Supporting Information

Channel-Opening Kinetic Mechanism for Human Wild-Type GluK2 and the M867I Mutant Kainate Receptor[†]

Yan Han, Congzhou Wang, Jae Seon Park, and Li Niu*

Department of Chemistry and Center for Neuroscience Research, University at Albany, SUNY, Albany, New York 12222

Table S1: Kinetic characterization of rat and human GluK2 receptors

Publication and References	Patch Configuration	Glutamate (mM)	k_{des} (s^{-1})	EC_{50} (mM)
This study with rat GluK2	Whole-cell	2-5	230 ± 32	0.26 ± 0.02
Paternain et al. (1998) *Neuropharm.* 37, 1249-59.	Whole-cell	3	81 ± 3	0.762 ± 0.052
Swanson et al. (1997) *Neuron* 19, 913-26.	Whole-cell	1	244 ± 12	—
Barberis et al. (2008) *J Neurosci* 28, 6402-6.	Whole-cell	1	222 ± 20	0.427 ± 0.023
Vivithanaporn etal. (2007) *J Neurosci* 27, 10423-33.	Whole-cell	10	238 ± 11	—
Paternain et al. (2000) *J Neurosci* 20, 196-205.	Whole-cell	10	92 ± 4.8	0.898
Zhang et al. (2008) *Mol Pharmacol* 74, 1163-9.	Whole-cell	10	189 ± 7	0.083 ± 0.022
Raymond et al. (1993) *Nature* 361, 637-41.	Whole-cell	—	—	0.273 ± 0.073
Heckmann et al. (1996) *Biophys J*. 71, 1743-50.	Outside-out	1-100	100 – 250[a]	~0.5
Traynelis et al. (1997) *J Physiol* 503.3, 513-31.	Outside-out	10	230 ± 25	0.5
Bufler et al. (1997) *Neurosci Lett*. 221, 173-6.	Outside-out	10	112 – 385[a]	—
Chaudhry et al. (2009) *PNAS USA* 106, 12329-34.	Outside-out	10	165 ± 14	—
Wong et al. (2007) *J Neurosci* 27, 6800-9.	Whole-cell[b]	10	253 ± 11	—
This study with human GluK2	Whole-cell	2-5	119 ± 20	0.15 ± 0.01
Barbon et al. (2008) *Neurosci Lett* 434, 77-82.	Whole-cell	1	167 ± 8[c]	—

Note:
[a]the authors reported a range of rate constants.
[b]all of the authors used HEK-293 cells except Wong et al. who used tsA201 cells.
[c]these authors measured the desensitization rate constant on human GluK2c isoform, whereas we used human GluK2a in our study.

Table S2: Nonlinear Fitting with Fixed k_{cl} Value for Wild-Type and M867I Mutant human GluK2

Fitting method	Initial values				outputs			
	k_{cl}[a] (s^{-1})	k_{op} (s^{-1})	K_1 (mM)	n	k_{op} (s^{-1})	K_1 (mM)	n	R^2
Simplex	310	500	1	1	1790	0.29	1.3	0.86
Newton	310	500	1	1	1874	0.19	1.7	0.88
Simplex	310	500	0.5	1	1727	0.17	1.8	0.87
Simplex	310	500	0.1	1	1357	0.07	2.8	0.88
Simplex	310	500	0.2	2.5	1516	0.1	2.3	0.87
Simplex	310	500	0.2	4	1788	0.18	1.7	0.87
Simplex	310	1000	0.5	1.5	1739	0.18	1.7	0.87
Newton	310	1000	0.5	1.5	1721	0.18	1.7	0.87
Simplex	310	1000	0.8	1.5	1759	0.27	1.3	0.86
Simplex	310	1000	0.1	1.5	1340	0.1	2.1	0.87
Simplex	310	1000	0.2	3	1598	0.17	1.7	0.87
Simplex	310	1500	0.3	2	1964	0.29	1.4	0.86
Newton	310	1500	0.3	2	1601	0.11	2.3	0.87
Simplex	310	1500	0.1	2	1417	0.1	2.2	0.87
Simplex	310	1500	0.2	4	2125	0.2	1.9	0.86
Simplex	310	2000	0.2	3	2476	0.25	1.8	0.86
Newton	310	2000	0.2	3	1818	0.18	1.8	0.87
Simplex	310	2000	0.2	1	1702	0.23	1.4	0.86
Simplex	310	2000	0.2	2	2100	0.21	1.8	0.86
Newton	310	2000	0.8	2	1895	0.2	1.7	0.87
Newton	310	2000	0.8	4	2100	0.21	1.8	0.87
Simplex	310	4000	0.1	4	1507	0.1	2.3	0.88
Newton	310	4000	0.1	4	1484	0.08	2.7	0.88
Newton	310	4000	0.5	4	1940	0.21	1.7	0.87
Newton	310	4000	0.5	1	1557	0.12	2.1	0.87
Simplex	310	5000	0.2	2.5	1546	0.11	2.2	0.87
Newton	310	5000	0.2	2.5	1652	0.16	1.8	0.87
Newton	310	5000	0.2	5	1652	0.16	1.8	0.87
Newton	310	5000	0.2	1	1741	0.15	1.9	0.87
Newton	310	5000	0.2	2	1601	0.11	2.3	0.87
Newton	310	5000	0.1	2	1601	0.11	2.3	0.87
Newton	310	5000	0.8	2	1498	0.1	2.4	0.88
Newton	310	5000	2	2	1482	0.1	2.4	0.88

[a] k_{cl} is fixed at 310 s^{-1} (see the text).

The table below summarizes the average value for each parameter.

	k_{cl}[a] (s^{-1})	k_{op} (x 10^3 s^{-1})	K_1 (mM)	n
Average	310	1.7	0.17	1.9

Table S3: Nonlinear Fitting with Fixed k_{cl} Value for Wild-Type Rat GluK2

Fitting method	Initial values				outputs			
	k_{cl}[a] (s^{-1})	k_{op} (s^{-1})	K_1 (mM)	n	k_{op} (s^{-1})	K_1 (mM)	n	R^2
Simplex	400	3000	0.1	1	5394	0.24	2.1	0.96
Newton	400	3000	0.2	2	5560	0.27	2.0	0.96
Simplex	400	3000	0.3	1	6046	0.3	2.0	0.96
Simplex	400	3000	0.5	2	5501	0.24	2.1	0.96
Simplex	400	3000	0.3	3	5286	0.25	2.0	0.96
Newton	400	3000	0.4	4	6565	0.33	1.9	0.95
Newton	400	4000	0.1	1	9303	0.6	1.6	0.95
Newton	400	4000	0.2	2	5556	0.24	2.2	0.96
Simplex	400	4000	0.3	1	4166	0.15	2.5	0.96
Simplex	400	4000	0.5	2	5898	0.34	1.8	0.95
Simplex	400	4000	0.3	3	5482	0.26	2.0	0.96
Simplex	400	5000	0.4	4	9611	0.45	1.9	0.94
Newton	400	5000	0.1	1	4711	0.18	2.4	0.96
Simplex	400	5000	0.2	2	4670	0.21	2.1	0.96
Newton	400	5000	0.3	1	5724	0.25	2.1	0.96
Simplex	400	6500	0.5	2	7530	0.49	1.6	0.95
Simplex	400	6500	0.3	3	7719	0.31	2.2	0.95
Newton	400	6500	0.4	4	5554	0.27	2.0	0.96
Newton	400	6500	0.1	1	5736	0.29	2.0	0.96
Simplex	400	6500	0.2	2	5264	0.2	2.4	0.96
Newton	400	7500	0.3	1	5724	0.25	2.1	0.96
Simplex	400	7500	0.5	2	7500	0.34	2.0	0.95
Newton	400	7500	0.3	3	5178	0.23	2.1	0.96
Newton	400	7500	0.4	4	7209	0.41	1.8	0.95
Newton	400	7500	0.1	1	6426	0.36	1.8	0.95
Simplex	400	8500	0.2	2	6357	0.24	2.3	0.95
Simplex	400	8500	0.3	1	7044	0.41	1.8	0.95
Simplex	400	8500	0.5	2	7044	0.41	1.8	0.95
Newton	400	8500	0.3	3	5410	0.25	2.1	0.96
Newton	400	8500	0.4	4	6157	0.33	1.9	0.96

[a] k_{cl} is fixed at 400 s^{-1} (see the text).

The table below summarizes the average value for each parameter.

	k_{cl}[a] (s^{-1})	k_{op} (s^{-1})	K_1 (mM)	n
Average	400	6200	0.30	2.0

Table S4: Fitting with k_{cl} and K_1 Fixed for Wild-Type and M867I Mutant human GluK2

Fitting method	Initial values				outputs		
	k_{cl}[a] (s^{-1})	K_1 (mM)	k_{op} (s^{-1})	n	k_{op} (s^{-1})	n	R^2
Simplex	310	0.17	500	1	1719	1.8	0.87
Newton	310	0.17	500	2	1725	1.8	0.87
Simplex	310	0.17	500	4	1489	1.6	0.86
Newton	310	0.17	1000	1	1725	1.8	0.87
Simplex	310	0.17	1000	5	1477	1.6	0.86
Simplex	310	0.17	2000	2	2000	2.0	0.86
Newton	310	0.17	2000	4	1725	1.8	0.87
Simplex	310	0.17	4000	1	1708	1.8	0.87
Simplex	310	0.17	4000	4	2429	2.3	0.83
Simplex	310	0.17	5000	2	1785	1.8	0.87
Simplex	310	0.17	5000	3	2418	2.3	0.83

The table below summarizes the average value for each parameter.

	k_{cl}[a] (s^{-1})	K_1[a] (mM)	k_{op} (s^{-1})	n
Average	310	0.17	2000	1.9

Table S5: Fitting with k_{cl} and K_1 Fixed for Wild-Type Rat GluK2

Fitting method	Initial values				outputs		
	k_{cl}[a] (s^{-1})	K_1 (mM)	k_{op} (s^{-1})	n	k_{op} (s^{-1})	n	R^2
Simplex	400	0.30	3000	1	6615	2.0	0.95
Newton	400	0.30	3000	2	6045	2.0	0.96
Simplex	400	0.30	4000	4	6066	2.0	0.96
Newton	400	0.30	4000	1	6045	2.0	0.96
Simplex	400	0.30	5000	5	6052	2.0	0.96
Simplex	400	0.30	6500	2	6734	2.1	0.95
Newton	400	0.30	6500	4	6045	2.0	0.96
Simplex	400	0.30	7500	1	6284	2.0	0.95
Simplex	400	0.30	7500	4	9703	2.5	0.92
Simplex	400	0.30	8500	2	7251	2.1	0.95
Simplex	400	0.30	8500	3	9617	2.4	0.92

The table below summarizes the average value for each parameter.

	k_{cl}[a] (s^{-1})	K_1[a] (mM)	k_{op} (s^{-1})	n
Average	400	0.30	7,000	2.1

FIGURE S1

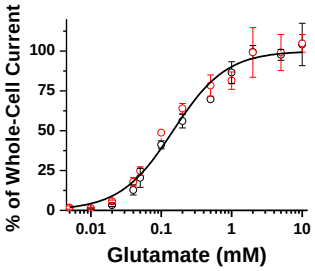

FIGURE S1. Fitting of dose-response relationship of the wild-type and mutant M867I human GluK2 to the Hill equation. Percentage of the whole-cell current of the wild-type and the mutant GluK2 as a function of glutamate concentration was obtained in the whole-cell recording. The black circles stand for the data of wild type hGluK2, and the red circles are from the mutant hGluK2 (M867I). Each data point is an average of at least three measurements from three different cells. The solid line is the best fit of the combined data based on Hill equation. EC_{50} of 0.15 ± 0.01 mM and the Hill coefficient of 1.13 ± 0.09 were obtained.

FIGURE S2

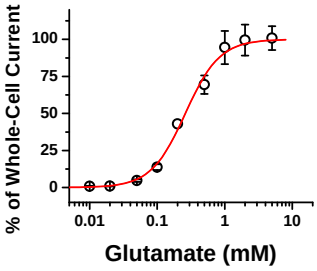

FIGURE S2. Fitting of dose-response relationship of the wild-type rGluK2 to the Hill equation. Percentage of the whole-cell current of the wild-type rGluK2 as a function of glutamate concentration was obtained by using the whole-cell recording. Each data point is an average of at least three measurements from three different cells. The solid line is the best fit of the data based on the Hill equation. EC_{50} of 0.26 ± 0.01 mM and the Hill coefficient of 1.71 ± 0.14 were obtained.

FIGURE S3

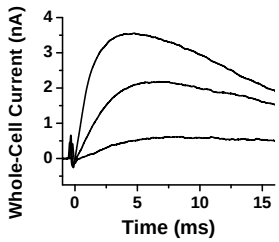

FIGURE S3. Raw laser-pulse photolysis traces of wild type human GluK2. All the traces are collected from the same cell. The laser pulse was triggered at time zero. The currents are shown reversed. From the top to the bottom, the glutamate concentrations are 0.30, 0.14 and 0.04 mM, and the k_{obs} are 962, 617 and 426 s^{-1}. The current amplitudes are 3.54, 2.19 and 0.62 nA.