Energy for Wild-Type Acetylcholine Receptor Channel Gating from Different Choline Derivatives

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Supplementary Information

Standard procedure for quaternization of amines

Reactions were carried out under an atmosphere of dry argon. Anhydrous acetonitrile and other (reagent grade) solvents were obtained commercially. The tertiary amine substrates, bromoalkanes, trimethylamine, and methyl tosylate were obtained from Aldrich. NMR spectra were recorded, using acetonitrile-d_3 as solvent, on a Bruker Avance 250 spectrometer. Spectra were calibrated to residual proton resonances from the solvent. Elemental analyses were performed by Atlantic MicroLabs, Atlanta, GA.

Quaternization was carried out in anhydrous acetonitrile (1 mmol/mL). To create a tosylate salt form, the tertiary amine was treated with methyl tosylate (1 equivalent). To create a bromide salt form, the bromoalkane was treated with anhydrous trimethylamine (1.5 equivalent). After stirring at room temp for 1-2 h, the reaction mixture was diluted 3-fold with diethyl ether. The white solid was collected and washed on the filter with a small volume of diethyl ether, then dried under vacuum for 2 h. Recrystallization from ethyl acetate/ethanol gave the product as colorless crystals.

3-hydroxypropyltrimethylammonium tosylate (3OH-PTMA): Prepared from 0.88 g (8.5 mmol) of 3-dimethylamino-1-propanol as described above. Yield: 1.13 g (3.9 mmol, 46%); ^{1}H NMR (250 MHz, acetonitrile-d_3) δ: 7.59 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 3.40-3.60 (m, 2H), 3.55 (t, J = 5.7 Hz, 2H), 3.30-3.45 (m, 3H: OH and CH_2), 3.01 (s, 9H), 2.32 (s, 3H), 1.80-1.95 (m, 2H); elemental analysis calculated for $C_{13}H_{23}NO_4S{\cdot}½\ H_2O$: C, 52.33; H, 8.11; N, 4.69; Found: C, 52.46; H, 8.13; N, 4.79.

4-hydroxybutyltrimethylammonium tosylate (4OH-BTMA): Prepared from 0.88 g (7.5 mmol) of 4-dimethylamino-1-butanol as described above. Yield: 1.30 g (4.3 mmol, 57%); ^{1}H NMR (250 MHz, acetonitrile-d_3) δ: 7.59 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 3.40-3.60 (m, 2H), 3.20-3.35 (m, 3H: OH and CH_2), 3.01 (s, 9H), 2.32 (s, 3H), 1.70-1.85 (m, 2H), 1.48 (5tet, J = 6.8 Hz, 2H); elemental analysis calculated for $C_{14}H_{25}NO_4S{\cdot}½\ H_2O$: C, 53.82; H, 8.39; N, 4.48; Found: C, 54.34; H, 8.22; N, 4.61.

2-hydroxypropyltrimethylammonium tosylate (2OH-PTMA): Prepared from 2.0 g (19.4 mmol) of 1-dimethylamino-2-propanol as described above. The product is a racemic mixture of the R and S-form of 2OH-PTMA.Yield: 4.04 g (14.0 mmol, 72%); ^{1}H NMR (250 MHz, acetonitrile-d_3) δ: 7.59 (d, J = 8.1 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 3.40-3.48 (m, 4H), 3.15-3.35 (m, 3H: OH and CH_2), 3.10 (s, 9H), 2.33 (s, 3H), 1.14 (d, J = 6.3 Hz, 3H).

Ethyltrimethylammonium bromide (ETMA): Prepared from 1.46 g (13.4 mmol) of 1-bromoethane as described above. Yield: 1.04 g (6.2 mmol, 46%); ^{1}H NMR (250 MHz, acetonitrile-d_3) δ: 3.43 (q, J = 7.3 Hz, 2H), 3.09 (s, 9H), 1.29 (t, J = 7.3 Hz, 3H); elemental analysis calculated for $C_5H_{14}BrN{\cdot}½\ H_2O$: C, 33.91; H, 8.54; N, 7.91; Found: C, 34.12; H, 8.43; N, 7.94.

Propyltrimethylammonium bromide (PTMA): Prepared from 1.35 g (11.0 mmol) of 1-bromopropane as described above. Yield: 0.81 g (4.5 mmol, 41%); ^{1}H NMR (250 MHz, acetonitrile-d_3) δ: 3.20-3.30 (m, 2H), 3.06 (s, 9H), 1.65-1.85 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); elemental analysis calculated for $C_6H_{16}BrN$: C, 39.57; H, 8.86; N, 7.69; Found: C, 38.66; H, 8.96; N, 7.58.

Butyltrimethylammonium tosylate (BTMA): Prepared from 2.0 g (19.8 mmol) of dimethylbutylamine as described above. Yield: 4.09 g (14.3 mmol, 72%); ^{1}H NMR (250 MHz,

acetonitrile-d_3) δ: 7.59 (d, *J* = 8.1 Hz, 2H), 7.15 (d, *J* = 8.1 Hz, 2H), 3.10-3.25 (m, 2H), 3.01 (s, 9H), 2.33 (s, 3H), 1.60-1.75 (m, 2H), 1.34 (6tet, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H).

Table S1: Observed and corrected rate/equilibrium constants of choline derivatives

Agonist	f_2	b_2	E_2^{obs}	bkgd	E_2^{corr}	n
ACh[a]					25.4	
TMA[a]					2.54	
ETMA[b]	866±165	2571±666	0.34±0.15	1.36	0.25±0.11	14
PTMA[b]	879±227	2242±318	0.39±0.38	1.36	0.29±0.28	8
BTMA[c]	2554±204	3259±340	0.78±0.19	0.32	2.44±0.18	2
choline[a]					0.05	
3OH-PTMA[b]	564±51	2764±521	0.20±0.04	1.36	0.15±0.03	7
4OH-BTMA[b]	2060±375	2132±330	0.97±0.47	1.36	0.71±0.34	10
chlorocholine[b]	652±145	2520±678	0.26±0.16	1.36	0.19±0.12	9
2OH-PTMA[d]	531±90	1843±297	0.29±0.11	14.3	0.02±0.01	8
cholamine pH9.0[d]	1580±105	2693±700	0.59±0.14	14.3	0.04±0.01	4
cholamine pH6.1[e]	88±18	2542±464	0.03±0.01	34.4	0.0012±0.001	8
betaine[f, -100mV]	2288±465	2123±745	1.08±0.33	6.8E4	1.6E-5±9.3E-6	7
betaine[b+f, +100mV]	1826±300	2179±576	0.84±0.30	9.2E4	9.0E-6±3.3E-6	4

f_2, diliganded opening rate constant (s^{-1}); b_2, diliganded closing rate constant (s^{-1}); E_2^{obs}, measured diliganded gating equilibrium constant ($=f_2/b_2$); bkgd, the net fold-change in E_0 of the background; E_2^{corr}, diliganded gating equilibrium constant corrected to the reference condition (-100 mV, wt; $= E_2^{obs}$/bkgd); n, number of patches.

[a] Measurements from (1).
Background perturbations: [b] εS450A, [c] εS450W+αD97I, [d] εL269F, [e] εL269F+αD97N, [f] α(D97A+Y127+S269I); V_m=+100 mV except where specified (for fold-changes in E_0 see Table S2).

Table S2: Locations and previously-published effects of mutations on E_2

Perturbation	Location	$E_0^p/E_0^{wt,\ -0.1V}$	Reference
αD97N	Loop A	2.4	(2)
αD97I	Loop A	0.4	(2)
αDYS	ECD	68,000	(3)
εL269F	M2	179	(4)
εS450A	M4	17	(5)
εS450W	M4	9.9	(5)
+100 mV	TMD	0.08	(6)

All mutations change E_2 by an equivalent change in E_0 and do not affect ΔG_B.
ECD: Extracellular domain, TMD, transmembrane domain; M2, M4: transmembrane segments 2 and 4; DYS: αD97A+αY127F+αS269I.

Table S3: Sequence alignment of AChR and AChBP

Channel	#	Sequence
AChR mouse α1	17	SVVRPVEDHREIVQVTVGLQLIQLINVDEVNQIVTTNVRLKQQWVDYNLKWN
AChR mouse δ	19	KDLRPVARKEDKVDVALSLTLSNLISLKEVEETLTTNVWIDHAWVDSRLQWD
AChBP *Aplysia*	17	SPMYPGPTKDDPLTVTLGFTLQDIVKADSSTNEVDLVYYEQQRWKLNSLMWD
AChBP *Lymnaea*	15	RPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVFWQQTTWSDRTLAWN
AChR mouse α1	69	PDDYGGVKKIHIPSEKIWRPDVVLYNNADGDFAIVKFTKVLLDYTGHITWTP
AChR mouse δ	71	ANDFGNITVLRLPPDMVWLPEIVLENNNDGSFQISYACNVLVYDSGYVTWLP
AChBP *Aplysia*	69	PNEYGNITDFRTSAADIWTPDITAYSSTR-PVQVLSPQIAVVTHDGSVMFIP
AChBP *Lymnaea*	67	SSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVSDGEVLYMP
AChR mouse α1	121	PAIFKSYCEIIVTHFPFDEQNCSMKLGT**W**TYDGSVVAINPESDQP-------
AChR mouse δ	123	PAIFRSSCPISVTYFPFDWQNCSLKFSSLKYTAKEITLSLKQEEENNRSYPI
AChBP *Aplysia*	120	AQRLSFMCDPTGVDS-EEGATCAVKFGSWVYSGFEIDLKTDTDQV-------
AChBP *Lymnaea*	116	SIRQRFSCDVSGVDT-ESGATCRIKIGSWTHHSREISVDPTTENSD------
AChR mouse α1	166	-----DLSNFMESGEWVIKEARGWKHWVF**Y**SCCPTTP**Y**LDITYHFVMQRLP
AChR mouse δ	175	EWIIIDPEGFTENGEWEIVHRAAKLNVDPSVPMDSTNHQDVTFYLIIRRKP
AChBP *Aplysia*	164	-----DLSSYYASSKYEILSATQTRQVQHYSCCP-EPYIDVNLVVKFRERR
AChBP *Lymnaea*	161	-----DSEYFSQYSRFEILDVTQKKNSVTYSCCP-EAYEDVEVSLNFRKKG

In bold, residues of the aromatic triad that provide most of the ΔG_B energy for ACh.

Table S4: Ligand-receptor energies of 2BYQ-based AChR complexes with TMA and choline

Energy, kcal/mol [a]	TMA	Choline
Total ligand-receptor	-9.7	-8.4
Aromatic triad [b]	-6.1	-5.5
αW149	-2.2	-2.6
αY190	-2.4	-1.9
αY198	-1.5	-1.0
αY93	-0.7	-0.8
δW57	-0.7	-0.6
δL121	-0.8	-0.5

[a] Average of the five best ligand-receptor energies of TMA and choline
[b] Aromatic triad consists of αW149, αY190, and αY198.

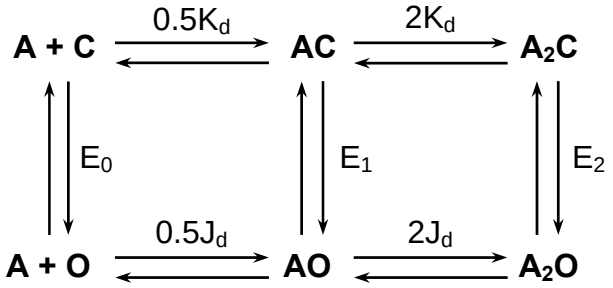

Figure S1. Thermodynamic cycle for AChR. A is the agonist, C is the 'closed' ground state ensemble (lower agonist affinity and ionic conductance) and O is the 'open' ground state ensemble (higher agonist affinity and ionic conductance). Next to the arrows are the salient equilibrium constants. E_0 is for unliganded gating, E_1 is for monoliganded gating, E_2 is for diliganded gating, K_d is the dissociation constant for agonist binding to C, J_d is the dissociation constant for agonist binding to O. In adult neuromuscular AChRs the two binding sites have approximately the same low and high affinities for ACh and for choline. Without an external energy source the net energy change, $E_2/E_0 = (K_d/J_d)^2$. The free energy for gating provided by the affinity change for each bound agonist molecule is ΔG_B (kcal/mol)=$-0.59\ln(K_d/J_d)$ or $-0.59(\sqrt{(E_2/E_0)})$.

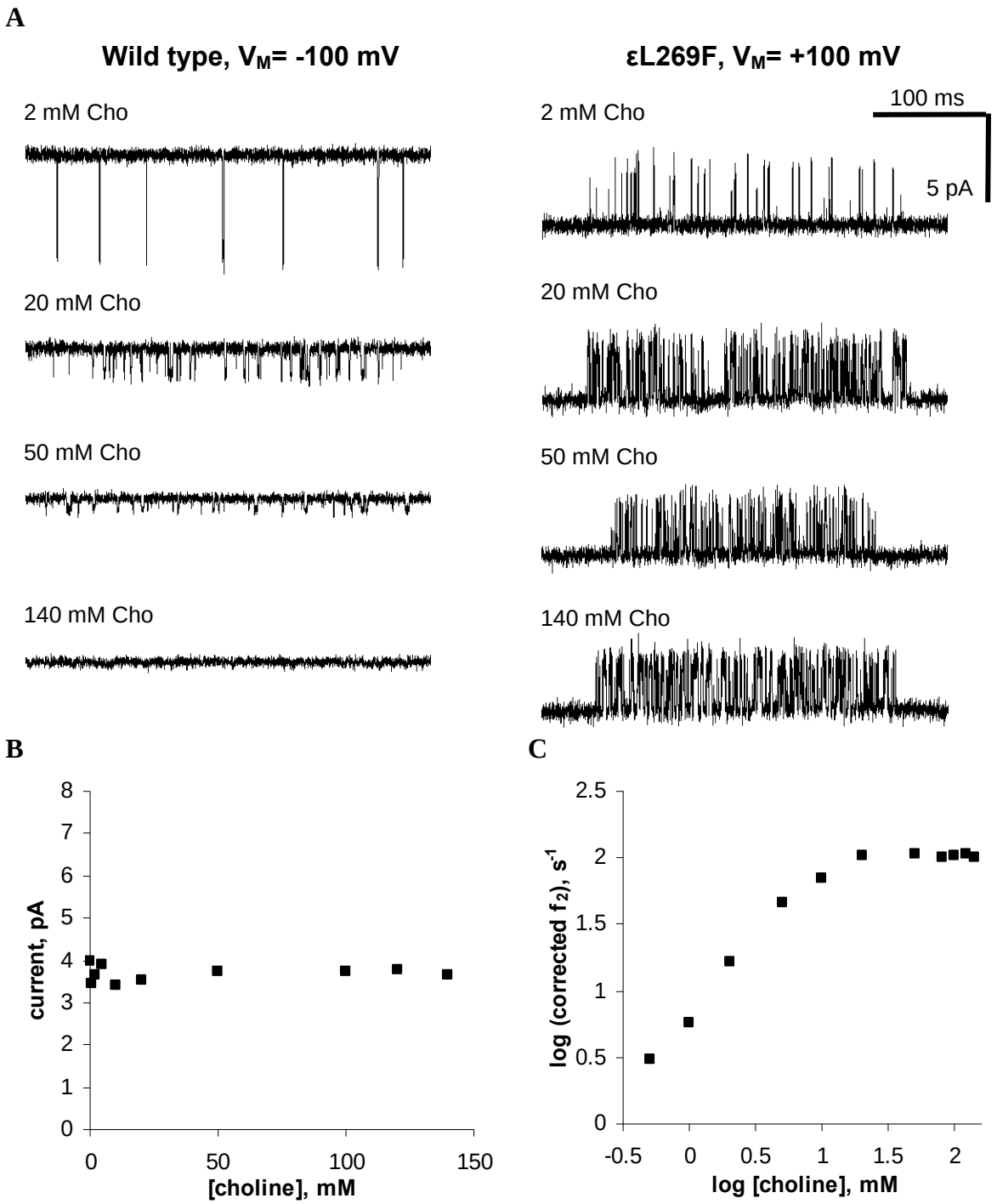

Figure S2. AChR currents activated by choline. A, AChR current clusters at different [choline] measured at a membrane potential (V_m) of -100 mV (wt AChRs, left) or +100 mV (εL269F background, right). **B**, At +100mV, the current amplitude does not change up to 140 mM choline, indicating that channel-block by the agonist was effectively-eliminated by the +200 mV depolarization. **C**, The background- and voltage-corrected, intracluster apparent opening rate reaches a plateau ~20 mM choline, signifying saturation of the transmitter binding sites.

SUPPORTING REFERENCES

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