RESOLUTE

Functional Assays for Solute Carrier Transporters

Impedance-based transporter assay for SLC29A1 using U2OS cells

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Assay description

The Impedance-based 'transporter activity through receptor activation (TRACT) assay detects transport activity of the Equilibrative nucleoside transporter (ENT1, SLC29A1) via GPCR-mediated changes in cell morphology. The assay is based on the principal that Co-expressed GPCR-SLC pair share a common agonist/substrate. Activation of a GPCR will result in changes in cell morphology that are detected as changes in cellular impedance using the xCELLigence RTCA system. Active SLCs transport part of the substrate into the cell leaving a lower extracellular concentration of agonist to activate the GPCR and consequently an attenuated GPCR-mediated response can be observed which reflects SLC activity (Fig. 1). Treatment with ENT1 inhibitors will enhance the GPCR response which is a measure of SLC inhibition. The TRACT assay can be used to determine the potency (EC_{50}) of (endogenous) G protein-coupled receptor (GPCR) agonists on JumpIn-ENT1 cells and enables the determination of inhibitory potency (IC_{50}) of ENT1 inhibitors.

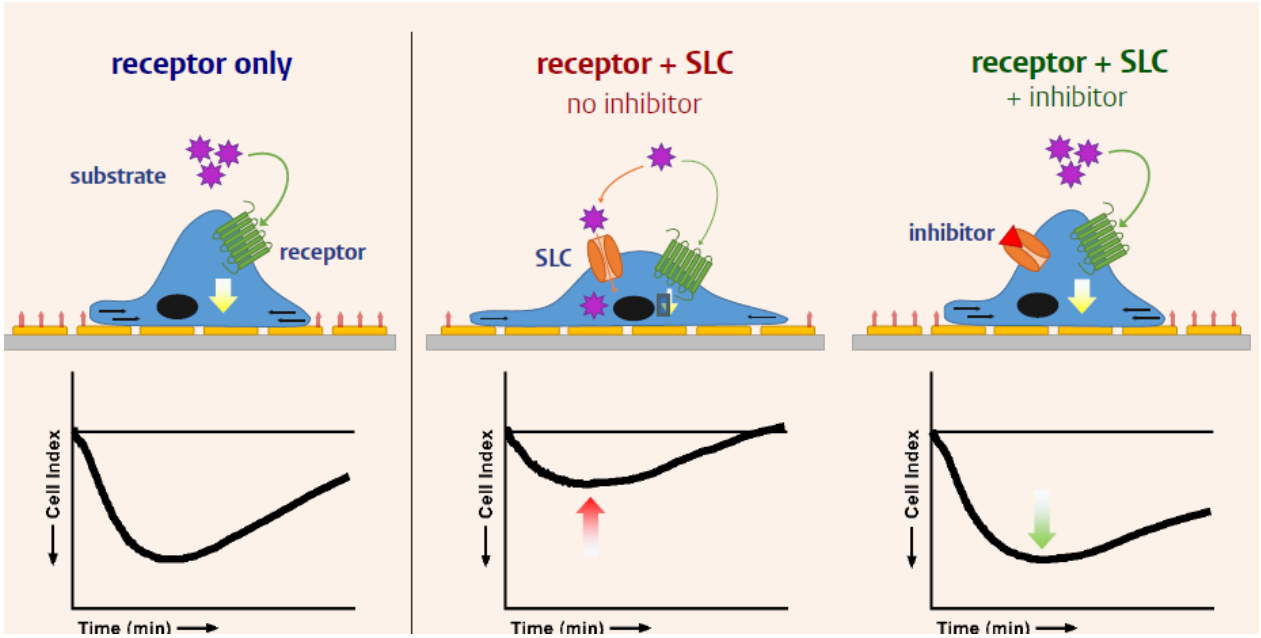


Figure 1. Principal of a Impedance-based TRACT assay that relies on a shared substrate/agonist for the SLC-GPCR pair*. 1) Activation of a GPCR by a agonist results in changes in cell morphology which can be detect using an xCELLigence real-time cell analyzer (RTCA) system as changes in cellular impedance. 2)Upon co-expression of a SLC, the shared substrate can be taken up by the SLC resulting in a reduced extracellular substrate/agonist concentration thereby attenuating the GPCR response. 3) Inhibition of the SLC by an inhibitor restores GPCR action to level without SLC present.*

Assay protocol

Detection principle: Label-free whole-cell assays were performed using the xCELLigence real-time cell analyser (RTCA) system. In short, the RTCA system measures the electrical impedance generated by cells adhering to gold-coated electrodes embedded on the bottom of microelectronic E-plates. Variations in number, degree of adhesion, cellular viability and morphology of cells result in relative changes in impedance.
Cell seeding & monitoring: U2OS cells were harvested by re-suspending in cell culture medium after brief trypsini- zation (treatment with trypsin/EDTA for about 5 min) and centrifuged once at 200 × g (1500 rpm) for 5 min. Background impedance was measured after the addition of 40 µL culture media to 96 well E-plates. Cells were seeded by adding 50 µL of cell suspension containing 20,000 cells per well. After resting at room temperature for at least 30 min, the E-plate was placed into the recording station situated in a 37 °C and 5% CO2 incubator. Impedance was measured every 15 minutes overnight.

Cell pre-treatment: Cells were pre-treated by an ENT1 inhibitor or vehicle control (0.25% dimethylsulfoxide (DMSO) in Phosphate-buffered saline (PBS)) in 5 µl volume using a VIAFLO 96 handheld electronic 96 channel pipette (INTEGRA Biosciences, Tokyo, Japan) after 18 h. CI was recorded for at least 30 minutes with a recording schedule of 15 second intervals for 20 minutes, followed by intervals of 1 minute.
Cell treatment: Cells were stimulated with adenosine (10−4.5 or 10−6 to 10−3.5 M depending on the assay) or vehicle control (0.125% DMSO in PBS) in 5 µL and CI was recorded for at least 90 minutes with a recording schedule of 15 second intervals for 20 minutes, followed by intervals of 1 minute for 10 minutes and finally 5 minutes. In all cases, final well volumes and DMSO concentrations upon cell and ligands addition were 100

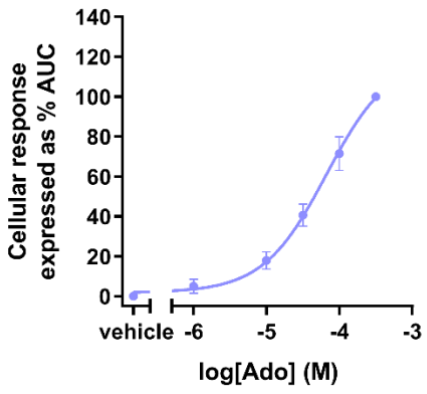
Data analysis: RTCA software 2.0 (ACEA Biosciences, Inc.) was used to obtain the experimental data. All data were analysed using GraphPad Prism 7.00 (GraphPad software, San Diego, CA, USA). After sub- tracting baseline (vehicle control), ligand responses were normalized at the time of ligand addition to obtain Δ Cell Index (Δ CI) values to correct for ligand-independent responses. Cells that were not pre-treated were used as control (vehicle 1) for the ENT1 inhibitor addition, while cells that were not treated with adenosine (vehicle 2) were used as control for the adenosine addition. The time of normalization was either 18 h or 18 h 30 min after cell seeding for analysis of ENT1 inhibitor/ARs antagonist or adenosine effects, respectively. The absolute values of Total Area Under the Curve (AUC) up to 90 min after adenosine addition were exported to Graphpad Prism for further analysis yielding concentration–response curves. pIC_{50} values of ENT1 inhibitors were obtained using non-linear regression curve fitting of Total AUC data into a sigmoidal dose-response curve with variable slope.

Additional information

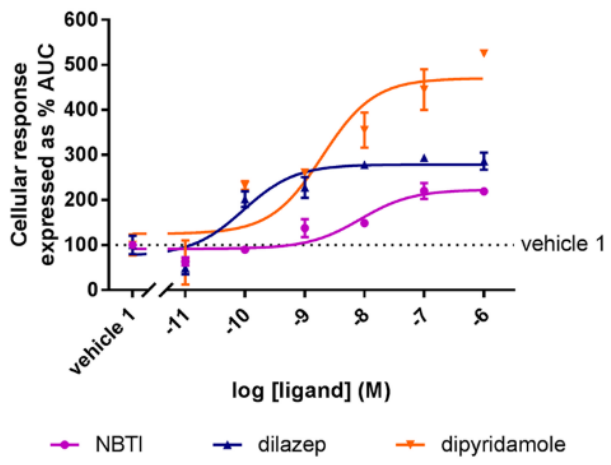
Target data

SLC	SLC29A1
Synonyms	Equilibrative nucleoside transporter-1 (ENT1)
SLC sub-family	Solute carrier family 29
UniProt ID	Canonical form (Q99808)
RESOLUTE Cell ID	In-house cell lines used

Assay data



Compound name (1)	Adenosine
PubChem CID	60961
Vendor (catalogue #)	Sigma 01890
Mode of action	Substrate
Standard value type (i.e EC50, PoC, etc)	EC_{50} = 63 µM

	**Compound name (2)**	NBTI
	PubChem CID	65407
	Vendor (catalogue #)	Sigma #N2255
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 2.6 nM
	Compound name (3)	dilazep
	PubChem CID	3074
	Vendor (catalogue #)	GIFT from Asta-Werke (Degussa Pharma Gruppe)
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 0.1 nM
	Compound name (4)	dipyridamole
	PubChem CID	3108
	Vendor (catalogue #)	Gift from Janssen pharmaceutics
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 8.1nM
	Compound name (5)	draflazine
	PubChem CID	60849
	Vendor (catalogue #)	Gift from Janssen pharmaceutics
	Mode of action	Inhibitor
	Standard value type (i.e EC50, PoC, etc)	IC_{50} = 5.3nM

Discussion

The impedance-based TRACT assay displays a shift in EC_{50} upon doxycycline-induction of ENT1 indicating that Adenosine is transported into the cell resulting in a lower extracellular concentration of Adenosine and consequently a reduced GPCR activation.

Cross references

- RESOLUTE report at Zenodo.
- https://doi.org/10.1038/s41598-019-48829-3
- https://doi.org/10.1016/j.bcp.2019.113747