Case Study

Development of High-Throughput Electrophysiological Assays for PepT1 Drug Discovery

Introduction

The human Peptide Transporter 1 (hPepT1, SLC15A1) plays a key role in the uptake of a wide range of substrates, including artificial di- and tri-peptides (e.g., Gly-Sar), active peptide-based drugs (e.g., cephalosporins, penicillins), and ester prodrugs (e.g., ACE inhibitors, protease inhibitors, valacyclovir, L-DOPA). Owing to its broad substrate specificity and expression in the intestinal epithelium, hPepT1 is being actively explored as a target for drug development and delivery, particularly in antiviral, anticancer and metabolic disease applications.

Using solid-supported membrane (SSM)-based electrophysiology, Sygnature Discovery has developed a robust hPepT1 assay for detailed functional and pharmacological characterization. SSME technology provides precise, reliable assessment of transporter activity and kinetic profiling across a range of substrates and pH conditions, enabling high-throughput compound screening and confident decision-making in drug discovery programs.

Methods

Membrane vesicles were produced from HEK cells expressing hPepT1. Untransfected HEK cells served as negative controls. Cell disruption was performed via nitrogen decompression and membrane fractions were isolated through sucrose gradient centrifugation (Figure 1).

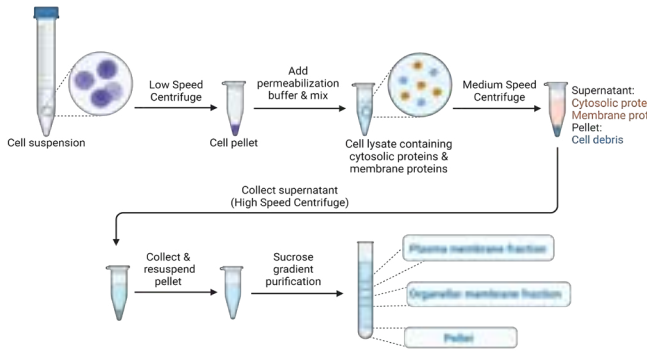

Figure 1.
SURFE²R SSM-Based Electrophysiology Membrane Preparation.

Membrane fractions were immobilized on sensors for electrophysiological analysis using the SURFE²R 96SE platform.

Transport activity was initiated through rapid solution exchange across the sensor surface. A non-activating (NA) buffer established a strong baseline at 0 mV. Introduction of activating (A) buffer containing endogenous substrate (Gly-Gly) created a concentration gradient, driving substrate and charge translocation via the transporter. As electrochemical equilibrium was reached, current decayed before reapplication of the NA buffer expelled accumulated charge, producing the off-signal response and restoring baseline conditions (Figure 2).

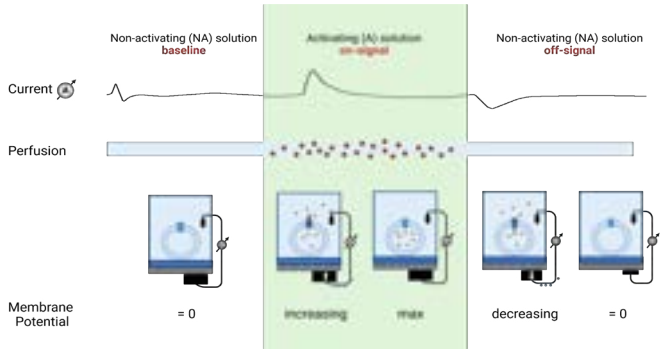

Figure 2.
Schematic illustrating SURFE²R assay. Transport is initiated by rapid perfusion of the sensor with NA buffer followed by A buffer containing 20 mM Gly-Gly.

Current traces reflected real-time electrogenic transport. and demonstrate robust signal generation in hPepT1-expressing membranes versus control samples, confirming transporter-specific activity (Figure 3).

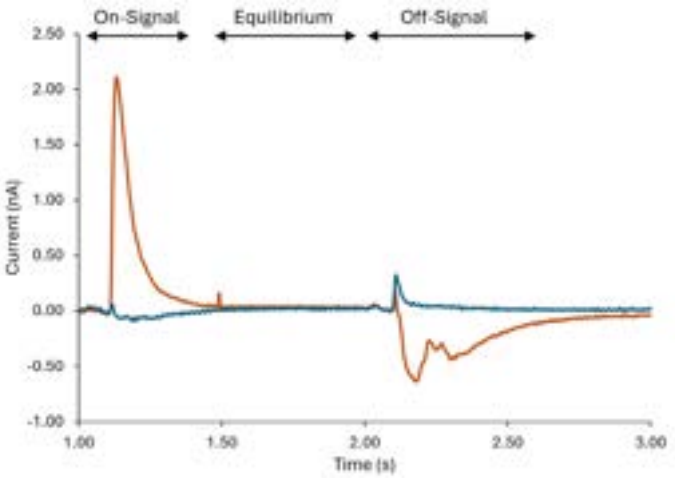

Figure 3.
Representative SSME traces for hPepT1 (red) vs control (blue) membranes. H^+/peptide influx generates a positive on-signal between 1.1-1.3 s. Once the electrochemical equilibrium is reached, the translocation is seized (1.5 - 2.0 s). This is followed by efflux and a negative off-signal (2.0 - 2.5 s). Measurements were performed at pH 7.2 using 20 mM Gly-Gly.

Substrate Specificity and pH Dependence

Given the broad range of potential hPepT1 substrates, assessing the apparent affinity (K_m) of different peptide substrates provides important insights into substrate-transporter interactions and supports the rational design of peptide-based drugs. To evaluate the substrate selectivity of hPepT1, we measured peptide transport kinetics for a panel of Gly-X substrates using the SURFE²R 96SE platform.

EC_{50} and apparent K_m were determined from dose-response curves recorded at pH7.2 (Figure 4). The EC_{50} values obtained were 4.3 mM for Gly-Sar, 8.9 mM for Gly-Gly and 10.5 mM for Gly-Gly-Gly, with corresponding K_m values of 3.2 mM, 6.8 mM and 9.0 mM, respectively.

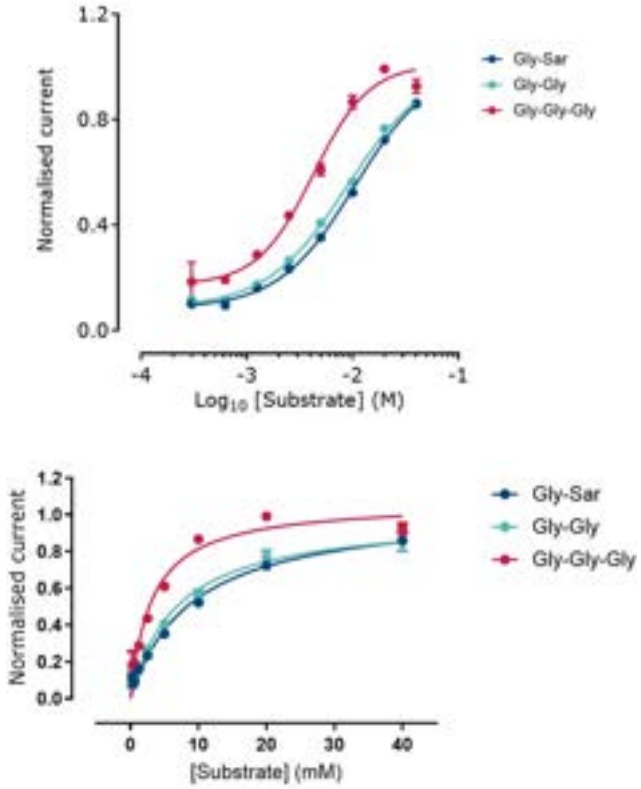

Figure 4.

Dose-response curve and substrate affinity of the Gly-Sar, Gly-Gly and Gly-Gly-Gly (40 mM-0.1 mM) in PepT1 at pH 7.2.

Peptide translocation mediated by hPepT1 is strictly coupled to protons. To investigate the influence of pH on transport activity, we examined the response to 20 mM Gly-Gly across a physiological pH range (5.0-9.0). We observed that the peak amplitude, which reflects the binding/translocation, increased with rising pH (Table 1).

In addition, the decay, proportional to the total charge translocated, decreased at both extremes of the pH spectrum, particularly under more acidic conditions. This finding was confirmed by calculating the steepness of the slope with the Levenberg-Marquardt algorithm in OriginLab Pro Software. The calculated decay constant increased steadily from pH 5.0 to neutral pH before dropping again under alkaline conditions (Figure 5).

Table 1.

Peak amplitude (pA) and decay constant (k) calculated for measurements at each pH and averaged.

pH	Average Response (pA)	S.D	Average Decay Constant (k)	S.D
5	366.8	99.46	7.9	0.30
5.5	460.3	166.90	11.6	0.27
6.0	896.1	202.34	16.7	0.21
6.5	1500.0	328.10	20.3	0.15
7.0	2037.9	386.63	21.0	0.14
7.5	2264.4	316.69	19.8	0.13
8	2469.0	322.02	16.1	0.11
8.5	2632.1	412.38	14.8	0.10
9.0	2590.3	339.72	13.4	0.09

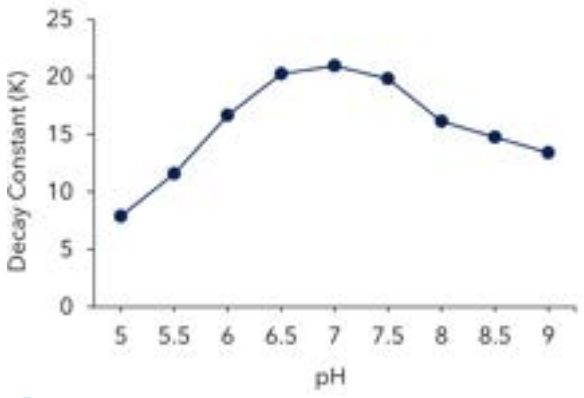

Figure 5.

Averaged decay constant response to each pH tested.

To illustrate these findings, representative peak traces recorded at different pH values were vertically shifted to align with the response observed at pH 7 (Figure 6). This visualisation demonstrates that under non-optimal pH conditions, both Gly-Gly binding and subsequent translocation are substantially reduced compared with neutral pH.

We further investigated the peptide concentration dependence at different pH values using the SURFE^{2}R 96SE platform to determine whether the K_m values for Gly-Gly are pH-dependent. Gly-Gly concentrations ranging from 0.1 mM to 40 mM were tested (Figure 7). Based on the decay constant data, which suggestedthat transport dominated over binding even at pH values near 6.5 and 7.0, we included an additional pH 6.7 condition in our studies. The results showed that K_m values increased with rising pH, ranging from 1.2 mM at pH 6.0 to 16.3 mM at pH 8.0.

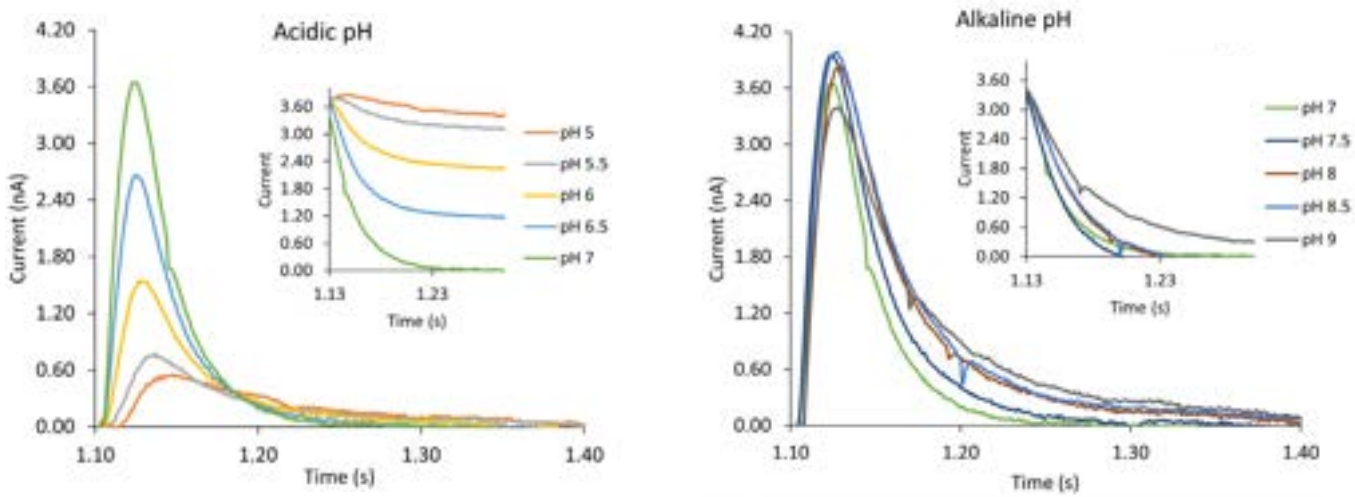

Figure 6.
Representative traces recorded for PepT1 at pH 5.0-9.0 at 20 mM Gly-Gly.

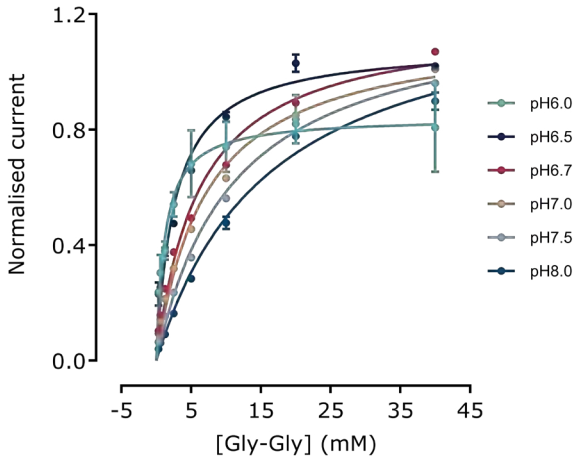

Figure 7.
Impact of pH on substrate affinity. Dose response curves for Gly-Gly at various pH values. Concentration dependent peak current obtained for a given sensor were fitted using a Hill equation, normalized to respective I_{MAX} and then averaged across sensors.

Dose Response of Gly-Gly

Based on the decay constant and substrate affinity across different pH values, which suggested an optimal range between pH 6.5 and 7.0, pH 6.7 was selected as the standard assay condition for subsequent studies. Prior to compound testing, EC_{50} values for Gly-Gly were measured across four days using the SURFE²R 96SE platform.

Consistent dose-response curves were observed, with EC_{50} values falling within a 3-6 mM range (Figure 8).

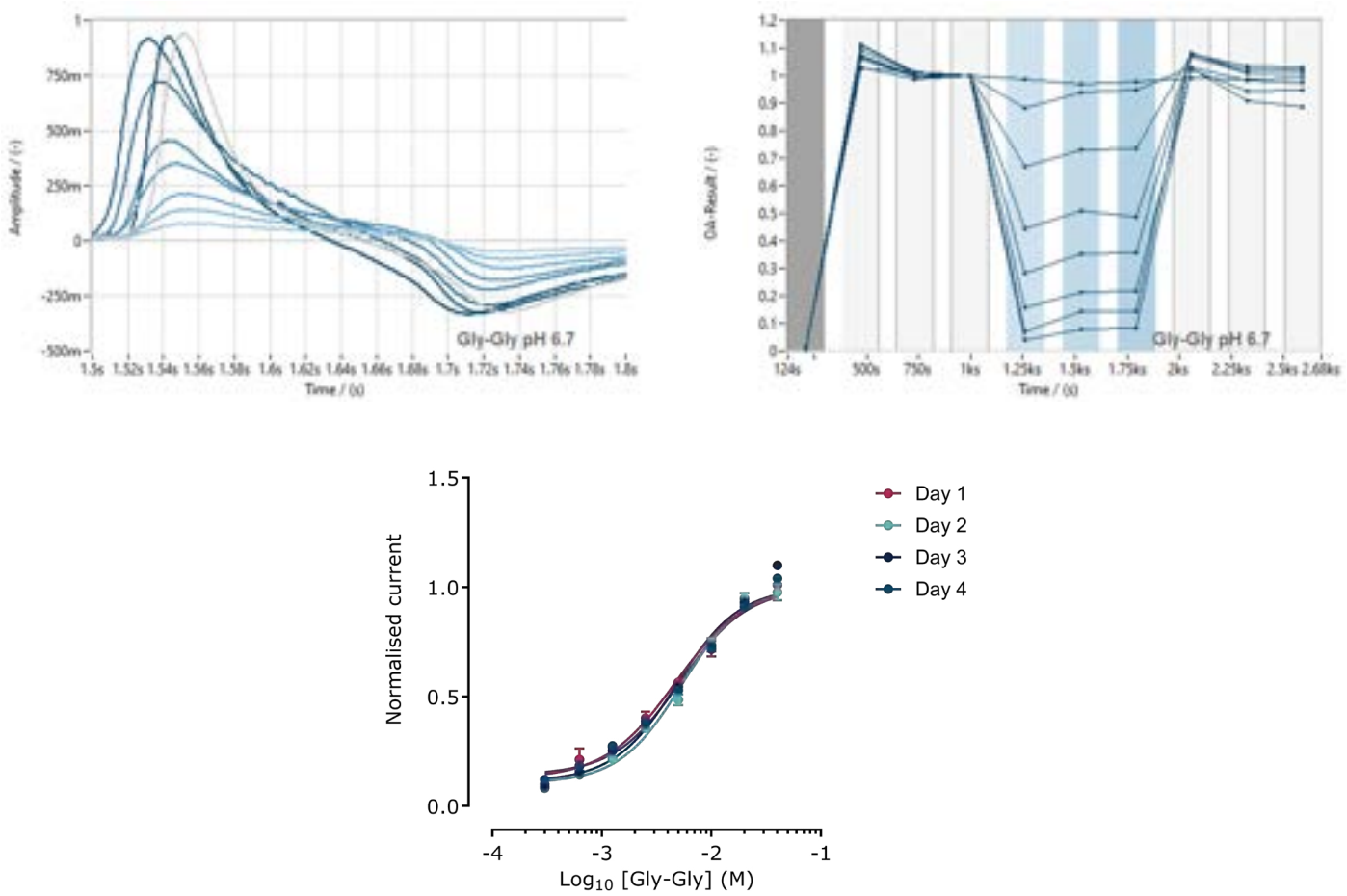

Figure 8.

Normalised current traces obtained upon Gly-Gly jumps (40 mM - 0.3 mM) (top left). Graphical user interface data analysis software used on the SURFE²R 96SE depicting online analysis (OA) (top right); reproducibility of dose-response curve of the Gly-Gly (40 mM - 0.1 mM) in PepT1 at pH 6.7 over 4 separate experimental days (bottom).

Assay Validation of Known Inhibitors

To validate the suitability of the PepT1 assay for hit identification screening, three known inhibitors of H^+/peptide transport, Glibenclamide, Valaciclovir and Zinc Chloride, were assessed. Compounds were profiled in parallel using the SURFE^{2}R 96SE platform.

The inhibitor assays followed a three-step protocol, illustrated in Figure 9 with $ZnCl_2$ as an example:

1. A reference peak current (I_{ref}) was recorded using substrate alone.
2. The inhibitor was applied to the NA solution and co-applied with the substrate to the A solution; the reduced current (I) was measured and the percentage inhibition was calculated as the ratio I/I_{ref}.
3. A final substrate-only step confirmed reversibility of inhibition.

The resulting IC_{50} values were 63 µM for Glibenclamide, 333 µM for Valaciclovir and 2.2 mM for Zinc Chloride (Figure 9).

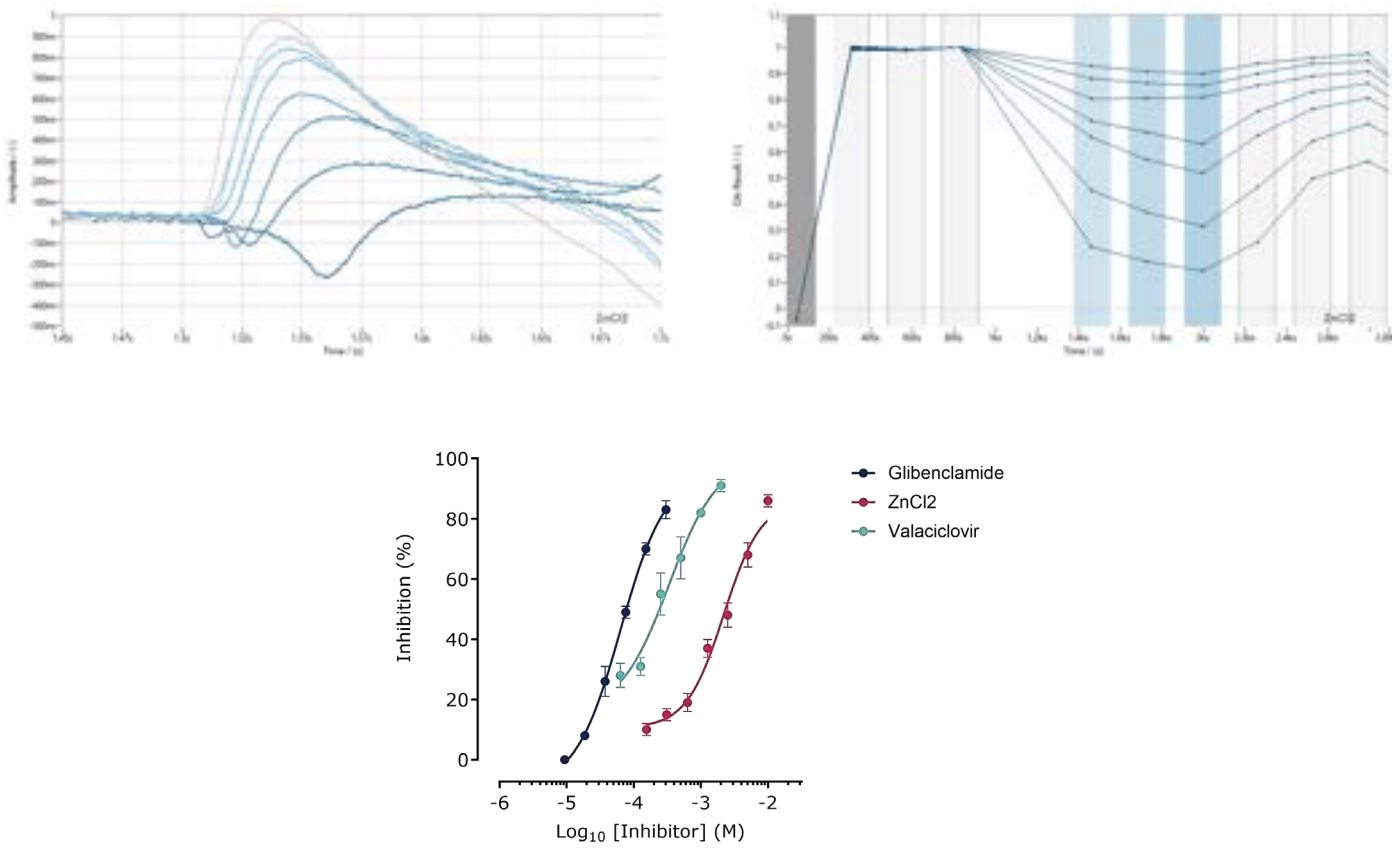

Figure 9.

Normalised current traces in the presence of increasing concentrations of $ZnCl_2$ (left) and corresponding time course (right). Glibenclamide, $ZnCl_2$ and Valaciclovir block of 10 mM Gly-Gly jumps in PepT1 (100% = full block, 0% = no inhibitor) (bottom).

Glibenclamide

IC_{50} determination at pH 6.7 showed consistent results across four independent days, demonstrating high assay reproducibility (Figure 10).

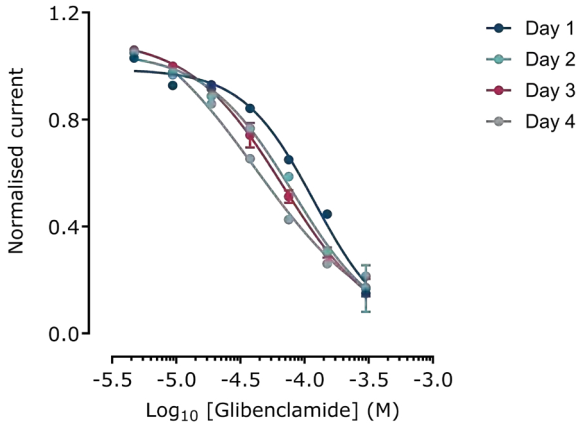

Figure 10.

Reproducibility data for PepT1 inhibitor Glibenclamide obtained with SURFE²R 96SE over multiple days at pH 6.7 (1=no inhibitor, 0=full block).

Zinc Chloride

Similar to Glibenclamide, reproducibility testing over four days showed good reproducibility (Figure 11), confirming the assay stability for inhibitor profiling.

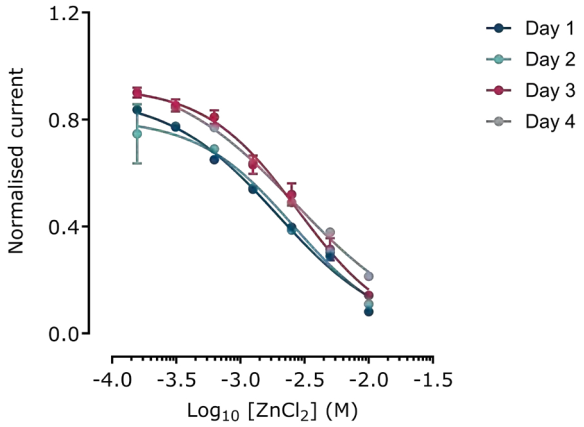

Figure 11.

Reproducibility data for PepT1 inhibitor $ZnCl_2$ obtained with SURFE²R 96SE over multiple days at pH 6.7 (1=no inhibitor, 0=full block).

Valaciclovir

Valaciclovir only produced a partial block under standard conditions, leaving approximately 25% of the current at the highest concentration tested (Figures 9 and 12). In Figure 7 we demonstrated that increasing pH reduced the apparent substrate affinity, which may also impact inhibitor efficacy depending on their mechanism of action. Valaciclovir, for example, is described as a competitive inhibitor that binds to the same site as Gly-Gly and may therefore interact preferentially with the protonated carrier.

To enhance the likelihood of achieving full inhibition, lowering the Gly-Gly concentration to approximately its EC_{50} may be beneficial due to the competitive nature of this inhibitor. To test this hypothesis, we recorded IC_{50} curves for Valaciclovir at pH 6.7 and pH 7.5 using 5 mM Gly-Gly in the activating buffer. As shown in Figure 13, both substrate concentration and the protonated state of the binding carrier contribute to achieving a full block.

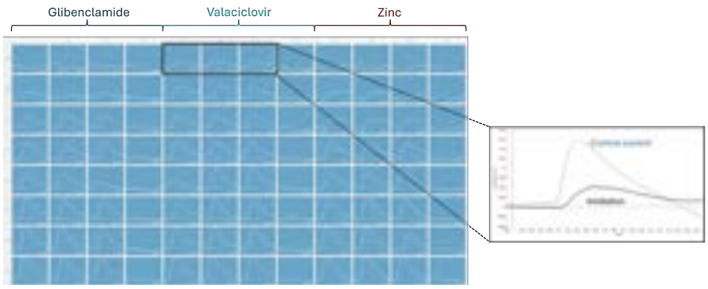

Figure 12.

Screenshot of one experiment on the SURFE2R 96SE showing the control current (blue, ref) and current in the presence of the inhibitor (black, refl). Columns 4, 5 and 12 contained membrane from UT cells as controls.

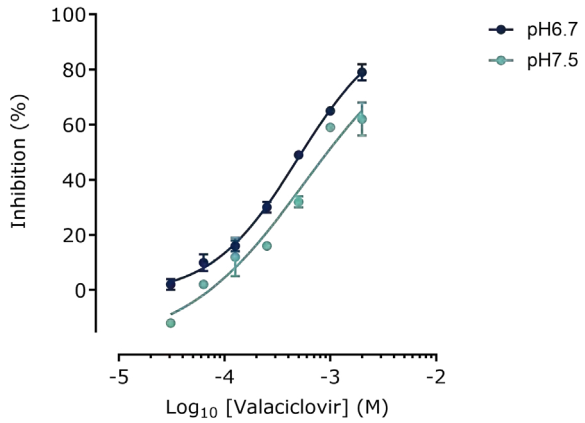

Figure 13.

Valaciclovir block of 5 mM Gly-Gly at pH 6.7 and pH 7.5. IC_{50} values were 490 µM at pH6.7 and 560 µM at pH 7.5.

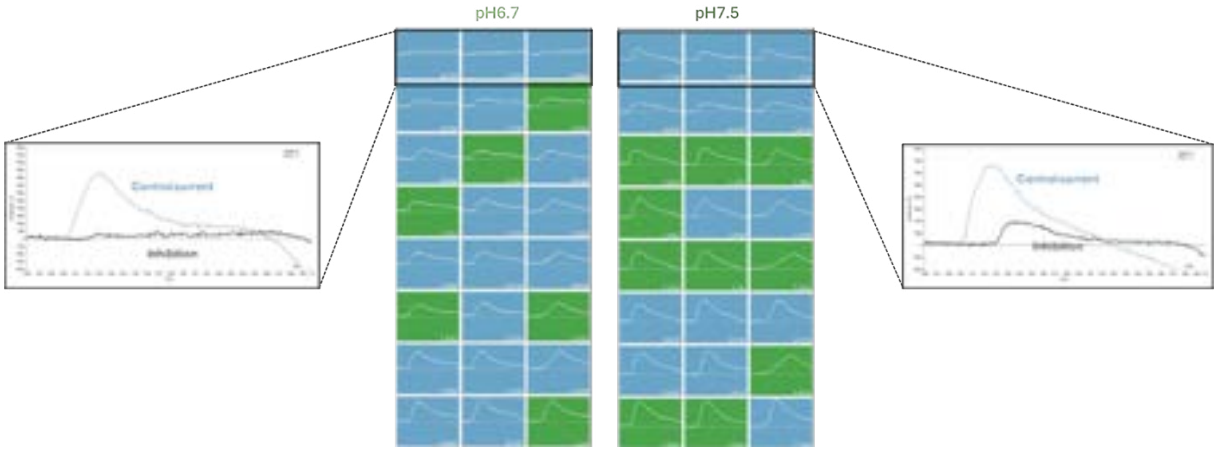

Figure 13 *cont.*
Screenshot of the SurfControl Software showing the control current in blue (ref) and the current in presence of highest concentration of Valaciclovir in black (Ref I).

Conclusion

This study demonstrates the successful development and validation of a solid-supported membrane-based electrophysiology assay for the human peptide transporter hPepT1 using the SURFE^{2}R 96SE platform. Transport of model peptide substrates was reliably detected across a physiological pH range, with kinetic parameters (EC_{50} and K_m) aligning with literature values and revealing pH-dependent transport characteristics.

The assay was further validated using known hPepT1 inhibitors, producing IC_{50} values consistent with literature. Mechanistic analysis of Valaciclovir confirmed a competitive, protonation-sensitive mode of action. Reproducibility studies also confirmed the platform's reliability for day-to-day compound profiling to support lead optimization campaigns.
Together, these findings confirm establishment of a robust, high-throughput compatible assay for hit identification and characterisation of PepT1-modulating compounds, supporting transporter research and early-stage drug discovery.

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