0026-895X/99/010001-07$3.00/0

MOLECULAR PHARMACOLOGY, **55:**1–7 (1999).

Identification of a New Amino Acid Residue Capable of Modulating Agonist Efficacy at the Homomeric Nicotinic Acetylcholine Receptor, $\alpha 7$

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Received May 26, 1998; accepted October 19, 1998

This paper is available online at http://www.molpharm.org

ABSTRACT

Neuronal nicotinic receptors (nAChRs) have been implicated in pathology associated with neurological diseases and aberrant cognitive states such as addiction and schizophrenia. The design of subtype-specific cholinergic drugs is dependent on identification of key amino acids that play a significant role in determining subunit-specific agonist efficacy. 1,1-Dimethyl-4-phenylpiperazinium (DMPP) and a series of piperazium (PIP)-derived cholinergic agonists (1,1 dimethyl-4-acetylpiperizinium iodide, EthylPIP, PropylPIP, and ButylPIP) were used to identify a site (position 84) in homomeric neuronal nAChRs, which is a partial determinant of pharmacological specificity. In contrast to absolutely conserved amino acids within the nicotinic superfamily, the amino acid in position 84 can be polar or nonpolar. The addition of one methylene to PropylPIP to form ButylPIP eliminated channel activation of but not binding to the chick $\alpha 7$ homomeric nAChR (leucine in position 84). In rat $\alpha 7$ (glutamine in position 84), ButylPIP was an agonist. 1,1-Dimethyl-4-phenylpiperazinium, a structural analog of ButylPIP, activates the rat $\alpha 7$ but is a weak partial agonist of the chick $\alpha 7$. Mutation of the chick $\alpha 7$ (L84Q) restored activation by ButylPIP, and the corresponding mutation in rat $\alpha 7$ (Q84L) abolished activation by ButylPIP. These mutations had moderate effects on the apparent affinity for acetylcholine, increasing its affinity for chick $\alpha 7$ and decreasing it for rat $\alpha 7$. Thus, the amino acid in position 84 (a residue on the periphery of the highly conserved loop A of the cys-loop superfamily of receptors) can potentially be exploited to produce subtype-specific drugs and can provide insights into the structure of the binding domain.

Nicotinic acetylcholine receptors (nAChR) are ligand-gated cation channels found at the vertebrate neuromuscular junction, nerve cell membranes, and electroplaque of the electric fish. The skeletal muscle and the electroplaque receptor is composed of four different subunits ($\alpha_2\beta\gamma\delta$) arranged in a rosette forming an integral membrane ion channel (Unwin, 1993). Neuronal nAChRs are pentameric receptors consisting of a homomeric array of one type of subunit ($\alpha 7$, $\alpha 8$, or $\alpha 9$) or a heteromeric array of two different subunits (e.g., $\alpha 2\beta 4$, $\alpha 3\beta 2$). Sequence comparisons suggest that the homomeric nAChR forms are ancestral to the nicotinic family (Ortells and Lunt, 1995). Although a variety of subtypes are present, the two most abundant neuronal nAChRs in vertebrate brain are receptors containing the $\alpha 7$ subunit and the $\alpha 4\beta 2$ receptor (Galzi and Changeux, 1996). Because of the importance of these receptors in a variety of normal (Wonnacott, 1997) and pathological processes (Lindstrom, 1997), as well as in the effects of drugs of abuse (Gotti et al., 1997), the development of subtype-specific cholinergic agents is of considerable interest.

Although pharmacological differences between skeletal muscle and ganglionic nAChRs have been long known (Taylor, 1996), the therapeutic potential of exploiting differences in neuronal nAChRs was recognized following the identification of the different neuronal subtypes (Sargent, 1993) and the finding that nicotinic receptors are important in a number of pathological processes (Lindstrom, 1997). The specific combination of α and β subunits can affect the affinity for a particular agonist. For example, the efficacy of cytisine is dictated almost exclusively by the type of β subunit present in a receptor oligomer (Papke et al., 1991). In the case of the homomeric $\alpha 7$, $\alpha 8$, and $\alpha 9$ receptors, pharmacological differences between these subtypes and between versions of these receptors in different species can be used to identify regions of the receptors involved in pharmacological specificity. For example, the rat and chick $\alpha 7$ nAChR, which are more than 87% identical in primary structure, exhibit dramatically different efficacy for the cholinergic agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP). Thus, these pharmacological differences between highly homologous subunits can be used to

This work was supported by National Institutes of Health Grant R01 NS18660 (to R.E.O.) and predoctoral Grant F31 DC00151 and training Grant T32 GM8210 (to R.W.V.).

ABBREVIATIONS: nAChRs, neuronal nicotinic receptors; DMPP, 1,1-dimethyl-4-phenylpiperazinium; PIP, piperazinium; HPIP, 1,1 dimethyl-4-acetylpiperizinium iodide.

determine both the portions of the receptor that control affinity and/or efficacy to specific agonists. In particular, a combination of site-directed mutagenesis with structure-activity relationships of cholinergic agonists has the promise of producing highly specific drugs for particular subtypes.

The optimal geometric requirements for an agonist of the nAChR are a cationic head (e.g., a quaternary ammonium group) and a hydrogen bond acceptor (e.g., a carbonyl oxygen) separated by 5.9 Å (Beers and Reich, 1970). We have developed a series of cholinergic agonists with channel blocking properties that have proven useful in studying the ion channel of the skeletal muscle nAChR (Carter and Oswald, 1993). These compounds are based on the structure of a synthetic agonist, 1,1-dimethyl-4-acetylpiperazinium iodide (HPIP) (Spivak et al., 1986), which is held by its ring structure in approximately the correct configuration for binding (the quaternary amine is 6.1 Å from the van der Waals extension of the carbonyl oxygen). By adding methylene groups to the acetyl moiety of HPIP (Fig. 1), the off-rate of channel blockade of the skeletal muscle nAChR is decreased and the position of blockade within the channel is modulated (Carter and Oswald, 1993). Activation of the skeletal muscle channel, however, is essentially identical for the entire series. Unlike the skeletal muscle nAChR, the rat and chick forms of the homomeric α7 nAChR are differentially activated by compounds in the piperazinium (PIP) series, and in an analogous fashion, they are also differentially activated by the ganglionic cholinergic agonist DMPP. The PIP ring of DMPP is identical with the PIP series, and the PIP series, in effect, substitutes a carbonyl and aliphatic side chain of varying length for the phenyl ring of DMPP. By focusing on nAChR subtypes that could be formed from a single subunit, we minimized the complexity of the multisubunit composition of the binding site and the species differences. In addition, the efficacy of DMPP for homomeric receptors is well known (Séguéla et al., 1993; Elgoyhen et al., 1994, Gerzanich et al., 1994; Chavez-Noriega et al., 1997), and using this information, we postulated that position 84 of α7 receptors may be a critical determinant of DMPP, and by extension, PIP efficacy. We show here that the PIP compounds can be explicitly "tuned" to activate a receptor with a leucine in position 84 but not one with a glutamine in that same position, suggesting a potential strategy for producing subtype specific cholinergic agonists.

Materials and Methods

Materials. DMPP was purchased from Sigma Chemical Company (St. Louis, MO). HPIP was synthesized as described by Spivak et al. (1986). The longer chain derivatives of HPIP were synthesized in the same manner with the appropriate anhydride substituted (equal number of moles) for acetic anhydride: propionic anhydride for EthylPIP, butyric anhydride for PropylPIP, valeric anhydride for ButylPIP, hexanoic anhydride for PentylPIP, and heptanoic anhydride for HexylPIP (Carter and Oswald, 1993). The syntheses were verified by ^{1}H NMR spectroscopy at 500 MHz (Varian Unity 500 Spectrometer; Varian Instruments, San Fernando, CA). The rat α7 DNA was provided by Dr. Ron Lukas (Barrow Neurological Inst., Phoenix, AZ), the chick α7 was provided by Dr. Marc Ballivet (Université de Genève, Geneva, Switzerland), and the chick α7 L247T mutant was from Dr. Jean-Luc Galzi (Institut Pasteur, Paris).

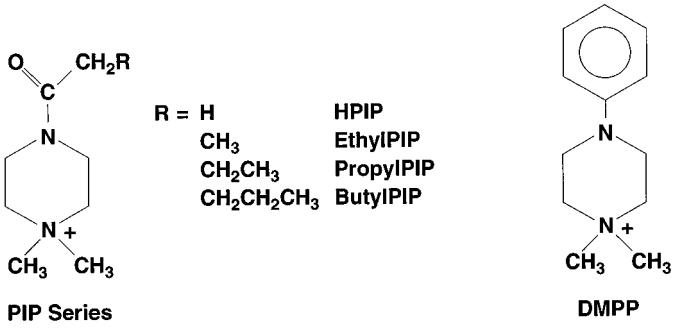


Fig. 1. Structures of DMPP and PIP compounds.

Mutagenesis. The overlapping polymerase chain reaction (PCR) method was used to generate single point mutations in the chick α7 and the rat α7 sequence. The overlapping primers used for the chick α7 mutant were 5′-CCTGATGGACAGATTTGGAAG-3′ (sense) and 5′-CTTCCAAATCTGTTCATCAGG-3′ (antisense) L84Q. For the rat α7, the overlapping primers were 5′-CCAGATGGCCTGATTTGGAA-3′ (sense) and 5′-TTCCAAATCAGGCCATCTGG-3′ (antisense) Q84L. For the chick α7, the PCR fragments were cut with *Sac*II and *Bgl*II and exchanged for wild-type chick α7 in the pOEV vector, and then subcloned into the pCEP4 vector with the restriction enzymes *Xho*I and *Kpn*I. In rat α7, PCR fragments were cut with *Kpn*I and *Sac*I and exchanged for wild-type rat α7 in the pCEP4 vector. The PCR products containing mutations were selected and verified by DNA sequencing.

Recording from Oocytes. For DNA injection, the cDNAs were subcloned into the oocyte expression vector pCEP4 (Invitrogen, San Diego, CA) using the *Xho*I and *Kpn*I sites. For RNA injections, cDNA was cloned into the SP65 vector and the AmpliScribe T7 or SP6 High Yield Transcription Kit (Epicentre Technologies, Madison, WI) was used to transcribe RNA. Plasmids were propagated in the *Escherichia coli* host (DH5α strain) and prepared using a Stratagene Midi Plasmid Quik kit (La Jolla, CA). A 10-nl sample of DNA (1–2 ng of plasmid DNA) was injected into the nucleus 3 to 4 days before recording. In the case of RNA injection, a 50-nl sample (approximately 8 ng of RNA per subunit) was injected into the cytoplasm 2 to 3 days before recording. Two-electrode voltage clamp measurements were made at room temperature in oocyte saline solution with 200 μM $CaCl_2$ (the low concentration of calcium minimizes the calcium-activated chloride channel activation) using a Turbo Tec 01C amplifier. The voltage electrodes were filled with 3 M KCl and had a resistance of 0.5 to 2 MΩ. The current electrode was filled with 250 mM CsCl, 250 mM CsF, and 100 mM EGTA, pH 7.3. The resistance of the current electrode was between 0.3 and 2 MΩ. Saline was allowed to flow over the oocytes at a rate of 4.5 ml/min in a Lucite chamber with a total volume of 300 μl. Agonist solutions were applied to the oocyte from a blunt pipette (1.5-mm diameter) placed 0.5 mm above the oocyte. In this manner, flows of 9 ml/min could be achieved with good mechanical stability of the oocyte. Judging from the rise time of the current following ACh application to oocytes injected with mouse muscle nAChR subunits (more slowing desensitizing than α7 nAChRs), the solutions were changed within approximately 0.5 s. Agonist application was initiated by a computer-triggered stream-switching valve, and data were collected on-line using software developed in the laboratory.

Except when measuring dose-response curves, agonist concentrations were kept 5- to 10-fold greater than the EC_{50} so that changes in agonist efficacy could be assessed directly. Because of the relatively low affinity of the homomeric α7 receptors for cholinergic agonists, the concentrations of the longer PIP compounds that are capable of channel blockade overlap the concentrations for which they act as agonists (Carter and Oswald, 1993). Likewise, homomeric α7 receptors have extremely fast kinetics, so desensitization and activation cannot be temporally separated, even with rapid application of agonists (Niu et al., 1996). Because of these complexities, the term agonist efficacy is used here to indicate a summation of activation, desensitization, and channel blockade.

Results

Differential Efficacy of Agonists on Chick and Rat α7 Homomeric Acetylcholine Receptors. DMPP and the PIP series of cholinergic agonists (Fig. 1) were used to activate both rat and chick α7 homomeric acetylcholine receptors. As shown in Fig. 2A, all were able to activate the rat α7 nAChR. Activation and desensitization by ButylPIP was considerably slower than the other PIP derivatives and the dose-response curve was bell shaped with a maximum near 1 mM (Fig. 5A), similar to other agonists that can both activate the receptor and block the ion channel. The reason for these differences in kinetics is not clear, although in skeletal muscle nAChR (a receptor for which channel blockade by ButylPIP has been demonstrated; Carter and Oswald, 1993), similar slow kinetics were observed (R. Vazquez, unpublished results). As reported previously (Gerzanich et al., 1994), DMPP exhibits a dramatically decreased efficacy on the chick α7 nicotinic receptor (3% of the current observed with saturating concentrations of ACh; Fig. 2B). In the case of the PIP series, the first three compounds in the series (HPIP, EthylPIP, and PropylPIP) were activators of the chick α7. However, ButylPIP, which has one more methylene group than PropylPIP, was completely inactive.

The addition of one methylene group either 1) resulted in the loss of binding to the chick α7 nAChR, 2) resulted in the conversion of an agonist to an antagonist, or 3) dramatically increased the ability of the compound to block the channel. As shown in Fig. 2B, ButylPIP was capable of inhibiting the activation of the chick α7 nAChR by acetylcholine, suggesting that it does interact with the receptor. As previously shown by Bertrand et al. (1992), a channel mutant of the chick α7 nAChR (L247T) can be activated by competitive antagonists such as D-tubocurarine. ButylPIP can also activate this mutant (Fig. 3A). These results suggest that ButylPIP can interact with the chick α7 nAChR and suggest, but do not prove, that the effect is at the agonist binding site. Previous results on the mouse skeletal muscle nAChR (Carter and Oswald, 1993), however, has shown that the PIP series are potent voltage-dependent channel blockers. At negative membrane potentials, significant blockade is observed (with the off-rate of the blockade decreasing with increasing numbers of methylene groups), but at positive potentials, no blockade is observed. Although the chick α7 nAChR is inwardly rectifying due to intracellular Mg^{++} block (Forster and Bertrand, 1995), outward current can be

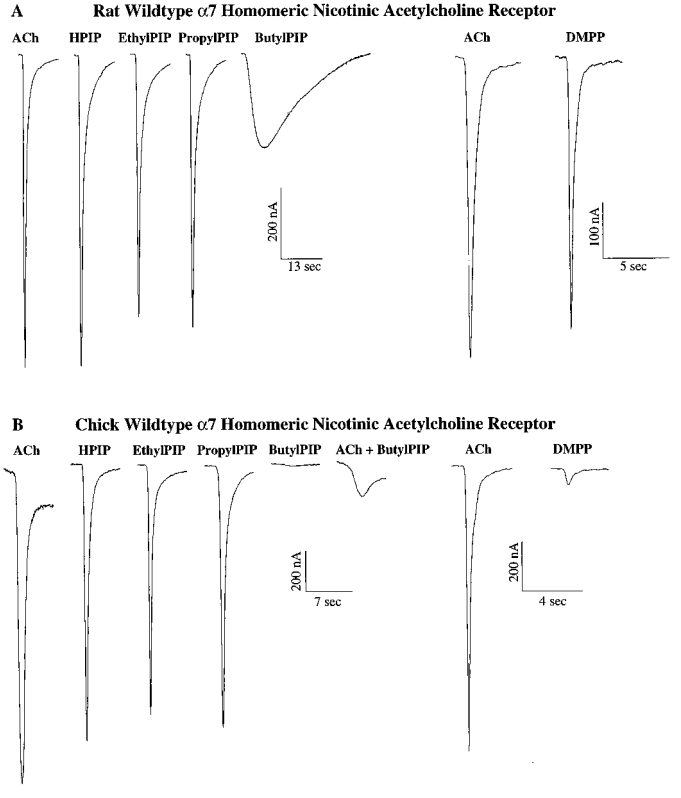


Fig. 2. Responses of rat α7 (A) and chick α7 nAChRs (B) to the PIP compounds and DMPP. For each oocyte, the ACh control (1 mM) is shown as is the corresponding calibration. All compounds were applied at 1 mM and are representative of four determinations for the PIP series and three determinations for DMPP (A), and eight determinations for the PIP series and six determinations for DMPP (B). The response to 1 mM ACh in the presence of 1 mM ButylPIP was 24 ± 4% of the response to 1 mM ACh alone. Unless otherwise noted, oocytes were clamped at −120 mV in this and all subsequent figures.

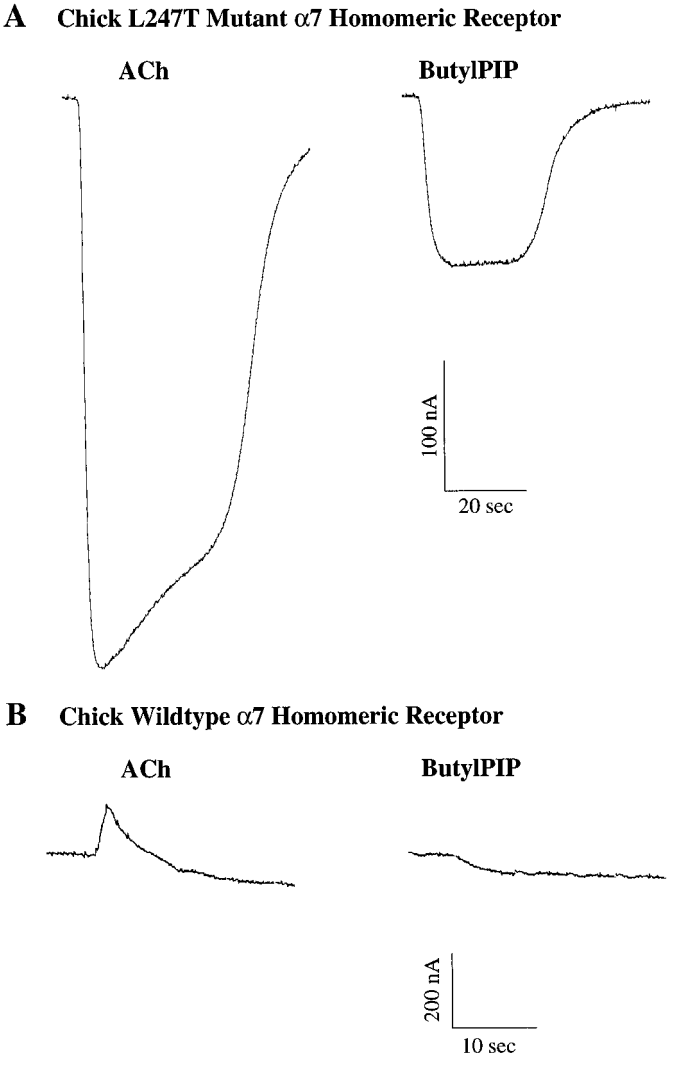


Fig. 3. A, response to 1 mM ACh and ButylPIP of the chick L247T mutant α7 receptor (representative of eight determinations). B, responses of the chick wild-type α7 homomeric receptor to 1 mM ACh and 1 mM ButylPIP with the oocyte clamped at +60 mV. EDTA (100 nl of 8 mM) was injected into the oocyte 15 min before recording (representative of five determinations).

observed by injecting EDTA into the oocyte. As shown in Fig. 3B, acetylcholine can, but ButylPIP cannot, activate an outward current, suggesting that the lack of response to ButylPIP is most likely due to lack of activation at the agonist binding site. That is, ButylPIP is a competitive antagonist at the agonist binding site but the shorter chain PIP derivatives both bind to and activate chick α7. On the other hand, rat α7 is activated by all of the PIP compounds.

Identification of an Amino Acid Partially Controlling Affinity for ACh, DMPP, and PIP Compounds. A sequence alignment of nAChR α subunits revealed very high homology between the chick and rat α7 nAChRs in the N-terminal extracellullar domain. However, a number of differences exist that may explain the differential efficacy of DMPP and ButylPIP. By comparing the efficacy of DMPP for all α7, α8, and α9 homomeric nAChRs, a glutamine in position 84 was strongly correlated with high efficacy for DMPP and a leucine in this position was correlated with low efficacy (Fig. 4). Other differences between the chick and rat α7 subunits showed no correlation when compared across α7, α8, and α9 sequences for which activation by DMPP had been reported. The L84Q mutant of chick α7 and the Q84L mutant of rat α7 were constructed to determine whether this site at least partially determines the efficacy for DMPP and the PIP compounds. As shown in Fig. 5A, both mutants exhibited a moderate change in affinity for ACh. In the case of the rat Q84L mutation, the EC_{50} for ACh increased (260 μM for the mutant versus 150 μM for wild type). The chick L84Q mutation exhibited a decreased EC_{50} for ACh (180 μM for the mutant versus 270 μM for wild type). Thus, although the mutations result in modest symmetrical changes in the affinity for acetylcholine, they do not produce a major change in the normal function of the two receptors.

As predicted, the chick L84Q mutant is activated with greater efficacy by DMPP than is wild-type chick α7 nAChR (compare Fig. 5, A and C with Fig. 2B). DMPP at 1 and 10 mM activated approximately 30% (Fig. 5A) of the current observed with saturating concentrations of ACh (relative to 3% in the wild-type receptor). This gain of function was not to the same level as wild-type rat α7 nAChR, in which DMPP activated approximately >80% of the current observed with ACh (Figs. 2A and 5A), suggesting that sites in addition to position 84 are involved in determining the relative affinity of DMPP for chick versus rat α7 homomeric receptors. The mutation had no effect on activation of chick α7 nAChR for HPIP, EthylPIP, and PropylPIP—all three activated the receptor to the same level as ACh. Unlike wild type, however, ButylPIP can activate the mutant chick α7 nAChR. Although the true level of channel activation cannot be determined due to presumed channel blockade, the level of activation at 1 mM was approximately half that observed for the wild-type rat α7 nAChR. In both cases, the activation was relatively slow, as noted above. Thus, the conversion of L to Q in position 84 is consistent with greater efficacy for both ButylPIP and DMPP.

The mutation of Q to L at position 84 in rat α7 nAChR was predicted to decrease activation by DMPP and ButylPIP. As shown in Fig. 5B, DMPP activated Q84L rat α7 nAChR to a level of 50% of that observed in the presence of saturating ACh. This was consistently lower than the activation of wild type, but greater than that observed for chick α7 nAChR. As expected, responses to HPIP, EthylPIP, and PropylPIP were unaffected by the mutation. The mutation, however, completely abolished the response to ButylPIP. However, the responses to ACh were inhibited by ButylPIP in the rat Q84L mutant, indicating that it does bind to this mutant receptor and can act as a competitive antagonist (Fig. 5B). Placing L in position 84 seemed to abolish activation by ButylPIP and resulted in a 2-fold or less decrease in efficacy of DMPP as well as an increase in the EC_{50}. Although the magnitude

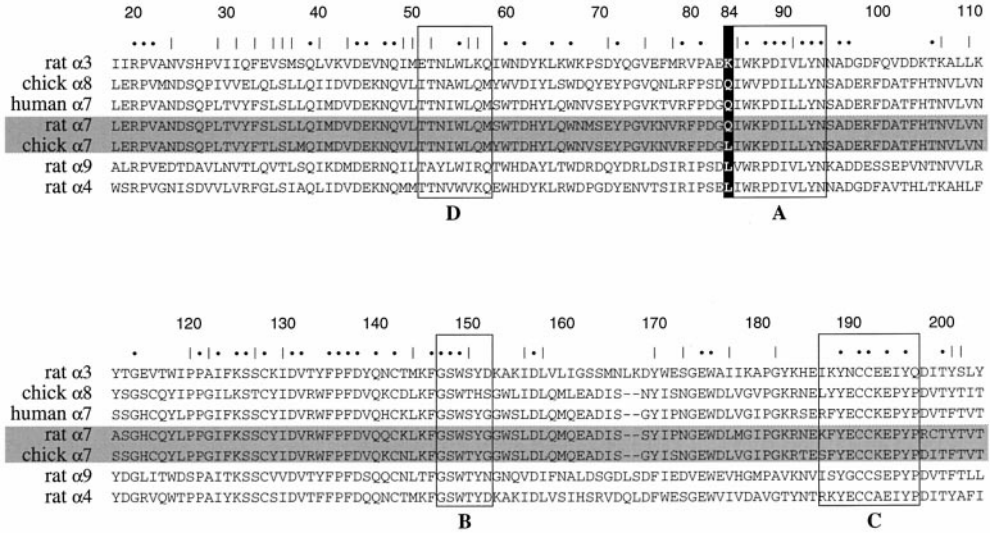
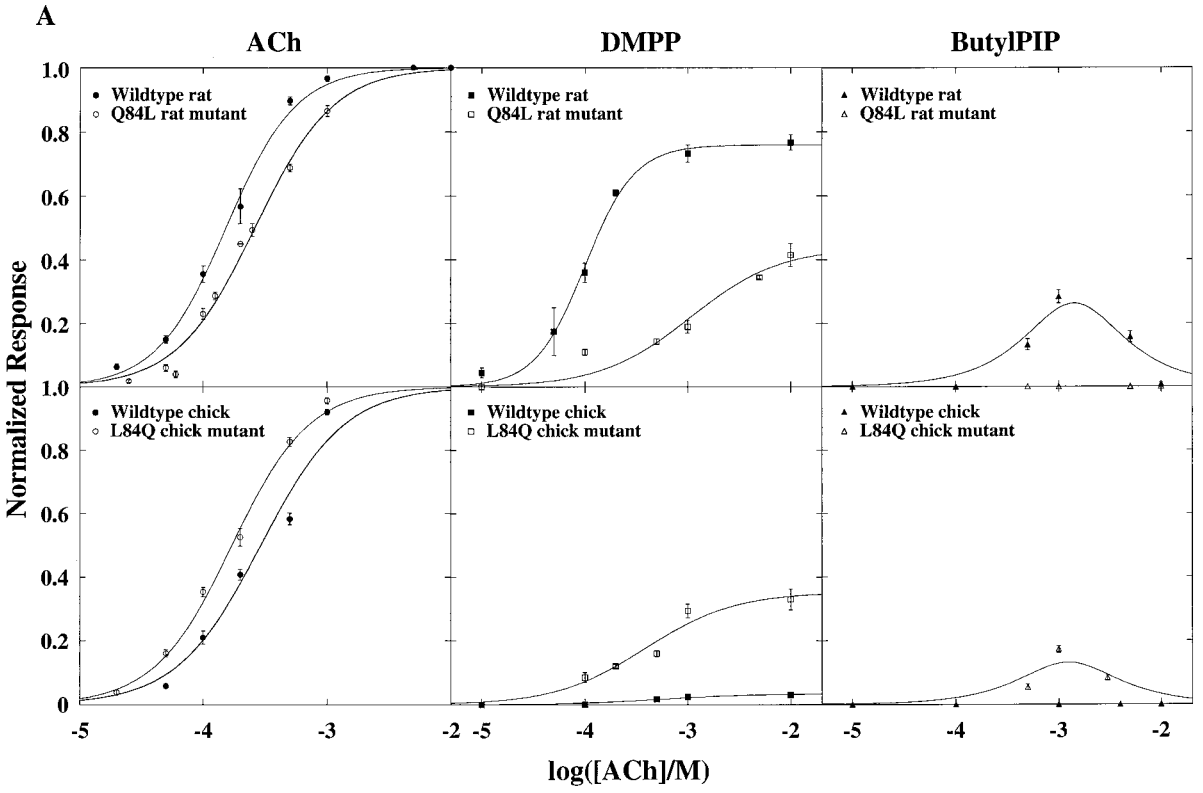
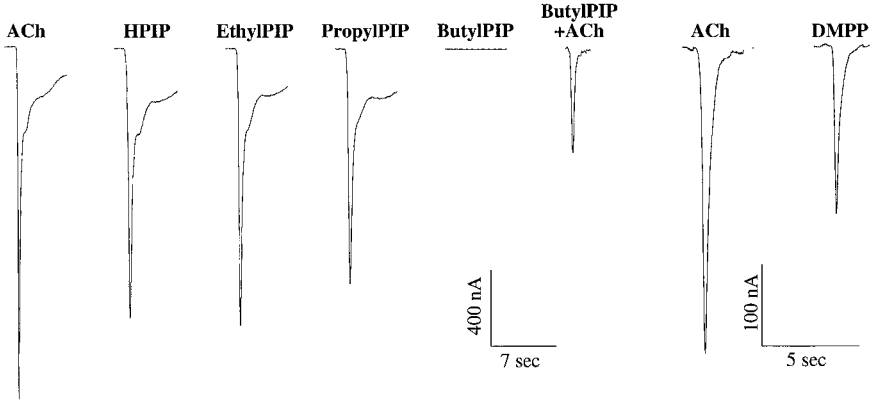
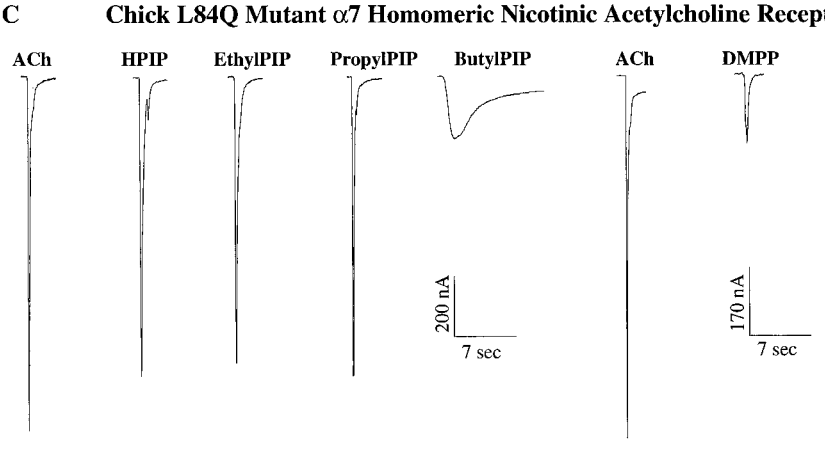


Fig. 4. Alignment of several neuronal nAChR subunits. The boxes indicate the positions of conserved sequences thought to participate in the acetylcholine binding site. DMPP is a full agonist for rat α3 (this includes $\alpha3\beta2$ and $\alpha3\beta4$; Luetje and Patrick, 1991), chick α8, human α7, and rat α7 (Chavez-Noriega et al., 1997; Gerzanich et al., 1994; Séguéla et al., 1993). In these cases, position 84 is Q or K. In the cases of chick α7 (Gerzanich et al., 1994), rat α9 (Elgoyhen et al., 1994), and rat α4 (this includes $\alpha4\beta2$ and $\alpha4\beta4$; Luetje and Patrick, 1991), DMPP is a weak partial agonist activating less than 20% of the maximal current induced by acetylcholine.

Fig. 5. A, Dose-response curves for acetylcholine, DMPP, and ButylPIP. Each point in the dose-response curve to acetylcholine is an average of two to six determinations, with the error bars representing the S.E.M. Lines are a nonlinear least squares fit of the entire data set to the Hill equation for ACh and DMPP. Hill coefficients ranged from 1.3 to 1.55 for ACh and were 1 for DMPP. The fit for ButylPIP included a term for inhibition as well as activation. Also shown are responses of the rat α7 Q84L mutant (B) and chick α7 L84Q mutant (C) to acetylcholine, the PIP compounds, and DMPP. For individual traces, the ACh control (1 mM) is shown as is the corresponding calibration. All compounds in individual traces were applied at 1 mM and are representative of four to five determinations. The response to 1 mM ACh in the presence of 1 mM ButylPIP was 35 $\pm$ 3% of the response to 1 mM ACh alone (the trace shown was from a separate oocyte but was normalized to the response to ACh alone in that same oocyte).


A
ACh
DMPP
ButylPIP
Wildtype rat
Q84L rat mutant
Wildtype chick
L84Q chick mutant
Normalized Response
log([ACh]/M)


B
Rat Q84L Mutant α7 Homomeric Nicotinic Acetylcholine Receptor
ACh
HPIP
EthylPIP
PropylPIP
ButylPIP
ButylPIP +ACh
ACh
DMPP
400 nA
7 sec
100 nA
5 sec


C
Chick L84Q Mutant α7 Homomeric Nicotinic Acetylcholine Receptor
ACh
HPIP
EthylPIP
PropylPIP
ButylPIP
ACh
DMPP
200 nA
7 sec
170 nA
7 sec

varied, the effects of the chick L84Q and the rat Q84L mutants were consistent across all three types of agonist (PIP compounds, DMPP, and ACh).

Discussion

The acetylcholine binding site on nicotinic acetylcholine receptors seems to consist of a number of highly conserved residues found in all subtypes (Fig. 4 and Galzi and Changeux, 1994). However, some compounds (e.g., DMPP, epibatidine, and anabaseine; Séguéla et al., 1993; Gerzanich et al., 1994, 1995, Kem et al., 1997) can distinguish different subtypes of nAChRs. We report here the role of position 84 in the species specificity of cholinergic agonists. In particular, the addition of one methylene group to PIP-based cholinergic agonist (PropylPIP versus ButylPIP) confers selectivity for the rat versus the chick form of the α7 nAChR and that selectivity is based largely on the amino acid in position 84.

Role of Position 84 in ACh Binding Site. Considerable evidence suggests that the binding site for acetylcholine exists at the interface between two subunits. In the skeletal muscle form of the receptor, it is the interfaces between the α and γ and the α and δ subunits that are thought to be the sites of ACh binding. Four regions (labeled loops A, B, C, and D, see Fig. 4) have been identified, based on photoaffinity labeling and site-directed mutagenesis, as potential contributors to the ACh binding site (Galzi and Changeux, 1994; Arias, 1997). Loops A through C are present in the α subunit of all subtypes. In the homomeric α7 receptors loop D is also on the α subunit (presumably forming a binding site with loops A through C of the adjacent subunit), but this loop resides on the γ and δ subunits of the skeletal muscle nAChR (Corringer et al., 1995). These loops consist of highly conserved residues that are presumed to be important for proper folding and many of the interactions with agonist. However, species and subtype specificity would result from variable residues rather than highly conserved residues. Loop C contains the vicinyl disulfide bond that is characteristic of nAChR α subunits. Labeling with sulfhydryl affinity reagents suggests that the carbonyl half of ACh is in the vicinity of this loop (Kao et al., 1984). Likewise, cross-linking (Chiara and Cohen, 1997; Dennis et al., 1988) and mutagenesis (Corringer et al., 1995) studies indicate that the quaternary amine interacts with loop D, which is presumably located on an adjacent subunit. Both loops A and B have been labeled with photoaffinity reagents directed to the ACh binding site (Dennis et al., 1988; Galzi et al., 1990; Cohen et al., 1991) and have residues that when mutated affect ACh binding and channel activation (O'Leary et al., 1994; Sine et al., 1994). Position 84 is at the edge of Loop A, near residues labeled by p-(*N*,*N*-dimethylamino)benzenediazonium fluoroborate (DDF) (Dennis et al. 1988; Galzi et al., 1990) and acetylcholine mustard (Cohen et al., 1991). Although most studies are consistent with the notion that the quaternary ammonium group faces the D loop and the carbonyl side of the molecule faces the C loop, the exact orientation is not known. A homology model of the *Torpedo* electroplaque nAChR (Tsigelny et al., 1997) places position 84 at the periphery of the binding site, near the central pore. If one makes the assumption that the carbonyl side of the agonist faces the C loop, then this model places loop D too far from the PIP side chain to interact directly. However, small changes in a few selected backbone torsion angles in the model results in a binding site into which ButylPIP can be docked with the alkyl group near position 84. This would suggest that a direct interaction between ButylPIP and position 84 is a strong possibility. The fact that position 84 is in the vicinity of but not within a conserved loop is consist with the fact that a mutation in this position has small but significant effects on ACh binding but can affect the interactions of larger cholinergic agonists to a much greater extent.

Possible Interaction between PIP Side Chains and Position 84. When position 84 is a leucine, activation by ButylPIP is prevented, but DMPP can still activate the α7, although with lower efficacy. Considering all other neuronal α subunits, it is only those subunits that have a nonpolar amino acid (leucine or methionine) in this position that exhibit a low efficacy for DMPP; DMPP is a full agonist for receptors formed from α subunits with polar or charged amino acids in this position (Fig. 4). Considering the data for the PIP compounds, one possibility is that the additional hydrophobic surface area afforded by the longer alkyl chain in ButylPIP versus PropylPIP allows a hydrophobic interaction with the leucine in position 84. If this is true, then the interaction with L84 would either prevent the formation of the open channel form of the receptor or could potentially produce a much more rapid desensitization. A charged or polar group in this position or a shorter chain length would then prevent this interaction and allow channel opening (or decrease the rate of desensitization).

Although DMPP was used to identify position 84 as a possible contributor to the differential activation of chick α7 by the PIP series, its subunit specificity is clearly different from that of the PIP series. The most striking example is the fact that DMPP does not activate skeletal muscle nAChRs, whereas the PIP series are all full agonists at micromolar concentrations (they are potent channel blockers at higher concentrations; Carter and Oswald, 1993). Likewise, the distinction between wild-type chick and rat α7 nAChR was much clearer using ButylPIP than DMPP, and the mutations (chick L84Q and rat Q84L) indicated that position 84 is almost entirely responsible for the differences in efficacy for ButylPIP between the two subtypes but that other sites are almost certainly involved for DMPP. Interestingly, Luetje et al. (1993) found that approximately 20% of the difference between α2 (M in position 84) and α3 subunits (K in position 84) in terms of activation by nicotine or inhibition by neuronal bungarotoxin resides in a portion of the subunits between the N-terminus and position 84. One possibility is that position 84 is in fact the residue responsible for these differences.

Implications for Development of Subunit-Specific Drugs. The fact that ButylPIP can clearly distinguish rat from chick α7 nAChR does not in itself demonstrate a subunit-specific drug. However, these two versions of α7 have the advantage of very high sequence identity (87%) and have led to the identification of position 84 as a crucial determinant of activation by ButylPIP. Considering first the homomeric nAChRs, only the α7 receptor from humans has been sequenced. However, in the chick, one could potentially develop a drug that would act as an agonist at the α8 homomeric receptor (Q in position 84) but not the α7 homomeric receptor (L in position 84). Perhaps, more importantly, given the prevalence of α7 and $\alpha4\beta2$ receptors in the human brain,

it may be possible to target a drug to α7 (Q in position 84) and not α4β2 (L in position 84 of the α4 subunit).

Summary. Using a series of systematically varying piperazium compounds and chick α7 nAChR, we identified a transition from full agonist activity (PropylPIP) to no agonist activity (ButylPIP) by the simple addition of one methylene group. In a closely related receptor, the rat α7 nAChR, both PropylPIP and ButylPIP are agonists. Using site-directed mutagenesis, we found that mutation of glutamine 84 to leucine in the rat α7 receptor abolished responses to ButylPIP but not PropylPIP. Likewise, mutating leucine 84 to glutamine in the chick α7 nAChR resulted in channel activation by ButylPIP. This is a clear interaction that structural modifications of an agonist can exploit partially conserved amino acid residues in the binding pocket to develop subunit and species specific cholinergic drugs.

Acknowledgments

We thank Mike Sutcliffe (University of Leicester), Jiancheng Cao, Li Niu, Grace Stafford, Nena Winand, and Greg Weiland for technical assistance and helpful discussions.

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