Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 434 (2008) 165–169
Neuroscience Letters
www.elsevier.com/locate/neulet

Properties of mutated murine α4β2 nicotinic receptors linked to partial epilepsy

Marcela Lipovsek [a], Paola Plazas [a], Jessica Savino [a], Alwin Klaassen [b], Jim Boulter [b], Ana Belén Elgoyhen [a,c], Eleonora Katz [a,d,*]

[a] *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Vuelta de Obligado 2490, Buenos Aires 1428, Argentina*
[b] *Department of Psychiatry and Behavioral Sciences, Hatos Research Center for Neuropharmacology, Brain Research and Molecular Biology Institutes, University of California, Los Angeles, CA 9005, USA*
[c] *Departamento de Farmacología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, Buenos Aires 1121, Argentina*
[d] *Dpto. de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Buenos Aires, Argentina*

Received 7 July 2007; received in revised form 13 December 2007; accepted 24 December 2007

Abstract

We characterized, by electrophysiological methods, two biophysical properties of murine recombinant α4β2 nicotinic acetylcholine receptors (nAChR) bearing a mutation (α4:+L264α4:β2 or α4:S252Fα4:β2) linked to autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). Sensitivity to acetylcholine (ACh) was increased by the S252F substitution expressed in heterozygosis (α4:S252Fα4:β2) but was markedly reduced when this mutation was expressed in homozygosis (S252Fα4:β2). ACh sensitivity was not altered by the +L264 insertion. Moreover, receptor desensitization was significantly increased by both mutations expressed in heterozygosis. These results are in general agreement to those of rat and human recombinant receptors bearing the same mutations, thus contributing to validate the use of *knock-in* mice harboring ADNFLE mutations as models to study this pathology.

Keywords: Ion channels; Nicotinic receptors; Epilepsy; Desensitization; ACh

Neuronal signaling and communication mainly depends upon the activity of ion channels. Mutations in the genes coding for some of these proteins have been linked to the occurrence of neurological diseases [23]. Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a form of familial monogenic epilepsy in which brief partial seizures occur during stage II of non-rapid eye movement sleep [3]. The finding of mutations in the genes coding for the α4 and β2 nAChR subunits in ADNFLE patients shows that alterations in these channels can severely affect brain function [8,13,19,20].

Nicotinic acetylcholine receptors are complexes of protein subunits that form an ion channel gated by the binding of the neurotransmitter acetylcholine (ACh) [10]. To date, several nAChR subunits have been cloned (α1–α10, β1–β4). These subunits co-assemble to form different homo or hetero-pentameric channels with characteristic physiological and pharmacological profiles [7]. Each subunit contains four transmembrane domains (TM1–TM4) and a large extracellular N-terminal domain containing the ligand-binding site [10]. All TM2 segments of each nAChR subunit form the walls of the pore and therefore the presence of specific aminoacids is crucial both for ion selectivity of the receptor and the energy level for allosteric transition [14].

Considerable clinical and genetic data now provide a strong link between the ADNFLE syndrome and six mutations located within the pore forming TM2 of the α4 (S252F, +L264, S256L, T265I; referring to the human α4 subunit gene numbering) and β2 (V278L, V287M) nAChR subunits [21]. Frontal lobe origin, adolescent onset and cluster of nocturnal, hyperkinetic motor seizures are signatures of ADNFLE, therefore, it was suggested that a common functional anomaly of α4- and β2-subunit containing nAChRs underlies this disorder. Studies in heterolo-

* Corresponding author at: Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Vuelta de Obligado 2490, Buenos Aires 1428, Argentina. Tel.: +54 1147 832871; fax: +54 1147 868578.
E-mail address: ekatz@dna.uba.ar (E. Katz).

0304-3940/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2007.12.061

gous expression systems, with both human and rat recombinant mutated α4 or β2 nAChR subunits, designed to discover a shared, altered anomaly that might explain the neuronal network dysfunction underlying ADNFLE seizures led to both "gain-of-function" [4,6,13] and "loss-of-function" [2,12,19] models. Recently, Klaassen et al. [11] based on studies performed with two mouse strains harboring the ADNFLE *Chrna4*^{S252F} and *Chrna4*$^{+L264}$ mutant alleles proposed a model of epileptogenesis in which ACh enhances GABAergic transmission. They postulate that asynchronously firing layer II/III pyramidal cells of the frontal cortex would be synchronized after recovery from a large GABAergic inhibition triggered by cholinergic activation of mutant nAChRs. In addition, Teper et al. [22], generated a mouse line harboring the S248F (S252F) mutation and report that these mice are partially protected from nicotine-induced seizures. Based on their studies, they conclude that the S248F mouse could provide a model for the paroxysmal dystonic element of ADNFLE semiology. To validate the use of these mouse models to study the pathophysiology of ADNFLE, we evaluated some biophysical characteristics of mouse recombinant mutated receptors (α4:+L264α4:β2 and α4:S252Fα4:β2) expressed in *Xenopus* oocytes and compared them to those reported for the human mutations.

For expression studies, the α4 (GeneBank AF225912), S252Fα4, +L264α4 and β2 (GeneBank AF299083) subunits were subcloned into the pSGEM plasmid derived from pGEMHE, a vector suitable for the expression in *Xenopus* oocytes [5]. Capped cRNAs were *in vitro* transcribed using the mMessage mMachine T7 Transcription Kit (Ambion Corporation, Austin, TX). Maintenance of *X. laevis* and cRNA injection of oocytes was as described [17]. Typically, oocytes were injected with 50 nl of RNAse-free water containing 0.01–1.0 ng of cRNA and maintained in Barth's solution at 17 °C. Each 50 nl of cRNA contained: α4 and β2 subunits in 1:1 molar ratio for the wild-type receptor; α4:α4*:β2 in a 1:1:2 molar ratio to simulate the expression of the mutated (α4*) heterozygous receptor and α4*:β2 in a 1:1 equimolar relation, to simulate the mutated homozygous situation.

Electrophysiological recordings were performed 2–6 days after cRNA injection under two-electrode voltage-clamp with a Geneclamp 500 amplifier (Molecular Devices, Sunnyvale, CA). During electrophysiological recordings, oocytes were superfused (∼10 ml/min) with frog saline (in mM): 115 NaCl, 2.5 KCl, 1.8 $CaCl_2$, and 10 HEPES buffer, pH 7.2. ACh (Sigma Chemical Co., St. Louis, MO, USA) was applied in the perfusion solution of the oocyte chamber. All experimental protocols were performed in accordance with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 80-23) revised 1978.

For the concentration–response curves to ACh, data were normalized to the maximal response in each oocyte and were referred to as a percentage of the maximal response. The mean and standard error of the mean of peak currents are represented. Agonist concentration–response curves were fitted with:

$$I = \frac{I_{max}}{1 + 10^{(\log EC_{50} - X)n}} \quad (1)$$

where I is the normalized peak inward current; I_{max} is the current evoked by the concentration of agonist eliciting a maximal response; EC_{50} the concentration of agonist inducing half-maximal current response and n is the Hill coefficient. For the heterozygote receptor formed by the α4, S252Fα4 and β2 subunits, concentration–response curves were fitted with the sum of three Hill equations:

$$I = \left(\frac{a}{1 + (b/x)^c}\right) + \left(\frac{d}{1 + (e/x)^g}\right) + \left(\frac{h}{1 + (i/x)^j}\right) \quad (2)$$

where I is the normalized peak inward current; a, d and h are the maximal responses achieved by the first, second and third components of the curve, respectively; b, e, i and c, g, j are the EC_{50} values and the Hill coefficients of each component of the curve, respectively.

Injection of *Xenopus* oocytes with mice cRNA encoding α4, either wild-type or mutated (α4*:+L264α4 or S252Fα4), together with β2 nAChR subunits resulted in the assembly of functional receptors. At a holding voltage of −70 mV, ACh evoked inward currents in oocytes expressing either wild-type, heterozygous or homozygous mutated receptors (Fig. 1, insets). The apparent affinity of the different receptors to ACh was analyzed by generating concentration–response curves. Data points obtained for the different ACh concentrations in oocytes injected with cRNA encoding the wild-type α4 and β2 subunits (1:1), were fitted by a Hill equation with an EC_{50} of 5.07 ± 2.4 μM, $n = 7$. Two possible arrangements of receptors with $\alpha4_3\beta2_2$ and $\alpha4_2\beta2_3$ stoichiometries have been described [1,15]. However, under our experimental conditions, only the one reported as

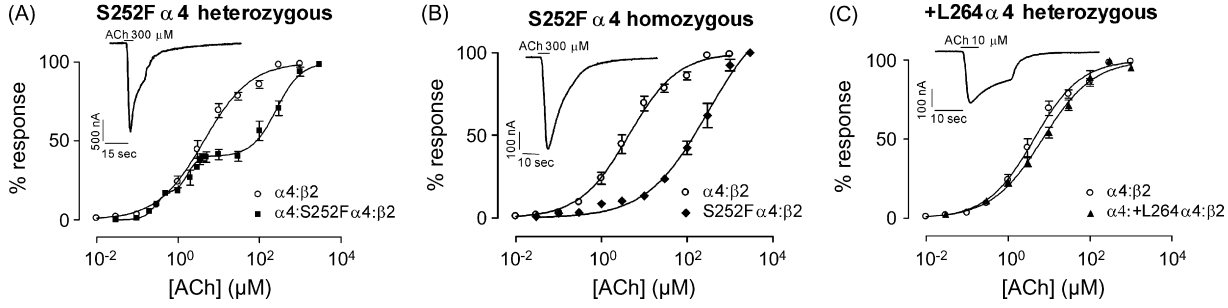

Fig. 1. Sensitivity to ACh. Concentration–response curves to ACh for: wild-type α4β2 and α4:S252Fα4:β2 (1:1:2 molar relation) (A); wild-type α4β2 and S252Fα4:β2 (B); wild-type α4β2 and α4:+L264α4:β2 (1:1:2 molar relation) (C) nAChRs. Insets: records of ACh-evoked currents in oocytes expressing the different receptor genotypes.

the high affinity population of receptors that corresponds to the $\alpha4_2\beta2_3$ stoichiometry [15] was observed.

If a mutation significantly affects the apparent affinity of the receptor for ACh, different populations of receptors should be evidenced in the concentration–response curve [15]. Data points from oocytes injected with cRNA encoding the α4, S252Fα4 and β2 subunits (1:1:2), were best fitted by the sum of three Hill equations, (Fig. 1a), with EC_{50s} of: 0.28 ± 0.03 μM, 2.35 ± 0.19 μM and 274.9 ± 17.1 μM (n = 3–7). Therefore, assuming a $\alpha4_2\beta2_3$ stoichiometry, three different types of receptors could have assembled: α4:β2, α4:α4*:β2 and α4*:β2. To associate each component of the curve with a population of receptors, oocytes were injected with α4*(S252Fα4), in the absence of wild-type α4, together with β2, thus simulating homozygosis. The concentration–response curve was best fitted by one Hill equation with an EC_{50} of 243.4 ± 40.5 μM (n = 3) (Fig. 1b). This EC_{50} is not significantly different from that obtained for the third component of the curve, suggesting that it derived from a nAChR population containing only α4* subunits (Fig. 1b). The second component of the curve illustrated in Fig. 1a, had an EC_{50} similar to that of the wild-type α4β2 (5.07 ± 2.4 μM, n = 7), indicating that it corresponds to receptors formed by wild-type α4 and β2 subunits. Finally, the remaining component (EC_{50}: 0.28 ± 0.03 μM; n = 3–7) could be attributed to the mutated heterozygous receptor formed by wild-type α4, S252Fα4 and β2 subunits. For heterozygous α4:+L264α4:β2 receptors, data points were fitted by one Hill equation with an EC_{50} of 7.40 ± 2.80; n = 4 which was not significantly different from that of the wild-type (Fig. 1c).

Neuronal nicotinic receptors desensitize upon a prolonged or a repetitive exposure to the agonist [18]. To study this process, we used two protocols modified from Bertrand et al. [2]. The first protocol consisted in applying different concentrations of ACh (0.03–300 μM) for 1 min (Fig. 2, right panels). The amplitudes of the currents measured between the peak and plateau (peak current) and between the plateau and baseline (plateau current), are a first estimate of the fraction of receptors that desensitize in the presence of the agonist. Differences in the desensitization pattern between the three types of receptors were observed when plotting these values as a function of the logarithm of the ACh concentration (Fig. 2, left panels). Peak and plateau currents of the wild-type α4β2 had similar amplitudes, being only significantly different at almost saturating ACh concentrations (ACh 300 μM; n = 3; $p < 0.0001$). A similar behavior was observed for the mutated heterozygous α4:+L264α4:β2 receptor (n = 4). The α4:S252Fα4:β2 receptors, however, presented the same plateau currents, which were smaller than the peak amplitudes, for all the ACh concentrations tested (n = 3), indicating a greater degree of desensitization.

The second protocol consisted of the application of test pulses of 1 μM ACh, for 5 s, at 1 min intervals. These trains lasted 12 min and were performed in the presence of fixed concentrations of ACh (0.1–10 μM). Representative records obtained with the wild-type α4β2 and the mutated (α4:+L264α4:β2 and α4:S252Fα4:β2) receptors are illustrated in Fig. 3. To analyze the dependency of the desensitization process with the ACh concentration during this prolonged exposure, the amplitude

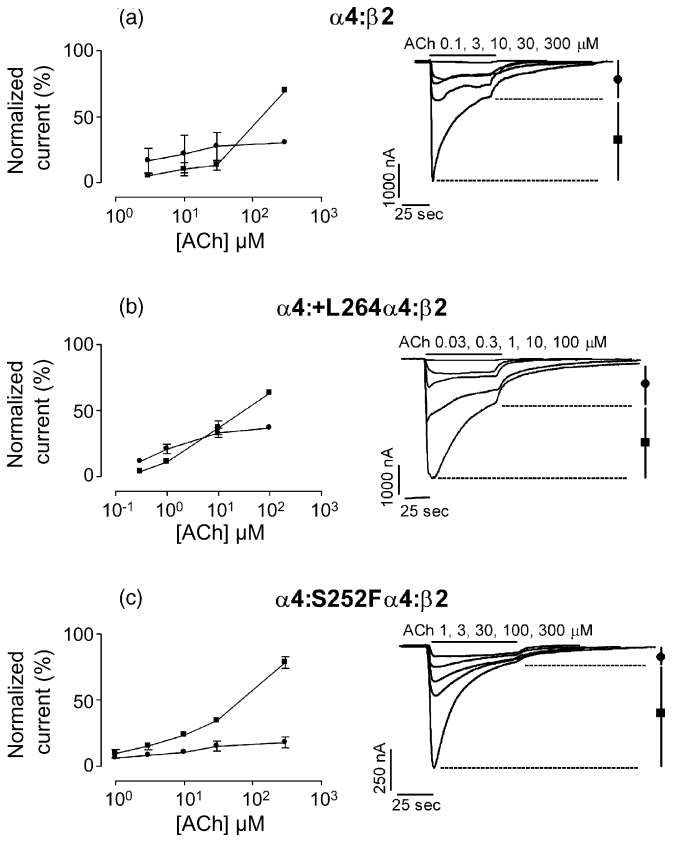

Fig. 2. Desensitization in the presence of ACh. Currents evoked by different concentrations of ACh (0.03–300 μM) for 1 min. Dotted lines indicate where peak and plateau current amplitudes were measured (Right panels). Plots of peak and plateau currents as a function of the logarithm of the ACh concentration (Left panels). Wild-type α4β2 (a), α4:+L264α4:β2 (b) and α4:S252Fα4:β2 (c) receptors.

of the last peak of the train, normalized to the amplitude of the control peak, was plotted as a function of the logarithm of the ACh concentration and was fitted by a Hill equation (Fig. 3). The IC_{50} values, corresponding to the concentration of ACh that caused 50% desensitization during a 12 min exposure, were 0.75 ± 0.16 μM (n = 3), 0.17 ± 0.02 μM (n = 2) and 0.062 ± 0.007 μM (n = 2) for the α4β2, the α4:+L264α4:β2 and the α4:S252Fα4:β2 receptors, respectively.

The wild-type receptor was desensitized only by ACh concentrations that were able to evoke a detectable response. The IC_{50} of the α4:+L264α4:β2 and that of the α4:S252Fα4:β2 were significantly lower compared to that of the wild-type receptor ($p < 0.05$). Desensitization of the α4:S252Fα4:β2 receptor, however, was much more pronounced than that of the α4:+L264α4:β2 receptor and was observed even at ACh concentrations that were not able to evoke detectable responses.

In the present work we studied the sensitivity to ACh and the desensitization pattern of α4β2 mouse recombinant receptors bearing two mutations in the α4 subunit that have been linked to the occurrence of ADNFLE [2,4,6,12,13,19,20].

The magnitude of the changes in the EC_{50} caused by the S252F substitution expressed in heterozygosis allowed the identification of three different populations of receptors in the concentration–response curve. Although this is consistent with

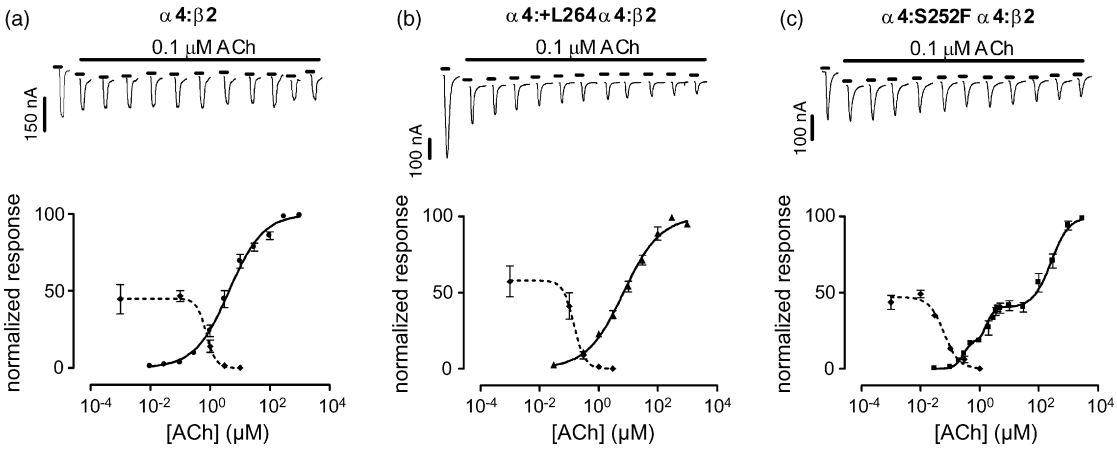

Fig. 3. Desensitization upon a prolonged exposure to ACh. Plots of the amplitude of the last peak of the train normalized to the amplitude of the control peak as a function of the logarithm of the ACh concentration. Concentration–response curves to ACh are plotted for comparison. Top panels: responses to test pulses of 1 μM ACh for 5 s, in the presence (12 min) of low concentrations of ACh (0.1–10 μM). Wild-type α4β2 (a), α4:S252Fα4:β2 (b) and α4:+L264α4:β2 (c) nAChRs.

the $\alpha4_2{:}\beta2_3$ stoichiometry found in *Xenopus* oocytes injected with equimolar amounts of α4 and β2 cRNA [1], we cannot disregard the possibility of the presence of a small population of $\alpha4_3{:}\beta2_2$ receptors [15] which could have been masked if more than one component had the same sensitivity to ACh.

The gate of the pore is possibly formed by V259 and L255 of the 5 subunits that assemble into a receptor complex [14]. Van der Walls interactions between these residues form two symmetric rings that stabilize the closed state. The rotation caused by the binding of ACh in the TM2 domains, separates the helixes from each other. This opens the pore and brings TM2 domains closer to TM1, TM3 and TM4 domains, establishing alternate hydrophobic interactions [14]. Changes in any of these hydrophobic residues for hydrophilic ones, destabilizes Van der Walls interactions favoring the open state, evidenced by an increase in the apparent affinity for the agonist [17]. The serine mutated in ADNFLE is just underneath the hydrophobic ring formed by L255. The replacement of this serine by a phenyalanine introduces an aromatic aminoacid that could interact with this ring. When only one of the two α4 subunits is mutated the interaction of phenyalanine with the ring could destabilize it, symmetry would be lost, favoring the open state. When both α4 subunits are mutated, the two aromatic residues interacting with the ring might not alter its symmetry and might even stabilize hydrophobic interactions thus favoring the closed state of the channel. These interactions could account for the shift in the EC_{50} to the left with the S252F mutation in heterozygosis and to the right in homozygosis. EC_{50} values reported for the human recombinant wild-type α4β2 nAChR are within the same range (2–4 μM) as that observed in the present work [19]. The decrease in the apparent affinity observed in the S252Fα4:β2 is greater than that reported for rat and human recombinant receptors in homozygosis [2,6,12,16,23]. The fact that the homozygous S252Fα4:β2 receptors, represented by the third component of the curve performed in heterozygosis, have an EC_{50} similar to that reported for $\alpha4_3\beta2_2$ receptors [15], reinforces the idea that receptors with this stoichiometry could also account for this subpopulation of receptors.

The increase in the apparent affinity of mouse α4:S252F:β2 receptors in heterozygosis is higher than that reported for human mutated receptors bearing the same mutation [9]. This might arise from the fact that we discriminated three components in the concentration–response curve, whereas in the mentioned work, this was not considered, therefore the apparent affinity is an average resulting from the different possible arrangements of receptors. Finally, there were no significant changes in the sensitivity of mouse recombinant α4:+L264α4:β2 receptors to ACh which contrasts with the increase in sensitivity to ACh reported for human α4:+L264α4:β2 receptors [19].

Mutations in TM2 can also alter transitions to desensitized states. The substitution of serine 252 by phenylalanine increased desensitization of the receptor in the presence of ACh, even at concentrations that were not able to evoke responses and this is similar to that observed for human receptors bearing the same mutations [2]. This suggests that this residue could be involved in the passage to a desensitized state. We simulated heterozygosis by equimolar injections of α4 and α*4 subunits, therefore, the calculated IC_{50} of desensitization is a compound value, influenced by the three types of possible arrangements of receptors in the membrane. In fact, the IC_{50} value obtained for mouse α4:S252Fα4:β2 nAChR (0.062 μM) was not as low as that reported for human S252Fα4:β2 recombinant receptors (28 pM) [2]. The discrepancy is most likely due to the fact that a non-conducting desensitized state is favored when the two phenylalanines are present in both α4 subunits. Even though the insertion of a leucine codon also increased the pattern of desensitization, the magnitude of this effect was much lower than that observed with the substitution. Recombinant human +L264α4:β2 receptors, however, did not desensitize more than wild-type ones [2].

It is intriguing that mutations that lead to the same disease behave in a different way with respect to ACh sensitivity and desensitization pattern. However, so far there is not a unifying theory for what causes ADNFLE and both the underlying mutations and mechanisms could be different. The present results are in general agreement with those reported for human recombi-

nant receptors bearing the same mutations, thus contributing to validate the use of *knock-in* mice harboring ADNFLE mutations [11,22] as models for the study of this pathology.

Acknowledgements

This work was supported by an International Research Scholar Grant from the Howard Hughes Medical Institute, a Research Grant from ANPCyT (Argentina) to A.B.E., a Research grant from Universidad de Buenos Aires (Argentina) to A.B.E. and E.K., a Research Grant from CONICET (Argentina) to E.K.

References

[1] R. Anand, W.G. Conroy, R. Schoepfer, P. Whiting, J. Lindstrom, Neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes have a pentameric quaternary structure, J. Biol. Chem. 266 (1991) 11192–11198.

[2] S. Bertrand, S. Weiland, S.F. Berkovic, O.K. Steinlein, D. Bertrand, Properties of neuronal nicotinic acetylcholine receptor mutants from humans suffering from autosomal dominant nocturnal frontal lobe epilepsy, Br. J. Pharmacol. 125 (1998) 751–760.

[3] R. Combi, L. Dalpra, M.L. Tenchini, L. Ferini-Strambi, Autosomal dominant nocturnal frontal lobe epilepsy—a critical overview, J. Neurol. 251 (2004) 923–934.

[4] M. De Fusco, A. Becchetti, A. Patrignani, G. Annesi, A. Gambardella, A. Quattrone, A. Ballabio, E. Wanke, G. Casari, The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy, Nat. Genet. 26 (2000) 275–276.

[5] A.B. Elgoyhen, D.E. Vetter, E. Katz, C.V. Rothlin, S.F. Heinemann, J. Boulter, Alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 3501–3506.

[6] A. Figl, N. Viseshakul, N. Shafaee, J. Forsayeth, B.N. Cohen, Two mutations linked to nocturnal frontal lobe epilepsy cause use-dependent potentiation of the nicotinic ACh response, J. Physiol. 513 (Pt 3) (1998) 655–670.

[7] C. Gotti, M. Zoli, F. Clementi, Brain nicotinic acetylcholine receptors: native subtypes and their relevance, Trends Pharmacol. Sci. 27 (2006) 482–491.

[8] S. Hirose, H. Iwata, H. Akiyoshi, K. Kobayashi, M. Ito, K. Wada, S. Kaneko, A. Mitsudome, A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy, Neurology 53 (1999) 1749–1753.

[9] V. Itier, D. Bertrand, Mutations of the neuronal nicotinic acetylcholine receptors and their association with ADNFLE, Neurophysiol. Clin. 32 (2002) 99–107.

[10] A. Karlin, Emerging structure of the nicotinic acetylcholine receptors, Nat. Rev. Neurosci. 3 (2002) 102–114.

[11] A. Klaassen, J. Glykys, J. Maguire, C. Labarca, I. Mody, J. Boulter, Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 19152–19157.

[12] A. Kuryatov, V. Gerzanich, M. Nelson, F. Olale, J. Lindstrom, Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca2+ permeability, conductance, and gating of human alpha4beta2 nicotinic acetylcholine receptors, J. Neurosci. 17 (1997) 9035–9047.

[13] T. Leniger, C. Kananura, A. Hufnagel, S. Bertrand, D. Bertrand, O.K. Steinlein, A new Chrna4 mutation with low penetrance in nocturnal frontal lobe epilepsy, Epilepsia 44 (2003) 981–985.

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

[15] M. Moroni, I. Bermudez, Stoichiometry and pharmacology of two human alpha4beta2 nicotinic receptor types, J. Mol. Neurosci. 30 (2006) 95–96.

[16] B. Moulard, F. Picard, S. le Hellard, C. Agulhon, S. Weiland, I. Favre, S. Bertrand, A. Malafosse, D. Bertrand, Ion channel variation causes epilepsies, Brain Res. Brain Res. Rev. 36 (2001) 275–284.

[17] P.V. Plazas, E. Katz, M.E. Gomez-Casati, C. Bouzat, A.B. Elgoyhen, Stoichiometry of the {alpha}9{alpha}10 nicotinic cholinergic receptor, J. Neurosci. 25 (2005) 10905–10912.

[18] M.W. Quick, R.A. Lester, Desensitization of neuronal nicotinic receptors, J. Neurobiol. 53 (2002) 457–478.

[19] O.K. Steinlein, A. Magnusson, J. Stoodt, S. Bertrand, S. Weiland, S.F. Berkovic, K.O. Nakken, P. Propping, D. Bertrand, An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy, Hum. Mol. Genet. 6 (1997) 943–947.

[20] O.K. Steinlein, J.C. Mulley, P. Propping, R.H. Wallace, H.A. Phillips, G.R. Sutherland, I.E. Scheffer, S.F. Berkovic, A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy, Nat. Genet. 11 (1995) 201–203.

[21] B. Sutor, G. Zolles, Neuronal nicotinic acetylcholine receptors and autosomal dominant nocturnal frontal lobe epilepsy: a critical review, Pflugers Arch. 442 (2001) 642–651.

[22] Y. Teper, D. Whyte, E. Cahir, H.A. Lester, S.R. Grady, M.J. Marks, B.N. Cohen, C. Fonck, T. McClure-Begley, J.M. McIntosh, C. Labarca, A. Lawrence, F. Chen, I. Gantois, P.J. Davies, S. Petrou, M. Murphy, J. Waddington, M.K. Horne, S.F. Berkovic, J. Drago, Nicotine-induced dystonic arousal complex in a mouse line harboring a human autosomal-dominant nocturnal frontal lobe epilepsy mutation, J. Neurosci. 27 (2007) 10128–10142.

[23] S. Weiland, D. Bertrand, S. Leonard, Neuronal nicotinic acetylcholine receptors: from the gene to the disease, Behav. Brain Res. 113 (2000) 43–56.