**EAAT3 INVESTIGATED USING SSM-BASED ELECTROPHYSIOLOGY**

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**EAAT3**

The excitatory amino acid transporter 3 (EAAT3, SLC1A3) is an electronegative secondary active cotransporter of the SLC-family (SLC1A3). Structure and function of this transporter are comparably well characterized. A crystal structure and other data from the glutamate transporter homologue GPr revealed, that the protein is a homo tetramer, with each monomer consisting of 7 TMDs and 2 helices. Driven by the concentration gradients of sodium and potassium, transports glutamate, aspartate and cysteine into the cell lumen. It is expressed throughout the CNS, but also in many organs such as kidneys, liver and lung, as well as in cardiac and skeletal muscle.

EAAT3 is involved in the neuro-reuptake and the homeostasis of glutamate. It plays a central role in the regulation of excitatory neurotransmission and synaptic plasticity. It also transports cytokines, necessary for the synthesis of glutathione and GABA, and seems to provide the main pathway for aspartate in some issues. Sensed to these physiological functions several connections of EAAT3 to severe neurological disorders like Huntington’s and Alzheimer’s disease, epalrestat and schizophrenia have been described, as well as metabolic disturbances concerning the maintenance of aspartate and cytokine levels.

Given all these implications of EAAT3, pharmacological modulation of this transporter is of interest. The unusually detailed characterization of the physiological and biochemical properties qualify the protein as a potential drug target. However, no selective inhibition of EAAT3 is known today. To facilitate the search for inhibitors, we developed an functional assay using SSM-based electrophysiology with high throughput and robustness.

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**Validation**

**Success rate:** The conductance, capacitance and current response in each well was analysed. A conductance below 200 nS or a capacitance below 15 nF indicates a healthy SSM and the respective wells were rejected. This resulted in a total success rate of 97.4 % (mean value of 960 individual sensors).

**Signal stability:** The activation cycle described above was repeated every 5 minutes for 45 minutes, followed by a reduced repetition frequency (30 min, 2 times 2 hours). After 2 hours, the sensor still generated a current response of the initial amplitude, demonstrating remarkable stability of the assay setup. A typical PBR/HH screening assay takes about 12.5 min.

**Comparison with other assay techniques:** EAAT3 has been investigated in the past using two-electrode voltage clamp with aspartate, patch clamp and uptake assays with immobilized substrates. To confirm validity of the SSM-based assay, IC50 values of 6 compounds were determined and compared to published values. Although the inhibitor potency is influenced by the glutamate concentration and the assay conditions, the IC50 correlate well.

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**SSM-based electrophysiology**

“Solid Supported Membrane (SSM) based electrophysiology” is a method which allows to resolve low amplitude electric events in biological membranes. This is why it enables real-time activity measurement of electrophoretic membrane proteins for which conventional electrophysiology is unsuitable, like many membrane transporters and pumps, or targets localized in intracellular or backward membranes. Unlike in coated-cell electrophysiology a large sensor coated with purified membrane vesicles or proteoliposomes is used, leading to a high signal current format. The method is established since the 90s and was used up to 96-well format recently.

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**Assay development**

EAAT3 was overexpressed in CHO cells. The cells were grown, harvested, lysed and the plasma membrane isolated by partial centrifugation and a density gradient centrifugation. The resulting membrane vesicles were loaded with glutamate.

At the day of the experiment an 10 µl aliquot of the membrane vesicles were diluted, diluted and adsorbed to a 96-well SSM sensor. During the procedure the vesicles were loaded with potassium.

During the experiment the sensor is perfused with a sodium buffer, leading to ion inward directing sodium gradient. When adding glutamate a transient current occurs. This current is caused by sodium and proton inward current while potassium moves out of the vesicles. The sensor is rinsed in potassium containing buffer to restore the ion gradients before the next activation cycle.

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**Conclusion**

We developed an assay to measure the activity of EAAT3. Both previous results in SSM-based electrophysiology,

We performed a screen of glutamate, determined IC50 for several known inhibitors and compared them with data derived from other techniques (TEVC, uptake), which shows good consistence.

The automated SSM-based assay has a throughput of about 500 data points during an 8 hour day (negative and positive control wells, 12 wells + 12 controls). This is sufficient for an secondary screening assay.

The method has some relevant advantages: in contrast to other assays no running cell culture is required. The read-out technique is direct and does not require radiactiv labeling.

We expect, that the developed EAAT3 assay can be transferred to other glutamate transporters without modification.

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**References:**

1. The last line of the Schematic diagram: "Analysis of EAAT3 INVESTIGATED USING SSM-BASED ELECTROPHYSIOLOGY."
2. The first line of the diagram: "Analysis of EAAT3 INVESTIGATED USING SSM-BASED ELECTROPHYSIOLOGY."
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