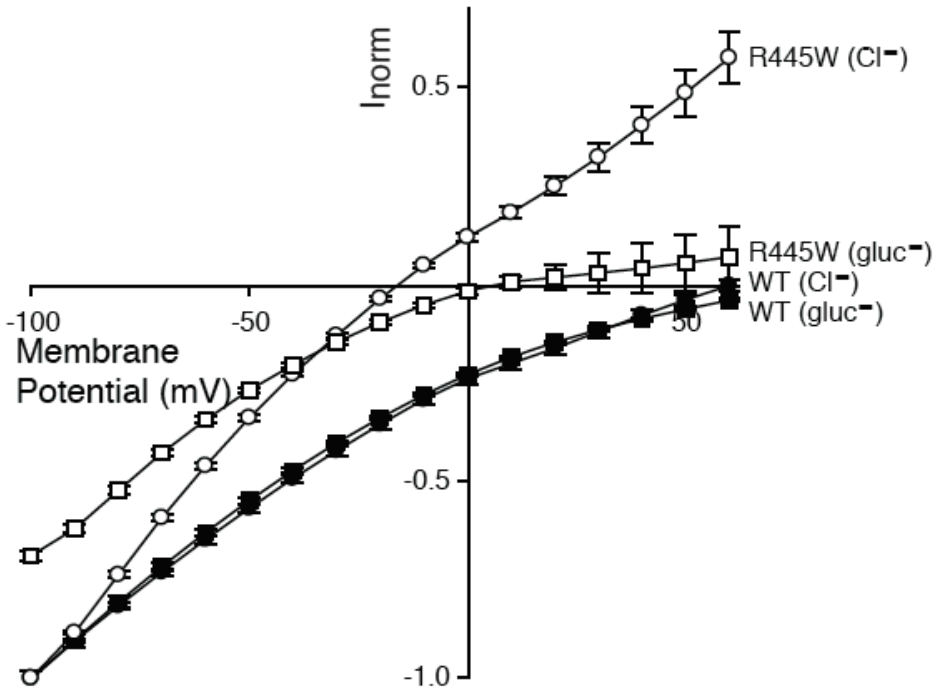
Supplemental Appendix



Supplemental Figure 1 Current versus membrane potential plots of conductance elicited by glutamate for SLC1A1 WT and R445W. Current (I_{norm}, y-axis) was normalized to the maximal current defined as unit I_{max}; membrane potential was measured in mV (x-axis). In WT SLC1A1, the glutamate-activated conductance has two components, an inwardly rectifying glutamate transport conductance and an uncoupled chloride conductance. L-glutamate (100 μM) was applied to *Xenopus laevis* oocytes expressing WT (filled symbols) or R445W (open symbols) cRNA in a chloride-containing buffer (circles) or chloride-free buffer containing the impermeant anion gluconate ($gluc^-$, squares). In WT, the replacement of chloride with gluconate ions did not significantly change the L-glutamate activated conductance as the contribution from chloride to the transport current of SLC1A1 was minimal. In contrast, when the chloride in the recording buffer was replaced with gluconate (which removes the uncoupled chloride current), the reversal potential of the L-glutamate activated conductance of R445W shifted to more depolarized potentials and the amplitude of the conductance at positive membrane potentials was greatly reduced. In *Xenopus*

laevis oocytes, the reversal potential for chloride ions (Cl^-) is ~ -24 mV (1), which was similar to the reversal potential observed for R445W (-16 ± 1 mV). Thus, the contribution of the uncoupled chloride conductance to the total transport current of R445W was reduced when chloride was replaced by gluconate. These data demonstrate that L-glutamate-activated conductance of R445W was mainly carried by the uncoupled chloride conductance, with a small contribution from transport, which is also consistent with a higher affinity and reduced rate of flux of substrate (Figure 3A, B and C) (2). This is also consistent with data in Supplemental Figure 1 which demonstrates that the proportion of current due to transport compared to the uncoupled anion current is reduced compared to WT. Furthermore, no glutamate-activated cation leak was observed from R445W, which differs from the large glutamate-dependent cation leak observed when R445 is mutated to serine (S), methionine (M) or glutamine (Q) (3). In the absence of glutamate, background leak current observed for oocytes expressing the R445W mutant was similar to that of oocytes expressing WT SLC1A1 (data not shown). Data were normalized to the glutamate-activated current at -100 mV in chloride buffer and represent mean ± s.e.m. of at least 3 oocytes.

Supplementary References

1. Barish, M.E. 1983. A transient calcium-dependent chloride current in the immature Xenopus oocyte. *J Physiol* **342**:309-325.

2. Fairman, W.A., Vandenberg, R.J., Arriza, J.L., Kavanaugh, M.P., and Amara, S.G. 1995. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* **375**:599-603.

3. Borre, L., and Kanner, B.I. 2004. Arginine 445 controls the coupling between glutamate and cations in the neuronal transporter EAAC-1. *J Biol Chem* **279**:2513-2519.

Primer name	Forward Primer	Reverse Primer	Amplicon Size (bp)	Comments
SLC1A1prom	TGAGTAGCCGAGCAAGAGG	ACAGGGGAGAGGTGAGAGC	743	
SLC1A1-5'UTR	TGAGTAGCCGAGCAAGAGG	ACAGGGGAGAGGTGAGAGC	572	
SLC1A1ex1	TCCCTCCTTCCTCGCTGGTTG	GCTACTTGCTTCCCTTTCTACCCC	669	
SLC1A1ex2	CCATAGACATAGATACAGGAGAACTC	TTAGGCTTTGTGTTCCCACCC	607	
SLC1A1ex3	CCATTGTCTGTAGATGAGAACGCC	AAGTCTGTCTGCCAGGTTCAAGTG	500	
SLC1A1ex4	ACACACACACAGACGCACACG	CCAATCCATCCCAGCATCTCTC	639	
SLC1A1ex5	AGGGGCCAGAAGAGAAACC	ACCAGAGGAAAAGCAAAGAGG	566	
SLC1A1ex6	TCTGCCTCGCTTTTAGTTCC	AGAGCACAGCCTTTTTCACC	540	
SLC1A1ex7	ATCTTCTCATTTCTGGACCTGTGC	CAGCAACAAAAGCCCCCAC	422	
SLC1A1ex8	CTTCAATTCCCATCCTGTGC	GGCTATTCTTGCTTGCTTGG	608	
SLC1A1ex9	GCAGGAATAGCACACACAGG	CAGGTCCAGGAATCAGAAGG	683	
SLC1A1ex10	GCTCAGGAAGGGGTTATGG	GAGTGATGGCTTTGGCTACC	646	
SLC1A1ex11	TGGAACAAGGGAGTAGGAAGG	GGAAAATTAAAGCAGCAACAGG	694	
SLC1A1ex12	GAGCCTTCAGTCAGTCAGCC	TTACCCCACACCCAAATCC	583	
SLC1A1-3'UTRa	TCTCATTCACCCAGACCTCAC	CAGTTACCAGCGTCCCAATAC	660	
SLC1A1-3'UTRb	TTTGGAAGAAAGGGAGAAGG	GATAACAGAAGTGCAAGGATGG	536	
SLC1A1-3'UTRc	TGGTGATACTCCAAGGTGG	AGCAGAATGACAAGCAGAGG	468	
SLC1A1-3'UTRd	CAGTGGTTCGGGGGAAATAG	CCCACACCCATCCAAGTAAG	720	
SLC1A1polyA	CACTTCCCTCATTCTTTCCC	GGGGTCTTTCTGTGTTGTCC	714	
I395del-RFLP	CGCTGTGCTGAAGAAAATAACC	GAGTGATGGCTTTGGCTACC	371	Wildtype allele (461 bp) is not digested by *BsaBI*. I395del allele (458 bp) is digested by *BsaBI* to give

R445W-RFLP	TTCTTTGGGAGAAGAGGAAGG	GGTCTGGGTGAATGAGATGG	481	245 and 213 bp fragments. Wildtype allele (481 bp) is digested by *AgeI* to give 252 and 229 bp fragments. R445W allele is not digested by *AgeI*.
SLC1A1-fl	CCTGCCACGCAAAACTACC	CTTACCCCACACCCAAATCC	1988	Amplified from human kidney cDNA, cloned into pGEM-T-Easy
SLC1A1-fl(EcoRI)	AATAGAATTCACAGCCATGGGGAAACCG	CTAGGAATTCTTACCCCACACCCAAATCC	1793	EcoRI ends, cloned into pcDNA3.1-HA

Supplementary Table 1 PCR primers used in this study (5'-3').