An amino acid exchange in the second transmembrane segment of a neuronal nicotinic receptor causes partial epilepsy by altering its desensitization kinetics

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Abstract **The α4 subunit of the neuronal nicotinic acetylcholine receptor is the first gene shown to be involved in a human idiopathic epileptic disease. A missense mutation, leading to the replacement of serine 248 by phenylalanine in the second transmembrane segment, had been detected in patients with autosomal dominant nocturnal frontal lobe epilepsy. The properties of the wild type receptor composed of α4 and β2 subunits and the mutant receptor where α4 subunits carried the mutation at serine 248 were compared by means of cDNA manipulation and expression in *Xenopus* oocytes. The mutant receptor exhibited faster desensitization upon activation by acetylcholine and recovery from the desensitized state was much slower than in the wild type receptor. We conclude that the reported mutation causes seizures via a diminution of the activity of the α4β2 neuronal nicotinic acetylcholine receptor.**

Key words: Acetylcholine receptor; Ion channel; Electrophysiology; Complementary DNA; *Xenopus laevis*; Partial epilepsy

1. Introduction

The neuronal nicotinic acetylcholine receptor (nAChR) belongs to the family of ligand-gated ion channels [1]. Several neuronal α and β subunits have been cloned that give rise to receptors with distinct electrophysiological and pharmacological features [1,2]. Among these, recombinant α7, α8 and α9 subunits can form functional homo-oligomers [3] whereas other α subunits may coassemble with at least one type of β subunit ([4], for review). The predominant nAChR subtype in rat and chicken brain contains α4 and β2 subunits and recent findings suggest that α5 subunits may also participate in the constitution of this receptor subtype [5,6].

In analogy to the pentameric nAChR of skeletal muscle it is assumed that neuronal receptors are formed by five subunits of as yet unknown stoichiometry. Each subunit folds with four putative transmembrane segments and the so-called TM2 segments form the walls of the channel pore [7–9]. Upon binding of ACh the pore-lining segments reorientate to allow cations to flow [10]. During prolonged exposure to ACh or repeated application of ACh at high frequency the nAChRs convert to a desensitized configuration which is unresponsive to added ACh, has a higher affinity to agonists and is more stable than the active states [11–13].

The gene encoding the α4 subunit of the human neuronal nAChR (CHRNA4) has been mapped to chromosome 20q13.2–q13.3 between the markers D20S20 and D20S24 [14]. Another marker, D20S19, has been localized adjacent to D20S20 and D20S24 and is tightly linked to the three brain-specific phenotypes benign familial neonatal convulsions (BFNC) [15,16], low-voltage EEG (LVEEG) [17] and autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [18]. ADNFLE is a partial epilepsy which typically begins in childhood and leads to clusters of brief seizures during light sleep or drowsing [19]. Recently, a mutation in the CHRNA4 gene was found in affected members of a large Australian pedigree in which the ADNFLE gene has been shown to be linked to chromosome 20q13.2–q13.3 [20]. A C to T transition in amino acid position 248 was detected leading to the replacement of serine by phenylalanine in the sixth residue of the second transmembrane segment (Fig. 1). The mutation was found in all clinically affected pedigree members, but not in 333 control individuals.

Interestingly, the mutated residue is located at a position termed the inner polar site (IPS) [21], which has been highly conserved not only during the evolution of all the agonist-binding nAChR subunits in different species but also in the $GABA_A$ and $5\text{-}HT_3$ receptor. In some receptor subunits serine is substituted by the polar threonine. Furthermore, Ser^{248} at position 6′ is located next to the most conserved residue in the TM2 sequence – Leu^{251} – separated only by one turn within a putative α-helical arrangement. According to the model proposed by Unwin [9], this Leu^{251} together with the leucine residues at homologous positions of the other subunits creates a tight hydrophobic ring at the most constricted part of the closed pore. Ser^{248} would be positioned immediately beneath this gate of the closed receptor in the lower part of the TM2 segment where small residues with hydroxyl groups alternate with bulky hydrophobic side-chains. A number of studies support the view that Ser^{248} is facing the pore in the mouse muscle and *Torpedo* electric organ nAChR [8]. The functional importance of the serine and threonine, respectively, at the sixth position of the TM2 segment has been emphasized based on experiments with site-directed mutagenesis in the α1 subunit of the mouse muscle nAChR and the α7 subunit of the chicken neuronal receptor. These results suggest that this position affects the affinity to ACh [22,23], desensitization properties [22], outward single-channel currents and concomitantly rectification [21].

Functional expression of recombinant neuronal nAChRs in *Xenopus* oocytes with characteristics similar to those of the native channels has been reported for several receptor subtypes [24,25]. In order to investigate the effects of the mutation $Ser^{248}Phe$ on the electrophysiological features of the human neuronal nAChR and to shed light on the mechanism by which it might cause seizures we introduced this amino acid

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exchange into the cDNA for the human α4 subunit. The mutated cDNA and the cDNA for the human β2 subunit were expressed in *Xenopus* oocytes to yield the mutant receptor, α4S248Fβ2. Changes in dose-response relations and desensitization kinetics in comparison to the wild type receptor, α4β2, were assessed by voltage-clamp experiments.

2. Materials and methods

2.1. Cloning of α4 and β2 subunit cDNAs and mutagenesis

The cDNA coding for the human neuronal nAChR β2 subunit [26] was obtained by amplification out of a human cerebral cortex cDNA pool (Human Brain Quick-Clone cDNA, Cerebral Cortex; Clonetech, Palo Alto, CA) by the use of primers flanking the translated region of the sequence (forward: GAGGCAGCGAGCTATGCCCA; reverse: TGGAGATGAGGAAGGGCCTCA). To get the 5′ part of the α4 subunit cDNA a 5′-RACE was performed using the Human Fetal Brain Marathon-Ready cDNA (Clonetech) and an exon-5 specific primer (GGGCATGGTGTGCGTGCGTG) in combination with the adaptor primer AP2 supplied with the kit. The PCR product was used for a nested PCR with two α4 subunit specific primers. The reverse primer was specific for sequences in exon 5 (CCACGATGACC-CACTCGCC) and the forward primer (CTAGATCTCGC-GAGGTGCGTG) bound to the 5′ region flanking the start codon. PCR generated a fragment of 667 bp including a unique *Pst*I site. The 3′ part was amplified employing the cDNA clone OS2-1-2 [14] and two α4 subunit specific primers (forward: GGCCGGA-CATCGTCTCTAC; reverse: GCCCCACAGAGTCCAGGGAG) yielding a cDNA fragment of 1622 bp containing the unique *Pst*I site as well. The two PCR fragments were ligated via the *Pst*I site and the complete α4 cDNA was amplified out of the ligation reaction with two primers flanking the coding sequence (forward: CGAGGTGCGGGATCCATGGAGCTAGG; reverse: CCGCATG-GATCCTGGCCCCGTGCAC). With the help of these two primers *Bam*HI sites were introduced at both ends of the α4 cDNA and restriction of the *Bam*HI sites yielded termini that were compatible with *Bgl*II termini. Both the α4 and the β2 cDNA were cloned into pSPOoD [27], a derivative of pSP64T [28], using the *Bgl*II restriction site.

For the introduction of the mutation S248F into the α4 subunit cDNA a 618 bp fragment of exon 5 was amplified from the genomic DNA of an ADNFLE patient belonging to the chromosome 20-linked Australian family [18] in which the mutation had been detected [20]. The PCR product included the TM2 segment with the C to T transition in the sixth amino acid position and was exchanged for the corresponding fragment of the wild type cDNA in pSPOoD using single-cut *Dra*III and *Mun*I sites. Plasmids containing the sequence of the mutant allele were identified by a PCR assay with one of the primers carrying a one-base mismatch near the 3′ end, creating a new *Hpa*II restriction site in the wild type but not in the mutant allele [20]. The cloning constructs were sequenced according to the Sanger dideoxy termination method (Sequenase Version 2.0 DNA Sequencing Kit; Amersham, Arlington Heights, IL) to verify that only the desired mutation was present.

2.2. In vitro transcription and expression in oocytes

Plasmid DNAs were linearized with *Sal*I and transcribed in vitro with SP6 polymerase [29]. *Xenopus laevis* oocytes were injected [30,31] with 10–15 ng cRNA in approximately 50 nl water encoding the nAChR β2 subunit and the wild type or mutant α4 subunit in a molar ratio 1:1 and incubated at 19 ± 1°C in OR2 medium (82.5 mM NaCl, 2.5 mM KCl, 1.0 mM Na_2HPO_4, 5.0 mM HEPES, 1.0 mM $MgCl_2$, 1.0 mM $CaCl_2$, 0.5 g/l PVP, pH 7.2 adjusted with NaOH) for 2–3 days prior to electrophysiological recordings. The day after injection oocytes were treated with 1 mg/ml collagenase type I in OR2 for 30 min at room temperature and washed three times with OR2. Afterwards the follicular cell layer was removed mechanically with forceps.

2.3. Electrophysiological measurements

Currents elicited in response to bath application of various acetylcholine (ACh) concentrations to oocytes expressing either wild type or mutant nAChRs were measured with a standard two micro-electrode voltage-clamp at a holding potential of −70 mV. Electrodes were filled with 3 M KCl and had resistances of 0.1–7 MΩ. Oocytes were continuously perfused at room temperature with a modified frog Ringer solution (Mg^{2+}-frog Ringer) without Ca^{2+} (135 mM NaCl, 5.4 mM KCl, 2.8 mM $MgCl_2$, 5 mM HEPES) to prevent activation of Ca^{2+}-activated chloride channels. ACh was diluted in perfusion solution and applied for 5 s except where otherwise stated. Oocytes were washed for 3–5 min – depending on ACh-concentration and receptor type – between exposures to ACh to minimize the desensitizing effect of each preceding pulse on the following measurement. Dose-response data from each oocyte were normalized to the response to 30 μM ACh and fit to the equation

$$\theta = [1 + (EC_{50}/[A])^n]^{-1}$$

where θ is the normalized current, EC_{50} is the concentration of ACh required to obtain half-maximal current, [A] is the concentration of ACh and n is the apparent Hill coefficient using a commercially available software package (Igor, Wave Metrics, Oswego, OR).

3. Results

3.1. The mutant receptor exhibits an accelerated desensitization

Both the wild type α4β2 and the mutant α4S248Fβ2 nAChR produced functional channels when expressed in *Xe-*

N-Terminus				1′	2′	3′	4′	5′	6′	7′	8′	9′	10′	11′	12′	13′	14′	15′	16′	17′	18′	19′	20′	C-Terminus
hα4	G	E	K	I	T	L	C	I	S	V	L	L	S	L	T	V	F	L	L	L	I	T	E	
hβ2	C	E	K	M	T	L	C	I		V	L	L	A	L	T	V	F	L	L	L	I	S	K	
mα1	G	**E**	K	M	**T**	**L**	S	I		V	**L**	L[34]	S	L	T	**V**	F	L	**L**	V	I	V	E	
mβ1	G	E	K	M	G	L	S	I	F	A	L	L[34]	T	L	T	V	F	L	L	L	L	A	D	
mγ	G	Q	K	C	T	V	A	T	N	V	L	L[34]	A	Q	T	V	F	L	F	L	V	A	K	
mδ	G	E	K	T	S	V	A	I		V	L	L[34]	A	Q	S	V	F	L	L	L	I	S	K	
cα7	G	E	K	I	S	L	G	I		V	L	L[32]	S	L	T	V[33]	F	M	L	L	V	A	E	
m5-HT_3	G	E	R	V	S	F	K	I		L	L	L[40]	G	Y	S	V	F	L	I	I	V	S	D	
dGABA	P	A	R	V	A[41]	L	G	V		T	V	L	T	M	T	T	L	M	S	S	T	N	A	
N-Terminus	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	C-Terminus

Fig. 1. Amino acid sequences of TM2 segments and neighboring residues of different ligand-gated ion channels. At the top the position of the residues in the TM2 segment is given with the terminology following that of Miller [44] which assigns the number 1′ to the residue at the putative N-terminus of the TM2 sequence – the cytoplasmic end. At the bottom the corresponding amino acid position is shown referring to the numbering of the *Torpedo* α subunit [45]. Residues marked in bold have been demonstrated to face the channel lumen in the mouse muscle receptor by Akabas et al. [46]. Boxes are drawn around small polar or neutral residues that are repeated at every fourth position in the amino terminal half. Filled boxes indicate the IPS [21]. Amino acids that have been shown to influence desensitization by experiments with site-directed mutagenesis are marked with superscript numbers indicating the corresponding reference. The serine that was substituted by phenyalanine in this study is highlighted by a circle. hα4: human neuronal nAChR α4 subunit; hβ2: human neuronal nAChR β2 subunit; mα1, *Mus musculus* muscle nAChR α1 subunit; mβ1, *Mus musculus* muscle nAChR β1 subunit; mγ, *Mus musculus* muscle nAChR γ subunit; mδ, *Mus musculus* muscle nAChR δ subunit; cα7, *Gallus gallus* neuronal nAChR α7 subunit; m5-HT_3: *Mus musculus* 5-HT_3 receptor; dGABA, *Drosophila melanogaster* GABA receptor.

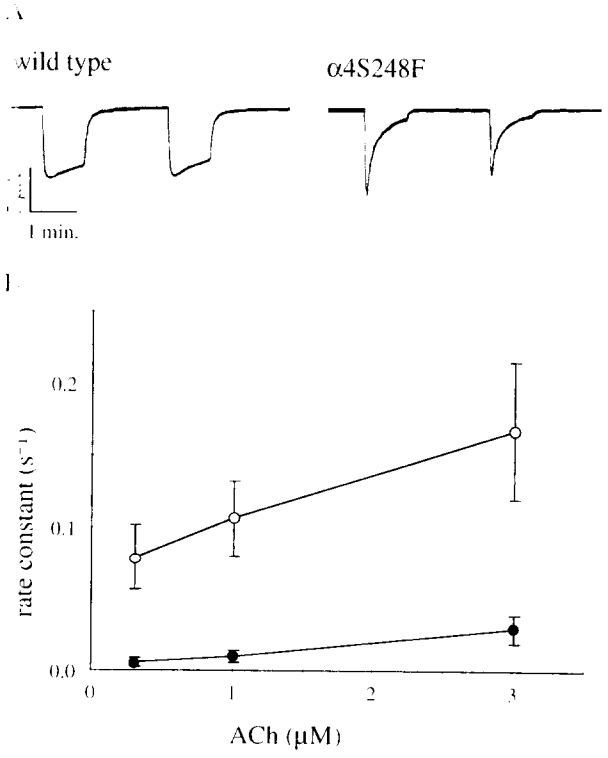

Fig. 2. Effect of the mutation on desensitization kinetics. A: Macroscopic currents elicited by twice bath application of 1 μM ACh for 1 min to oocytes with wild type or α4S248Fβ2 nAChRs. The interval between the two pulses was 2 min. Note the marked increase of desensitization for the α4S248Fβ2 nAChR. Recordings performed in several oocytes displayed identical features. B: Rate constants (s^{-1}) of current inactivation for wild type (●) and α4S248Fβ2 (○) nAChRs were estimated by a monoexponential fit to the time course for the reduction of the current amplitude during 1 min application of 0.3, 1 and 3 μM ACh concentrations and plotted against the ACh concentration. Each point represents the mean ± S.D. obtained in 2 (wild type) and 9 (mutant) cells from more than one batch of oocytes.

nopus oocytes. Comparing the magnitude of the macroscopic currents in oocytes expressing either wild type or mutant receptors revealed no significant differences. The current responses evoked by 1 μM ACh ranged between 1.65 ± 1.26 μA (n=13) for the wild type and 1.83 ± 1.99 μA (n=12) for the mutant receptor reflecting on the fact that the quantitative comparison of currents evoked by bath-applied agonist in whole cells displays a large variability. Fig. 2A shows, however, that the agonist-evoked current response of the α4S248Fβ2 nAChR differs from the current response observed for the α4β2 nAChR. At 1 μM ACh the initial response of the mutant receptor decreased by more than 85% within 1 min, while the decrease of the current response of the wild type receptor was much slower. Moreover, 2 min after the first application of ACh to the mutant receptor, followed by a wash step with Mg^{2+}-frog Ringer, the response to the second application of ACh at the same concentration was depressed by approximately 25%, whereas repetitive activation of the wild type receptor evoked comparable initial current amplitudes. These findings suggested that the α4S248Fβ2 receptor may become inactivated or desensitized upon interaction with agonist at a significantly faster rate than the wild type receptor. The different desensitization rates were estimated by a monoexponential fit to the time course for the reduction of the current amplitude during application of 0.3, 1 and 3 μM ACh concentrations (Fig. 2B). At 0.3 μM ACh desensitization for the wild type receptor was very slow ($t_{1/2}$ = 147 ± 71 s; n = 4) in contrast to the mutant receptor which showed already significant desensitization ($t_{1/2}$ = 10 ± 4 s; n = 18). Between 0.3 and 1 μM ACh the current through the mutant receptor inactivated ≥10 times faster than that of the wild type receptor. The increased rate constants observed at 3 μM and higher ACh concentrations (not shown), which are still significantly higher for the mutant receptor, indicate that both receptor subtypes show agonist-dependent desensitization which precludes an exact determination of dose-response relationships.

3.2. *The mutant and the wild type receptor recover at different rates from desensitization*

The traces shown in Fig. 2A gave a first indication of the difference in the rate of recovery from desensitization between the wild type and the mutant receptor. The time course of recovery T_P was analyzed by applying pairs of 5 s pulses of 1 μM ACh with varying interpulse intervals (Fig. 3). The concentration of 1 μM ACh was chosen since the wild type receptor showed moderate desensitization at this concentration, while desensitization of the mutant receptor was still slow enough to be measured. The interpulse intervals were varied between 10 and 120 s. With pauses shorter than 10 s no clear distinction between the first and the second pulse could be seen, perhaps due to the size and shape of *Xenopus* oocytes, which make a more rapid agonist application difficult. To minimize a desensitizing effect of the preceding double pulse on the following response to agonist application, cells were washed with Mg^{2+}-frog Ringer for 5 min to allow for complete recovery of the receptors. Fig. 3 shows that with 30 s pulse intervals the wild type receptor responds to both agonist pulses with a similar current amplitude displaying little or no desensitization. In case of the α4S248Fβ2 receptor the response to the second ACh pulse was reduced by approximately 60%. With a pulse interval of 120 s the second agonist pulse could evoke a current amplitude reaching about 90% of the amplitude of the current evoked by the first pulse. The presented data fitted an exponential function giving time

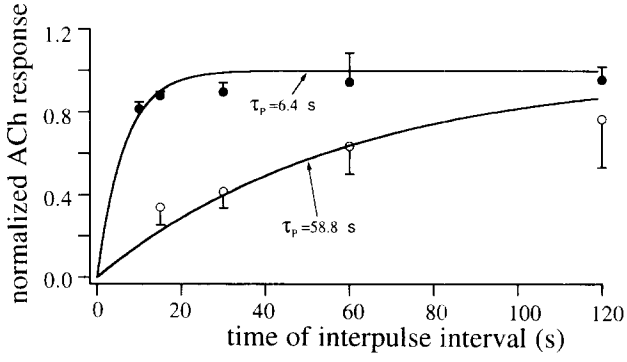

Fig. 3. Effect of the mutation on the time course of recovery from desensitization. Pairs of 5 s pulses of 1 μM ACh were applied to oocytes expressing wild type (●) or α4S248Fβ2 nAChRs (○) with varying interpulse intervals. The maximal amplitude of the response to the second pulse is divided by that of the response to the first pulse and plotted against the interval between the two pulses. Each point shows the mean ± half S.D. of the normalized responses of 5–15 oocytes from 2–3 separate sets of oocytes.

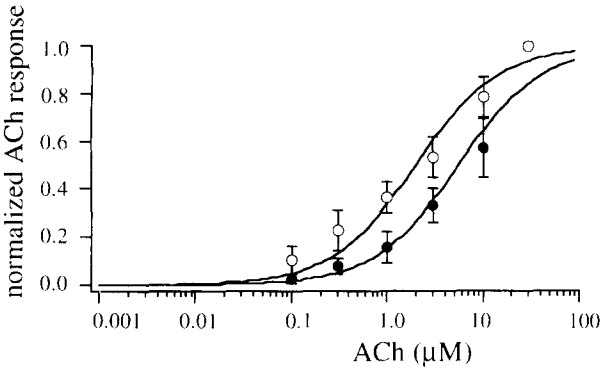

Fig. 4. Dose-response curves for oocytes with wild type (●) and α4S248Fβ2 (○) nAChRs for ACh in the range of 0.1–30 μM. Responses were normalized to the response of the same oocyte to 30 μM ACh and plotted against the logarithm of the ACh concentration. Each point represents the average ± S.D. of the normalized responses of 15–21 oocytes from 2–3 donors. The plots were fitted to the Hill equation assuming a Hill coefficient of 1 to give apparent EC_{50} values of 4.8 μM for the wild type and 2.1 μM for the α4S248Fβ2 nAChR.

constants for recovery of 6.4 s for the wild type and 58.8 s for the mutant receptor.

3.3. The mutation does not alter the apparent affinity for ACh

Effects due to mutations in the TM2 segment at positions 6′, 9′ and 13′ of the chicken α7 neuronal nAChR [22,32,33] and positions 9′ and 10′ of the mouse muscle nAChR [23,34,35] on desensitization kinetics have been reported to be accompanied by changes in the apparent affinity for agonist as compared to wild type receptors. It was therefore of interest to investigate whether the altered desensitization properties of the α4S248Fβ2 receptor, mutated at the 6′ position, were due to an increased affinity for ACh (Fig. 4). The rapid, extensive desensitization of the human α4β2 nAChR at high ACh concentrations, which is even more pronounced for the α4S248Fβ2 mutant receptor, makes the determination of the true peak current amplitude difficult and for this reason it was not possible to normalize data to the maximal response values. Thus, dose-response curves were constructed by normalizing all current response measurements to the response evoked by 30 μM, the highest concentration at which reliable standard values could be obtained. Even more pronounced desensitization was observed by Luetje and Patrick [24] for the rat α4β2 nAChR and current responses were normalized to the response evoked by 1 μM ACh in this case to generate dose-response curves. The agonist-induced activation of both the α4β2 and the α4S248Fβ2 nAChR was best fitted assuming a Hill coefficient of 1, a value which agrees with the observation that neuronal nAChRs are characterized by Hill coefficients of about 1.5 [1]. According to the dose-response curves generated for the two recombinant receptor subtypes there was no significant difference between the EC_{50} value of the α4β2 (4.8 μM) and the mutant α4S248Fβ2 nAChR (2.1 μM).

4. Discussion

In the present study we have expressed recombinant neuronal nAChRs composed of α4β2 subunits and mutated receptors containing α4S248F subunits in order to investigate functional differences with regard to agonist affinity and desensitization properties. Until now, the human α4β2 receptor has not been characterized in the oocyte expression system. Recently, the pharmacological properties of the human α4β2 receptor have been studied in human embryonic kidney 293 cells. The observed EC_{50} of ACh for cation efflux stimulation was 44 μM, which differs from the electrophysiologically determined EC_{50} value of 4.8 μM by a factor 9. Furthermore, desensitization was not pronounced and was only observed at much higher ACh concentrations [36]. This difference could be due to the different expression systems and assay methods used. For instance, the chicken α4β2 nAChR expressed in fibroblasts appears to have a lower time constant of desensitization and a 10-fold higher EC_{50} value than the receptor expressed in oocytes [37–39]. The EC_{50} values of recombinant rat and chicken α4β2 receptors determined in oocytes were 2 μM and 0.77 μM [24,39], respectively, and are in the same order of magnitude as the EC_{50} value determined for the wild type human α4β2 nAChR in the present study. The chicken receptor desensitized far more slowly than the human receptor whereas the desensitization of the rat receptor was even more pronounced [24,37]. These distinct properties could be caused by species differences: the TM2 segment of the human α4 subunit differs at one amino acid position (1′) from the rat α4 subunit, and the human β2 subunit shows differences at three positions (3′/9′/16′) compared with both the chicken and the rat β2 subunit.

The substitution of serine at position 6′ of the TM2 segment by phenylalanine increased the rate of inactivation or desensitization and prolonged significantly the time the receptor needed to recover to a conducting state. One could speculate that the wild type and the mutant receptor are activated initially with comparable efficiency. The hydrophobic phenylalanines, however, replacing the hydrophilic serines may interact with each other or additional nearby located hydrophobic side chains to reduce the water space. As a consequence a non-conducting state may be preferentially stabilized leading to the blockade of ion translocation and reducing the rate of recovery. Leonard et al. [21] proposed that serines at the IPS could be necessary for the stabilization of the open channel because of interactions between their OH-groups and water molecules either contacting other water molecules or hydrating a permeant ion. Thus, the removal of serines at this position could affect the stability of the open configuration leading to changes in gating kinetics. Mutation of threonine at the homologous position in the chicken α7 subunit to the strongly polar glutamine and aspartate led to a deceleration of desensitization [22]. Upon replacement of the leucine at position 9′ in the chicken α7 receptor the velocity of desensitization decreased with the size and hydrophobicity of the introduced residue with the most pronounced effect being caused by the introduction of serine [32]. In the serotonin $5\text{-}HT_3$ receptor, which is similar to the neuronal nAChR, hydrophobic substitution at position 9′ in the TM2 segment accelerated desensitization, whereas the introduction of the polar threonine at the same position led to a marked reduction of desensitization [40]. Taken together, these results support the view that the residues located within the TM2 segment contribute to the stabilization of different channel configurations [10]. Depending on their polarity and size they increase or decrease rates of desensitization both of several neuronal nAChRs and of the $5\text{-}HT_3$ receptor and thus influence desensitization properties. For the *Drosophila* GABA receptor, carrying an alanine to

serine mutation at position 2′ of the TM2 segment, it was shown that the markedly decreased desensitization was caused by a 5-fold stabilization of the channel open state and a 29-fold destabilization of the desensitized conformation [41]. Similarly, the leucine to threonine and leucine to serine mutations at position 9′ of the mouse muscle receptor have been found to lead to stabilization of the open state and a reduced stability of the closed state, respectively, accompanied by a decreased desensitization [34,35].

The great differences between the wild type α4β2 and the mutant α4S248Fβ2 nAChR concerning desensitization properties contrast with the almost identical dose-response relations. Effects on desensitization properties due to amino acid substitutions in the TM2 segment without changes of the apparent affinity for agonist have also been reported for the *Drosophila* GABA receptor and the mouse 5-HT_3 receptor [40,41]. In contrast, the mouse muscle nAChR with the serine to phenylalanine mutation at the IPS exhibited an 8-fold higher sensitivity to ACh as compared to the wild type receptor, without changes in desensitization properties [23]. Recently, Forman and Yellen have suggested that the mutation could have a similar effect on the neuronal nAChR and lead to hyperactivity of the α4S248Fβ2 nAChR [42]. This could not be confirmed by our results. The ≥10-fold accelerated desensitization and strongly delayed recovery of the mutant receptor lead to hypoactivity rather than to hyperactivity. Muscle and neuronal nAChRs have been shown to differ in their pharmacological and kinetic properties [25], suggesting that apparently modest differences between the primary structure of distinct receptor types may lead to profound differences in their behavior.

Taken together, our results indicate that the reported mutation in the TM2 segment of the nAChR α4 subunit causes seizures in ADNFLE patients by diminishing its activity: the receptor converts faster to a state in which it is unresponsive to ACh than the wild type receptor and remains longer in this conformation. It has been hypothesized that the predominant role of the nAChR in the brain could be that of a modifier of neuronal excitability [43]. The $Ser^{248}Phe$ mutation possibly disturbs the balance between inhibitory and excitatory synaptic transmission, thus lowering the seizure threshold. It remains to be investigated why this epileptogenic effect is restricted to the frontal lobe and shows a striking dependence on the brain state of vigilance.

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