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# Regions of β4·β2 subunit chimeras that contribute to the agonist selectivity of neuronal nicotinic receptors

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Fifteen chimeric nicotinic receptor β subunits were constructed consisting of N-terminal neuronal β4 sequences and C-terminal β2 sequences. Responses to cytisine, nicotine, or tetramethylammonium were compared to acetylcholine responses for these subunits expressed in *Xenopus* oocytes with α3 subunits. The results show that (i) two residues in the extracellular domain of chimeric β4·β2 subunits (108β2F/β4V, 110β2S/β4T) account for much of the relative cytisine sensitivity; and (ii) four extracellular residues of chimeric β4·β2 subunits (112β2A/β4V, 113β2V/β4I and 115β2S/β4R, 116β2Y/β4S) account for most of the relative tetramethylammonium sensitivity. The data did not permit localization of nicotine sensitivity to any particular region.

Nicotinic receptor; Chimera; Pharmacology; Ganglionic stimulant

## 1. INTRODUCTION

The recent cloning of multiple α and β subunits for neuronal nicotinic acetylcholine receptors (nAChR's) has enabled studies of nAChR's formed from a variety of α and β subtypes [1]. Expression of all possible combinations of the α and β subtypes in *Xenopus* oocytes shows that both subunits contribute to the relative sensitivity to ganglionic stimulants and neurotoxins [1,2], channel conductance and gating properties [3-5] of neuronal nAChR's. Receptors containing the β4 subtype generally give larger responses than receptors containing the β2 subtype to ganglionic stimulants such as cytisine (CYT) and nicotine (NIC) [2]. To localize the regions of β4 and β2 that contribute to agonist selectivity, we constructed chimeras of β4 and β2 consisting of N-terminal sequences from β4 varying length and appropriate C-terminal counterparts from β2. These were expressed in combination with α3 in *Xenopus* oocytes.

## 2. MATERIALS AND METHODS

Fifteen β4·β2 chimeras were constructed using a previously described PCR method [6] so that they contained an N-terminal end from β4 and a C-terminal end from β2. For example, the arrow labeled 7 in Fig. 1 denotes the chimera β4(7)·β2 that contains the 7 most N-terminal residues from β4 and the remaining 470 C-terminal residues from β2. mRNA for α3 and the β subunits was transcribed in vitro [7] and 18 ng of each subunit was coinjected into stage V or VI *Xenopus laevis* oocytes. The oocytes were incubated 2-7 days in a modified Barth's solution containing 5% horse-serum. We measured the peak current produced by bath application of 30 μM acetylcholine (ACh), 30 μM cytisine (CYT), 30 μM nicotine (NIC), and 100 μM tetramethylammonium (TMA) at a typical holding potential of −80 mV using a two-electrode voltage clamp. Receptors that gave ACh responses too large for accurate recording at −80 mV were measured at more depolarized potentials (−70 to −20 mV). ACh responses of the chimeric and wild-type (WT) receptors were typically in the 100-2,000 nA range. The recording solution contained 96 mM NaCl, 2 mM NaOH, 1 mM $MgCl_2$, and 5 mM HEPES (pH 7.4). External $Ca^{2+}$ was omitted to minimize the activation of the $Ca^{2+}$ activated $Cl^-$ conductance [8]. All recordings were made at ambient temperature (23-25°C).

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## 3. RESULTS

The region of β4 N-terminal to the first transmembrane region M1 (residues 1 to 214) was sufficient to confer complete β4-like CYT (Fig. 2) and TMA (Fig. 3), but not NIC (Fig. 4), sensitivity on the receptors containing the chimeric β4·β2 subunits. The relative responses of the α3β2 WT to CYT, TMA and NIC were 0.03 ± 0.01 (mean ± S.D., $n$ = 5), 0.60 ± 0.14 ($n$ = 5), and 0.25 ± 0.11 ($n$ = 5); the relative responses of the α3β4 WT to these same agonists were 2.47 ± 0.59 ($n$ = 7), 1.80 ± 0.47 ($n$ = 3), and 1.36 ± 0.80 ($n$ = 7). The relative responses of the α3β4(214)·β2 chimeric receptor, which contains most of the putative extracellular region of β4, were 2.38 ± 0.26 ($n$ = 2) to CYT, 1.98 ± 0.46 ($n$ = 6) to TMA, and 0.81 ± 0.09 ($n$ = 5) to NIC. Thus, the relative response of α3β4(214)·β2 to CYT and TMA did not differ substantially from the relative response of the α3β4 WT to these agonists.

Chimeras β4(105)·β2 and β4(109)·β2 differ by only one residue (108β4V/β2F); as do chimeras β4(109)·β2 and β4(111)·β2 (110β4T/β2S). These chimeras displayed dramatic differences in relative sensitivity to CYT when expressed with α3 (Fig. 2). Fig. 2 also shows that (*a*) all six chimeras with ≥ 111 N-terminal residues from β4

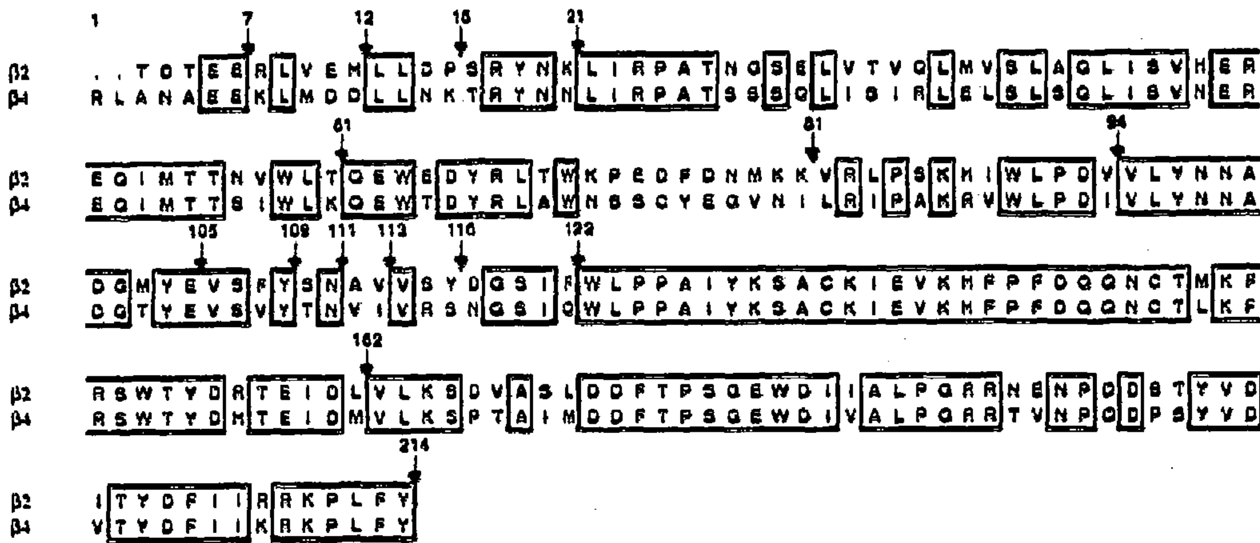

Fig. 1. Aligned sequences of the N-terminal regions of β2 [18] and β4 [19]. Downward arrows indicate the β4 to β2 transition point of each chimera. Numbering is based on the β4 sequence. Outlines denote identical residues. Shading denotes homologous residues.

(and the remaining C-terminal residues from β2) displayed ≥ 70% of the relative α3β4 WT response to CYT and (*b*) all eight chimeras with ≤ 105 N-terminal residues from β4 displayed ≤ 13% of the relative α3β4 WT response to CYT. The relative CYT response of the α3β2 WT was 1% of the relative CYT response of the α3β4 WT receptor. Thus, chimeric receptors containing β4 residues at 108 and 110 displayed a relative CYT sensitivity near that of the β4 WT while chimeric receptors containing β2 residues at these positions displayed a relative CYT sensitivity much nearer to that of the β2 WT.

Inclusion of two adjacent regions of β4 (112V, 113I and 115R, 116S) dramatically increased the relative sensitivity of the chimeric receptors to TMA. Fig. 3 shows that (*a*) all four chimeras with ≥ 116 N-terminal residues from β4 displayed ≥ 83% of the α3β4 WT relative response to TMA and (*b*) all eight chimeras with ≤ 111 N-terminal residues from β4 displayed ≤ 39% of the α3β4 WT relative response to TMA. The relative TMA response of the α3β2 WT was 33% of the relative response of the α3β4 WT to TMA. Thus, chimeric receptors containing β4 residues at 112, 113, 115 and 116 displayed a relative TMA sensitivity near that of the β4 WT while chimeric receptors containing β2 residues at these positions displayed a relative TMA sensitivity much closer to that of the β2 WT.

There also appears to be a transition in the relative CYT responses, but not for the TMA responses from values < 0.06 for α3β4(7)·β2 ($n$ = 5) and α3β4(12)·β2 ($n$ = 7) to values > 0.30 for α3β4(61)·β2 ($n$ = 5) (Fig. 2). However, our data are insufficiently precise to localize the important region.

There was no clearly demarcated zone responsible for the relative NIC sensitivity of β4 (Fig. 4). However, chimeras with transitions from β4 to β2 between 94β2V/β4I and 122β2F/β4Q displayed dramatic variations in the mean nicotine sensitivity when expressed with α3.

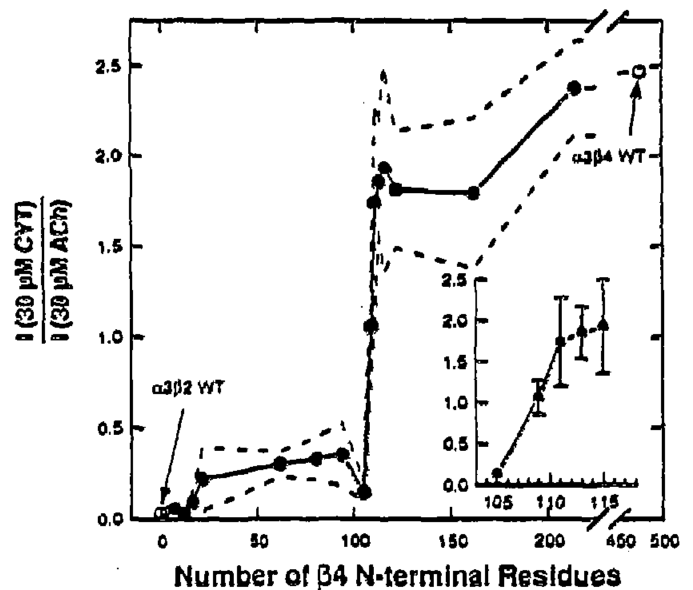


Fig. 2. Ratio of the CYT-induced current [I (30 μM CYT)] to the ACh-induced current [I (30 μM ACh)] versus the number of N-terminal residues from β4 in the β4·β2 chimera. Position zero and 475 (marked with open circles) correspond to the wild-type β2 and β4 subunits, respectively. Dotted lines above and below the circles denote ± one S.D. Sample sizes for individual data points were typically 3–8 oocytes. Inset shows the region with the most dramatic changes in relative CYT sensitivity in greater detail. Bars in inset denote ± one S.D.

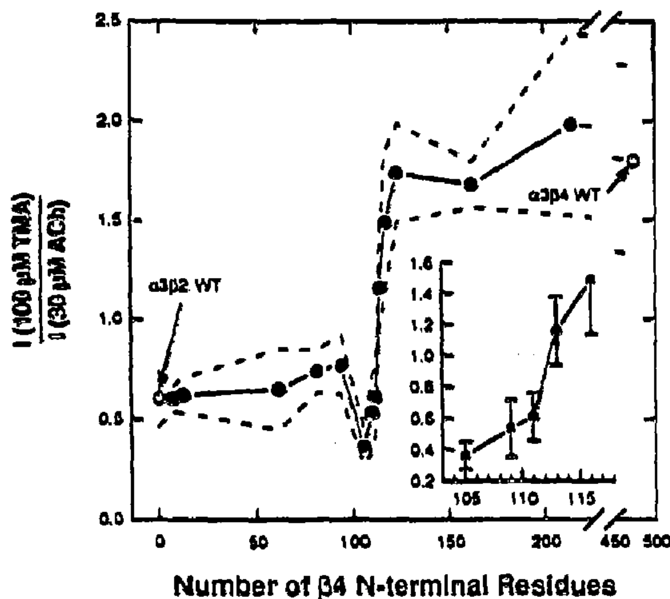


Fig. 3. Ratio of the TMA-induced current [I (100 μM TMA)] to the ACh-induced current [I (30 μM ACh)] versus the number of N-terminal residues from β4.

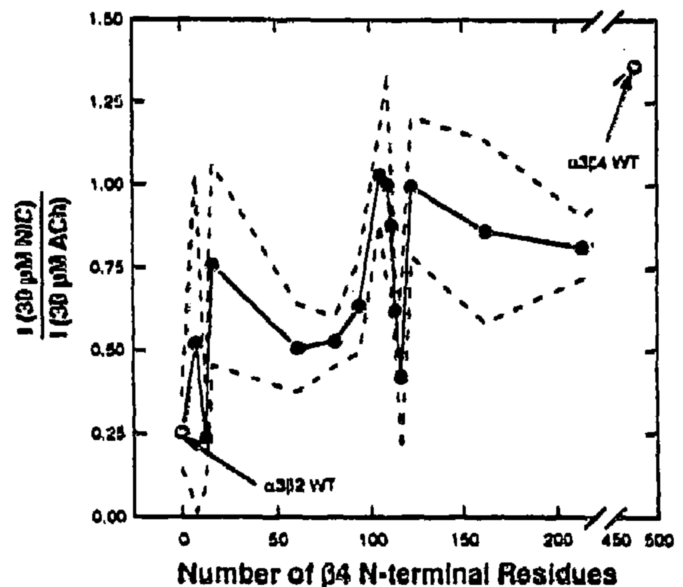


Fig. 4. Ratio of the NIC-induced current [I (30 μM NIC)] to the ACh-induced current [I (30 μM ACh)] versus the number of N-terminal residues from β4.

## 4. DISCUSSION

Our results are consistent with previous work showing that both the α and β subunits influence the pharmacological selectivity of the neuronal nAChR [2] and that non-α subunits from *Torpedo* nAChR's bind cholinergic ligands [9,10]. In addition to previously noted differences between the sensitivity of α3β2 and α3β4 to CYT. and NIC relative to ACh [2], we found that α3β4 is more sensitive than α3β2 to the ganglionic stimulant TMA. Two lines of evidence show that the greater relative response of α3β4 to CYT, TMA and NIC cannot be due solely to a decrease in the ACh response of α3β4. First, normalized to the NIC response, the CYT and TMA response was 0.05 and 0.42 for α3β2 but 1.37 and 0.76 for α3β4. Second, the present data show that distinct regions of β4 contribute to the relative CYT and TMA sensitivity. Nonetheless, differences in the ACh sensitivity of α3β2 and α3β4 may be responsible for part of the difference between the agonist selectivities of the two receptors.

Our data suggest that the region of the β subunit that is most critical for relative CYT and TMA sensitivity lies in the middle of the putative extracellular N-terminal sequence. Neuronal nicotinic receptors are thought to be composed of five subunits [11,12]. Each subunit contains four transmembrane repeats [13]. The portion of each subunit N-terminal to M1 forms the bulk of the extracellular portion of the receptor [13]. The M2 transmembrane segment [13] and possibly M1 [14] form the channel pore. Confirming an earlier report [15], we have shown that the chimeric receptor α3β4(214)-β2, containing all of the putative extracellular region N-terminal to M1, has complete β4-like relative sensitivity to CYT and to TMA.

The relative NIC response of α3β4(214)-β2 differed from that of α3β4 and the results did not reveal any single area in the β4-β2 chimeras responsible for relative NIC sensitivity. Thus, there may be (*a*) several regions of the β subunit involved in NIC selectivity; or (*b*) the chimeras may cause novel structural changes in the receptor which interfere with NIC responses.

Previous authors [2] suggested that the small CYT response of receptors containing α3β2 is due to open-channel block by CYT. However, in contrast to previous examples of agonist block of the nAChR [16,17], putative block of the ACh response of α3β2 by CYT is not voltage dependent [2]. If the channel-block hypothesis is correct, then our results suggest either (*a*) that the open-channel blocking site for CYT is in the extracellular portion of α3β2 or (*b*) that the regions we have identified in β4-β2 chimeras affect the structure of the channel indirectly. A more straightforward interpretation is that CYT, TMA and NIC may be more effective either (*a*) at binding to α3β4 than to α3β2 or (*b*) at inducing the conformational change that opens the α3β4 channel. In this case, the regions we identified probably influence one of these molecular events at the neuronal nAChR. Dose-response data and agonist competition experiments will be necessary to resolve these questions.

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