazawa A, Li J, Fujiyoshi Y (2002) Activation of the nicotinic acetylcholine receptor involves a switch in conformation of the a subunits. J Mol Biol 319:1165–1176

Williamson PTF, Grobner G, Spooner PJR, Miller KW, Watts A (1998) Probing the agonist binding pocket in the nicotinic acetylcholine receptor: a high-resolution solid-state NMR approach. Biochemistry 37:10854–10859

Williamson PTF, Watts JA, Addona GH, Miller KW, Watts A (2001a) Dynamics and orientation of $N^+(CD_3)(3)$-bromoacetylcholine bound to its binding site on the nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 98:2346–2351

Williamson PTF, Zandomeneghi G, Bonev B, Barrantes FJ, Watts A, Meier BH (2001b) Structural characterization of the M4-TMD of the nicotinic acetylcholine receptor by VASS NMR of ordered liquid crystalline phases. Biophys J 80:699

Williamson PTF, Ernst M, Meier BH (2003) MAS solid state NMR of isotopically enriched biological samples. In: Zerbe O (ed) BioNMR in drug research. Wiley-VCH, Weinheim, pp 243–282

Wishart DS, Sykes BD (1994) The C-13 chemical-shift index—a simple method for the identification of protein secondary structure using C-13 chemical-shift data. J Biomol NMR 4:171–180

Yao Y, Wang JM, Viroonchatapan N, Samson A, Chill J, Rothe E, Angliste J, Wang ZZ (2002) Yeast expression and NMR analysis of the extracellular domain of muscle nicotinic acetylcholine receptor alpha-subunit. J Biol Chem 277:12613–12621

Zandomeneghi G, Tomaselli M, Williamson PTF, Meier BH (2003a) NMR of bicelles: orientation and mosaic spread of the liquid crystal director under sample rotation. J Biomol NMR 25:113–123

Zandomeneghi G, Williamson PTF, Hunkler A, Meier BH (2003b) Switched-angle spinning applied to bicelles containing phospholipid-associated peptides. J Biomol NMR 25:125–132