Application Note

Channel: EAAC1
Cells: CHO
Tools: SURFE²R N1

Electrophysiological recordings of EAAC1 (SLC1A1) activity on Nanion's SURFE²R N1

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Summary

The excitatory amino acid carrier 1 (EAAC1; also known as EAAT3) is a sodium-dependent neuronal uptake transporter encoded by the slc1a1 gene[1]. It plays a major role in the reuptake of glutamate from the synaptic cleft, thereby maintaining a low extracellular concentration of glutamate and regulating the excitatory neurotransmission. EAAC1 is also involved in the uptake of aspartate and cysteine into the cells[1,2]. The transporter is highly expressed in mature neurons, where it is distributed in somata and dendrites[1,2].

EAAC1 functions as a co-transporter, coupling the uphill substrate transport into the cells to the electrochemical gradients of sodium and potassium. The stoichiometry of transport is 1 glutamate with 3 Na^+ and 1H^+ moving into the cell to 1 K^+ moving out of the cell[1,2]. Therefore EAAC1 is an electrogenic transporter, generating a net charge flow.

Dysfunction of glutamate transporters leads to increased extracellular glutamate levels, thereby causing neurotoxicity and neurodegeneration[2]. Regulatory mechanisms facilitating EAAC1 function are, therefore, interesting as targets for the treatment of neurodegenerative diseases[2].

Here we present EAAC1 activity measurements on the SURFE²R N1 instrument using purified plasma membrane of CHO cells expressing EAAC1.

Results

To activate transport on the SURFE²R N1, a sensor with attached EAAC1-containing membrane fragments was inserted into the device and perfused with a buffer containing NaCl and glutamate. When the substrate is present, Na^+ and K^+ movement across the membrane can be observed until an electrochemical equilibrium is reached. To generate Na^+ and K^+ gradients, necessary as a driving force, the sensor was flushed with KCl before and after the EAAC1 activation (Figure 1).

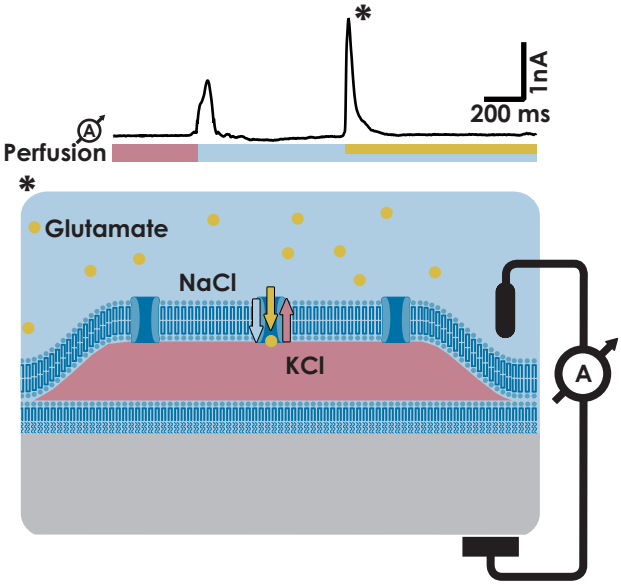

Figure 1: Typical EAAC1 current response on the SURFE²R N1. Glutamate was used as the substrate for EAAC1. When applying glutamate to the sensor, a transient current occurs (top).

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The stability of the current response was tested. Ten activation cycles on the same sensor were performed consecutively, showing a stable signal height after the second application (n = 5; Figure 2A).

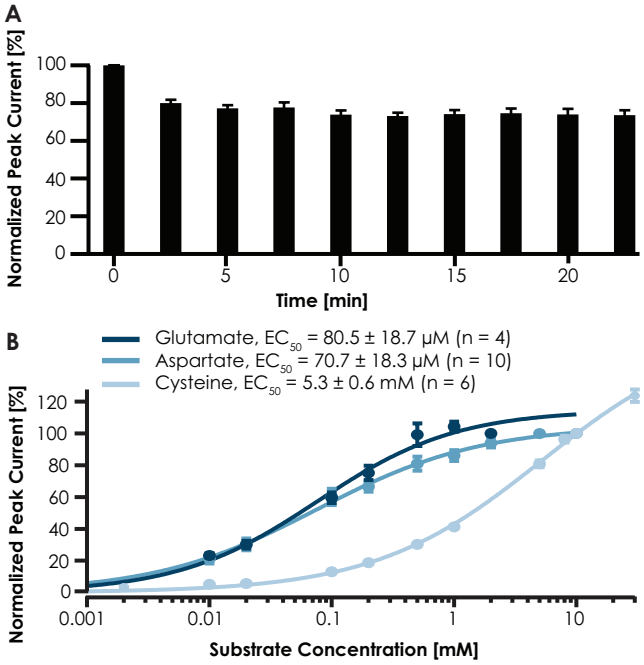

Figure 2: **A** Repeated measurements on sensors with 1 mM glutamate as the substrate (n = 5). **B** Concentration response curves for 3 different substrates shown overlaid. Increasing concentrations of the substrates were tested cumulatively on the same population of membrane fragments.

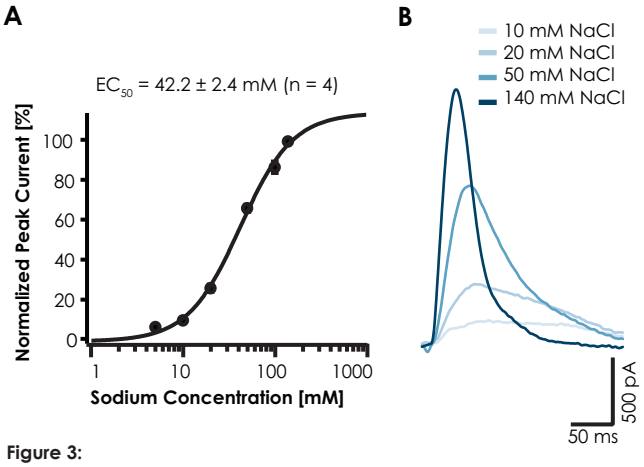

Figure 3:
EAAC1 is sodium-dependent, with an EC_{50} of 42.2 ± 2.4 mM (n = 4).

The apparent affinity of EAAC1 for the substrates glutamate, aspartate and cysteine was investigated. EC_{50} values of 80.5 ± 18.7 µM (n = 4) for glutamate, 70.7 ± 18.3 µM (n = 10) for aspartate and 5.3 ± 0.6 mM (n = 6) for cysteine were determined (Figure 2B). The Na^+ sensitivity of EAAC1 was investigated using buffers with different concentrations of Na^+, revealing a Na^+ EC_{50} of 42.2 ± 2.4 mM (n = 4; Figure 3).

In conclusion, the SURFE²R N1 can be used to reliably measure EAAC1 activity. This has important implications for drug discovery targeting EAAC1 for neurodegenerative diseases.

References

1. Zhou, Y & Danbolt, N.C. 2013. Front. Endocrinol. 4:165.
2. Aoyama, K & Nakaki, T. 2013. Amino acids. 45(1): 133–142

Methods

Plasma membrane preparation

According to the Nanion's standard procedure ("Quickguide Membrane Preparation from CHO cells"). Total protein concentration was between 5 - 10 µg/µl.

Buffers

EAAC1 experiments were performed by the exchange of a sodium and glutamate free ("resting") buffer for a sodium containing ("control") buffer and afterwards a substrate and sodium containing ("activating") buffer.
Control buffer contained: 140 mM KCl, 2 mM $MgCl_2$ 30 mM HEPES, pH 7.4 with NMG.
Activating buffer contained: 140 mM NaCl, 2 mM $MgCl_2$ 30 mM HEPES, pH 7.4 with NMG, x mM glutamate.

SURFE²R sensor preparation

According to the Nanion standard procedure "SURFE2R Sensor Preparation". Sensors are prepared in resting buffer, membrane is diluted 1:10 with resting buffer.

SURFE²R N1 measurement workflow

EAAC1 can be activated by providing glutamate, aspartate or cysteine as a substrate. A sodium gradient needs to be established in advance of substrate addition. Therefore, any 3-buffer Nanion standard protocol is suitable.

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