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Mutations at two distinct sites within the channel domain M2 alter calcium permeability of neuronal α7 nicotinic receptor

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ABSTRACT **The relative permeability for sodium, potassium, and calcium of chicken α7 neuronal nicotinic receptor was investigated by mutagenesis of the channel domain M2. Mutations in the "intermediate ring" of negatively charged residues, located at the cytoplasmic end of M2 (site 1), reduce calcium permeability without significantly modifying other functional properties (activation and desensitization) of the receptor; a similar change of ion selectivity is also noticed when mutations at site 1 are done in the context of a receptor mutant that conducts ions in a desensitized state. Moreover, mutations of two adjacent rings of leucines at the synaptic end of M2 (site 2) have multiple effects. They abolish calcium permeability, increase the apparent affinity for acetylcholine by 10- to 100-fold, augment Hill numbers (up to 4.6–5.0) of acetylcholine dose–response relationships, slow rates of ionic response onset, and lower the extent of desensitization. Mutations at these two topographically distinct sites within M2 selectively alter calcium transport without affecting the relative permeabilities for sodium and potassium.**

Influx of calcium (Ca^{2+}) through peripheral and brain nicotinic acetylcholine receptors (nAChRs) has been described in several preparations including muscle (1, 2), chromaffin cells (3, 4), cultured neurons from parasympathetic cardiac ganglia (5), rat medial habenular nucleus (6), and chicken ciliary ganglia (ref. 7; for review, see ref. 8). Neuronal nAChRs, in particular, exhibit a significant permeability to Ca^{2+} with associated P_{Ca}/P_{Na} close to 1 in parasympathetic cardiac neurons (5) or 2.5 in PC12 cells (9), which contrasts with a P_{Ca}/P_{Na} of 0.1–0.3 in skeletal muscle cells (1, 2, 10).

Recent molecular cloning studies have led to the identification of several genes encoding brain nAChR subunits that form functional acetylcholine (ACh)-gated ion channels upon expression in *Xenopus* oocytes (for review, see ref. 11). The α3–β4 hetero- (12) and the α7 homo- (13) oligomers display, for instance, significant Ca^{2+} permeabilities (3, 14, 15).

Chemical labeling (16–18) and site-directed mutagenesis (19–21) experiments have pointed to the critical role played by the M2 segments in ion permeation through nAChR channels (for review, see ref. 22). In particular, they led to the identification of several rings of amino acids that contribute to the characteristic pharmacological (21, 23) and conductance (20) properties of the ion channel, as its relative selectivity among monovalent cations (24–26). In chicken neuronal nicotinic α7 homooligomer, mutations of three rings of residues within M2 (T244Q or L247T or V251T) resulted in modifications of the response to ACh that were interpreted in terms of the conversion of a high-affinity closed desensitized state into a conducting state (23, 27, 28). In another study, the simultaneous introduction of three mutations in the M2 segment of the α7 receptor converted its ionic selectivity from cationic to anionic (14). For one of the mutations (E237A), indirect evidence suggested significant alterations of Ca^{2+} permeability (14).

In this study, we further investigate the monovalent vs. divalent cation selectivity of the α7 receptor. We show that a mutation at the cytoplasmic end of M2 (E237A) abolishes Ca^{2+} permeability without significantly affecting other properties of the pharmacological and physiological responses to ACh. We further identify another site of two adjacent amino acids, close to the extracellular end of M2 (Leu-254 and Leu-255), where mutations reduce Ca^{2+} permeability and affect other physiological properties of the response such as its apparent affinity for ACh and the rate of currents onset and desensitization.

MATERIALS AND METHODS

Mutagenesis. Mutants were prepared as described (23, 27). Their coding sequence was checked.

Electrophysiology. Oocytes were prepared, injected, and recorded as described (29). For voltage-clamp measurements, cells were incubated in OR2 medium (solution B, Table 1) and challenged by ACh application. Data from one oocyte are given in the figures and values (mean ± SEM) determined from 5 to 10 oocytes from more than one donor are given in Table 2.

Current–voltage (*I–V*) curves were obtained by subtracting passive membrane currents from currents evoked in the presence of ACh. ACh was applied at a concentration close to its EC_{50} value for each mutant.

Computations were done by the constant field theory, using the Goldman–Hodgkin–Katz equations for Na^+, K^+, Ca^{2+}, and Cl^- permeabilities extended from that described in ref. 30 for the *N*-methyl-D-aspartate receptor, to yield relative permeabilities. Ionic activities were used in these equations, instead of concentrations. Except for Mg^{2+}, activity coefficients for Na^+, Ca^{2+}, and K^+ were estimated as described (30, 31) and are given in Table 1. Effects of all ionic substitutions were fully reversible. 1,2-Bis(2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) injections were performed as described (14).

RESULTS

Unless otherwise indicated, all experiments described below were performed in the presence of internal BAPTA to suppress possible contribution of the Ca^{2+}-activated chloride currents as described (14, 15, 28, 31, 32).

***α7* Reversal Potential Is Sensitive to Calcium.** When expressed in *Xenopus* oocytes, α7 wild-type (WT) receptors elicit rapidly desensitizing (Fig. 1*A*) inwardly rectifying ACh-evoked currents. To evaluate the relative permeability of α7

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Abbreviations: ACh, acetylcholine; BAPTA, 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; E_{rev}, reversal potential; nAChR, nicotinic acetylcholine receptor; WT, wild type; *I–V*, current–voltage; DHβE, dihydro-β-erythroidine.

Table 1. Solution composition

Solution	Extracellular concentration, mM				Extracellular activity, mM		
	Na	K	Ca	Mannitol	γ_{Na}	γ_K	γ_{Ca}
A (2× Na)	168.75	2.5	2.5	0.0	129.90	1.92	1.30
B (1× Na)	86.25	2.5	2.5	0.0	66.40	1.92	1.43
C (0.02× Na)	1.75	2.5	2.5	82.5	1.35	1.92	2.05
D (10× K)	1.75	25.0	2.5	82.5	1.35	19.25	2.05
E (3× K)	1.75	7.5	2.5	82.5	1.35	5.77	2.05
F (1× K)	1.75	2.5	2.5	82.5	1.35	1.92	2.05
G (isoCaCl$_2$)	1.75	2.5	85.0	0.0	1.35	1.92	42.50
H (10× Ca)	1.75	2.5	25.0	82.5	1.35	1.92	15.50
I (8× Ca)	1.75	2.5	20.0	82.5	1.35	1.92	13.08
J (3× Ca)	1.75	2.5	7.5	82.5	1.35	1.92	5.55
K (1× Ca)	1.75	2.5	2.5	82.5	1.35	1.92	2.05
L (0.4× Ca)	1.75	2.5	1.0	82.5	1.35	1.92	0.88
M (0.1× Ca)	1.75	2.5	0.25	82.5	1.35	1.92	0.25

All solutions contain 0.5 μM atropine and 5 mM Hepes (pH 7.4); solutions A–G also contain 1 mM $MgCl_2$; solutions A and B also contain 1 mM phosphate.

WT to various monovalent and divalent cations, we measured reversal potentials, at 100 μM ACh, after changing the ionic composition of the external medium. As in studies on muscle nAChR (2), Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^- were taken into account as potentially permeant ions.

Fig. 2*A* shows that the reversal potential (E_{rev}) of the α7 WT nAChR (control; E_{rev} = −5.6 mV) shifts toward more positive potentials after a 2-fold increase of external sodium chloride concentration (2× Na; E_{rev} = 4 mV) or replacement of external NaCl with $CaCl_2$ (isoCaCl$_2$; E_{rev} = +28 mV), as expected if both Na^+ and Ca^{2+} were permeating through the channel. To further characterize these permeabilities, reversal potentials were measured in the presence of various concentrations of external Na^+ (1.35–129.9 mM), K^+ (1.92–19.25 mM), Ca^{2+} (0.25–13 mM; Fig. 2 *D–F*), and Mg^{2+}. In the low Na^+, low K^+ external solution K (Table 1), suppression of external Mg^{2+} did not shift the reversal potential to a significant extent (E_{rev} = 20 ± 2 mV in 1 mM Mg^{2+} and E_{rev} = 22 ± 3 mV in 0 mM Mg^{2+}), thus suggesting a negligible, if any, contribution of Mg^{2+} to ACh-evoked currents (data not shown). On the other hand, in low K^+ conditions, reversal potentials did not approach E_K as expected if only Na^+, K^+, and Ca^{2+} were permeant. A weak contribution of Cl^-, of the same amplitude as for parasympathetic cardiac ganglionic

Table 2. Affinity and permeabilities of WT and mutants

Type	ACh EC_{50}, μM	nH	DHβE/ ACh, %	P_{Na}/P_K	P_{Ca}/P_K	P_{Cl}/P_K
α7-WT	115.00	1.4	0.00	0.5	5.000	0.05
α7-E237A	194.00	1.4	0.00	0.5	<0.010	0.10
α7-E237, V251T	0.98	2.0	65.72	0.5	0.015	0.05
α7-V251T	0.63	2.0	66.10	0.3	5.000	0.05
α7-L254T	17.60	5.0	7.84	0.5	<0.010	0.08
α7-L254G	13.30	3.6	0.99	0.5	0.175	0.05
α7-L254Q	4.30	4.6	0.01	0.5	1.220	0.05
α7-L254R	1.65	3.0	1.57	0.5	<0.010	0.05
α7-L255T	2.60	2.2	0.14	0.5	<0.010	0.08
α7-L255G	19.00	4.5	0.00	0.5	<0.010	0.08
α7-L255Q	2.90	2.6	0.38	0.5	0.175	0.05
α7-L255R	17.60	3.2	0.37	0.5	<0.010	0.03

Summary of the data obtained in three to five oocytes, from several batches, for WT and mutant receptors. EC_{50} values for ACh and Hill numbers (nH) are given. The amplitude of DHβE-evoked currents (10 μM) is given relative to the maximal ACh-evoked current in the same oocyte. The relative permeabilities (Px) of Na, Ca, and K are given by taking P_K = 1.

(P_{Cl}/P_{Na} = 0.05) nAChRs (5), was found sufficient to account for the deviations detected at low K^+ and Na^+ concentrations. Thus, with only Na^+, K^+, Ca^{2+}, and Cl^- as permeant ions, a full set of permeability ratios could be determined, using the constant field theory (P_{Na}/P_K = 0.5, P_{Ca}/P_K = 5, P_{Cl}/P_K = 0.05; Table 2), that fitted the experimental data reported in Fig. 2 *A* and *D–F*.

From these data, it appears that the α7 nAChR channel, like several other neuronal nAChRs (3, 5, 6, 9), is significantly more permeable to Ca^{2+} than muscle nAChR (1, 2). Its selectivity toward the monovalent cations Na^+ and K^+, on the other hand, is comparable to that of endplate (1, 33) or *Torpedo* electric organ receptor channels (24).

Mutation of Site 1 at the Cytoplasmic End of M2 Selectively Alters Ca^{2+} Permeability. Mutation E237A was introduced at the N-terminal end of M2, at a site (site 1) referred to as the "intermediate ring of negatively charged residues" (20). E237A and WT yield functional nAChRs eliciting ACh-activated ionic currents of comparable amplitude, time course, and ACh sensitivity (in the presence of internal BAPTA; see ref. 14).

Ionic responses recorded at 200 μM ACh reverse at −24 mV under control conditions (Fig. 2*B*) and shift toward more positive potentials upon a 2-fold increase in external NaCl (2 × Na^+; E_{rev} = −3 mV). In contrast to WT, (*i*) replacement of NaCl with $CaCl_2$ displaced the reversal potential toward E_K (isoCaCl$_2$; E_{rev} = −56 mV) and (*ii*) increasing $CaCl_2$ concentration (from 0.25 to 15.5 mM in Fig. 2*E* in an external medium containing 1.35 mM Na^+ and 1.92 mM K^+) did not change the reversal potential. These two observations indicate that mutant E237A displays a negligible Ca^{2+} permeability. Indeed, the best fits for the *I–V* curves (Fig. 2*B*) and the reversal potentials measured under various ionic conditions (Fig. 2 *D–F*) are obtained using a value of P_{Na}/P_K identical to that of WT (0.5) and a low Ca^{2+} permeability relative to K^+ ($P_{Ca}/P_K < 0.01$).

The E237A mutation thus selectively reduces Ca^{2+} permeability of the open state of the α7 nAChR without affecting other properties of the pharmacological response. The lack of effects on P_{Na}/P_K permeability ratio is consistent with the data obtained with mutants of *Torpedo* (24) and muscle receptors (34), showing that the permeability for large monovalent cations (Rb^+, Cs^+, or $Tris^+$), but not that for Na^+, relative to K^+ is affected upon mutating this ring.

Ca^{2+} Permeability Through "Desensitized but Conducting" Mutants. Two α7 mutants (the single V251T and the double E237A,V251T mutants) have been shown (14, 28) to exhibit altered pharmacological responses to ACh, characterized by (*i*) 250-fold increased apparent affinities for ACh, (*ii*) the absence of ionic currents desensitization, and (*iii*) slow onsets of permeability changes. Also, dihydro-β-erythroidine (DHβE), a competitive antagonist of the WT, activated ion permeation through these mutant channels (refs. 14 and 28; see Table 2). The V251T mutant in addition displayed two cationic conductance levels (14). Thus, these data were interpreted, as for L247T (23, 27), by assuming that the mutation V251T renders conducting one of the desensitized states of the WT receptor. Accordingly, the two mutants would display a D* "desensitized but conducting" state (14), in addition to the WT conducting active (A) state.

Ionic responses of the V251T mutant were analyzed at 1 μM ACh, a concentration that selectively activates the D* state. The currents now reverse at −10 mV (data not shown) and shift toward more positive potentials upon a 2-fold increase in external NaCl (E_{rev} = −3 mV) or upon substitution of external NaCl by $CaCl_2$ (E_{rev} = 1.5 mV), indicating that this mutant channel is permeable to Ca^{2+}. The relative permeabilities determined from plots of reversal potential vs. concentration of Na^+, K^+, and Ca^{2+} yielded a P_{Na}/P_K of 0.3, ≈2-fold smaller than that of the WT, indicating that this mutation affects

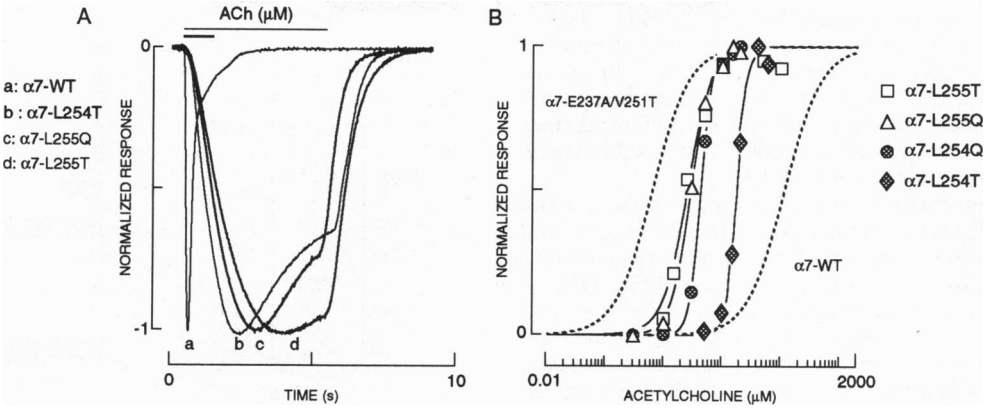

FIG. 1. (*A*) Time courses of ACh-evoked currents of the WT α7 and mutated receptors are superimposed. Agonist concentrations near the EC_{50} value of each mutant were chosen and cells were held at −100 mV. Duration of ACh applications was of 1 s and 5 s for the WT and mutated receptors, respectively. (*B*) ACh dose–response curves of mutants at positions 254 and 255 were determined. Dashed lines represent the dose–response curves of the WT and the double mutant E237A,V251T (taken from ref. 14). Continuous lines correspond to the empirical Hill equations, whose coefficients are indicated in Table 2. All data were obtained in the absence of BAPTA.

monovalent cation permeation. On the other hand, the permeability to Ca^{2+} relative to K^+ is high ($P_{Ca}/P_K = 5$) and cannot be distinguished from that of the WT receptor.

The ionic selectivity of the double E237A,V251T mutant was determined, at 1 μM ACh, using the same experimental protocol. As for mutant E237A, Ca^{2+} permeability was dramatically reduced. The relative permeability ratios determined, upon fitting *I–V* curves and reversal potentials vs. ionic concentration plots ($P_{Na}/P_K = 0.5$ and $P_{Ca}/P_K < 0.01$, Table 2), did not significantly differ from those of the single E237A mutant. The drastic changes in rectification, observed with the E237A mutant (Fig. 2*B*), might not be related to Ca^{2+} permeability since both a Ca^{2+}-impermeable E237A mutant and a Ca^{2+}-permeable V251T mutant (see ref. 14) show altered rectification properties.

Thus, the V251T mutation leads to a new conducting state, the ionic selectivity of which is comparable to that of the WT receptor. The E237A mutation, introduced at the cytoplasmic end of the channel domain, abolishes Ca^{2+} permeability and this mutation has the same effects on the low-affinity low-conductance (WT) and on the high-affinity high-conductance (V251T) open-channel states.

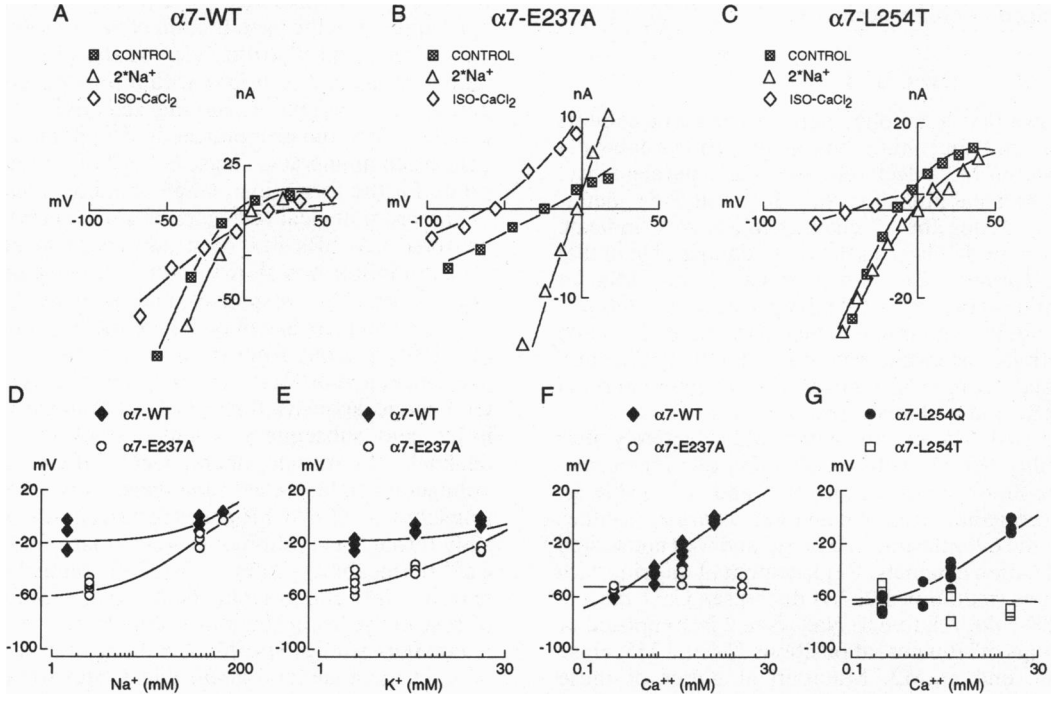

FIG. 2. Comparison of the ionic selectivity of the WT and two mutants. (*A–C*) *I–V* curves of the WT (*A*), E237A (*B*), and L254T (*C*) receptors recorded in three different ionic compositions. *I–V* relationships were determined first in a control condition by reporting the peak ACh-evoked current to agonist application as a function of the holding potential. Superfusion medium was then replaced with a solution containing twice as much Na^+ (2× Na^+) and the *I–V* relationship was determined again. Bathing medium was then replaced with a solution of isotonic Ca^{2+} ($ISO\text{-}CaCl_2$). At the end of each experiment, a control medium was again applied and another *I–V* curve was taken. Only cells that were stable throughout the entire experiment were considered in our analysis. (*C*) *I–V* curves were determined using ramp protocols (14). (*D–F*) Displacement of the reversal potential of the WT and mutant E237A as a function of the extracellular Na^+, K^+, and Ca^{2+} concentration is illustrated. *I–V* curves for several cells were measured using the same protocol as in *A* and the reversal potentials were reported as a function of the concentration of ionic medium on a logarithmic scale. (*G*) Reversal potential of L254Q and L254T mutants as a function of the extracellular Ca^{2+} concentration was determined and plotted. All concentrations were expressed in ionic activity, using the conversion in Table 1.

Mutations at the Synaptic End of M2 (Site 2) Alter Calcium Permeability and ACh Sensitivity. Using the shift of reversal potential in the presence of BAPTA as a test to screen mutations affecting Ca^{2+} permeability, we identified an additional site within M2 (site 2), composed of neighboring rings (Leu-254 and Leu-255), where mutations render ACh-evoked currents insensitive to internal BAPTA.

Mutation of Leu-254 and Leu-255 to Thr, Arg, Gln, or Gly (Table 2) was performed. All these mutations affected several properties of the ionic response to ACh as illustrated by (*i*) a higher apparent affinity for agonist (Table 2 and Fig. 1*B*), (*ii*) slower time courses of permeability changes (Fig. 1*A*), and (*iii*) lower extents of desensitization (Fig. 1*A*) at all ACh concentrations tested (data not shown). None of the mutants, however, was significantly activated by DHβE (see Table 2), indicating that alterations of receptor properties differ from those reported above for the V251T mutant or other mutations at positions 244 and 247 (see ref. 28). Also at variance with the mutants that become conducting in a desensitized state (T244Q, L247T, and V251T), mutations at positions 254 and 255 are accompanied by significant increases of apparent Hill numbers (up to 4.6–5, Table 2).

For all mutations at positions 254 and 255, the relative permeability ratios determined, in the presence of ACh at its EC_{50}, for Na^+ vs. K^+ (P_{Na}/P_K = 0.5) and for Cl^- vs. K^+ (P_{Cl}/P_K = 0.05–0.08) were comparable to those of WT receptor (Table 2). The *I–V* relationships recorded in control, 2× Na^+, and isoCaCl$_2$ of L254T mutant are illustrated in Fig. 2*C*. These data indicate that the selectivity for the monovalent ions Na^+ and K^+ was not affected. On the other hand, the relative permeability for Ca^{2+} (Fig. 2*G*) vs. K^+ was systematically altered and P_{Ca}/P_K ratios ranged from <0.01 (L254T and -R; L255T, -G, and -R) to 0.18 (L254G and L255Q) or 1.2 (L254Q). No mutation was found to leave the P_{Ca}/P_K unchanged at either positions.

DISCUSSION

This study shows that homooligomeric α7 receptor exhibits a high P_{Ca}/P_{Na} and that mutations at two topographically distinct sites within M2 selectively alter Ca^{2+} permeability.

The relative permeabilities for Na^+, K^+, and Ca^{2+}, determined upon expressing the α7 channel in oocytes, indicate that this WT nAChR displays a selectivity comparable to that of muscle (2), *Torpedo* (24), and neuronal (5) nAChRs for monovalent cations (P_{Na}/P_K = 0.5). Its permeability to Ca^{2+}, on the other hand, is comparable to that of the rat α7 homolog (15) and *N*-methyl-D-aspartate receptor (30) but significantly higher than that determined for muscle (2) and other neuronal (3, 5, 6) nAChRs and glutamate receptors (31, 35).

Mutations at two distinct sites within M2 selectively alter Ca^{2+} permeability (Fig. 3) without affecting relative permeabilities for the monovalent cations Na^+ and K^+ (Table 2). Site 1 is the intermediate ring of negatively charged residues (20), located at the cytoplasmic end of M2 and well conserved in ligand-gated cation channels. Replacement of this ring with a ring of alanines (mutation E237A) decreases Ca^{2+} permeability by >1000-fold, relative to Na^+. Site 2 is composed of two adjacent rings of leucines, at positions 254 and 255, close to the synaptic end of M2. Mutation at either of these positions reduces Ca^{2+} permeability by 10- to 1000-fold, depending on the nature of the amino acid (Fig. 3). This is in agreement with the notion, proposed for endplate channels (2, 33, 36), that divalent cations are not selected according to the same principles as monovalent cations. Interestingly, alignment of the M2 segment from nAChR with glutamate receptors reveals that Leu-254 is homologous to glutamate receptor Gln/Asn position where mutations to arginines alter Ca^{2+} permeability (35, 37) and Mg^{2+} block (38). Parallel observations are made here with the α7 nAChR since mutants

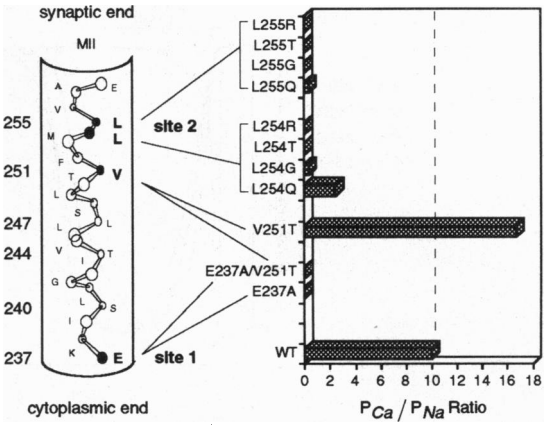

FIG. 3. Effects of the mutations on the Ca^{2+}/Na^+ permeability ratios. (*Left*) M2 segment of the WT receptor is schematized. Amino acids mutated in these experiments are indicated by boldface type. (*Right*) Representation of the P_{Ca}/P_{Na} for the diverse mutants.

with a glutamine at position 254 or 255 exhibit a higher Ca^{2+} permeability than with an arginine (Table 2 and Fig. 3).

It is not clear, at this stage, whether mutations at these two sites affect Ca^{2+} permeability in exactly the same manner. The most widely accepted model (39) posits that the channel lumen contains binding sites to which Ca^{2+} preferentially binds, resulting in its selective transport through the channel. Mutation of such sites would affect Ca^{2+} affinity and thus permeability. In agreement with this model, Ca^{2+}-permeable WT nAChRs exhibit a lower single-channel conductance in the presence of Ca^{2+} ions (6, 20, 40). In an alternative model, the channel is viewed as essentially nonselective for cations but acquires monovalent vs. divalent cation selectivity as a consequence of the introduction of barriers that repel calcium ions. Consistent with this view is the observation that mutations at distinct positions within the channel all alter Ca^{2+} permeation without modifying selectivity for monovalent cations. Also, the pronounced hydrophobic character of the concerned amino acid rings, in the WT, do not seem appropriate for the formation of Ca^{2+} binding sites.

The physiological relevance of Ca^{2+} permeability through neuronal nAChRs has been addressed in several reports. Calcium influx was shown to decrease γ-aminobutyric acid type A receptor responses and activate Ca^{2+}-dependent chloride channels in cell bodies of medial habenular neurons (6). Also, neurons from rat dorsolateral septal nucleus (41) and chicken short hair cells (42) become hyperpolarized by cholinergic agonists through a mechanism involving Ca^{2+} influx and subsequent stimulation of Ca^{2+}-activated K^+ channels. In muscle fibers, Ca^{2+} influx, mainly through voltage-dependent Ca^{2+} channels, was shown to repress transcription of nAChR genes in extrajunctional nuclei, possibly through activation of protein kinase C (43, 44). The low Ca^{2+} permeability of muscle nAChR channel thus prevents a massive Ca^{2+} entry that might interfere with the transcription of nAChR genes at the motor endplate.

Previous studies (14, 23, 27, 28) have revealed that mutations in the channel domain might alter not only ion selectivity but also sensitivity to agonist or antagonist and desensitization of agonist-evoked currents. These functional features were thus investigated with all the Ca^{2+} mutants studied. Two instances were encountered. Mutations at site 1 selectively affect Ca^{2+} permeability without changing other properties of the receptor. This holds for the active "A" state of the WT receptor as well as for the desensitized but conducting D* state of the V251T mutant and indicates that the E237A mutation yields the same phenotype independently of the type of state of the nAChR that is permeable to

ions. In contrast, mutations at site 2 (positions 254 and 255) reduce onset and desensitization rates of permeability responses, increase the apparent affinity for ACh, and alter cooperativity, in addition to affecting Ca^{2+} permeability. Still, the pharmacology of the mutant receptors appears "conventional" since the ACh-evoked currents of L254T and L255T mutants are still blocked by the competitive antagonist DHβE. At least two interpretations may be offered for the functional properties of site 2 mutants, both within the framework of the allosteric scheme (45). A first possibility is that one of the closed states becomes conducting as a consequence of the mutation, the observed changes simply revealing consequences of this new property. Such interpretation was proposed for T244Q, L247T, and V251T mutants (28). For Leu-254 or Leu-255 mutants, the possibility that one of the "fast" desensitized states of the WT becomes conducting might be considered on the basis of ACh sensitivity and kinetics of current onset and desensitization. This conducting state, however, would differ from the previously described D* state (14, 23, 27, 28), which in addition is activated by DHβE. An alternative interpretation is that, although keeping the intrinsic properties of the allosteric states, the mutations alter the coupling between the binding domain for nicotinic ligands and the ion channel and thus affect the access and/or properties of the A state (e.g., by changing the equilibrium constants between liganded open and closed states). Whatever the correct interpretation, our observations point to the possibility that, in agreement with the allosteric scheme, alterations in the apparent affinity for neurotransmitter or rate of current onset and desensitization may result from variations of amino acids from the channel domain and not only from differences in the ligand binding area.

A large diversity of functional properties has been reported for naturally occurring (46) or heterologously expressed (47–50) brain nicotinic, γ-aminobutyric acid type A, or glutamate receptors. Due to its homooligomeric structure, the α7 receptor may not reflect the complex organization and functional diversity of such receptors. Variations in the subunit composition may largely account for such diversity (47, 48). Yet, the wide variety of phenotypes obtained with α7 mutants offers plausible explanations for the occurrence of multiple conductance levels, differences in ion selectivity, in agonist/antagonist sensitivities, or in kinetic properties that are observed with various combinations of cloned subunits of ligand-gated ion channels. The functional properties of defined α7 mutants may thus serve as useful models for the whole superfamily of ligand-gated ion channels, as long as the three-dimensional structures of these molecules share common principles of functional organization.

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1. Bregetovski, P. D., Miledi, R. & Parker, I. (1979) *Nature (London)* **279**, 638–639.
2. Adams, D. J., Dwyer, T. M. & Hille, B. (1980) *J. Gen. Physiol.* **75**, 493–510.
3. Vernino, M. A., Luetje, C. W., Patrick, J. & Dani, J. A. (1992) *Neuron* **8**, 127–134.
4. Nooney, J. M., Peters, J. A. & Lambert, J. J. (1992) *J. Physiol. (London)* **445**, 503–527.
5. Fieber, L. A. & Adams, D. J. (1991) *J. Physiol. (London)* **434**, 215–237.
6. Mulle, C., Choquet, D., Korn, H. & Changeux, J. P. (1992) *Neuron* **8**, 135–143.
7. Vijayaraghavan, S., Pugh, C. P., Zhang, Z.-W., Rathouz, M. M. & Berg, D. K. (1992) *Neuron* **8**, 353–362.
8. Sargent, P. B. (1992) *Annu. Rev. Neurosci.* **16**, 403–443.
9. Sands, S. B. & Barish, M. E. (1991) *Brain Res.* **560**, 38–42.
10. Miledi, R. (1980) *Proc. R. Soc. London Ser. B* **209**, 447–452.
11. Deneris, E. S., Connoly, J., Rogers, S. W. & Duvoisin, R. (1991) *Trends Pharmacol. Sci.* **12**, 34–40.
12. Duvoisin, R. M., Deneris, E. S., Patrick, J. & Heinemann, S. (1989) *Neuron* **3**, 487–496.
13. Couturier, S., Bertrand, D., Matter, J. M., Hernandez, M. C., Bertrand, S., Millar, N., Valera, S., Barkas, T. & Ballivet, M. (1990) *Neuron* **5**, 845–856.
14. Galzi, J. L., Devillers-Thiéry, A., Hussy, N., Bertrand, S., Changeux, J. P. & Bertrand, D. (1992) *Nature (London)* **359**, 500–505.
15. Séguéla, P., Wadiche, J., Dineley-Miller, K., Dani, J. A. & Patrick, J. W. (1993) *J. Neurosci.* **13**, 596–604.
16. Giraudat, J., Dennis, M., Heidmann, T., Chang, J. Y. & Changeux, J. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2719–2723.
17. Hucho, F., Oberthür, W. & Lottspeich, F. (1986) *FEBS Lett.* **205**, 137–142.
18. Révah, F., Galzi, J. L., Giraudat, J., Haumont, P. Y., Lederer, F. & Changeux, J. P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4675–4679.
19. Imoto, K., Methfessel, C., Sakmann, B., Mishina, M., Mori, Y., Konno, T., Fukuda, K., Kurasaki, M., Bujo, H., Fujita, Y. & Numa, S. (1986) *Nature (London)* **324**, 670–674.
20. Imoto, K., Busch, C., Sakmann, B., Mishina, M., Konno, T., Nakai, J., Bujo, H., Mori, Y., Fukuda, K. & Numa, S. (1988) *Nature (London)* **335**, 645–648.
21. Leonard, R. J., Labarca, C. G., Charnet, P., Davidson, N. & Lester, H. A. (1988) *Science* **242**, 1578–1581.
22. Changeux, J. P., Galzi, J. L., Devillers-Thiéry, A. & Bertrand, D. (1992) *Q. Rev. Biophys.* **25**, 395–432.
23. Revah, F., Bertrand, D., Galzi, J. L., Devillers-Thiéry, A., Mulle, C., Hussy, N., Bertrand, S., Ballivet, M. & Changeux, J. P. (1991) *Nature (London)* **353**, 846–849.
24. Konno, T., Busch, C., Von Kitzing, E., Imoto, K., Wang, F., Nakai, J., Mishina, M., Numa, S. & Sakmann, B. (1991) *Proc. R. Soc. London Ser. B* **244**, 69–79.
25. Villarroel, A., Herlitze, S., Koenen, M. & Sakmann, B. (1991) *Proc. R. Soc. London Ser. B* **243**, 69–74.
26. Imoto, K., Konno, T., Nakai, J., Wang, F., Mishina, M. & Numa, S. (1991) *FEBS Lett.* **289**, 193–200.
27. Bertrand, D., Devillers-Thiéry, A., Revah, F., Galzi, J. L., Hussy, N., Mulle, C., Bertrand, S., Ballivet, M. & Changeux, J. P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1261–1265.
28. Devillers-Thiéry, A., Galzi, J. L., Bertrand, S., Changeux, J. P. & Bertrand, D. (1992) *NeuroReport* **3**, 1001–1004.
29. Bertrand, D., Cooper, E., Valera, S., Rungger, D. & Ballivet, M. (1991) *Methods Neurosci.* **4**, 174–193.
30. Mayer, M. L. & Westbrook, G. L. (1987) *J. Physiol. (London)* **394**, 501–527.
31. Egebjerg, J. & Heinemann, S. F. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 755–759.
32. Miledi, R. & Parker, I. (1984) *J. Physiol. (London)* **357**, 173–183.
33. Lewis, C. A. & Stevens, C. F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6110–6113.
34. Cohen, B. N., Labarca, C., Czyzyk, L., Davidson, N. & Lester, H. A. (1992) *J. Gen. Physiol.* **99**, 545–572.
35. Gasic, G. P. & Heinemann, S. (1992) *Curr. Opin. Cell Biol.* **4**, 670–677.
36. Dani, J. A. & Eisenmann, G. (1987) *J. Gen. Physiol.* **89**, 959–983.
37. Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J. P., Günther, W., Seeburg, P. H. & Sakmann, B. (1992) *Science* **257**, 1415–1419.
38. Mori, H., Masaki, H., Yamakura, T. & Mishina, M. (1992) *Nature (London)* **358**, 673–675.
39. Hess, P. & Tsien, R. W. (1984) *Nature (London)* **309**, 453–456.
40. Lewis, C. A. (1979) *J. Physiol. (London)* **286**, 417–445.
41. Wong, L. A. & Gallagher, J. P. (1991) *J. Physiol. (London)* **436**, 325–346.
42. Fuchs, P. A. & Murrow, B. W. (1992) *J. Neurosci.* **12**, 800–809.
43. Klarsfeld, A., Laufer, R., Fontaine, B., Devillers-Thiéry, A., Dubreuil, C. & Changeux, J. P. (1989) *Neuron* **2**, 1229–1236.
44. Huang, C. F., Tong, J. & Schmidt, J. (1992) *Neuron* **9**, 671–678.
45. Monod, J., Wyman, J. & Changeux, J. P. (1965) *J. Mol. Biol.* **12**, 88–118.
46. Mulle, C., Vidal, C., Benoit, P. & Changeux, J. P. (1991) *J. Neurosci.* **11**, 2588–2597.
47. Luetje, C. W. & Patrick, J. (1991) *J. Neurosci.* **11**, 837–845.
48. Couturier, S., Erkman, L., Valera, S., Rungger, D., Bertrand, S., Boulter, J., Ballivet, M. & Bertrand, D. (1990) *J. Biol. Chem.* **265**, 17560–17567.
49. Sigel, E., Baur, R., Trube, G., Möhler, H. & Malherbe, P. (1990) *Neuron* **5**, 703–711.
50. Nakanishi, S. (1992) *Science* **258**, 597–603.