

α -Conotoxins EpI and AuIB switch subtype selectivity and activity in native versus recombinant nicotinic acetylcholine receptors

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Abstract The *Xenopus laevis* oocyte expression system was used to determine the activities of α -conotoxins EpI and the ribbon isomer of AuIB, on defined nicotinic acetylcholine receptors (nAChRs). In contrast to previous findings on intracardiac ganglion neurones, α -EpI showed no significant activity on oocyte-expressed $\alpha 3\beta 4$ and $\alpha 3\beta 2$ nAChRs but blocked the $\alpha 7$ nAChR with an IC_{50} value of 30 nM. A similar IC_{50} value (103 nM) was obtained on the $\alpha 7/5HT_3$ chimeric receptor stably expressed in mammalian cells. Ribbon AuIB maintained its selectivity on oocyte-expressed $\alpha 3\beta 4$ receptors but unlike in native cells, where it was 10-fold more potent than native α -AuIB, had 25-fold lower activity. These results indicate that as yet unidentified factors influence α -conotoxin pharmacology at native versus oocyte-expressed nAChRs.

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Key words: $\alpha 7$ nicotinic acetylcholine receptor; Intracardiac ganglia; α -Conotoxin EpI; α -Conotoxin AuIB; *Xenopus laevis* oocyte

1. Introduction

To date eight α and three β subunits ($\alpha 2$ – $\alpha 7$, $\alpha 9$, $\alpha 10$, $\beta 2$ – $\beta 4$) of the nicotinic acetylcholine receptor (nAChR) have been cloned from sensory and neuronal mammalian cells. These have the potential to assemble in a great diversity of nAChR subtypes with different pharmacological and functional properties [1]. Numerous neuronal nAChR subtypes have been identified in native tissues and in several cells the presence of more than one subtype has been demonstrated. To decipher the composition and function of the various nAChR subtypes, selective ligands are needed to correlate the pharmacological properties of defined heterologously expressed subunit combinations with native receptors. α -Conotoxins, small disulfide-rich peptides from the venom of the predatory ma-

rine snails of the genus *Conus*, are amongst the most selective inhibitors of nAChR subtypes to be identified [2] (Fig. 1).

Differences in single channel conductance, kinetics and relative agonist potencies have been observed between heterologously expressed nAChRs and nAChRs characterised in native tissues [3] and even upon heterologous expression of the same subunit combination in different expression systems distinct properties were observed [4]. In order to define structure–activity relations for the design of optimised synthetic peptides, it is therefore important to compare activity data generated in a common system.

Because of its highly reproducible and efficient expression and the apparent absence of endogenous nAChR subunits, the *Xenopus* oocyte expression system is widely used to study defined subunit combinations of the nAChR. In fact, most neuronal active α -conotoxins were initially characterised on oocyte-expressed nAChR subtypes. Here we show that subtype selectivity of α -conotoxin EpI is switched from $\alpha 3\beta 2$ / $\alpha 3\beta 4$ -selective to $\alpha 7$ -selective in native versus oocyte-expressed receptors, respectively. In addition, significant differences in activity between two isomers of α -conotoxin AuIB that act on the same receptor exist between native and oocyte-expressed $\alpha 3\beta 4$ receptors.

2. Materials and methods

2.1. Peptide synthesis

AuIB and its isomer were assembled on Boc-Phe-OCH₂-Pam resin using standard BOC-Chemistry, deprotected and cleaved from resin as described previously [5]. The globular and ribbon isomers were formed by oxidation of the fully reduced purified [reversed-phase high-performance liquid chromatography (RP-HPLC)] peptide at a concentration of 0.08 mg/ml in 20% isopropanol in 0.1 M NH₄HCO₃ (pH 8.0). A third isomer, most probably a beads isomer, formed in very small amounts and was found to be unstable. The oxidised peptides were purified by preparative RP-HPLC using a C18 Vydac column.

EpI and [Y¹⁵]EpI were made by Fmoc chemistry as described [6]. HPLC-purified reduced peptides (20 μ M) were oxidised in 100 mM ammonium bicarbonate at pH 7.5–8 with stirring for 24–48 h at room temperature. The oxidised peptides were purified by preparative RP-HPLC. Peptides were quantified initially in triplicate by amino acid analysis, then subsequently by RP-HPLC using an external reference standard for each peptide.

2.2. Functional characterisation

2.2.1. Plasmids and cRNA preparation. Plasmids containing cDNA encoding rat $\alpha 3$, $\alpha 4$, $\alpha 7$, $\beta 2$, and $\beta 4$ nAChR subunits were provided by J. Patrick (Baylor College of Medicine, Houston, TX, USA) and subcloned into the oocyte expression vector pNKS2 [7].

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Abbreviations: nAChR, nicotinic acetylcholine receptor; ACh, acetylcholine; α -BTX, α -bungarotoxin

cRNA was synthesised using the Message Machine kit (Ambion, Austin, TX, USA). cDNAs for chicken $\alpha 7$ and mouse 5HT₃ subunits were provided by M. Ballivet and D. Julius. The $\alpha 7/5HT_3$ chimera was constructed according to Eisele et al. [8] and cloned into the inducible vector pcDNA4/TO [9].

2.2.2. Two-electrode voltage-clamp recording on oocyte-expressed nAChRs. Oocytes were prepared and two-electrode voltage-clamp recordings were performed as previously described [10]. cRNA-injected oocytes were clamped at a holding potential of -70 mV and acetylcholine (ACh)-activated membrane currents were recorded using a two-electrode virtual ground circuit on a GeneClamp 500B amplifier (Axon Instruments Inc., Union City, CA, USA), filtered at 200 Hz and digitised at 1 kHz using a Digidata 1322A interface and v8.2 Clampex software (Axon Instruments Inc.). The perfusion medium was manually switched between ND96 (96 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES at pH 7.4) with or without agonist using a Valve Driver II (General Valve Corporation, Fairfield, NJ, USA). A fast and reproducible solution exchange (< 300 ms) for agonist application was achieved using a 50 μ l funnel-shaped oocyte chamber combined with a fast solution flow (~ 150 μ l/s) fed through a custom-made manifold mounted immediately above the oocyte.

One μ M ACh (Sigma) was used to activate $\alpha 4\beta 4$ subunits, 100 μ M ACh was used to activate $\alpha 3\beta 2$, $\alpha 3\beta 4$ and $\alpha 4\beta 2$ subunits, and 100 μ M nicotine (Sigma) was used to activate $\alpha 7$ nAChRs. Agonist pulses were applied for 2 s in 4-min intervals. After each application, the cell was superfused for 1 min and the flow was then stopped until the agonist was re-applied. Peptide was applied when responses to three consecutive agonist applications differed by less than 10%. After the 1-min agonist washout step was stopped, 5.5 μ l of a 10-fold-concentrated peptide solution were pipetted directly into the static bath, mixed by repeated pipetting, and incubated for 3 or 5 min prior to addition of agonist. Dose–response curves were fitted to the data by the equation: % response = $100 / (1 + ([\text{toxin}] / IC_{50})^{n_H})$, using Prism software (GraphPad v 3.0 for Macintosh, San Diego, CA, USA).

2.2.3. Patch-clamp recordings on chimeric $\alpha 7/5HT_3$ nAChRs. The chimera $\alpha 7/5HT_3$ was expressed in TRex-293 cells (Invitrogen) which are derived from HEK293 cells. Expression of the $\alpha 7/5HT_3$ chimera was induced by the addition of tetracycline 24–48 h prior to experiments. Whole-cell current recordings were performed, using an LM-EPC-7 patch-clamp system (List, Darmstadt, Germany), on nAChR-expressing HEK293 cells cultured 3 days on fibronectin-coated coverslips. The bathing solution was composed of (mM): NaCl 145; KCl 5; MgCl₂ 1; CaCl₂ 2; D-glucose 10; HEPES 10 (pH 7.3; 300 mOsm), and the internal pipette solution contained (mM): CsCl 140 (equilibrated with CsOH); EGTA 11; HEPES 10; MgCl₂ 1, (pH 7.3; 300 mOsm). The patch microelectrodes were made from borosilicate glass (external diameter 1.6 mm), and the pipette resistance was measured as 3–7 M Ω when filled with the internal solution. After formation of a high-resistance seal with the cell membrane, capacitance transients were minimised using the C-Fast facility of the system. No additional capacitance and serial resistance compensation was applied. All experiments were performed at room temperature at a holding potential of -70 mV. Coverslips were incubated with 5 ml of bathing solution with selected concentrations of EpI for 5 min. Then whole-cell currents of $\alpha 7/5HT_3$ were induced by fast application of 3 μ M nicotine dissolved in external solution, using a U-shaped tube positioned near the investigated cell, at a flow rate of 1.0 ml/min. The agonist pulses were for 2 s in 1-min intervals. Signals were filtered at 3.15 kHz (Bessel), digitised to 10 kHz and analysed on a PC using the pClamp software package version 6.03 (Axon Instruments, Foster City, CA, USA). Dose–response curves were fitted to the data by using Prism software (GraphPad v 4.0 for Windows, San Diego, CA, USA).

3. Results and discussion

3.1. EpI switches subtype selectivity on oocyte-expressed versus native nAChRs in intracardiac ganglia

EpI was originally characterised in dissociated neurones of parasympathetic intracardiac ganglia, which are believed to contain primarily $\alpha 3\beta 2^*$ and $\alpha 3\beta 4^*$ nAChRs (* indicates the possible inclusion of further subunits [11]), and on bovine chromaffin cells, a model for $\alpha 3\beta 4^*$ nAChRs. EpI was found

α -Conotoxin	Sequence	Oocyte-expressed	Selectivity
ImI	GCCSDPRCAWR---C*	$\alpha 7$	$\alpha 7$
ImII	ACCSDRRCRW---C*	$\alpha 7$	$\alpha 7$
MII	GCCSNPVCHLEHSLNC*	$\alpha 3\beta 2$, $\alpha 6\beta 2$	$\alpha 3\beta 2$, $\alpha 6\beta 2$
AuIB	GCCSYPPCFATNPD-C*	$\alpha 3\beta 4$	$\alpha 3\beta 4$
EpI	GCCSDPRCNMNPDYC*	$\alpha 7$	$\alpha 3\beta 2$, $\alpha 3\beta 4$
PnIA	GCCSLPPCAANNPDYC*	$\alpha 3\beta 2 > \alpha 7$	$\alpha 3\beta 2 > \alpha 7$
[A10L]PnIA	GCCSLPPCALNPNPDYC*	$\alpha 7 > \alpha 3\beta 2$	$\alpha 7 > \alpha 3\beta 2$
PnIB	GCCSLPPCALSNPDYC*	$\alpha 7 > \alpha 3\beta 2$	$\alpha 7 > \alpha 3\beta 2$
GIC	GCCSHFACAGNNQHIC*	$\alpha 3\beta 2$	
GID	IRDYCCSNPACRVNNOHVC	$\alpha 3\beta 2 \approx \alpha 7 > \alpha 4\beta 2$	
AuIB (ribbon)	GCCSYPPCFATNPD-C*	$\alpha 3\beta 4$	$\alpha 3\beta 4$

Fig. 1. Comparison of sequences and activity of EpI and AuIB and other neuronal active α -conotoxins. Asterisks indicate C-terminal amidation. Sulfated Tyr are underlined, γ = γ -carboxyglutamic acid, and O=hydroxyproline. Note that PnIA, [A10L]PnIA, and PnIB were originally synthesised and characterised as unsulphated peptides, although the native toxins are sulphated.

to block $\alpha 3\beta 2^*$ and $\alpha 3\beta 4^*$ nAChRs but failed to inhibit an α -bungarotoxin (BTX)-sensitive current believed to represent an $\alpha 7^*$ receptor [6]. EpI shares the N-terminal eight amino acid residues with the $\alpha 7$ -selective α -conotoxin ImI (Fig. 1) and comparison of their 3D structures showed identical structures for the first loop [12]. To determine the selectivity of EpI and to provide a common basis for comparison with other neuronal active α -conotoxins, we determined EpI activity on defined oocyte-expressed nAChRs. In contrast to previous findings in the native systems [6], EpI showed no or very low activity on oocyte-expressed $\alpha 3\beta 2$ or $\alpha 3\beta 4$ receptors. It blocked, however, homomeric $\alpha 7$ receptors with an IC_{50} value of 30 nM (Fig. 3, Table 1). Even 100 nM EpI, which caused a 75% block of the $\alpha 7$ receptor in the oocyte system, did not inhibit the α -BTX-sensitive receptor in intracardiac ganglia [6]. The non-sulphated analogue of EpI, which blocked the native $\alpha 3\beta 4/\alpha 2\beta 4$ with slightly higher potency than EpI, was three-fold less potent on oocyte-expressed $\alpha 7$ receptors (Fig. 3, Table 1).

The α -BTX-sensitive current in intracardiac ganglia shows an unusually slow inactivation and rapid recovery from α -BTX-block, in contrast to the 'classical' $\alpha 7$ current found for example in chicken ciliary ganglia, PC12 cells and hippocampus neurones [13]. Together with the lack of sensitivity to EpI, this might indicate that another subunit than $\alpha 7$ accounts for this current. However, the fact that an anti- $\alpha 7$ mAb selectively inhibited the α -BTX-sensitive current identified in intracardiac ganglia argues against this possibility, and for the presence of an $\alpha 7^*$ nAChR with unusual kinetics [13]. Interestingly, an $\alpha 7$ splice variant ($\alpha 7-2$) recently identified in intracardiac ganglia is able to form functional homomeric channels that resemble the α -BTX-sensitive current in these cells [14] and might account for the distinct α -conotoxin pharmacology. Since EpI was able to inhibit currents that were maximally inhibited by the $\alpha 7$ -selective antagonists [A10L]PnIA and α -BTX [15], the receptor targeted by EpI in intracardiac ganglia is clearly different from known $\alpha 7^*$ receptors.

3.2. Native AuIB and the ribbon isomer of AuIB switch relative activities in oocyte-expressed versus native $\alpha 3\beta 4^*$ nAChRs in intracardiac ganglia

The lack of activity of EpI at oocyte-expressed $\alpha 3\beta 2$ and

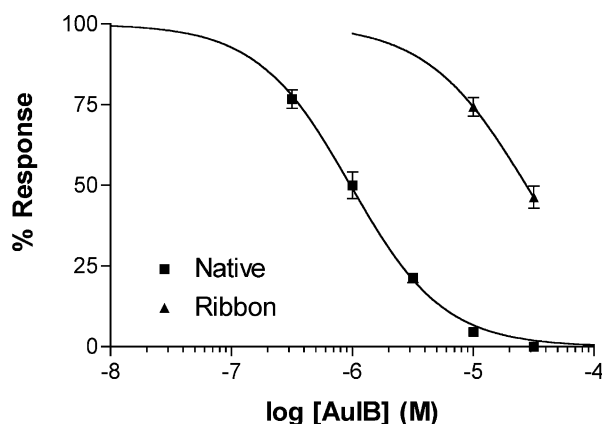


Fig. 2. Comparison of activity of native and ribbon AuIB at $\alpha 3\beta 4$ nAChRs expressed in *Xenopus* oocytes. Oocytes were injected with cRNA encoding $\alpha 3$ and $\beta 4$ subunits and voltage clamped 2–10 days after injection. Responses to 100 μ M ACh after 5 min incubation in the presence of peptide are shown as percentage of control responses. Error bars are S.E.M. and $n=3-5$ for each data point. IC_{50} values and Hill slope coefficients are summarised in Table 1.

$\alpha 3\beta 4$ nAChRs also suggests that the native receptors behave significantly differently to the oocyte-expressed receptors. However, the $\alpha 3\beta 2$ -selective PnIA and the $\alpha 3\beta 4$ -selective AuIB both inhibited the respective currents in oocytes and in intracardiac ganglia and the reported IC_{50} values for PnIA in intracardiac ganglia cells (14 nM) and on the oocyte-expressed $\alpha 3\beta 2$ combination (9.6 nM) are similar. This indicates that at least $\alpha 3\beta 2^*$ receptors have matching properties in oocytes and native tissues. In contrast, AuIB blocked the current in intracardiac ganglia with an IC_{50} value of 1.2 nM [5], which is about 500-fold lower than the IC_{50} values of 750 nM [16] and 966 nM (this study) found in oocytes (Fig. 2, Table 1). Surprisingly, ribbon AuIB, an isomer with the disulfide connectivity 1–4 and 2–3 instead of 1–3 and 2–4, was even more active (IC_{50} 0.1 nM) on the ganglia cells [5]. Its IC_{50} value on oocyte-expressed $\alpha 3\beta 4$ was 27.5 μ M, about 30 times higher than that for native AuIB and even 3000 000 times higher than that determined on native receptors (Fig. 2, Table 1). The unexpected activity of the ribbon isomer was explained by a higher structural flexibility of the molecule possibly allowing a more complementary fit in the binding site [5]. To check whether this could also change the subtype selectivity, we tested ribbon AuIB on oocyte-expressed nAChRs. Like native AuIB, 10 μ M ribbon AuIB did not cause significant inhibition of $\alpha 3\beta 2$, $\alpha 4\beta 2$, $\alpha 4\beta 4$, and $\alpha 7$ receptors (data not shown). AuIB was originally identified on oocyte-expressed receptors [16] (Fig. 1) and in various subsequent studies it was successfully used to define native $\alpha 4\beta 3^*$ nAChRs. The surprisingly large differences in activity of AuIB

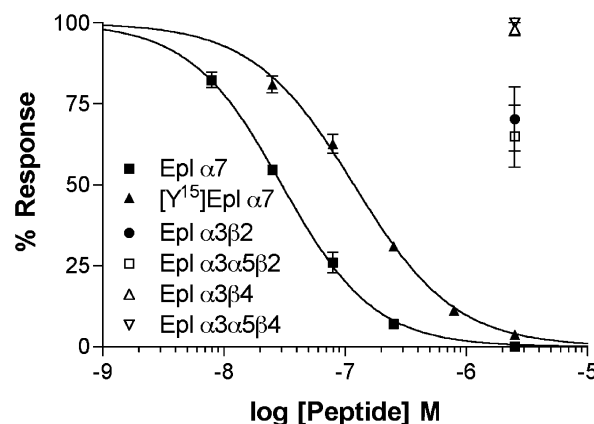


Fig. 3. Activity of EpI and $[Y^{15}]$ EpI on oocyte-expressed nAChRs. Oocytes were injected with cRNA encoding the indicated subunits and voltage clamped 2–10 days after injection. Responses to 100 μ M nicotine after 3 min incubation in the presence of peptide are shown as percentage of control responses. Error bars are S.E.M. and $n=3-5$ for each data point. IC_{50} values and Hill slope coefficients are summarised in Table 1.

and its ribbon isomer found in this study suggest that the supposed $\alpha 3\beta 4^*$ nAChR in intracardiac ganglia differs from those in oocytes and other tissues, e.g. rat medial habenula neurones and chick ciliary ganglion neurones, where similar potencies as in the oocyte system were observed [17,18].

3.3. Do $\alpha 3\beta 4$ and $\alpha 7$ subunits in intracardiac ganglia neurones combine with other subunits?

A possible explanation for these inconsistent findings could be that alternately transcribed isoforms or other combinations than the assumed $\alpha 3\beta 2$ and $\alpha 3\beta 4$ receptors account for the observed phenotype in native cells. A single-cell PCR study on intracardiac ganglia neurones [19] revealed the presence of eight different subunits ($\alpha 2$ – $\alpha 5$, $\alpha 7$ and $\beta 2$ – $\beta 4$) that were heterogeneously expressed in nine examined cells. Overall, the $\alpha 3$, $\beta 2$, $\beta 4$ and $\alpha 7$ subunits appeared to dominate whereas $\alpha 2$, $\alpha 4$ and $\beta 3$ were only detected in one or two cells. The genes encoding $\alpha 3$, $\alpha 5$, and $\beta 4$ are clustered and heterologous expression of trimeric $\alpha 3\alpha 5\beta 2$ and $\alpha 3\alpha 5\beta 4$ combinations has been described [20,21]. Therefore, the $\alpha 3\alpha 5\beta 4$ combination was expressed in oocytes to test whether the $\alpha 5$ subunit was able to confer EpI sensitivity or the increased sensitivity to the ribbon AuIB isomer. However, no differences as compared to the $\alpha 3\beta 4$ combinations were found. Likewise, the $\alpha 3\alpha 5\beta 2$ combinations did not show an altered EpI sensitivity (Fig. 3).

Although several studies indicate a homopentameric assembly of native $\alpha 7$ receptors [22] there is also increasing evidence for heteromeric $\alpha 7$ complexes and recently, the coassembly of $\alpha 7$ and $\beta 2$ receptors has been described [23]. To test whether

Table 1

Comparison of IC_{50} values and Hill slope coefficients (in brackets) at oocyte-expressed $\alpha 7$ and $\alpha 3\beta 4$ receptors and the assumed corresponding receptors in intracardiac ganglia

α -Conotoxin	Oocytes			Intracardiac ganglion		Reference
	$\alpha 7$ (nM)	$\alpha 3\beta 2$ (nM)	$\alpha 3\beta 4$ (nM)	$\alpha 7^*$ (nM)	$\alpha 3\beta 2^*/\alpha 3\beta 4^*$ (nM)	
EpI	30 (1.1)	–	–	Inactive at 100	1.6	this study, [6]
$[Y^{15}]$ EpI	118 (1.0)	–	–	inactive at 100	0.4	this study, [6]
nAuIB	–	–	966 (1.1)	–	1.2	this study, [5]
rAuIB	–	–	27,500 (1.1)	–	0.1	this study, [5]

the $\beta 2$ subunit could account for the 'EpI resistance' of the native $\alpha 7$ receptor this combination was also tested in oocytes. Despite a strong decrease in the current, coexpression of the $\beta 2$ subunit caused no difference in EpI sensitivity (data not shown).

3.4. Do oocyte-expressed $\alpha 7$ nAChRs have a different EpI sensitivity than $\alpha 7$ receptors expressed in mammalian cells?

The above results suggest that so far unidentified subunits or splice [14] variants participate in the formation of EpI-resistant $\alpha 7^*$ receptors or that the functional properties of the receptor are modified in a cell-specific way [13]. Different properties of recombinant nAChRs in oocytes and mammalian cells have been reported [4]. Factors that could modify receptor properties include interactions with proteins such as adaptor proteins, cytoskeletal elements or other membrane proteins. Since the oocyte is of non-neuronal origin, interacting proteins might either be absent or not sufficiently expressed to modulate the highly overexpressed nAChRs in oocytes effectively. Alternatively, the different lipid composition of the oocyte membrane could influence receptor function. Finally, a difference in maturation and folding events or post-translational processing between the oocytes and mammalian cells could result in different tertiary structures that exhibit distinct binding and signal transduction properties. To exclude the possibility that the observed differences in EpI sensitivity are due to oocyte-specific modification of the $\alpha 7$ receptor, EpI was also tested on the HEK293 derived TRex-293 cell line stably transfected with a $\alpha 7/5HT_3$ chimeric nAChRs. This chimera represents a common model for the $\alpha 7$ receptor which has been difficult to express as a functional homopentamer [24]. The IC_{50} value obtained on these cells was similar (103 nM) to that obtained on oocyte-expressed receptors (Fig. 4). This small difference can be explained by different measuring protocols, toxin application systems and different stock solutions. Another explanation would be that the rat $\alpha 7$ receptor could be slightly more sensitive to EpI than the chicken isoform used for construction of the chimera.

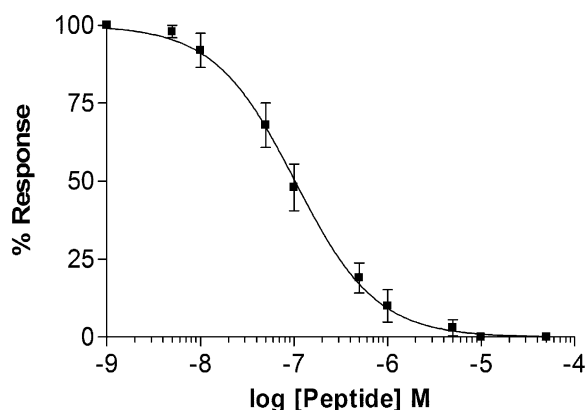


Fig. 4. Activity of EpI on TRex-293 cells stably transfected with $\alpha 7/5HT_3$ chimeric nAChR. Cells were cultured on the coverslips for 3 days before whole-cell recordings were performed at holding potential of -70 mV. Responses to the agonist, nicotine, were measured after 5 min incubation in the presence of α -conotoxin. Data are expressed as percentage of control responses. Error bars are S.E.M. and $n=8-10$ for each data point. The IC_{50} value was 103 nM and the Hill slope coefficient was 1.4.

Table 2

Correlation of the length and hydrophobicity of the amino acid residue in position 10 with the selectivity of 4/7 α -conotoxins for oocyte-expressed $\alpha 7$ receptors [10,28,29]

α -Conotoxin	$\alpha 3\beta 2$ (nM)	$\alpha 7$ (nM)	$\alpha 3\beta 2/\alpha 7$ Ratio	Consensus Sequence	Reference
GIC	1.1	n.d.		CA C NNQ	[28]
PnIA	9.6	252	0.04	CA A NNP	[29]
[N11S]PnIA	241	1710	0.14	CA A SNP	[29]
[R12A]GID	10	48	0.2	CA V NNQ	[10]
GID	3.1	4.5	0.7	CR V NNQ	[10]
[A10L]PnIA	99	12.6	7.9	CA L NNP	[29]
PnIB	1970	61	32	CA L SNP	[29]
EpI	>4000	30	>100	CN M NNP	This study

Corresponding residues in PnIA

8 9 10 11 12 13

3.5. Comparison of the activities of EpI and other α -conotoxins

In this study, EpI has been identified as a potent and selective inhibitor of the recombinant $\alpha 7$ nAChRs as well as the $\alpha 7/5HT_3$ chimera. In this respect, EpI has closest similarity to the 3/4 α -conotoxin ImI with which it also shares the first eight amino acids. Interestingly, ImI seems to have a different binding mode than the 4/7 α -conotoxins [25,26]. However, EpI also shows a consensus sequence found in the second loop of many 4/7 α -conotoxins [10]. Comparison with the other 4/7 α -conotoxins (Table 2) supports the previously found importance of a long hydrophobic side chain in position 10 for $\alpha 7$ selectivity and might indicate that EpI shares a common binding mode with these 4/7 α -conotoxins rather than with ImI. Very recently, the 3/4 α -conotoxin ImII, which has nine out of 12 amino acids in common with ImI, was identified [27]. ImII inhibits the $\alpha 7$ receptor with similar activity as ImI and EpI but, in contrast to these, does not compete with α -BTX binding, indicating that it binds to a different binding site. Thus the similar peptides ImI, ImII and EpI seem to represent important key structures to study the different binding modes and binding sites of neuronal active α -conotoxins.

In conclusion, this study confirms that subunit combinations in native tissues are much more complex than assumed and/or that the pharmacology of nAChR receptors might be modulated by factors other than subunit stoichiometry and assembly. Understanding the cause of the observed differences between heterologously expressed and native nAChRs is crucial for future studies that use heterologous expression systems to identify selective ligands for the characterisation of native receptors. A thorough comparison and characterisation of various α -conotoxins in different systems is therefore essential to prove the utility of the oocyte system and the validity of correlations based on the characterisation of oocyte-expressed subunit combinations. Nevertheless, the activity data obtained from oocyte-expressed receptors provide an extensive and valuable basis for structure activity studies.

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