Key Residues in the Nicotinic Acetylcholine Receptor β2 Subunit Contribute to α-Conotoxin LvIA Binding

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Background: Toxins such as LvIA can help elucidate the physiological roles of nAChr subtypes.

Results: Three residues in the β2 subunit were identified as critical to LvIA binding.

Consequence: The β complementary subunit plays a crucial role in the subtype selectivity of α-conotoxin LvIA.

Significance: This study provides new insights into the unique selectivity of LvIA and more broadly into toxin-receptor interactions.

α-Conotoxin LvIA (α-CTx LvIA) is a small peptide from the venom of the carnivorous marine gastropod Conus lividus and is the most selective inhibitor of αβ2 nicotinic acetylcholine receptors (nAChRs) known to date. It can distinguish the αβ2 nAChR subtype from the α6β2* (* indicates the other subunit) and α3β4 nAChR subtypes. In this study, we performed mutational studies to assess the influence of residues of the β2 subunit versus those of the β4 subunit on the binding of α-CTx LvIA. Although two β2 mutations, αβ2[F119Q] and α3β2[T59K], strongly enhanced the affinity of LvIA, the β2 mutation αβ2[V111I] substantially reduced the binding of LvIA. Increased activity of LvIA was also observed when the β2-T59L mutant was combined with the α3 subunit. There were no significant difference in inhibition of αβ3β[T59I], αβ3β[Q34A], and αβ3β[K79A] nAChRs when compared with wild-type αβ3β nAChR. α-CTx LvIA displayed slower off-rate kinetics at αβ3β[F119Q] and αβ3β[T59K] than at the wild-type receptor, with the latter mutant having the most pronounced effect. Taken together, these data provide evidence that the β2 subunit contributes to α-CTx LvIA binding and selectivity. The results demonstrate that Val111 is critical and facilitates LvIA binding; this position has not been previously identified as important to binding of other 4/7 framework α-conotoxins. Thr59 and Phe119 of the β2 subunit appear to interfere with LvIA binding, and their replacement by the corresponding residues of the β4 subunit leads to increased affinity.

Nicotinic acetylcholine receptors (nAChRs), which comprise many different molecular subtypes, are ligand-gated ion channels that are activated by the endogenous neurotransmitter acetylcholine (ACh) or exogenous nicotine (1, 2). nAChRs are found in the neuromuscular junction, and in peripheral and central nervous systems throughout the animal kingdom, and play important roles in regulating synaptic transmission (3–9). Neuronal nAChRs are pentameric membrane-bound proteins, which are made up of α (α2–α10) and β (β2–β4) subunits (10).

Pharmacological properties of the heteromeric nAChRs are influenced by the presence of β2 and/or β4 subunits (11). This study is part of an ongoing effort to elucidate the physiological role of each subtype of nAChR and the key binding residue determinants for selective ligands (12).

α-Conotoxins (α-CTxs) are a rich source of highly selective ligands that discriminate among different nAChR subtypes (13–15). α-CTxs contain two conserved disulfide bridges and are classified into several structural subfamilies according to the number of residues in the two backbone loops bracketed by cysteine residues, the largest subfamilies being the 4/7, 4/6, 4/5, 4/4, 4/3, and 3/5 α-CTxs. The 4/7 α-CTxs are able to discriminate between diverse neuronal α-β nAChR subunit combinations and stoichiometries (16, 17).

Previous research revealed that some of the 4/7 α-CTxs, such as α-CTx MII, PnIA, and BuIA, bind to a small conserved cleft of the α3β2 nAChR, and the β2 subunit contributes to binding and selectivity (18, 19). This cleft contains the ligand-accessible residues β2 Leu121, Val111, Phe119, and Thr59, which act as a common binding site/pocket for 4/7 α-CTxs (18, 19). α-CTx MII from Conus magus is a potent antagonist of α3β2 and α6β2* (* indicates the other subunit) nAChRs (20). The α-CTx PnIA is selective on the α3β2 and α7 nAChRs (21). α-CTx BuIA from Conus bullatus blocks both β2* (* indicates the other subunit) and β4* (* indicates the other subunit) nAChRs, and kinetically distinguishes between them with long and short off-times.

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§ The abbreviations used are: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; CTx, conotoxin.
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(22). The 4/7 α-CTx LtIA targets a novel microsite and has a shallow binding site on the α3β2 nAChR that includes β2 Lys79 outside of the cleft (23). This indicates that different key residues of the β2 subunit are targeted by different α-CTx to block the α3β2 nAChR (19, 24).

α-CTx LvIA from Conus lividus was recently characterized and has high affinity for α3β2 nAChRs, with an IC50 of 8.7 nm (25). LvIA is notable for its ability to selectively block the α3β2 nAChR versus α6/α3β2β3 or α3β4 nAChRs. The residues in the β2 subunit that contribute to α-CTx LvIA binding to the α3β2 nAChR remain unknown. We therefore performed a mutational study of the α3β2 nAChR in which we assessed the influence of residues that line the β2 subunit on the binding of α-CTx LvIA.

EXPERIMENTAL PROCEDURES

Materials—Reagents for peptide synthesis were from GL Biochem (Shanghai, China). Reversed-phase HPLC analytical Vydac column (10 × 100 mm) was from Grace Vydac (Hesperia, CA). Clones of rat α3, β2, and β4 cDNAs were kindly provided by S. Heinemann (Salk Institute, San Diego, CA).

Peptide Synthesis—A two-step oxidation protocol was used to synthesize α-CTx LvIA as described previously (25). Because this protocol worked well, we did not attempt a simpler one-step oxidation approach. In this protocol, linear (see Fig. 1A) and folded (see Fig. 1B) peptides were purified by HPLC on a reversed-phase C18 Vydac column. HPLC elution conditions included a linear gradient of 0–40% solvent B over 40 min. Solvent A was 0.075% trifluoroacetic acid in H2O. Solvent B was 0.05% TFA, 90% acetonitrile in H2O. Absorbance was monitored at 214 nm.

Mutagenesis and Construction of Chimeric β2 Point Mutation Receptors—Point mutants of nAChR β2 subunit cDNA (see Table 1) were created using PCR and the QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s instructions. Primers that contained the desired point mutation as well as at least 15 bases on either side of the mutation were synthesized. The mutagenic primers were extended by PCR, DpnI was used to digest the mutagenic PCR products, and the wild-type fragment was inserted into DH5α competent cells. The molecular model of the complex between acetylcholine-binding protein (AChBP) and conotoxin PnIA variant (PDB identifier 2br8) as templates, as described previously (25). The molecular model was refined by a 30-ns explicit water molecular dynamics simulation carried out with the GROMACS 4.6.5 (26) package and the ff03 force field (27), using a procedure described previously (28, 29). All the models of complexes involving β2 subunit mutants were generated by substituting residue side chains using Modeler 9v14 (30). This procedure refines the positions of the substituted side chain atoms as well as those of the neighbor residues using a conjugate gradient minimization followed by a short molecular dynamics simulation. The molecular models were refined by a 2-ns explicit water molecular dynamics simulation, and the simulations of the T59K, V111I, and F119Q mutants were extended to 10 ns.

RESULTS

Chemical Synthesis of α-CTx LvIA—α-CTx LvIA linear peptide (Fig. 1A) was successfully synthesized with Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry, in which Cys residues were orthogonally protected using acid-labile S-trityl and acid-stable S-acetamidomethyl groups. The acid-labile groups (tri-
highlighted in Fig. 2. We created point mutations of the LvIA-binding pocket in a previous modeling study (25) are subsequent experiments.

The mass of the synthesized fully folded peptide was utilized in all sequence (calculated average mass, 1679.9 Da; observed, 1679.7). This synthesized fully folded peptide was utilized in all experiments.

Absorbance was monitored at 214 nm. Flow rate was 0.75 ml/min.

Ferricyanide was used to close the first disulfide bridge, Cys1–Cys3 and Cys2–Cys4 disulfide bonds (Fig. 1B). The fully folded peptide of this synthesized intermediate shown in A, D, HPLC chromatograms of oxidized and folded α-CTX LvIA. Peptides were analyzed on a reversed-phase analytical Vydac C18 HPLC using a linear gradient of 0–40% Solvent B over 40 min, where Solvent A = 0.075% TFA and Solvent B = 0.05% TFA, 90% acetonitrile in H2O. Absorbance was monitored at 214 nm. Flow rate was 0.75 ml/min. AU, absorbance units.

Effect of Mutations of the β2 Subunit on Block by α-CTX LvIA—Previous studies using molecular modeling of related toxins suggested the nAChR positions that form the ligand-binding pocket of α-conotoxins (23, 31, 32). The residue positions of the β2 and β4 subunits that were suggested to form the LvIA-binding pocket in a previous modeling study (25) are highlighted in Fig. 2. We created point mutations of the β2 subunit where residues in this pocket were replaced with those found in the homologous position of the β4 subunit. These mutant receptors were then tested to determine toxin potency differences (Table 1). Seven nAChR β2 mutants were created, including Q34A, T59I, T59K, T59L, K79A, V111I, and F119Q. The concentration-response block by α-CTX LvIA on α3β2 nAChR and wild-type and mutant α3β2 nAChRs was investigated (Table 1 and Fig. 3). The potency at wild-type α3β2

**TABLE 1** IC50 and Hill slope values for block of nAChRs by α-CTX LvIA

| Subtypes | IC50 | Ratio | Hill slope |
|----------|------|-------|------------|
| α3β2     | 8.67 | 1     | 1.17 (0.88–1.46) |
| α3β4     | 148  | 17    | 1.14 (0.72–1.55) |
| α3β2[F119Q] | 0.58 | 0.07  | 1.12 (0.79–1.44) |
| α3β2[T59K] | 0.96 | 0.11  | 0.80 (0.47–1.13) |
| α3β2[T59L] | 2.03 | 0.23  | 1.07 (0.77–1.37) |
| α3β2[Q34A] | 8.64 | 1.0   | 0.90 (0.22–1.58) |
| α3β2[K79A] | 10.8 | 1.3   | 0.86 (0.43–1.30) |
| α3β2[V111I] | 15.2 | 1.5   | 1.15 (0.43–1.86) |

Numbers in parentheses are 95% confidence intervals.

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| α3β2[Q34A] | 8.64 | 1.0   | 0.90 (0.22–1.58) |
| α3β2[K79A] | 10.8 | 1.3   | 0.86 (0.43–1.30) |
| α3β2[V111I] | 15.2 | 1.5   | 1.15 (0.43–1.86) |
nAChR was 17-fold greater than that at wild-type α3β4 nAChR. Among the seven α3β2 mutants, LvIA had the lowest activity on α3β2[V111I], with an IC50 of 126 nM, which is similar to the 148 nM IC50 on wild-type α3β4. Thus, the β2 subunit mutation V111I reduced the binding of LvIA to α3β2 nAChR by 15-fold. There were no significant differences in LvIA potency on mutants α3β2 Q34A, K79A, or T591 when compared with activity at wild-type α3β2 nAChR (Table 1, Fig. 3).

Increased potency of α-CTX LvIA was observed at α3β2[F119Q], α3β2[T59K], and α3β2[T59L] nAChRs. LvIA potently blocked ACh-evoked currents of these nAChRs with IC50 values of 0.58, 0.96, and 2.03 nM, respectively (Table 1). The ratio between the IC50 values of wild-type and mutant α3β2 nAChRs was 15 and 11 for α3β2[F119Q] and α3β2[T59K], respectively. The mutation T59L resulted in only a small increase (~4.3-fold) of LvIA potency. The most LvIA-sensitive mutant, α3β2[F119Q], had a potency 2 orders of magnitude higher than that of the least sensitive mutant, α3β2[V111I] (Table 1, Fig. 3B).

LvIA is 255-fold less potent at α3β4 than at α3β2[F119Q], and 154-fold less potent at α3β4 than at α3β2[T59K]. Although introducing the β4 residues Lys and Gln at positions 119 and 59, respectively, increased the potency of LvIA for α3β2, this CTx is more potent at wild-type α3β2 than α3β4. A synergistic effect of binding site positions displaying different residues between the two subtypes might explain why LvIA is more active at α3β2 than at α3β4.

Mutations of the β2 Subunit Affect Recovery Time after Block by α-CTX LvIA—The α3β2 receptor mutants affected not only the potency of LvIA but also its recovery (Table 2, Fig. 4). α-CTX LvIA (10 nM) blocked wild-type α3β2 nAChRs versus mutant receptors α3β2[F119Q], α3β2[T59K], and α3β2[V111I] to different degrees (Fig. 4). α-CTX LvIA at 10 nM blocked ~55% current of wild-type α3β2 but produced little or no block of α3β2[V111I] (Fig. 4, A and B). In contrast, complete block of ACh-evoked currents was obtained with 10 nM α-CTX LvIA on mutant receptors α3β2[F119Q] and α3β2[T59K] (Fig. 4, C and D).

We compared the recovery time (>95% initial current) after block by 10 μM toxin for the wild-type and mutant receptors (Table 2). Recovery of wild-type α3β2 nAChR was complete within 2 min after toxin washout. Thus, the t1/2 was estimated to be <30 s, and this time scale is beyond the resolution of the experimental setup. Similarly the off-rates for α3β2[T59I], α3β2[K79A], α3β2[V111I], and α3β2[Q34A] were also rapid (full recovery in 1–3 min). The recovery time of wild-type α3β4 nAChR was 20–26 min, which is slower than that of α3β2[F119Q] (10–12 min), but much faster than α3β2[T59K].

The three α3β2 mutations, T59K, T59L, and F119Q, affected the off-rates of LvIA significantly, as evidenced by the corresponding receptors having much slower reversible block by LvIA than wild-type α3β2 nAChR or mutants T59I, K79A, V111I, and Q34A. The recovery times of mutants α3β2[T59L] and α3β2[F119Q] were 6–9 and 10–12 min, respectively (Fig. 4C, Table 2), whereas α3β2[T59K] displayed the slowest recovery time, with less than ~3% recovery 20 min after washout (Table 2). Even at low concentrations of LvIA (10–100 nM), α3β2[T59K] recovered very slowly from block. At 10 nM LvIA concentration, α3β2[T59K] recovered to 28 ± 3.5% current 20 min after washout, and at 100 nM concentration, only 13 ± 2% current was recovered 20 min after washout (Fig. 4D).

Molecular Modeling—A molecular model of the interactions between LvIA and the wild-type α3β2 nAChR showed that the β2 subunit positions considered for mutations are all potentially in contact with the conotoxin with the exception of position 34, as shown in Fig. 5A. LvIA had similar activity at wild-type and α3β2 Q34A nAChR, in agreement with the absence of interaction of this position. The wild-type β2 residue Lys79 can form a surface salt bridge with LvIA Asp11, and this interaction was found to be stable over a 30-ns molecular dynamics simulation (Fig. 5B). The three other substituted positions, i.e. positions 59, 111, and 119, are at least partly buried at the interface with LvIA. The change of activity of LvIA correlated with a change of buried surface solvent-accessible surface area at the interface for mutants of positions 59 and 119 (Fig. 5C). The molecular model did not provide a simple explanation for the decreased activity of LvIA at the V111I mutant, and we propose that this mutation could potentially result in conformational changes that cannot be modeled using short molecular dynamics simulations. The other mutated positions, i.e. 59 and 119, are located in β-strands, and the residues chosen for substitu-

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**TABLE 2**

| nAChR subtype | T95 | nAChR subtype | T95 |
|---------------|----|---------------|----|
| α3β2          |     | α3β2[T59L]    |   6–9 |
| α3β2[K79A]    | <2 | α3β2[F119Q]   | 10–12 |
| α3β2[V111I]   | <1 | α3β2[T59K]    | 20–26 |
| α3β2[Q34A]    | 2–3| α3β2[T59I]    | >=20

*Time to 95% recovery after toxin washout.

< 3% recovery after 20 min; concentration of α-CTX LvIA was 10 μM.
tions are not likely to disrupt this β-strand secondary structure. The 10-ns simulations of complexes incorporating the mutations T59K, V111I, or F119Q resulted in similar binding modes with no change of binding site conformation (Fig. 6).

**DISCUSSION**

Neuronal nAChRs are widely expressed in the CNS and peripheral nervous system in adults and during development, but the identification of which subtype is expressed in which nervous cell is challenging (33–38). LvIA is the first ligand to be highly specific for nAChR, and it could potentially be used in physiological studies of this receptor (25). We sought here to gain further insights into the binding interactions of LvIA at this receptor through mutations of positions that have been shown to be important for the binding of other α-CTxs and ligands (24, 39). These studies have shown that competitive nicotinic ligands of...
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nAChRs generally bind to both the α subunits and the β subunits that form a ligand-binding interface (18, 32).

We investigated the influence of seven α3β2 nAChR mutants on the binding of α-CTx LvIA. These residues were chosen based on previous findings with the related α-CTx LtiA (23). Four mutants, α3β2[F119Q], α3β2[T59K], α3β2[T59L], and α3β2[V111I], had significantly different sensitivity than the wild-type receptor to α-CTx LvIA (Tables 1 and 2; Figs. 3 and 4). The three other mutations, Q34A, K79A and T59I, had little or no detectable effect on α-CTx LvIA activity (Tables 1 and 2; Figs. 3 and 4). α-CTx LvIA at 10 nM blocked ~55% of the current of wild-type α3β2 with rapid reversibility but blocked >95% of the current of α3β2[F119Q] and α3β2[T59K]; the block of the latter two nAChRs had much slower reversibility after toxin washout when compared with that observed for the wild-type α3β2 nAChR (Fig. 4). The substitution of Phe119 of the β2 subunit by Gln, which is present in the homologous position of the β4 subunit (α3β2[F119Q]), resulted in a 15-fold increase in α-CTx LvIA potency. The mutation T59K caused an 11-fold increase sensitivity for α-CTx LvIA, partly due to a decrease in off-rate (Fig. 4D). A similar finding has been reported for the 4/4 α-CTx BuIA (19). The potency of BuIA at α3β2[F119Q] and α3β2[T59K] increased 8- and 20-fold, respectively, when compared with wild-type α3β2, with very slow off-rates. However, BuIA had a faster off-rate but similar IC50 at α3β2[V111I] versus wild-type α3β2, in contrast to LvIA, which has a 15-fold decrease in potency at α3β2[V111I] when compared with wild-type α3β2 (Table 3) (19). Thus, we suggest that BuIA and LvIA have overlapping, yet distinct binding interactions with the receptor. Overall our data suggest that the three positions on the receptor, 59, 111, and 119, are key to LvIA binding. Of course, because we examined only a finite number of mutations, we cannot exclude the possibility that other positions might also be important.

As far as ligand residues contributing to binding are concerned, the highly conserved Ser-Xaa-Pro motif in the first loop of α-CTxs contains a small α-helix important for nAChR binding. α-CTx LtiA is atypical because it lacks this Ser-Xaa-Pro motif and has been suggested to bind a novel microsite on the α3β2 nAChR (19). α-CTx LtiA potentially interacts with β2 Phe119 and β2 Lys79 because the Phe119 and Lys79 mutants disrupted LtiA binding (19), but mutations of these positions were without effect for activity of α-CTxs MII, PnIA, and GID (18). By contrast, the mutation F119Q increased affinity of LvIA, and the mutation K79A did not affect LvIA activity. The mutation V111I in the β2 subunit was previously reported to have only a small effect on the activity of 4/7 α-CTx MII, PnIA, GID (18), ***TABLE 3***

| Toxin   | Amino acid sequencea | α3β2 | α3β2[F119Q] | α3β2[T59K] | α3β2[K79A] | α3β2[Q34A] |
|---------|----------------------|------|-------------|------------|------------|------------|
|         | IC50 IC50            | Ratio| IC50 IC50 | Ratio      | IC50 IC50 | Ratio IC50 |
| LvIA    | CCCSPFLGDHVPE       | 8.67 | 126 14.5    | 0.58 0.07 | 0.96 0.11 | 10.8 1.3   | 8.64 1.0   | This work |
| LtiA    | GCCYRACAGIH          | 9.79 | 28.2 2.9    | 9190 939  | 7.2 0.7   | 195 19.9   | 35.7 3.65  | (23)       |
| BuIA    | GCCSPFLGY--          | 5.72 | 8.98 1.57   | 0.74 0.13 | 0.24 0.04 | ND ND      | ND ND ND   | (19)       |
| MII     | GCCSPFLGSLN         | 3.5 | ND ND ND    | ND ND     | 14 4.0    | ND ND ND   | ND ND ND   | (18, 24)  |

*a Conserved amino acids are shaded in light grey. Conserved cysteine residues are boldface and boxed. Disulfide connectivity of these α-conotoxins is Cys4–Cys3 and Cys3–Cys5. + C-terminal amide.

b Data for LtiA, BuIA, and MII are from previous studies of these toxins tested on the indicated receptors expressed in Xenopus oocytes.

c IC50 in nM.

d Ratio of IC50 of mutant α3β2 nAChR/IC50 wild-type α3β2.

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**FIGURE 6.** Molecular dynamics simulation of LvIA/α3β2 incorporating β2 subunit mutations T59K, V111I, or F119Q. A, overlay of the conformation of the binding sites after 10-ns simulations, with the α3 subunit in green, the β2 subunit in blue, and conotoxin LvIA in white. The side chains of LvIA as well as the mutated side chains Lys57–63, Ile111, and Gln119 are in stick representation. B, backbone root mean square deviation (RMSD) over the 10-ns simulations from the starting conformation of the β2 subunit binding site. This binding site is defined here as including the β1 (positions 32–40), β2 (positions 57–63), β5’ (positions 109–113), and β6 (positions 116–120) strands.

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Note: The text continues with more detailed analysis and discussion of the results.
and Lt1A (23) (Table 3). By contrast, this mutation decreased LVIα activity by 15-fold. LVIα displays the conserved Ser-Xaa-Pro motif in its first loop, suggesting that it adopts a similar binding mode to most 4/7 α-CTx. The sequence in the second loop of α-CTx LVIα is therefore probably a determinant of its unique selectivity and distinct binding site. Molecular models indeed suggest that this second loop, especially residues Asn9, Val10, Asp11, and Pro13, interacts with the β2 subunit. Interestingly, the 4/7 α-CTx PeIA also potently blocks α3β2 nAChRs and has similar residues in its second loop (31).

The mutant K79A does not show a significant difference in activity from the wild-type nAChR, but the molecular models suggest that the Lys79 residue establishes a stable charge interaction with LVIα Asp11 (Fig. 5B). It has been proposed that surface salt bridges can have little contribution to binding affinity because the favorable charge-charge interaction can be counterbalanced by the negative entropic effect of restraining the conformation of the side chain (40). This compensation of enthalpy for entropy components between apo and bound states is a potential explanation for the innocuous nature of the K79A substitution. Indeed, the Lys79 side chain is highly exposed to the solvent and should therefore have considerable conformational freedom in the absence of the toxin. The side chain of Lys79 was restrained during the molecular simulations of the bound toxin, suggesting a significant entropic cost to the immobilization of the side chain.

The molecular models suggest that substitutions at positions 119 and 59 increase the solvent-accessible surface area buried at the interface, and this increase correlates with higher affinities of LVIα observed experimentally (Fig. 5C). In particular, the three mutations, T59I, T59L, and T59K, incrementally introduce longer side chains at position 59, and they result in increasing inhibitory potency of LVIα. The introduction of a positively charged Lys at position 59 results in the burial of a positively charged group, which could be detrimental to binding, but this residue can potentially interact with the negatively charged β2 subunit Glu61, which is proximally located (Fig. 5A). The F119Q mutation resulted in better complementarity at the interface by creating further interactions, especially with LVIα residues Val10 and Pro13, resulting in the largest buried surface area among all mutants in this study, in agreement with the highest inhibitory activity of LVIα among all mutants.

It is interesting to compare the trends in LVIα binding to α3β2 versus α3β4 relative to the individual residue substitutions at the three key positions of 59, 111, and 119. In principle, the decreases in IC50 values associated with the T59K and F119Q substitutions should more than compensate for the increased IC50 associated with the V111I substitution. Nevertheless, LVIα is 17-fold more potent at α3β2 than at α3β4. The non-additivity of the single point mutant effects can probably be explained by the spatial organization of these positions because the side chain at position 119 is sandwiched by those of positions 111 and 59. The β4 subunit, which displays bulkier side chains at these positions than the β2 subunit, should present a different interface to LVIα than the β2 subunit single point mutants.

In conclusion, we have identified three residues in the nAChR β2 subunit that are key to the binding interaction of LVIα with the α3β2 nAChR. Furthermore, molecular modeling indicates that the sequence of residues in the second loop of LVIα is particularly important for high affinity for the α3β2 nAChR. These findings help provide insights into the unique selectivity profile of this toxin. Understanding interactions between different α-CTx and α3β2 nAChR should further help to elucidate the molecular pharmacology of this subtype.

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