RESOLUTE

Functional Assays for Solute Carrier Transporters

Impedance-based phenotypic for SLC1A3 using HEK JumpIn SLC1A3 OE cells

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Assay description

Human SLC1A3 (solute carrier family 1 member 3) or EAAT1 (excitatory amino acid transporter 1), is a high affinity sodium-, proton-, and potassium-dependent glutamate transporter located in the plasma membrane.The phenotypic impedance-based assay to measure excitatory amino acid transporter (EAAT1, SLC1A3) enables detection of EAAT1 activity by uptake-induced changes of cell morphology.The mechanism behind the glutamate-induced response in dox-treated JumpIn-EAAT1 cells was attributed to initial cell swelling and subsequent spreading.

Assay protocol

General

Label-free whole-cell assays, based on cell-induced changes in impedance, were performed using the xCELLigence real-time cell analyzer (RTCA) system (ACEA Biosciences, San Diego, CA, USA). In short, cells are cultured in medium on gold-plated electrodes in microwell E-plates. Assays were performed at 37 °C and 5% CO_2 in 96-well PET E-plates in a total volume of 100 µl per well. Prior to cell seeding, the baseline impedance was measured in the recording station in 40 µl (two compound additions) or 45 µl (one compound addition) medium in the presence (+) or absence (–) of 1 µg/ml dox. All compounds were diluted in phosphate-buffered saline (PBS) and added in 5 µl per addition using a VIAFLO 96 handheld electronic 96 channel pipette (INTEGRA Biosciences, Tokyo, Japan). When DMSO was used as a solvent for a compound, the final amount of DMSO was kept at 0.1% per well and was included in the vehicle (PBS/DMSO). All conditions were tested at two technical replicates per plate.

Assay procedures

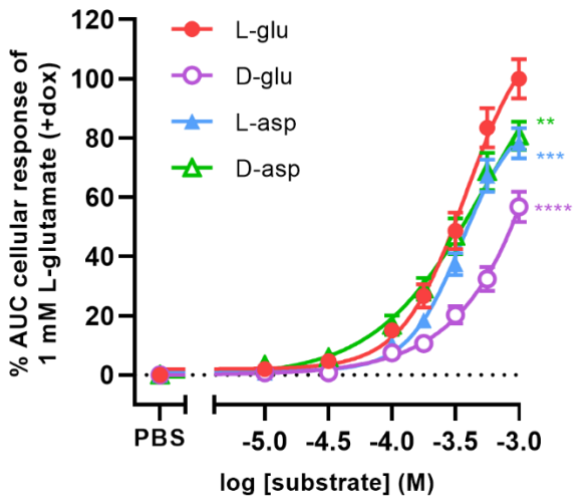
On the day of the experiment, JumpIn-EAAT cells grown to 70-80% confluence were trypsinized (0.25% trypsin in PBS/EDTA), counted and seeded in the E-plate in a volume of 50 µl at 60,000 cells/well. Transiently transfected JumpIn-EAAT1 cells were detached using only PBS/EDTA. The E-plate was left to rest at room temperature for 30 min prior to replacement in the recording station at 37 °C and 5% CO_2. Cell growth was recorded overnight for 22 hr. If a pretreatment was required for the experiment, the cells were pretreated after 22 h with either a concentration of inhibitor or vehicle (PBS/DMSO) and cells were monitored for 60 min. For EAAT inhibition experiments increasing concentrations (1 nM – 10 µM) of UCPH-101 and TFB-TBOA were used. After the pretreatment, cells were stimulated with substrate or vehicle (PBS). For EAAT inhibition experiments, cells were stimulated with a submaximal concentration of L-glu, i.e. 1 mM L-glu for EAAT1 inhibition. After stimulation the impedance was measured for at least 2 h.

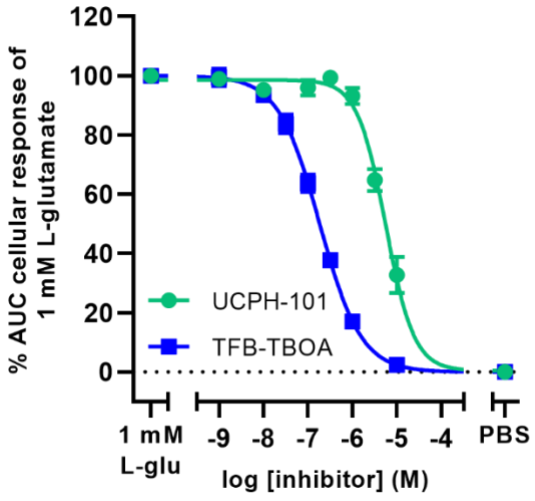
Additional information

Target data

SLC	SLC1A3
Synonyms	EAAT1 (excitatory amino acid transporter 1)
SLC sub-family	Solute carrier Family 1, (Glutamate transporter subfamily)
UniProt ID	Canonical form isoform-1 (i.e P43003-1)

Assay data





Compound name (1)	L-glutamic acid monosodium salt monohydrate
PubChem CID	33032
Vendor (catalogue #)	Sigma #49621
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 398 μM
Compound name (2)	D-glutamate
PubChem CID	23327
Vendor (catalogue #)	Sigma #G1001
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = <1000 μM
Compound name (3)	L-aspartic acid monosodium salt monohydrate
PubChem CID	5960
Vendor (catalogue #)	Sigma #A6683
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 398 μM
Compound name (4)	D-aspartate
PubChem CID	83887
Vendor (catalogue #)	Sigma #219096
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 398 μM
Compound name (5)	UCPH-101
PubChem CID	25223366
Vendor (catalogue #)	Santa cruz biotechnology #sc-361391
Mode of action	Inhibitor
Standard value type (i.e EC50, PoC, etc)	IC_{50} = 6.3 μM
Compound name (6)	TFB-TBOA
PubChem CID	52941382
Vendor (catalogue #)	Axon medchem #2640
Mode of action	Inhibitor
Standard value type (i.e EC50, PoC, etc)	IC_{50} = 0.2 μM

Cross references

- RESOLUTE report at Zenodo.
- https://doi.org/10.3389/fphar.2022.872335