

Human $\alpha 6\beta 4$ nicotinic acetylcholine receptor: heterologous expression and agonist behavior provide insights into the immediate binding site

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Supplemental Tables

Supplemental Table 1: The μM EC_{50} values and Hill coefficients obtained for human $\alpha 6\beta 4$ or $\alpha 6\text{L}9\text{S}\beta 4$ receptors, with or without the chaperone BARP elicited by ACh.

Receptor	Ratio	EC_{50}	pEC_{50} ($\text{CI}_{95\%}$) ^a	Hill	n^b	I_{max} μA
$\alpha 6\beta 4\text{BARP}$	1:1:5	NE	NE	NE	NE^*	NE
	1:10:5	NE	NE	NE	NE^*	NE
Receptor	Ratio	EC_{50}	pEC_{50} ($\text{CI}_{95\%}$) ^a	Hill	n^b	I_{max} μA
$\alpha 6\text{L}9\text{S}\beta 4$	10:1	NE	NE	NE	NE^{**}	NE
	1:10	NE	NE	NE	NE^{\dagger}	NE
Receptor	Ratio	EC_{50}	pEC_{50} ($\text{CI}_{95\%}$) ^a	Hill	n^b	I_{max} μA
$\alpha 6\text{L}9\text{S}\beta 4\text{BARP}$	1:1:1	1.6 ± 0.4	$-0.2(-0.1 \text{ to } -0.4)$	1.2 ± 0.3	$2^{\dagger\dagger}$	0.21-0.22
	1:1:5	0.5 ± 0.1	$0.3(0.2 \text{ to } 0.5)$	1.3 ± 0.1	8	1.27-19.74
	1:10:1	2.8 ± 0.5	$-0.4(-0.3 \text{ to } -0.6)$	1.2 ± 0.5	13	0.17-0.74
	1:10:5	0.6 ± 0.1	$0.2(0.2 \text{ to } 0.3)$	1.2 ± 0.2	15	0.37-2.46

^a pEC_{50} corresponds to $(-1 * \log \text{EC}_{50})$; ^b number of oocytes recorded per condition; * 20 oocytes were impaled; **23 oocytes were impaled; † 15 oocytes were impaled; †† 10 oocytes were impaled, number of oocytes with detectable currents. Data presented as Mean \pm SD.

Supplemental Table 2: Agonist efficacies relative to ACh for human $\alpha 6\beta 4$ receptors.

	Agonist	Efficacy to ACh %	n^a
1	Cyt	61	8
	Var	45	6
	Cho	35	6
	CCho	28	8
	Nic	40	6
	Derivative	Efficacy to ACh %	n^a
2	F	33	10
3	Et	29	9
4	C(CH ₃)=CH ₂	15	10
5	iPr	47	9
7	Tolyl	18	7
8	OMe	31	8
9	NH ₂	32	9
13	Br/Et	88	10
16	9-Br	81	10

$\alpha 6\beta 4$ receptors were expressed as mentioned in methods, by using two-electrode voltage clamp electrophysiology the potency of diverse agonist versus the relative efficacy to ACh was obtained. ^a corresponds to the number of cells.

Supplemental Table 3: The μM EC_{50} values and Hill coefficients for cytisine and various derivatives for WT and mutated $\alpha 6\beta 4$ receptors, and agonist volumes.

	$\alpha 6\beta 4$	EC_{50}^a	$\text{pEC}_{50} (\text{CI}_{95\%})^b$	Hill	n^c	$\text{Imax } \mu\text{A}$	Fold shift	Volume (\AA)*
1	Cyt	0.5±0.2	0.3(0.2 to 0.3)	1.4±0.2	10	0.223-1.268	1	198
6	Me	0.7±0.3	0.2(0.1 to 0.2)	1.8±0.4	8	0.077-1.579	1.4	216
3	Et	0.6±0.3	0.2(0.1 to 0.3)	2.0±0.4	9	0.112-1.129	1	234
4	C(CH ₃)=CH ₂	2.4±0.6***	-0.4(0.3 to 0.4)	2.0±0.5	12	0.066-1.548	4.8	249
5	iPr	4.8±0.5***	-0.7(0.6 to 0.7)	1.4±0.9	6	0.053-0.728	9.6	252
	$\alpha 6\text{T180S}\beta 4$	EC_{50}^a	$\text{pEC}_{50} (\text{CI}_{95\%})^b$	Hill	n^c	$\text{Imax } \mu\text{A}$	Fold shift	Volume (\AA)*
1	Cyt	1.6±0.5	-0.2(-0.1 to -0.3)	2.0±1.0	7	0.182-2.095	1	198
6	Me	7.4±1.6***	-0.9(-0.8 to -1.0)	1.6±0.3	8	0.054-1.345	4.6	216
3	Et	7.7±0.9***	-0.9(-0.8 to -0.9)	1.4±0.2	9	1.638-22.591	4.8	234
4	C(CH ₃)=CH ₂	10.5±1.6***	-1.0(-0.9 to -1.0)	1.8±0.5	6	0.050-0.196	6.6	249
5	iPr	7.8±1.5***	-0.9(-0.8 to -1.0)	1.8±0.3	9	0.067-0.643	4.8	252
	$\alpha 6\text{T228P}\beta 4$	EC_{50}^a	$\text{pEC}_{50} (\text{CI}_{95\%})^b$	Hill	n^c	$\text{Imax } \mu\text{A}$	Fold shift	Volume (\AA)*
1	Cyt	0.8±0.2	0.1(0.0 to 0.2)	1.2±0.3	7	0.098-2.410	1	198
6	Me	1.2±0.7	-0.1(-0.1 to -0.2)	0.9±0.3	7	0.342-10.520	1.5	216
3	Et	0.2±0.1***	0.6(0.5 to 0.8)	0.9±0.3	8	1.638-22.591	0.3	234
4	C(CH ₃)=CH ₂	2.1±0.4***	-0.3(-0.2 to -0.4)	1.5±0.6	8	0.356-5.881	2.6	249
5	iPr	0.8±0.2	0.1(0.1 to 0.2)	1.6±0.5	8	0.468-20.456	1.0	252
	$\alpha 6\text{N221E}\beta 4$	EC_{50}^a	$\text{pEC}_{50} (\text{CI}_{95\%})^b$	Hill	n^c	$\text{Imax } \mu\text{A}$	Fold shift	Volume (\AA)*
1	Cyt	0.2±0.0	0.8(0.7 to 0.9)	1.3±0.3	8	0.199-3.471	1	198
6	Me	1.1±0.3***	-0.1(-0.0 to -0.1)	1.3±0.3	7	0.488-2.032	5.5	216
3	Et	2.8±0.8***	-0.4(-0.4 to -0.5)	1.1±0.3	10	0.047-4.016	14	234
4	C(CH ₃)=CH ₂	4.4±1.6***	-0.6(-0.5 to -0.8)	1.4±0.1	8	0.067-0.755	22	249
5	iPr	3.5±0.4***	-0.5(-0.5 to -0.6)	1.3±0.2	17	0.141-9.868	17.5	252

Mutated and WT $\alpha 6\beta 4$ receptors were expressed in *Xenopus laevis* oocytes, and the potency of diverse cytisine derivatives with increasing bulk sizes was obtained. Folds shifts were compared versus cytisine as the parent compound for each receptor subtype expressed. * Data of total ligand volume was previously published (Blom et al., 2019). Agonist EC_{50} values are represented as Mean \pm SD. *** p

<0.01, *** p <0.001 compared to cytosine EC₅₀, t-test; ^bpEC₅₀ corresponds to (-1*logEC₅₀); ^ccorresponds to the number of cells.