

Molecular interaction of α -conotoxin RgIA with the rat α 9 α 10 nAChR

Layla Azam, Athanasios Papakyriakou, Marios Zouridakis, Petros Giastas, Socrates J. Tzartos and J. Michael McIntosh

Molecular Pharmacology

Q9JLB5	ACH10_RAT	1	MGTRSHYLDLGFLLLFLPA---ECLGAEGRLAHKLFRDLFANYTSALRPVADTDQTLNV	57
Q9GZ26	ACH10_HUMAN	1	MGLRSHHLSLGLLLLFLPLA---ECLGAEGRALAKLFRDLFANYTSALRPVADTDQTLNV	57
P43144	ACHA9_RAT	1	--MNRPHSCLSFQWYFAASGIRAVETANGKYAOKLFSDLFEDYSSALRPVEDTAVLVN	58
Q9UGM1	ACHA9_HUMAN	1	--MNVSHSCISFCWIYFAASRLAAETADGKYAOKLFNDLFEDYSNALRPVEDTDKVLNV	58
			. : : : : : : : : . : : * * * * * : * : * * * * * * * * . * * *	
Q9JLB5	ACH10_RAT	58	TLEVTLAQIIDMERNQVLTLYLWIRQEWTDAYLHWDPKAYGDLDAIRIPSRVWRPDIV	117
Q9GZ26	ACH10_HUMAN	58	TLEVTLAQIIDMERNQVLTLYLWIRQEWTDAYLHWDPKAYGDLDAIRIPSRVWRPDIV	117
P43144	ACHA9_RAT	59	TLQVTLAQIIDMERNQILTAYLWIRQIWHDAYLTWDRDQYDRLDISIRIPSDLVWRPDIV	118
Q9UGM1	ACHA9_HUMAN	59	TLQITLSQLTKMERNQILTAYLWIRQIWHDAYLTWDRDQYDGLDSIRIPSDLVWRPDIV	118
			* * : *	
Q9JLB5	ACH10_RAT	118	LYNKADTPPPASASTNVVVRHGDGAVRWDAAPITRSSCRVDVSAFPFDAQRCGLTFGSWTH	177
Q9GZ26	ACH10_HUMAN	118	LYNKADAQPFGSASTNVVLRHGDGAVRWDAAPITRSSCRVDVSAFPFDAQRCGLTFGSWTH	177
P43144	ACHA9_RAT	119	LYNKADDESSEPVNTNVVLRDGLITWDSAPITKSSCVVDVTYFPFDSQQCNLTFGSWTY	178
Q9UGM1	ACHA9_HUMAN	119	LYNKADDESSEPVNTNVVLRDGLITWDSAPITKSSCVVDVTYFPFDDQQCNLTFGSWTY	178
			* * * * * : : * * * * * * * * : *	
Q9JLB5	ACH10_RAT	178	GGHQLDVRPRGTASLADFVENVWVRVLMGPARRRVLTYGCCSEYPYDVTFTLLRRRAA	237
Q9GZ26	ACH10_HUMAN	178	GGHQLDVRPRGAAAASLADFVENVWVRVLMGPARRRVLTYGCCSEYPYDVTFTLLRRRAA	237
P43144	ACHA9_RAT	179	NGNQVDIFNALDSGDLSDFIEDVEVEVHGMPAVKNVISYGCCSEYPYDVTFTLLRRRSS	238
Q9UGM1	ACHA9_HUMAN	179	NGNQVDIFNALDSGDLSDFIEDVEVEVHGMPAVKNVISYGCCSEYPYDVTFTLLRRRSS	238
			* * : * * : : *	
Q9JLB5	ACH10_RAT	238	AYVCNLLLPCVFLISLLAPLAFHLPADSGETVSLGVTVLLALTFVQLILAESMPPAESVPL	297
Q9GZ26	ACH10_HUMAN	238	AYVCNLLLPCVFLISLLAPLAFHLPADSGETVSLGVTVLLALTFVQLILAESMPPAESVPL	297
P43144	ACHA9_RAT	239	FYIVNLLIPCVLISFLAPLSFYLPAAASGETVSLGVTITLLAMTVFQLMVAEIMPASENVPL	298
Q9UGM1	ACHA9_HUMAN	239	FYIVNLLIPCVLISFLAPLSFYLPAAASGETVSLGVTITLLAMTVFQLMVAEIMPASENVPL	298
			* : *	
Q9JLB5	ACH10_RAT	298	IGKYYMATMTMTVTFSTALITLIMNLHYCGPNAPVPAWARVLLLGHLAGLCVRRERGEPC	357
Q9GZ26	ACH10_HUMAN	298	IGKYYMATMTMTVTFSTALITLIMNLHYCGSPVRVPAWARALLLGHLAGLCVRRERGEPC	357
P43144	ACHA9_RAT	299	IGKYYIATMALITASTALTIMVMNIHFCGAEARVPVHAKVILKYSRILFVYDVGESG	358
Q9UGM1	ACHA9_HUMAN	299	IGKYYIATMALITASTALTIMVMNIHFCGAEARVPVHARVVILKYSRVLFFYDVGESG	358
			* * * * * : * * * * * * * * : *	
Q9JLB5	ACH10_RAT	358	GQSKPLESAPSLQ-----PPP-----ASPAGPC-HEPRCL	386
Q9GZ26	ACH10_HUMAN	358	GQSRPPELSPSPQ-----SPE-----GGAGPPAGPC-HEPRCL	389
P43144	ACHA9_RAT	359	LSPRHSQEPEQVTKVYSKLPESNLKTSRNKDLSRKKKVRKLLKNDLGYQGGIPONTDSYC	418
Q9UGM1	ACHA9_HUMAN	359	LSPHHSRERDHLTKVYSKLPESNLKAAARNDLSRKKDMNKRLLKNDLGYCGKQGNPQEAESYC	418
			. : *	
Q9JLB5	ACH10_RAT	387	CHQEALLHHIASIASTFRSHRAAQRHRHEDWKRLARVMDRFFLGIFFCMALVMSLIVLVQA	446
Q9GZ26	ACH10_HUMAN	390	CRQEALLHHVATIANFRSHRAAQRCHDWRKRLARVMDRFFLAIFFSMALVMSLIVLVQA	449
P43144	ACHA9_RAT	419	ARYEALTKNIEYIAKCLKDHKATNSKGSEWKKVAKVIDRFFMWIFFAMVFMVTILIIARA	478
Q9UGM1	ACHA9_HUMAN	419	AQYKVLTRNIEYIAKCLKDHKATNSKGSEWKKVAKVIDRFFMWIFFIMVFMVTILIIARA	478
			. : : * : : * * . : * * * : : : *	
Q9JLB5	ACH10_RAT	447	L	447
Q9GZ26	ACH10_HUMAN	450	L	450
P43144	ACHA9_RAT	479	D	479
Q9UGM1	ACHA9_HUMAN	479	D	479

Figure S1. Sequence alignment of human and rat α 9 and α 10 nAChR subunits color-coded by similarity and topological domain (red for signal peptide residues).

Molecular interaction of α -conotoxin RgIA with the rat $\alpha 9\alpha 10$ nAChR

Layla Azam, Athanasios Papakyriakou, Marios Zouridakis, Petros Giastas, Socrates J. Tzartos and J. Michael McIntosh

Molecular Pharmacology

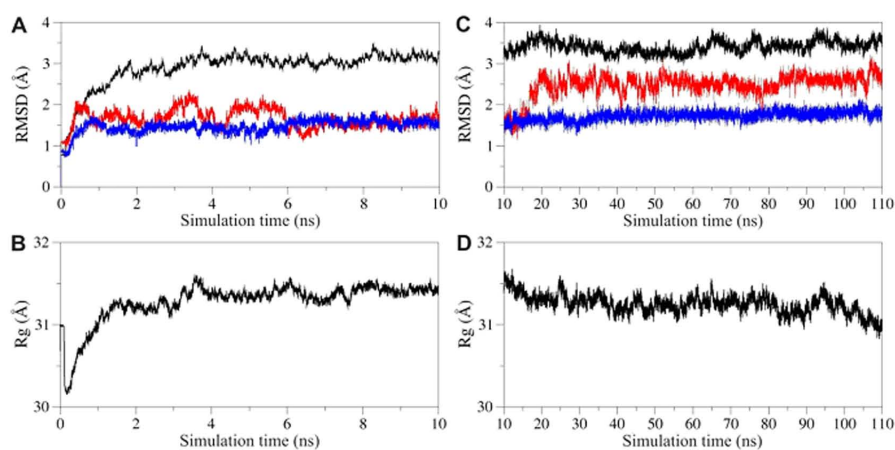


Figure S2. (A) Positional root-mean-square deviation (RMSD) from the initial model of rat $(\alpha 9)_2(\alpha 10)_3$ ECD complex with RgIA during the equilibration phase, calculated for the backbone atoms of the receptor (black line) and the two bound α -conotoxin ligands (red and blue lines). (B) Radius of gyration (Rg) of the receptor during the course of the equilibration phase of the MD simulations. (C) and (D) are the same as (A) and (B), respectively, during the course of the 100-ns production MD runs.

Molecular interaction of α -conotoxin RgIA with the rat $\alpha 9\alpha 10$ nAChR

Layla Azam, Athanasios Papakyriakou, Marios Zouridakis, Petros Giastas,
Socrates J. Tzartos and J. Michael McIntosh

Molecular Pharmacology

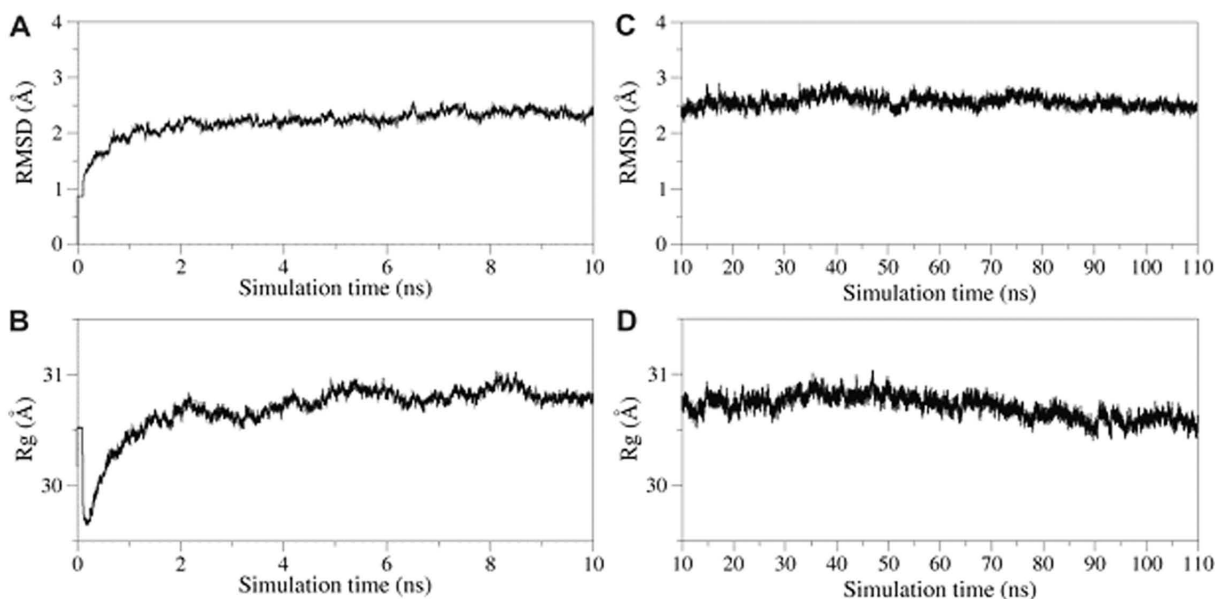


Figure S3. (A) Positional root-mean-square deviation (RMSD) of the backbone atoms of the receptor from the initial model of rat $(\alpha 9)_2(\alpha 10)_3$ ECD complex with ACh during the equilibration phase. (B) Radius of gyration (R_g) of the receptor during the course of the MD simulations. (C) and (D) are the same as (A) and (B), respectively, during the course of the 100-ns production MD runs.

Molecular interaction of α -conotoxin RgIA with the rat $\alpha 9\alpha 10$ nAChR

Layla Azam, Athanasios Papakyriakou, Marios Zouridakis, Petros Giastas, Socrates J. Tzartos and J. Michael McIntosh

Molecular Pharmacology

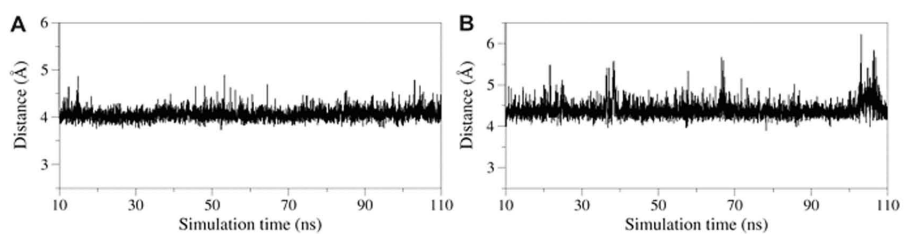


Figure S4. Distance between D201-C γ of $\alpha 10(+)$ subunit and R7-C ζ of RgIA as a function of simulation time taken from : (A) the MDs of the native receptor, and (B) the MDs of the $\alpha 9\alpha 10$ P200Q mutant.

Molecular interaction of α -conotoxin RgIA with the rat $\alpha 9\alpha 10$ nAChR

Layla Azam, Athanasios Papakyriakou, Marios Zouridakis, Petros Giastas,
Socrates J. Tzartos and J. Michael McIntosh

Molecular Pharmacology

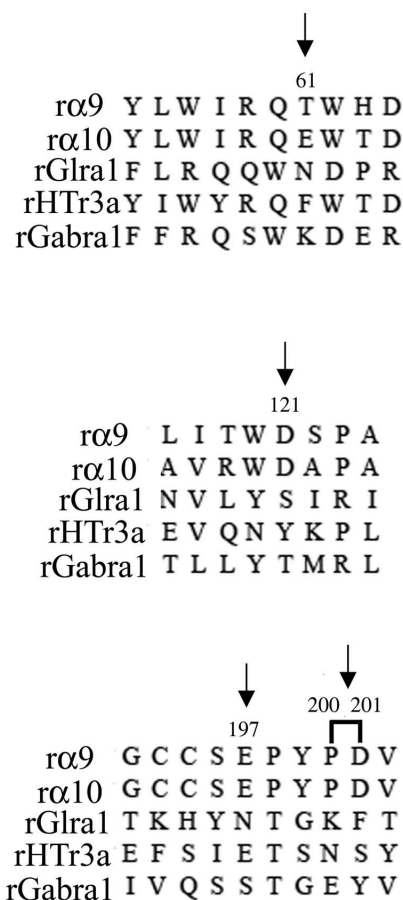


Figure S5. Alignment of critical α -CTx RgIA binding residues in ECDs of the rat $\alpha 9$ and $\alpha 10$ nAChR subunits with other rat Cys-loop ligand-gated ion channels. These other pentameric channels include glycine (Gla1), 5-HT₃ (HTr3a) and GABA_A (Gabra1). The sequence alignment among the rat ECDs was performed with MacVector 10.5.1 ClustalW alignment, using the UNIPROT accession codes P43144 for rat $\alpha 9$, Q9JLB5 for rat $\alpha 10$, P35563 for 5HT3A, P62813 for GABA_A alpha1 subunit and P07727 for Glycine receptor alpha1 subunit. Note that few of the residues are conserved in the non-nicotinic receptors. Numbering is for the $\alpha 9$ and $\alpha 10$ nAChR subunits.