Case Study

Advanced Electrophysiology Solutions for PAT-1 Drug Discovery

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Introduction

The protein-coupled amino acid transporter PAT-1 (SLC36A1) plays a critical role in neurotransmitter precursor transport and the uptake of small neutral amino acids such as glycine, alanine, and proline, as well as certain pharmacologically active compounds. This transporter is essential for amino acid absorption and broader metabolic and neurological health, utilizing a proton gradient to facilitate amino acid uptake at a 1:1 H^+ to amino acid ratio.

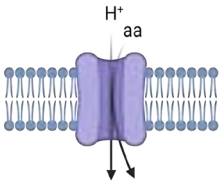

Figure 1.

Schematic diagram representing PAT-1 utilizing the proton gradient across the membrane to drive the uptake of amino acids into cells.

PAT-1 Therapeutic Potential

PAT-1's involvement in nutrient absorption and neurotransmitter precursor transport highlights its potential as a novel therapeutic target. Modulating PAT-1 activity offers opportunities to address various conditions, including:

- Hartnup Disease
- Metabolic Disorders
- Gastrointestinal Diseases
- Neurological Disorders

Supporting Your Research

Sygnature Discovery's advanced cell-based assay capabilities and state-of-the-art SURFE^{2}R platforms enable the exploration of the therapeutic potential of PAT-1. The SURFE^{2}R N1 platform facilitates low-throughput, customizable assays, while the SURFE^{2}R 96SE platform supports high-throughput studies, delivering robust solutions for investigating PAT-1 activity under diverse conditions and supporting the advancement of novel transporter-targeted therapies.

Methods

HEK cells overexpressing PAT-1 were generated by Sygnature Discovery and harvested in PBS at 80-90% confluency. Cells not expressing PAT-1 served as negative controls.

Parental cells were lysed using Nitrogen decompression in a cell disruption buffer (10 mM Tris, pH 7.5, 250 mM sucrose, Complete™ protease inhibitor cocktail). Membranes were prepared through centrifugation and sucrose gradient ultracentrifugation and diluted in storage buffer (5% glycerol, 0.2 mM DTT). Protein concentration was assessed using Bradford Assay and the samples were flash frozen in 10 µL aliquots, and stored at -80°C.

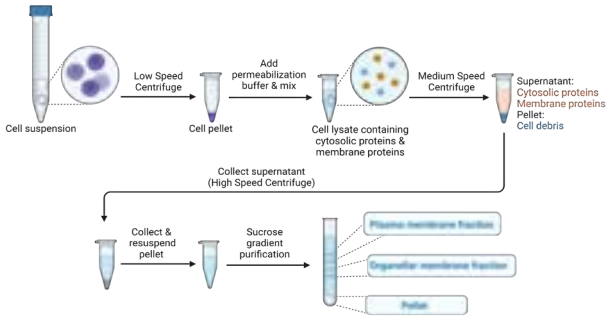

Figure 2.

SURFE^{2}R SSM-Based Electrophysiology Membrane Preparation.

Buffers

PAT-1 experiments were performed by exchanging substrate-free (non-activating) buffer for a substrate containing (activating) buffer.

Non-activating buffer: 80 mM L-Leucine, 100 mM KCl, 30mM MES, 2 mM $MgCl_2$ (pH 5.5-7.0 KOH).

Activating buffer: 80mM L-Proline/L-Glycine, 100 mM KCl, 30 mM MES, 2 mM $MgCL_2$ (pH 5.7-7.0 KOH).

Electrogenic events were measured using SURFE^{2}R N1 and SURFE^{2}R 96SE SSM-based electrophysiology platforms in which charge translocation is triggered by rapidly perfusing the sensor with an activating substrate resulting in a gradient across the membrane (figure 3). Using this technology the current's size and shape provides information about speed of transport, coupling ratio and substrate affinity.

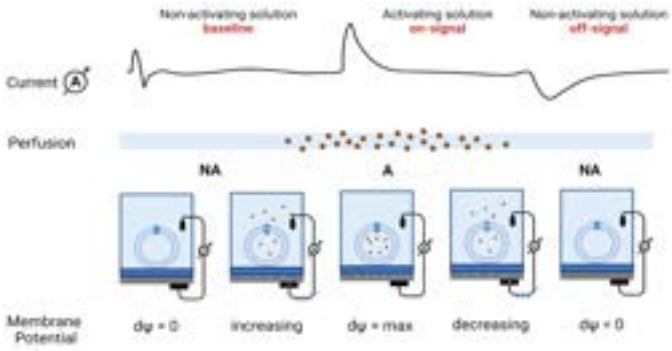

Figure 3.

Transport activity is triggered by perfusion of the membrane coated sensor with a substrate-containing solution (activating solution). Ionic background is adapted to the transporter properties.

Assay Development

The SURFE²R N1 SSM technology was employed for initial PAT-1 characterization and assay development. This small-scale platform enabled a flexible and thorough investigation of PAT-1, facilitating a refined approach to subsequent assay development.

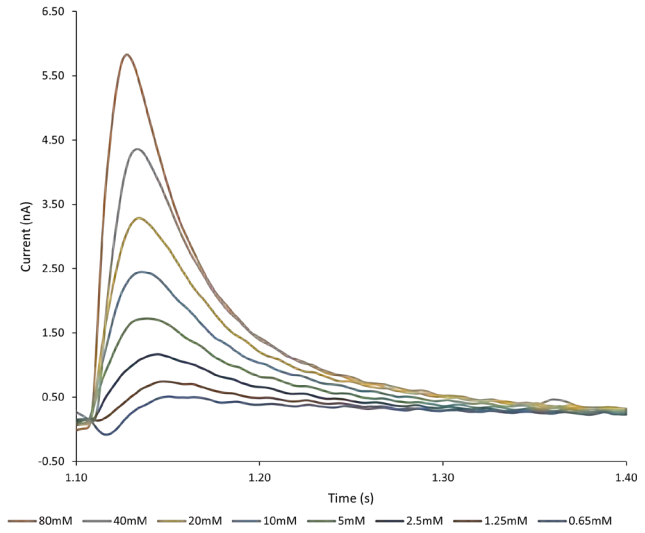

Figure 4.

Raw representative current traces obtained on SURFE²R N1 upon proline jumps (80 mM-0.65 mM) from an individual sensor.

Building on the initial validation of PAT-1 activity using the SURFE²R N1 platform, the assay was then transitioned to the high-throughput SURFE²R 96SE platform. The 96SE platform supports increased throughput while enabling the simultaneous testing of multiple variables, including endogenous substrates and pH conditions. Measurement across differing pH conditions is particularly relevant for PAT-1, as it functions as an H^+-driven transporter, making pH an essential factor in accurately assessing its activity.

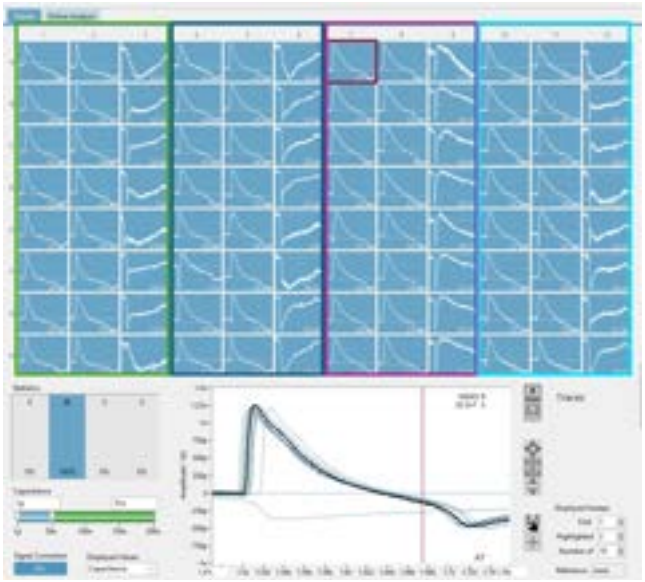

Figure 5.

Screenshot of one experiment on the SURFE²R 96SE comparing membranes overexpressing PAT-1 and UT sample. Signal generated upon repetitive perfusion with activating buffer (80 mM L-Proline) at different pH conditions. Green (pH 5.5), blue (pH 6), purple (pH 6.5), turquoise (pH 7.0).

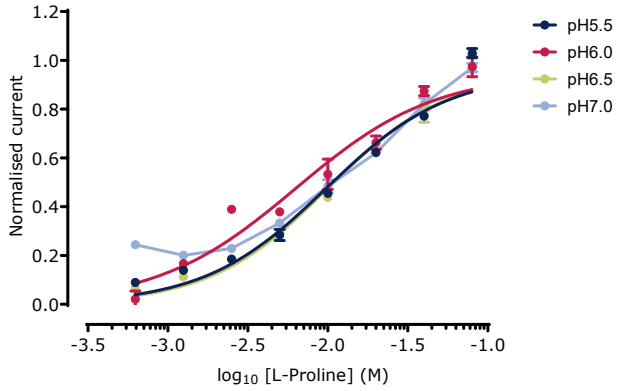

Figure 6.

Concentration response curves showing activation of PAT-1 by L-Proline under different pH conditions.

The SURFE²R 96SE platform further enables rapid assessment of different endogenous substrates (Figure 7) and demonstrates reproducibile activation within experiments (Figures 8–10).

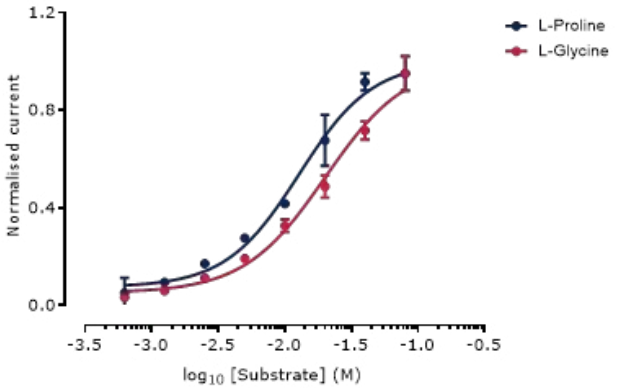

Figure 7.

Concentration response curves showing activation of PAT-1 by L-Glycine and L-Proline at pH 6.5. EC_{50} values were 14 mM and 10 mM, respectively.

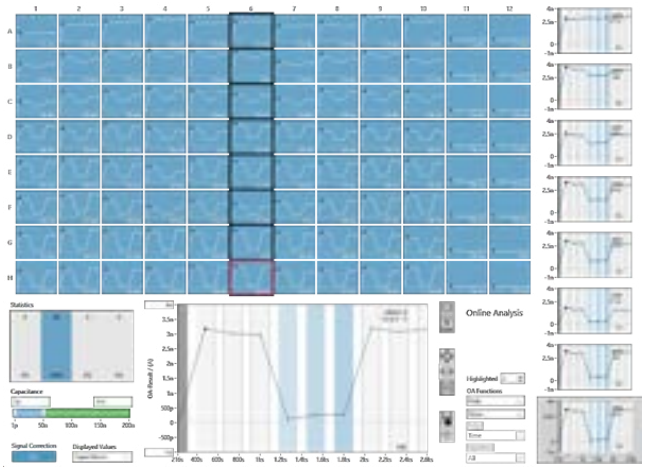

Figure 8.
Screenshot of one experiment on the SURFE²R 96SE showing concentration-dependent activation of PAT-1. PAT-1 was activated by 80 mM L-Proline followed by a subsequent range of L-Proline concentrations and finally a repeat application of 80 mM L-proline. Columns 1-10 contained membranes overexpressing PAT-1, columns 11 and 12 contained membrane from UT cells.

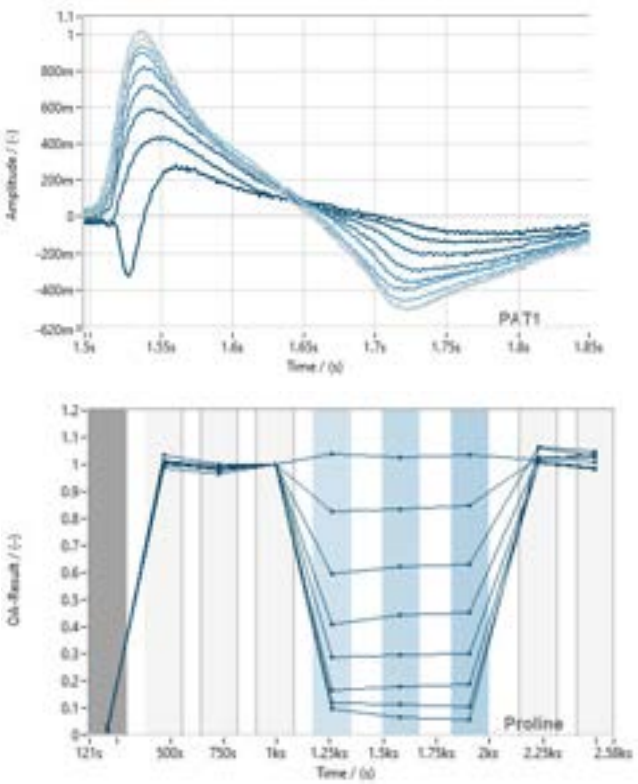

Figure 9.
Current traces (top) and corresponding time-course (bottom) showing PAT-1 activity in the presence of increasing concentrations of L-Proline.

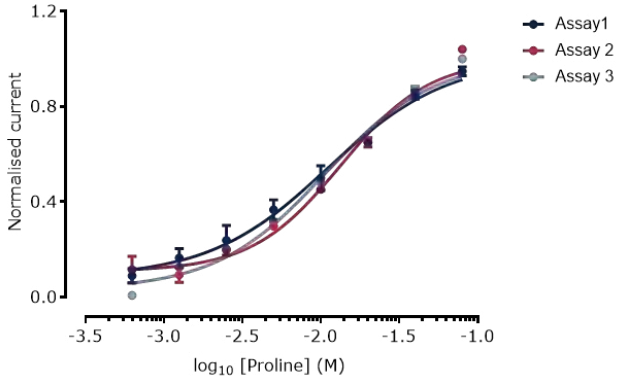

Figure 10.
Concentration response curves showing reproducibility of L-Proline EC_{50} across multiple days. EC_{50} values were consistently ~10 mM.

The SURFE²R N1 platform offers exceptional flexibility for testing diverse conditions with minimal sample requirements, while the SURFE²R 96SE platform is optimized for higher throughput needs. Both platforms are designed for seamless integration, allowing data and methodologies to transition effortlessly between them (Figure 11).

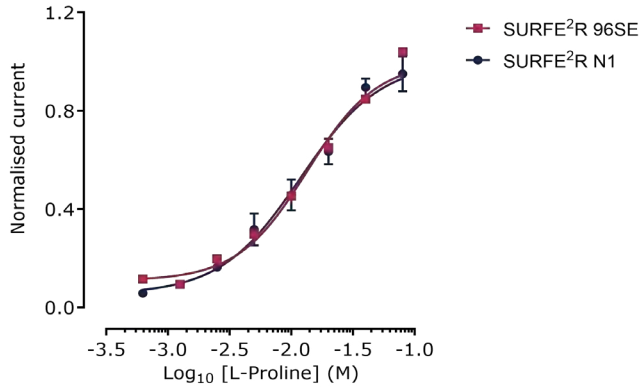

Figure 11.
Concentration response curve for activation of PAT-1 by L-Proline on SURFE²R N1 and 96SE at pH6.5. The curve was fit using GraphPad Prism revealing comparable EC_{50} values of 11 mM and 13 mM L-Proline on N1 and 96SE respectively.

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