Application Note

Channel: PepT1
Cells: CHO
Tools: SURFE²R N1 & N96

Electrophysiological recordings of PepT1 (SLC15A1) activity on Nanion's SURFE²R

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Summary

The human peptide transporter PepT1 is an uptake transporter responsible for initial absorption and renal reabsorption of dietary oligopeptides[1]. It is primarily located in the plasma membranes of enterocytes of the small intestine as well as the renal proximal tubular cells[1]. PepT1 functions as a co-transporter, coupling the uphill peptide transport into the cells to the electrochemical proton gradient. Due to the movement of protons, PepT1 is an electrogenic transporter. PepT1 shows a very high capacity but a low affinity and substrate specificity[1]. Its ability to transport a large range of compounds has enabled the rational design of drugs and pro-drugs (e.g. penicillins, ACE inhibitors) which have good oral bio-availability using delivery via PepT1[1]. Designing pro-drugs with higher affinity for PepT1 is a successful strategy to increase the bio-availability of poorly absorbed drugs.

Here we present electric real-time PepT1 activity measurements on the SURFE²R instruments using purified plasma membranes of CHO cells over-expressing PepT1. Peptide transport was activated on the SURFE²R N1 using a sensor with attached PepT1-containing membrane fragments which was inserted into the device. This was perfused with a buffer containing the dipeptide glycyl-glycine as the substrate. The data presented here show activation of PepT1 by glycly-glycine and inhibition by Lys[Z(No2)]-Val on the SURFE²R N1 and scale-up of the assay on the SURFE²R N96.

Results

Glycine, which does not act as a substrate for PepT1, was first used as a control in order to separate possible mechanical disturbances (perfusion) from the signal. The substrate, glycyl-glycine, was then added causing proton movement across the membrane which was observed until electrochemical equilibrium was reached (Fig. 1A).

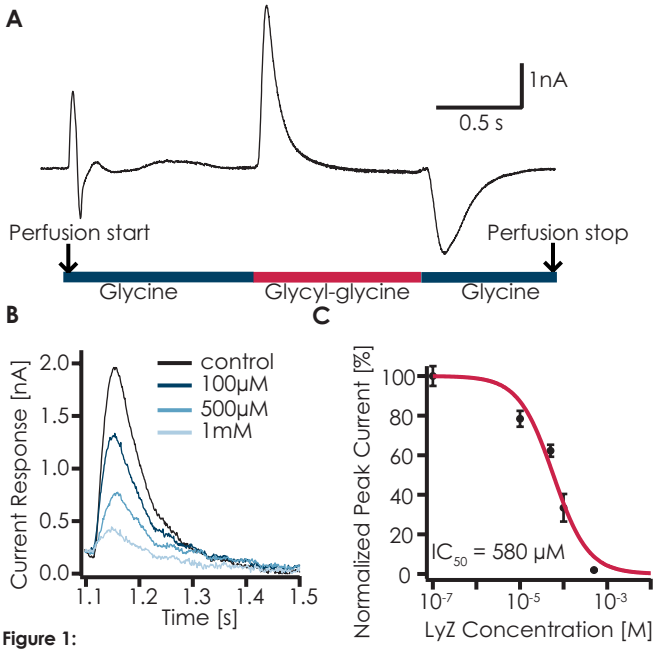

Figure 1:
A PepT1 current responses on the SURFE²R N1 were activated by glycyl-glycine but not glycine. **B** Inhibition of PepT1 proton current by Lys[Z(No2)]-Val. Increasing concentrations of the inhibitor were tested cumulatively on the same population of membrane fragments **C** Concentration response curve for Lys[Z(No2)]-Val giving an IC_{50} of 580 ± 34 µM (n = 5).

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The rationally designed oligopeptide Lys[Z(No2)]-Val was tested as an inhibitor of PepT1. By adding different concentrations of the peptide via the buffer solution an IC_{50} of 580 ± 34 µM (n = 5) was determined (Fig. 1B & C).

To increase throughput, PepT1 measurements were also performed on the SURFE²R N96 in 96-well-plate format (Fig. 2).

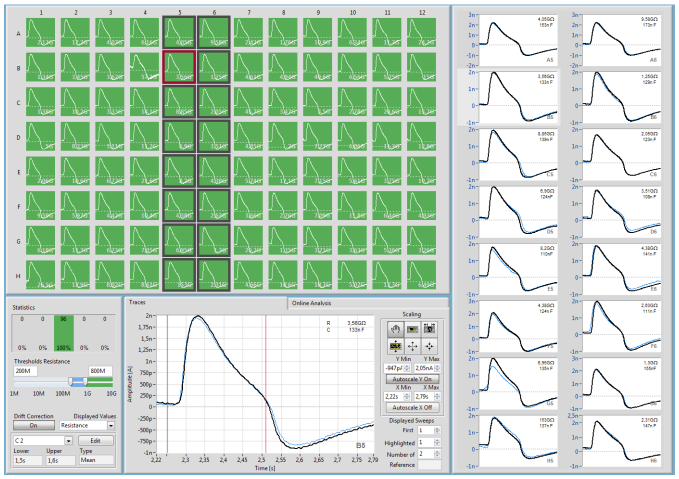

Figure 2:
Screenshot of the data acquisition software of the SURFE²R N96 showing PepT1 activation by glycyl-glycine.

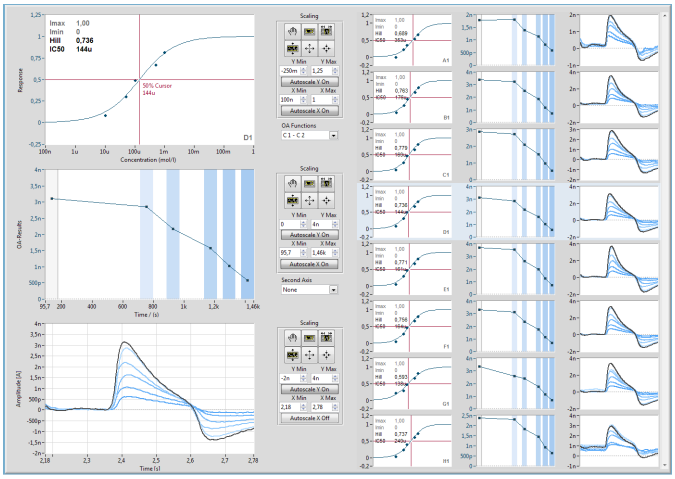

Figure 3:
Screenshot of the analysis software, DataControl 96 showing inhibition of PepT1 by Lys[Z(No2)]-Val. 96 individual concentration-response curves were generated.

PepT1 activity was inhibited by Lys[Z(No2)]-Val on the SURFE²R N96. An individual IC_{50} determination was performed in each well. The data were automatically analyzed with Nanion's DataControl 96 (Fig. 3).

In conclusion, the SURFE²R N1 and N96 platforms can be used to measure activation and inhibition of transporters such as PepT1. The ability to reliably record peptide transporters is critical for the rational design of compounds to improve oral bio-availability.

References

1. Daniel, H & Kottra, G. 2004. Pflugers Arch. 447(5):610-8

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

PepT1 experiments were performed by the exchange of a substrate-free ("control") buffer for a substrate-containing ("activating") buffer. Control buffer contained: 140 mM KCl, 25 mM HEPES, 25 mM MES, 4 mM $MgCl_2$, 20 mM glycine, pH 6.7 with KOH. Activating buffer contained: 140 mM KCl, 25 mM HEPES, 25 mM MES, 4 mM $MgCl_2$, 20 mM Glycyl-glycine, pH 6.7 with KOH. Both buffers were prepared from a 2 x stock solution.

SURFE²R sensor preparation

According to the Nanion's standard procedure "SURFE²R Sensor Preparation". Sensors were prepared in control buffer, membrane was diluted 1:50 with control buffer.

SURFE²R N1 measurement workflow

PepT1 can be activated by providing glycyl-glycine as a substrate, no further driving force is necessary. Therefore any standard two step protocol is suitable.

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