Erdem *et al.: Intrinsic Activity*, 2019; **7**(Suppl.1):A3.13
doi:10.25006/IA.7.S1-A3.13
published online: 11 September 2019

INTRINSIC ACTIVITY

Joint Meeting of the Austrian Neuroscience Association (16th ANA Meeting) and the Austrian Pharmacological Society (25th Scientific Symposium of APHAR)

Innsbruck, 25–27 September 2019

MEETING ABSTRACT

A3.13

Glycine transporter 1 and glycine transporter 2: a comparison of their transport kinetics

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Background: Members of the transporter family SLC6 translocate their cognate substrate together with Na^+ and Cl^-. Kinetic models exist for the transporters of GABA (GAT1/SLC6A1) and the monoamines dopamine (DAT/SLC6A3) and serotonin (SERT/SLC6A4). We posited that the transport cycle of each SLC6 transporter reflects the physiological requirements they operate under. We tested this hypothesis by analyzing the transport cycle of glycine transporter 1 (GlyT1/SLC6A9) and glycine transporter 2 (GlyT2/SLC6A5). GlyT2 is the only SLC6 family member known to translocate glycine, Na^+, and Cl^- in a 1:3:1 stoichiometry. We aimed to identify partial reactions in real time by electrophysiological recordings.

Methods: We transiently transfected GFP-tagged GlyT1b and GlyT2a in COS-7 cells and recorded glycine-induced currents in the whole-cell patch-clamp configuration. The cells were challenged with glycine utilizing a rapid application device which allowed for complete solution exchange within 100 ms.

Results: We found that both GlyTs have a high transport capacity driven by rapid return of the empty transporter, after releasing Cl^- on the intracellular side. Highly cooperative binding of cosubstrate ions and substrate further enhanced rapid cycling in both isoforms so that their forward transport mode was maintained even under conditions of high intracellular Na^+ or Cl^-. Most importantly, differences in the transport cycle of GlyT1 and GlyT2 resulted from the kinetics of charge movement and the voltage-dependent rate-limiting reactions: GlyT1 kinetics were governed by transition of the substrate-bound transporter from outward- to inward-facing conformations, whereas GlyT2 kinetics were governed by Na^+ binding (or a related conformational change). Kinetic modeling showed that the kinetics of GlyT1 are ideally suited for supplying the extracellular glycine levels required for NMDA receptor activation.

Discussion: Based on our data we conclude that GlyT1 and GlyT2 operate under distinct kinetic rules. In addition to the established differences in their stoichiometry, this might be important to meet the specific requirements in astrocytes and neurons, respectively.

Acknowledgements: We thank Shreyas Bhat and Verena Burtscher for discussions and comments on the data.

Keywords: glycine transporter – GlyT – transport – current – transport cycle

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