

The intracellular domain of homomeric glycine receptors modulates agonist efficacy

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Supporting Information

Figure S1

Figure S2

Table S1

	Signal peptide	
human $\alpha 1$ GlyR	MYSFNTLRRLYLWETIVFFSLAASKEAEAARSAPKPMSPSDFLDKLMGRTSGYDARIRPNF	60
zebrafish $\alpha 1$ GlyR	---MFALGIYLWETIVFFSLAASQAAA-ARKAASPMPPSEFLDKLMGKVSVDARIRPNF	56
zebrafish $\alpha 1$ GlyREM	---MFALGIYLWETIVFFSLAASQAAA-A-----PSEFLDKLMGKVSVDARIRPNF	48
zebrafish $\alpha 1$ GlyR Δ ICD	---MFALGIYLWETIVFFSLAASQAAA-ARKAASPMPPSEFLDKLMGKVSVDARIRPNF	56
human $\alpha 1$ GlyR Δ ICD	MYSFNTLRRLYLWETIVFFSLAASKEAEAARSAPKPMSPSDFLDKLMGRTSGYDARIRPNF	60
hu $\alpha 1$ GlyR Δ ICD + zf TM4	MYSFNTLRRLYLWETIVFFSLAASKEAEAARSAPKPMSPSDFLDKLMGRTSGYDARIRPNF	60
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human $\alpha 1$ GlyR	KGPPVNVSCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYNEYPDSDLDDPSMLDS	120
zebrafish $\alpha 1$ GlyR	KGPPVNVTCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYSEYPDSDLDDPSMLDS	116
zebrafish $\alpha 1$ GlyREM	KGPPVNVTCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYSEYPDSDLDDPSMLDS	108
zebrafish $\alpha 1$ GlyR Δ ICD	KGPPVNVTCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYSEYPDSDLDDPSMLDS	116
human $\alpha 1$ GlyR Δ ICD	KGPPVNVSCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYNEYPDSDLDDPSMLDS	120
hu $\alpha 1$ GlyR Δ ICD + zf TM4	KGPPVNVSCNIFINSFGSIAETTM DYRVNIFLRQQWNDPRLAYNEYPDSDLDDPSMLDS	120
	# #	
human $\alpha 1$ GlyR	IWKPDLLFANEKGAHFHEITTDNKLRLRISRNGNVLYSIRITLTLACPMDLKNFPMDVQTC	180
zebrafish $\alpha 1$ GlyR	IWKPDLLFANEKGANFHEVTDNKLRLRISKNGNVLYSIRITLVLACPMDLKNFPMDVQTC	176
zebrafish $\alpha 1$ GlyREM	IWKPDLLFANEKGANFHEVTDNKLRLRISKNGNVLYSIRITLVLACPMDLKNFPMDVQTC	168
zebrafish $\alpha 1$ GlyR Δ ICD	IWKPDLLFANEKGANFHEVTDNKLRLRISKNGNVLYSIRITLVLACPMDLKNFPMDVQTC	176
human $\alpha 1$ GlyR Δ ICD	IWKPDLLFANEKGAHFHEITTDNKLRLRISRNGNVLYSIRITLTLACPMDLKNFPMDVQTC	180
hu $\alpha 1$ GlyR Δ ICD + zf TM4	IWKPDLLFANEKGAHFHEITTDNKLRLRISRNGNVLYSIRITLTLACPMDLKNFPMDVQTC	180
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human $\alpha 1$ GlyR	IMQLESFGYTMNDLIFEWQEQGAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	240
zebrafish $\alpha 1$ GlyR	IMQLESFGYTMNDLIFEWDEKQAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	236
zebrafish $\alpha 1$ GlyREM	IMQLESFGYTMNDLIFEWDEKQAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	228
zebrafish $\alpha 1$ GlyR Δ ICD	IMQLESFGYTMNDLIFEWDEKQAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	236
human $\alpha 1$ GlyR Δ ICD	IMQLESFGYTMNDLIFEWQEQGAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	240
hu $\alpha 1$ GlyR Δ ICD + zf TM4	IMQLESFGYTMNDLIFEWQEQGAVQVADGLTLPQFILKEEKDLRYCTKHYNIGKFTCIEA	240
	# #	
	TM1 TM2	
human $\alpha 1$ GlyR	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	300
zebrafish $\alpha 1$ GlyR	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	296
zebrafish $\alpha 1$ GlyREM	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	288
zebrafish $\alpha 1$ GlyR Δ ICD	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	296
human $\alpha 1$ GlyR Δ ICD	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	300
hu $\alpha 1$ GlyR Δ ICD + zf TM4	RFHLERQMGYYLIQMYIPSLLVILVLSWVSWINMDAAPARVGLGITTTLTMTTQSSGSR	300
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	TM3 ICD	
human $\alpha 1$ GlyR	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSRQHKE LLRFRRRRRHKEDEAGEG	360
zebrafish $\alpha 1$ GlyR	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSRQHKE LLRFRRRRRHKEDEAGDG	356
zebrafish $\alpha 1$ GlyREM	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSR-----AGT-----	327
zebrafish $\alpha 1$ GlyR Δ ICD	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSR-----AGT-----	335
human $\alpha 1$ GlyR Δ ICD	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSR-----AGT-----	339
hu $\alpha 1$ GlyR Δ ICD + zf TM4	SLPKVSVYKAIIDIMWAVCLLFFVFSALLEYA AVNFVSR-----AGT-----	339
	##	
	TM4	
human $\alpha 1$ GlyR	RFNFSAYGMGPAQLQAKDGSVKGANNSTNTNPPAPSKSPEEMRKLFIQRAKKIDKISR	420
zebrafish $\alpha 1$ GlyR	RFSFAAYGMGPAQLQAKDGMAIKGNNNNAPTST-NPPEKTVEEMRKLFI SRAKRIDTVSR	415
zebrafish $\alpha 1$ GlyREM	-----KLFISRAKRIDTVSR	343
zebrafish $\alpha 1$ GlyR Δ ICD	-----KLFISRAKRIDTVSR	351
human $\alpha 1$ GlyR Δ ICD	-----KLFIQRAKKIDKISR	355
hu $\alpha 1$ GlyR Δ ICD + zf TM4	-----KLFISRAKRIDTVSR	355
	# # #	
	C-term	
human $\alpha 1$ GlyR	IGFPM AFLIFNMFYWIYKIVRREDVHNQ	449
zebrafish $\alpha 1$ GlyR	VAFPLVFLIFNIFYWITYKIIRSEDIHKQ	444
zebrafish $\alpha 1$ GlyREM	VAFPLVFLIFNIFYWITYKLVPRGS---	368
zebrafish $\alpha 1$ GlyR Δ ICD	VAFPLVFLIFNIFYWITYKIIRSEDIHKQ	380
human $\alpha 1$ GlyR Δ ICD	IGFPM AFLIFNMFYWIYKIVRREDVHNQ	384
hu $\alpha 1$ GlyR Δ ICD + zf TM4	VAFPLVFLIFNIFYWITYKIIRSEDIHKQ	384
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Figure S 1. Clustal W alignment of GlyR constructs used in this work. Differences between human $\alpha 1$ GlyR and zebrafish $\alpha 1$ GlyR outside the ICD are marked with #. The amino acids involved in glycine binding are highlighted in green. The AGT linker used to replace the native ICD in some of the GlyR constructs is the same as used by Du et al. for zebrafish $\alpha 1$ GlyR_{EM} and is highlighted in yellow (1).

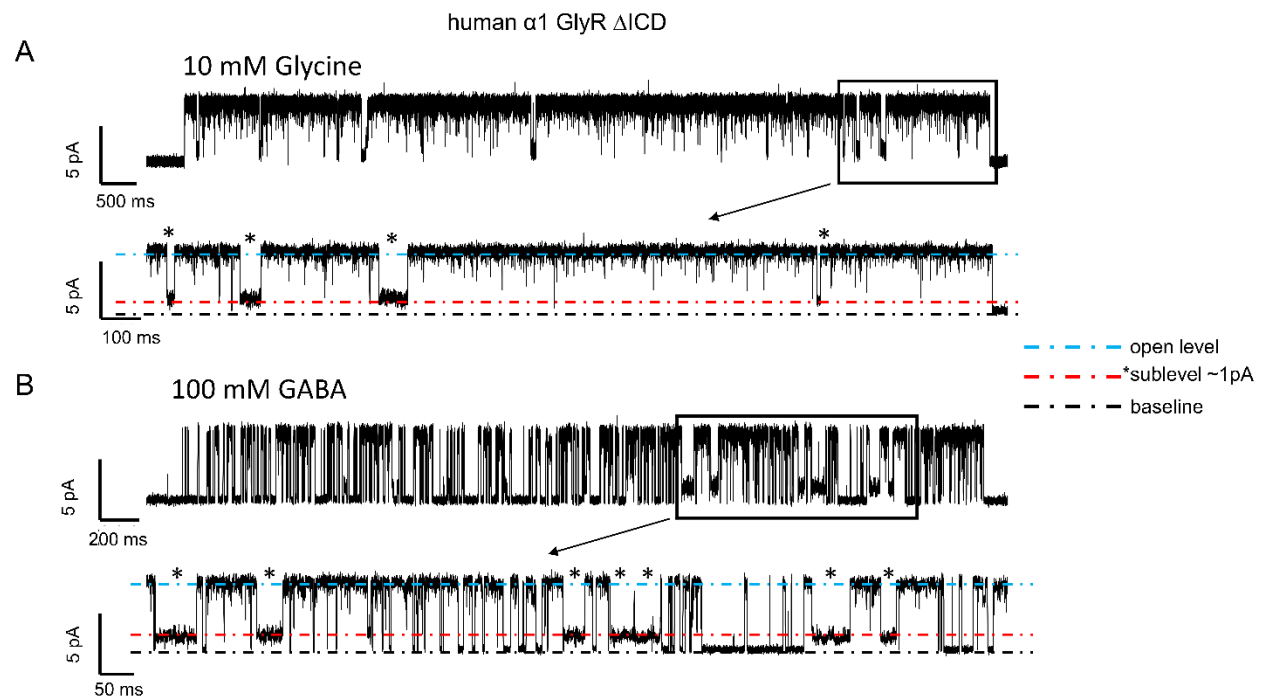


Figure S 2. Sublevel of amplitude observed in human $\alpha 1$ GlyR Δ ICD. A Cluster of single channel activity evoked by saturating concentration of Glycine (top trace). The area enclosed with a rectangle is presented in the bottom trace. The events that are not reaching full closure are annotated with *. B) Cluster of single channel activity evoked by saturating concentration of GABA (top trace). The area enclosed with a rectangle is presented in the bottom trace. The events that are not reaching full closure are annotated with *.

Table S 1. Pairwise comparison of parameters from whole-cell and single-channel recording. P values are estimated using two tail randomization test (10000 randomization iterations).

GlyR pair	parameter	Agonist (p value)			
		GABA	Taurine	Balanine	Glycine
human $\alpha 1$ GlyR & zebrafish $\alpha 1$ GlyR_{EM}	EC ₅₀	<10 ⁻⁴	0.0004	0.0006	0.0010
	I _{rel}	<10 ⁻⁴	<10 ⁻⁴	<10 ⁻⁴	/
	maxP _{oepn}	<10 ⁻⁴	<10 ⁻⁴	<10 ⁻⁴	0.3396
human $\alpha 1$ GlyR & human $\alpha 1$ GlyR Δ ICD	EC ₅₀	0.0093	0.0146	0.0235	0.0049
	I _{rel}	0.0001	<10 ⁻⁴	0.0061	/
	maxP _{oepn}	<10 ⁻⁴	<10 ⁻⁴	0.0004	0.3397
zebrafish $\alpha 1$ GlyR & zebrafish $\alpha 1$ GlyR_{EM}	EC ₅₀	<10 ⁻⁴	0.0037	0.0344	0.0111
	I _{rel}	<10 ⁻⁴	0.0008	0.0003	/
	maxP _{open}	<10 ⁻⁴	<10 ⁻⁴	0.0503	0.2548
human $\alpha 1$ GlyR & zebrafish $\alpha 1$ GlyR	EC ₅₀	0.0006	0.0004	0.0003	0.1384
	I _{rel}	<10 ⁻⁴	0.0266	0.0143	/
	maxP _{oepn}	<10 ⁻⁴	<10 ⁻⁴	0.0006	0.6696
human $\alpha 1$ GlyR Δ ICD & zebrafish $\alpha 1$ GlyR_{EM}	EC ₅₀	<10 ⁻⁴	0.0002	0.0014	0.0084
	I _{rel}	<10 ⁻⁴	0.0036	0.0004	/
	maxP _{oepn}	<10 ⁻⁴	0.0203	0.0151	0.1084
human $\alpha 1$ GlyR Δ ICD & hu$\alpha 1$ GlyR Δ ICD+ TM4 zf	EC ₅₀	0.0009	0.9264	0.1040	0.0223
	I _{rel}	0.2456	0.7757	0.9124	/
	maxP _{open}	0.6194	0.7895	0.2263	0.0054
zebrafish $\alpha 1$ GlyR_{EM} & zebrafish $\alpha 1$ GlyR Δ ICD	maxP _{open}	0.8052	0.5591	0.6066	0.0454

References

1. Du, J., Lu, W., Wu, S., Cheng, Y., and Gouaux, E. (2015) Glycine receptor mechanism elucidated by electron cryo-microscopy. *Nature* **526**, 224-229