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Gain of function mutation of the α7 nicotinic receptor: distinct pharmacology of the human α7V274T variant

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Abstract

In the human α7 nicotinic receptor, valine-274 in the pore-lining transmembrane-2 region was mutated to threonine to produce the variant human α7V274T, which was evaluated electrophysiologically following expression in *Xenopus laevis* oocytes. Inward current rectification was strong in human α7V274T as in the human α7 wild type nicotinic receptor. However, human α7V274T was 100-fold more sensitive to the nicotinic receptor agonists acetylcholine, (−)-nicotine and 1,1-dimethyl-4-phenylpiperazinium. Choline also activated human α7V274T (EC_{50} = 12 μM) and was 82-fold more potent than at human α7 wild type nicotinic receptor. (−)-Cotinine, (2,4)-dimethoxybenzylidene anabaseine (GTS-21) and 2-methyl-3-(2-(*S*)-pyrrolidinylmethoxy)pyridine (ABT-089), weak partial agonists at human α7 wild type, were much stronger agonists at human α7V274T with EC_{50} values of 70 μM, 4 μM and 28 μM and fractional activation values of 93%, 96% and 40%, respectively. However, (−)-lobeline, a human α7 wild type nicotinic receptor antagonist, and dihydro-β-erythroidine, which activates chick mutagenized α7 nicotinic receptors, had only weak agonist-like activity at human α7V274T (≤ 4% of the maximal acetylcholine response). Methyllycaconitine, mecamylamine, *d*-tubocurarine and dihydro-β-erythroidine retained antagonist activity and, indeed, appeared to be at least as potent at human α7V274T as at human α7 wild type. These results support and extend the concept that human nicotinic receptor pharmacology can be profoundly altered by single amino acid changes in the pore-lining segment.

Keywords: Nicotinic receptor; (Human); Oocyte, *Xenopus laevis*; Dihydro-β-erythroidine; Methyllycaconitine; Mecamylamine

1. Introduction

Although the physiological roles of many nicotinic acetylcholine receptors in the central nervous system remain to be elucidated, variants and spontaneous mutations of some nicotinic receptor have been identified and in some cases related to specific syndromes. In addition to mutations in neuromuscular nicotinic receptor subunits linked to slow channel myasthenic syndrome (Engel et al., 1998), these include mutations in the M2 region of the α4 nicotinic receptor subunit linked to instances of autosomal dominant nocturnal frontal lobe epilepsy (Kuryatov et al., 1997; Steinlein et al., 1997), and possible polymorphism in the α7 nicotinic receptor related to schizophrenia (Freedman et al., 1997). Other examples among ligand-gated ion channels (Vafa and Schofield, 1998) include mutation of the glycine receptor associated with human startle disease (Lynch et al., 1997) and mutation of the GABA receptor associated with resistance to insecticides and other channel blockers (Zhang et al., 1994; Pan et al., 1997).

With regard to α7 nicotinic receptor in particular, site-directed mutagenesis studies have identified several amino acid residues in the putative pore-lining second transmembrane region (M2) of the chick nicotinic receptor variants which appear to be involved in the rapid form of desensitization (Revah et al., 1991; Devillers-Thiéry et al., 1992). As a result, certain wild-type nicotinic receptor antagonists acted like agonists at the chick mutant nicotinic receptor, suggesting that some antagonists and partial agonists may act by stabilizing the desensitized state without first activating the channel (Palma et al., 1997). However, this has

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been based upon studies of the chick α7 nicotinic receptor, which is pharmacologically similar but not identical to mammalian α7 nicotinic receptor (Peng et al., 1994). Furthermore, while mutagenesis of the chick α7 generally reduced the rate of desensitization, similar mutagenesis of the 5-HT_3 M2 segment increased or decreased the rate of desensitization depending upon the amino acid substitution (Yakel et al., 1993). Thus, it is important to know whether the results with chick α7 nicotinic receptor generalize to other species and to other nicotinic receptor subunits.

The present report describes a human α7 nicotinic receptor in which valine-274 was changed to threonine (human α7V274T), homologous to the chick α7V251T mutant, also referred to as 'α7-4' (Galzi et al., 1992). The present focus is on the effects of this mutation on nicotinic receptor pharmacology, including several classic nicotinic receptor agonists and antagonists which were not explored with the chick nicotinic receptor: the selective α7 activator choline (Papke et al., 1996), the partial agonists (−)-cotinine, GTS-21 and ABT-089, and the mixed-function nicotinic receptor agonist/α7 antagonist (−)-lobeline (Briggs and McKenna, 1998). Portions of these experiments have been published in abstract form (McKenna et al., 1997).

2. Methods and materials

2.1. Human α7 wild-type cDNA

The human α7 nicotinic receptor cDNA (Doucette-Stamm et al., 1993) was modified to include the complete human signal peptide (MRCSPGGVWLALAASL-LHVSLQGEF) as reported by Elliott et al. (1993). The following oligonucleotide was synthesized: 5′GG-GGCAGCA *CTCGAG* CCC **ATG** AGGTGTAGCCCCGG-GGAGTGTGGCTGGCACTGGCAGCATCTCTCCTGC-ACGTGTCCCTGCAAGGCGAGTTCCAGAGG-AAGCTTTACAAGGAGGGG-3′. This oligonucleotide contains an *Xho*I restriction site (italics) and an ATG initiation codon (bold) followed by the next 28 codons of the human α7 nicotinic receptor cDNA sequence. It encodes the complete signal peptide and extends to the *Hin*dIII site (underlined) present in the α7 nicotinic receptor cDNA. The reverse complement of this oligonucleotide also was synthesized and the oligonucleotides were annealed together, digested with *Xho*I and *Hin*dIII, and then ligated into a pBluescript vector containing the human α7 cDNA previously digested with *Xho*I and *Hin*dIII. This created a new cDNA encoding a full length human α7 nicotinic receptor. The sequence of the new cDNA was confirmed by dideoxy sequencing. The cDNA was excised from pBluescript with *Xho*I and *Not*I, the 5′ overhangs were filled-in with Klenow polymerase, linked with *Bst*XI adapters, digested with *Bst*XI, and ligated into the *Bst*XI site of the pRcCMV vector (Invitrogen). The orientation of the insert in the expression vector was determined by restriction analysis with enzymes cutting the α7 nicotinic receptor cDNA at asymmetrical positions.

2.2. Human α7V274T mutant cDNA

Valine-274 was changed to threonine in the human α7 wild-type cDNA described above using site-directed mutagenesis (Ho et al., 1989). The strategy used two polymerase chain reaction (PCR) steps followed by restriction digest and subcloning the mutated fragment into the wild type human α7 cDNA. In the first step, PCR was used to introduce the mutation in both DNA strands. The longer 5′ fragment was generated using the forward external primer 5′-GTTTGGGTCCTGGTCTTACG-3′ and the reverse internal primer 5′-GCAGCATGAAGGTGGTAAGAGAG-3′ bearing the mutation. The shorter 3′ fragment was generated using the forward internal primer 5′-CTCTCTTAC-CACCTTCATGCTGC-3′, also bearing the mutation, and the reverse external primer 5′-GTACTGCAGCACGAT-CACCG-3′. The conditions for PCR consisted of 100 ng input α7 DNA, 2 × Pfu buffer, 100 ng of each primer pair and 0.625 U Pfu enzyme (Stratagene, La Jolla, CA). Reactions were carried out in a Perkin-Elmer 9600 for 20 cycles at 95°C for 24 s, 60°C for 22 s then 72°C for 78 s. The products were combined with the external primers in the second PCR step, reamplifying the sequence and generating a longer product which included the mutation. This was digested with *Kpn*I and *Eco*RV, gel-purified, and ligated into the wild type human α7 cDNA previously digested with *Kpn*I and *Eco*RV. Dideoxy sequencing of the final cDNA showed the presence of the desired mutation and that no other mutation had been introduced during the PCR process.

2.3. Expression and measurement in Xenopus oocytes

The preparation of *Xenopus laevis* oocytes, injection with RNA or DNA, and measurement of α7 nicotinic receptor responses using two-electrode voltage-clamp followed procedures described previously for the wild-type human α7 nicotinic receptor (Briggs et al., 1995; Briggs and McKenna, 1998) except that atropine was not routinely present in the bathing solution. Oocytes were maintained at 17–18°C in normal Barth's solution (90 mM NaCl, 1 mM KCl, 0.66 mM $NaNO_3$, 0.74 mM $CaCl_2$, 0.82 mM $MgCl_2$, 2.4 mM $NaHCO_3$, 2.5 mM sodium pyruvate, and 10 mM Na–HEPES buffer, final pH 7.55) containing 100 μg/ml gentamicin. Responses were measured at a holding potential of −60 mV in modified Barth's solution containing 10 mM $BaCl_2$ and lacking $CaCl_2$ and $MgCl_2$ to prevent activation of Ca^{2+}-dependent secondary responses. In some experiments, as indicated, responses were measured in OR-2 solution (82.5 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 5 mM Na–HEPES buffer, final pH 7.4) containing 0.5 μM

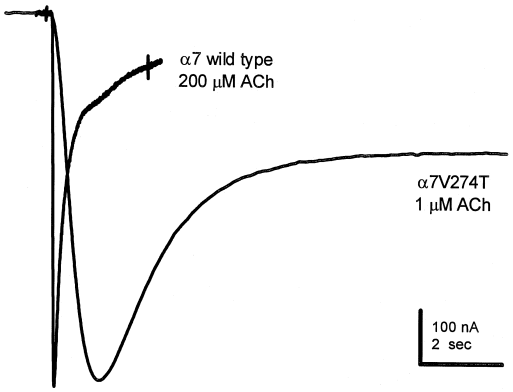

Fig. 1. Activation and decay rate of human α7V274T compared to wild-type human α7 wild type nicotinic receptor. Human α7V274T and human α7 wild type responses to approximate EC_{50} concentrations of acetylcholine (1 μM and 200 μM, respectively) were matched for similar amplitude and are shown synchronized to the beginning of acetylcholine application and adjusted for equivalent baseline holding current. Acetylcholine was applied to human α7V274T for 10 s and to human α7 wild type for 2.5 s. Brief spike-like tics near the beginning and end of the human α7 wild type trace are electrical artifacts marking the opening and closing of the agonist application valve. Calibration lines indicate 100 nA and 1 s for both responses.

atropine (Galzi et al., 1992). Agonist was applied briefly using a computer-controlled solenoid valve and a push/pull applicator positioned to within 200–400 μM from the oocyte and responses were recorded by computer in synchrony with agonist application. Responses were quantified by measuring the amplitude of the current and were normalized to the maximal response to acetylcholine (10 μM for human α7V274T, 10 mM for human α7 wild type) determined in the same oocyte before and after the experimental trials.

2.4. Materials

Acetylcholine chloride, 3-aminobenzoic acid ethyl ester methanesulfonate (tricaine), atropine sulfate, choline chloride, collagenase Type 1A, (−)-cotinine (no salt), 1,1-dimethyl-4-phenylpiperazinium (DMPP) iodide, gentamicin, (−)-lobeline hydrochloride, mecamylamine hydrochloride, (−)-nicotine tartrate and *d*-tubocurarine chloride were obtained from Sigma (St. Louis, MO, USA). Dihydro-β-erythroidine hydrobromide and methyllycaconitine citrate were obtained from Research Biochemicals International (Natick, MA, USA). 2-Methyl-3-(2-(*S*)-pyrrolidinylmethoxy)pyridine (ABT-089) was synthesized at Abbott Laboratories (Lin et al., 1997). (2,4)-Dimethoxybenzylidene anabaseine dihydrochloride (GTS-21) also was synthesized at Abbott Laboratories, according to the methods described by Zoltewicz et al. (1993). GTS-21 was prepared as the dihydrochloride and was analyzed by nuclear magnetic resonance (NMR), mass spectrometry, and elemental analysis.

3. Results

Human α7V274T responses activated and decayed slowly compared to the human α7 wild type responses, as with chick nicotinic receptor (Galzi et al., 1992). An example is shown in Fig. 1, where the human α7V274T nicotinic receptor response to 1 μM acetylcholine is compared to the human α7 wild type nicotinic receptor response to 200 μM acetylcholine. At the higher agonist concentrations, human α7V274T responses decayed in the continued presence of agonist, albeit more slowly than human α7 wild type. This may be due to receptor desensitization, blockade of the channel by the agonist, or some other inhibitory process.

Human α7 wild type nicotinic receptor, like other nicotinic receptors, exhibits strong inward current rectification (Briggs et al., 1995). Similarly, human α7V274T rectified strongly in the presence of 10 mM Ba^{2+} or in a Ca^{2+}-containing solution (Fig. 2).

Agonist concentration–response curves for human α7V274T and human α7 wild type are shown in Fig. 3, and the parameters extracted from fitting the Hill equation to the data are given in Table 1. Acetylcholine, (−)-nicotine and 1,1-dimethyl-4-phenylpiperazinium (DMPP) were two orders of magnitude more potent at human α7V274T than at human α7 wild type, and the EC_{50} values at human α7V274T were similar, within a factor of about 2, to the desensitization IC_{50} values at human α7 wild type (Briggs and McKenna, 1998). The Hill coefficients for acetylcholine and DMPP at human α7V274T (1.8 and 2.9, respectively) were higher than at human α7 wild type (0.9

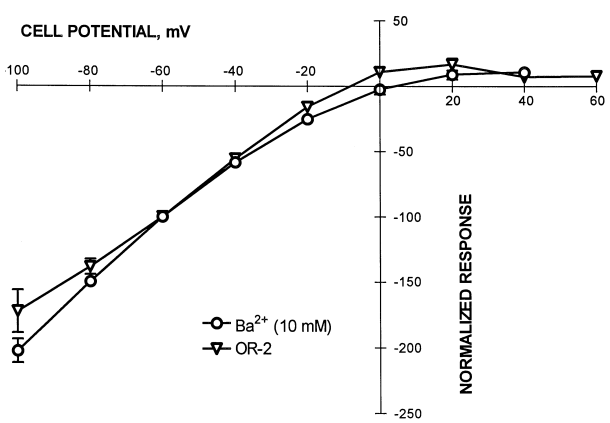

Fig. 2. Inward current rectification of the human α7V274T. Responses to 10 μM acetylcholine were measured at various cell potentials in modified Barth's solution containing 10 mM $BaCl_2$ ($n = 4$) or in OR-2 solution ($n = 3$) containing 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 0.5 μM atropine (Galzi et al., 1992). The cell potential was set before acetylcholine was applied and the responses at the various cell potentials were normalized to the response at −60 mV in each oocyte. Inward currents at −60 mV in 10 mM Ba^{2+} Barth's were −178, −999, −1520 and −1300 nA; in OR-2 they were −487, −497 and −1864 nA. Data are shown as mean ± S.E.M.

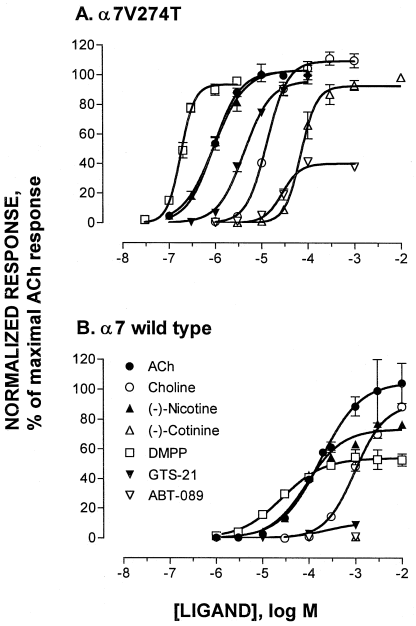

Fig. 3. Human α7V274T and human α7 wild type agonist concentration–response relationships. Responses were normalized to the maximal response to acetylcholine (10 μM acetylcholine for human α7V274T, 10 mM acetylcholine for human α7 wild type) in each oocyte. The curves show the Hill equation fitted to the data. Values for (−)-cotinine are shown for human α7V274T, but not for human α7 wild type. All concentration–response parameters and the number of oocytes used for each curve are presented in Table 1. Data for most of the human α7 wild type responses, except choline, were published previously (Briggs et al., 1995; Briggs and McKenna, 1996, 1998) and are shown here for comparison with human α7V274T; human α7 wild type data for GTS-21 and ABT-089 were recomputed relative to acetylcholine.

and 1.0, respectively). However, the Hill coefficients for (−)-nicotine were similar at the two receptors. Choline also acted as an agonist at both receptors. Its EC_{50} value at human α7 wild type was 982 μM, but at human α7V274T the EC_{50} for choline was 12 μM.

Human α7 wild type is inhibited by desensitization following exposure to low concentrations of acetylcholine or other agonists sufficient to elicit a response of only about 1% (Briggs and McKenna, 1998). In contrast, human α7V274T did not appear to be desensitized following several minutes exposure to 0.3 μM acetylcholine, which itself elicited a 6% ± 1% ($n = 4$) response when applied as an agonist. The response to 1 μM acetylcholine applied on top of 0.3 μM acetylcholine was 95% ± 5% ($n = 4$) of the control response to 1 μM acetylcholine applied alone. These results are consistent with the idea, developed from chick α7 nicotinic receptor (Bertrand and Changeux, 1995), that the V274T mutation generates a non-desensitizing nicotinic receptor, or one in which the channel is conducting in the receptor-desensitized state as well as the activated state.

Partial agonists were also evaluated at human α7V274T (Fig. 3 and Table 1). DMPP itself acted as a partial agonist (54%) at human α7 wild type, but was a full agonist (94%) at human α7V274T. GTS-21, ABT-089 and (−)-cotinine, the main metabolite of (−)-nicotine in man (Benowitz, 1996), were weak partial agonists at human α7 wild type with fractional activation values of 1–10% at a concentration of 1000 μM. At human α7V274T, however, their activities were dramatically increased. (−)-Cotinine and GTS-21 acted as full agonists at human α7V274T with fractional activation values of 93% and 96%, and EC_{50} values of 70 μM and 4.3 μM, respectively. ABT-089 was a partial agonist at human α7V274T, but with ~40-fold greater fractional activation than at human α7 wild type and an EC_{50} value of 28 μM.

(−)-Lobeline, found to be an antagonist at human α7 wild type with an IC_{50} value of 8.5 μM (Briggs and

Table 1
Comparison of agonist concentration–response parameters at human α7V274T mutant and α7 wild-type nicotinic receptors

	Human α7 wild type				Human α7V274T			
	EC_{50} (μM)	n_H	Fractional activation (%)	N	EC_{50} (μM)	n_H	Fractional activation (%)	N
Acetylcholine	177 ± 32	1.0 ± 0.2	105 ± 6	9	1.02 ± 0.04	1.8 ± 0.2	101 ± 1	16
(−)-Nicotine	91 ± 13	1.1 ± 0.2	74 ± 3	6	0.94 ± 0.12	1.3 ± 0.2	103 ± 4	4
DMPP	26 ± 4	1.0 ± 0.1	54 ± 2	6	0.18 ± 0.008	2.9 ± 0.3	94 ± 2	7
Choline	982 ± 88	1.3 ± 0.1	90 ± 3	8	12 ± 0.9	1.9 ± 0.2	109 ± 2	7
(−)-Cotinine			1.0 ± 0.3[a]	3	70 ± 6	2.5 ± 0.4	93 ± 3	5
GTS-21			8.9 ± 0.7[a]	3	4.3 ± 0.3	1.5 ± 0.1	96 ± 2	6
ABT-089			1.1 ± 0.1[a]	9	28 ± 3	2.3 ± 0.4	40 ± 1	5
(−)-Lobeline			0.15 ± 0.07[a]	6			3.0 ± 0.3[b]	3

All data are normalized to the response to acetylcholine at a maximal concentration (10 mM for human α7 wild type, 10 μM for human α7V274T) and are shown as mean ± S.E.M.

Human α7 wild type results for all compounds except choline were previously published (Briggs et al., 1995; Briggs and McKenna, 1996, 1998) and are reproduced here for comparison; human α7 wild type data for GTS-21 and ABT-089 were recomputed relative to acetylcholine.

[a]The fractional activation values for (−)-cotinine, GTS-21, ABT-089 and (−)-lobeline at human α7 wild type nicotinic receptor reflect response amplitudes measured at 1000 μM; the concentration–response curve parameters could not be determined.

[b]The fractional activation value for (−)-lobeline at human α7V274T reflects the response at a concentration of 30 μM. Responses to < 10 μM and ≥ 100 μM (−)-lobeline were smaller.

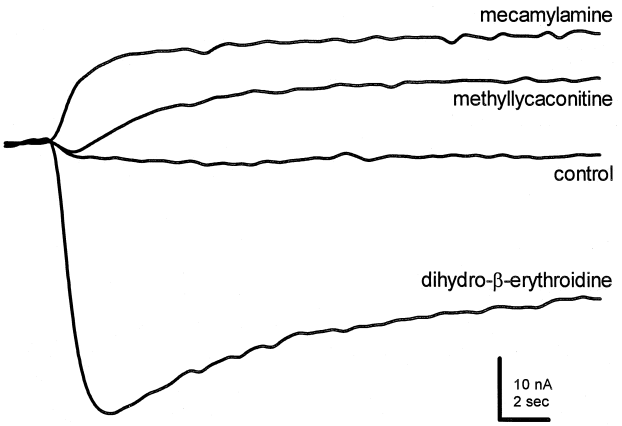

Fig. 4. Human α7V274T responses to nicotinic receptor antagonists applied as agonists. Traces, all from one oocyte, compare responses to mecamylamine (10 μM), methyllycaconitine (10 nM), dihydro-β-erythroidine (10 μM) and bathing solution (control) applied for 20 s each. The small no-agonist control responses, observed in human α7V274T but not human α7 wild type oocytes, were subtracted from agonist responses when data were tabulated. Calibration lines represent 10 nA and 2 s for all traces.

McKenna, 1998), had little effect at human α7V274T, eliciting only a 3% response at a concentration of 30 μM (Table 1).

At human α7V274T, mecamylamine (10 μM) and the α7-selective antagonist methyllycaconitine (10 nM) themselves consistently elicited small outward currents ranging in amplitude from 0.9% to 12.4% of the maximal inward current response to acetylcholine (Fig. 4 and Table 2). This is opposite in direction to the inward current elicited by agonists and likely is due to inhibition of spontaneously active channels, as has been observed with chick mutant α7 nicotinic receptors (Bertrand et al., 1992; Bertrand et al., 1997). Unlike the chick mutants, however, the nicotinic receptor antagonist dihydro-β-erythroidine (10 μM) activated only very small agonist-like inward current responses at human α7V274T (Fig. 4), ranging from 2.8% to 6.9% of the response to 10 μM acetylcholine (Table 2), and *d*-tubocurarine (1 μM) did not elicit agonist-like inward currents but did elicit small outward currents (3–5% of the maximal inward current response to acetylcholine) in two of four human α7V274T oocytes.

At the wild-type human α7 nicotinic receptor under similar conditions, neither dihydro-β-erythroidine (10 μM), methyllycaconitine (10 nM), mecamylamine (10 μM) nor *d*-tubocurarine (1 μM) elicited any significant inward or outward current response (Table 2). The muscarinic receptor antagonist atropine (2 μM) applied as an agonist had little effect at either nicotinic receptor.

Additionally, the antagonism of the response to acetylcholine at both human α7V274T and human α7 wild type was evaluated using two concentrations of acetylcholine: one near the EC_{50} value (1 μM for human α7V274T and 200 μM for human α7 wild type) and one near the maximal response level (10 μM for human α7V274T and 10 mM for human α7 wild type). Data are shown in Table 2. Dihydro-β-erythroidine, *d*-tubocurarine, methyllycaconitine and mecamylamine acted as antagonists at both nicotinic receptors. Methyllycaconitine was most potent, blocking human α7V274T as well as human α7 wild type at a concentration of 10 nM. Surprisingly, however, mecamylamine (10 μM), dihydro-β-erythroidine (10 μM) and *d*-tubocurarine (1 μM) each appeared to inhibit the mutant human α7V274T more strongly than human α7 wild type, an effect not anticipated simply from the theory of a conducting desensitized state.

Atropine (2 μM) inhibited the human α7V274T response to 1 μM acetylcholine by 28%, but had little effect on the human α7 wild type response to 200 μM acetylcholine. This inhibition by atropine did not appear to be due to an endogenous muscarinic component (Dascal and

Table 2
Effects of cholinergic antagonists at human α7V274T mutant and α7 wild-type nicotinic receptors

Receptor	Ligand	Activation[a]	% Inhibition	
		% of 10 μM acetylcholine	1 μM Acetylcholine	10 μM Acetylcholine
α7V274T	dihydro-β-erythroidine (10 μM)	4 ± 1 (4)[b]	69 ± 5 (4)[c]	52 ± 6 (4)[c]
	d-tubocurarine (1 μM)	−2 ± 1 (4)	99 ± 1 (4)[c]	97 ± 3 (3)[c]
	methyllycaconitine (10 nM)	−4 ± 2 (7)[b]	103 ± 1 (5)[c]	95 ± 3 (7)[c]
	mecamylamine (10 μM)	−1.9 ± 0.2 (4)[c]	101 ± 1 (4)[c]	53 ± 2 (4)[c]
	atropine (2 μM)	0.1 ± 0.1 (4)	28 ± 7 (5)[b]	13 ± 5 (6)[b]
		% of 10 mM acetylcholine	200 μM Acetylcholine	10 mM Acetylcholine
α7 Wild type	dihydro-β-erythroidine (10 μM)	−0.2 ± 0.1 (5)	41 ± 10 (4)[b]	23 ± 2 (4)[c]
	d-tubocurarine (1 μM)	−0.1 ± 0.1 (5)	28 ± 2 (4)[c]	25 ± 3 (4)[c]
	methyllycaconitine (10 nM)	−0.2 ± 0.4 (3)	100 ± 0.5 (4)[c]	99 ± 0.4 (4)[c]
	mecamylamine (10 μM)	−0.3 ± 0.2 (3)	82 ± 1 (3)[c]	85 ± 3 (3)[c]
	atropine (2 μM)	0.2 ± 0.5 (3)	4 ± 3 (3)	12 ± 3 (3)[b]

Data are shown as mean ± S.E.M. (*n*).
[a]Positive values for % activation indicate inward current (normalized to acetylcholine), negative values indicate outward current.
[b]$P < 0.05$ compared to 0 (Student's two-tailed *t*-test).
[c]$P < 0.005$ compared to 0.

Landau, 1980) because the human α7V274T response was completely blocked by the nicotinic receptor antagonist mecamylamine (10 μM) in three oocytes where the response was partially inhibited by atropine.

4. Discussion

While mutations in several regions of the nicotinic receptor may alter channel gating (Croxen et al., 1997; Ortiz-Miranda et al., 1997; Wang et al., 1997; Zhang and Karlin, 1997; Bouzat et al., 1998), the putative transmembrane segment M2 generally is accepted as the pore-lining region of the complex (Labarca et al., 1995; Unwin, 1998). Mutagenesis studies of neuronal nicotinic receptors have focused upon chick α7 nicotinic receptor (Bertrand and Changeux, 1995) and recently human α4 (Weiland et al., 1996; Kuryatov et al., 1997). However, chick and mammalian α7 nicotinic receptors are known to differ markedly in their activation by DMPP (Peng et al., 1994), and GTS-21 appears to be a weaker partial agonist at human than at rat α7 nicotinic receptor (Briggs et al., 1995; De Fiebre et al., 1995). Thus, the potential that the pharmacological effects of homologous nicotinic receptor mutations may differ among species needs to be considered.

Human α7V274T, like the homologous chick α7V251T, was slowly- or non-desensitizing; the response decay observed in the continued presence of agonist may have been due to channel block. Human α7V274T also was about two orders of magnitude more sensitive to acetylcholine than human α7 wild type, again similar to the corresponding chick α7 nicotinic receptors. However, other α7 nicotinic receptor agonists were not evaluated at chick mutant α7 nicotinic receptors. (−)-Nicotine, DMPP and choline, like acetylcholine, were about two orders of magnitude more potent at human α7V274T than at human α7 wild type, demonstrating that this enhanced potency was not peculiar to acetylcholine but could be generalized to other α7 nicotinic receptor agonists. Indeed, the EC_{50} values for acetylcholine, (−)-nicotine and DMPP at human α7V274T were similar to their desensitization IC_{50} values at human α7 wild type (Briggs and McKenna, 1998). These observations are consistent with the idea that human α7V274T may be non-desensitizing or may have a conducting channel in the receptor-desensitized state. Single-channel analysis would be required to evaluate this further.

Human α7V274T, like human α7 wild type (Briggs et al., 1995), exhibited strong inward current rectification in Ba^{2+}- or Ca^{2+}-containing media when the cell potential was changed before application of acetylcholine. Under similar conditions, chick α7V251T, unlike chick α7 wild type, did not appear to rectify (Galzi et al., 1992). Although Ca^{2+} influx through α7 nicotinic receptor can activate Ca^{2+}-dependent Cl^- channels, the overall response would be expected to remain inwardly rectifying when the cell potential is changed before nicotinic receptor activation, as in the present study and in Galzi et al. (1992), because the Ca^{2+}-dependent Cl^- current is secondary to the nicotinic receptor current. Additionally, chick α7V251T exhibited little rectification even in the presence of external isethionate and internal BAPTA to block Ca^{2+}-dependent Cl^- currents (Galzi et al., 1992). This would suggest the possibility of a difference between human α7V274T and chick α7V251T in their rectification properties. However, chick α7V251T rectification has been observed under a different voltage protocol in which the cell potential was changed after peak nicotinic receptor activation by acetylcholine (Forster and Bertrand, 1995). A possible explanation, requiring further evaluation, is that rectification may depend upon the allosteric state and that the population of allosteric states may change as the response evolves, leading changes in rectification during the response.

The nicotinic receptor antagonists methyllycaconitine and mecamylamine consistently elicited small outward currents in oocytes expressing human α7V274T but not those expressing human α7 wild type. These outward currents likely were due to inhibition of open human α7V274T, as with related chick mutant α7 nicotinic receptors (Bertrand et al., 1997). The mutant α7 nicotinic receptor may produce a relatively large current in the absence of exogenous agonist due to an increase in the number of spontaneous openings compared to the wild-type α7 nicotinic receptor, due to an ability of the mutant but not the wild type α7 nicotinic receptor to conduct current upon spontaneous transitions to the desensitized state, or due to an endogenous release of choline sufficient to activate the mutant nicotinic receptor but not the less sensitive wild-type α7 nicotinic receptor.

Other nicotinic receptor antagonists such as dihydro-β-erythroidine, *d*-tubocurarine and hexamethonium have been reported to act as agonists at non-desensitizing chick α7 mutant nicotinic receptors such as α7V251T and α7L247T, possibly because these ligands stabilize a desensitized state that is conducting in the mutants an non-conducting in wild-type (Bertrand et al., 1992; Devillers-Thiéry et al., 1992). At human α7V274T, however, dihydro-β-erythroidine was only a very weak agonist with a 4% response at 10 μM, and *d*-tubocurarine did not activate inward currents at the human α7V274T in contrast to the full agonist-like response at chick α7L247T nicotinic receptor. Furthermore, these ligands functioned as antagonists at the human α7V274T variant and appeared to be at least as potent at human α7V274T as at human α7 wild type. A similar effect on antagonist potency has not been described using chick nicotinic receptor.

Thus, this mutation of the human α7 nicotinic receptor produced effects that were both similar and unexpectedly different from models (Edelstein and Changeux, 1996) based upon chick α7 nicotinic receptor. These differences

may relate to species differences in the effects of these mutations on α7 nicotinic receptor function, or possibly to species differences in the interaction between ligand and receptor. Further evaluation of human α7 mutant nicotinic receptors and comparison of the effects of specific mutations across different species should help clarify nicotinic receptor structure, function and pharmacology.

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